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AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT UNDER THE CARTAGENA PROTOCOL ON BIOSAFETY
First meeting
Montreal, 20-24 April 2009

COMPILATION OF VIEWS AND/OR INFORMATION IN PREPARATION FOR THE AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT

Note by the Executive Secretary

INTRODUCTION

1. At its fourth meeting, the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP), in its decision BS-IV/11, established an Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety. The AHTEG was mandated to meet twice prior to the fifth meeting of the Parties, in October 2010, within an interval of not less than ten months, and perform the tasks outlined in the annex to decision BS-IV/11, in order to achieve the proposed outcomes.

2. In the same decision, the Parties invited Parties, other Governments and relevant organizations to submit to the Executive Secretary information relevant to the work of the AHTEG on Risk Assessment and Risk Management, particularly on existing guidance documents on risk assessment.

3. In light of the above, the Secretariat sent out a notification 1/ to Parties, other Governments and relevant organizations, on 23 October 2008. The compilation of submissions, received by the Secretariat to date, presented in the annex serves as one of the inputs for the deliberations of the AHTEG during its first meeting in Montreal, from 20 to 24 April 2009.

4. The contributions have been reproduced in the form and language in which they were received.

1/ Notification 2008-140 (SCBD/BS/MPDM/ABw/65409).

/…

Annex

COMPILATION OF SUBMISSIONS PROVIDED BY PARTIES, OTHER GOVERNMENTS AND RELEVANT ORGANIZATIONS ON INFORMATION RELEVANT TO THE WORK OF THE AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT

CONTENTS

I. SUBMISSIONS FROM PARTIES

A. EUROPEAN COMMUNITY

EU2009.CZ

České předsednictví v Radě EU

Czech Presidency of the Council of the EU

Présidence tchèque du Conseil de l'UE

2009

Prague, 29 January 2009

Risk Assessment and Risk Management

First meeting of AHTEG on Risk Assessment and Risk Management

EU coordinated response to the notification No. 2008-140:

General guidance

- Directive 2001/18/EC of 12 March 2001 on the deliberate release of genetically modified organisms and repealing Council Directive 90/220/EEC, including Annex II (Principles for environmental risk assessment), Annex VI (Guidelines for assessment report) and Annex VII (Monitoring plan).
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II (Principles for environmental risk assessment) to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.
- Guidance document of the Scientific Panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal (2006) 99,1-100, updated in 2008.
- Guidance document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use by the Scientific Panel on Genetically Modified Organisms (GMO) EFSA Journal (2006) 374,1-115.
- Codex Alimentarius. Codex principles and guidelines on foods derived from biotechnology(2003):

  Principles for the risk analysis of foods derived from modern biotechnology, CAC/GL 44-2003

  Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms, CAC/GL 46-2003

Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants CAC/GL 45-2003, including Annex 1 (Assessment of possible allergenicity), Annex 2 (Food safety assessment of foods derived from recombinant-DNA plants modified for nutritional and health benefits) and Annex 3 (Food safety assessment in situations of low-level presence of recombinant-DNA plant material in food), (2003, Annexes 2 and 3 adopted 2008).

Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals, CAC/GL 68-2008

http://www.fao.org/ag/agn/agns/biotechnology_detection_en.asp

http://www.fao.org/ag/agn/agns/biotechnology_labelling_en.asp.

- Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation: Rome.
- Communication from the Commisssion on the Precautionary Principle, COM (2000) 1 final. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:52000DC0001:EN:HTML
- Points to Consider for Consensus Documents on the Biology of Cultivated Plants No 35, 2006, ENV/JM/MONO(2006)1
- An Introduction to the Biosafety Consensus Documents of OECD's Working Group for Harmonisation in Biotechnology No. 32, 2005, ENV/JM/MONO(2005)5
- Revised 2006: OECD Guidance for the Designation of a Unique Identifier for Transgenic Plants No. 23, 2006, ENV/JM/MONO(2002)7/REV1
- OECD, 1993. Safety Considerations for Biotechnology: Scale-up of Crop Plants
- an den Eede et al. (2004): The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. Food and Chemical Toxicology 42, 1127-1156

Guidance on specific aspects of the risk assessment process,

- Module II: Herbicide Biochemistry, Herbicide Metabolism and the Residues in Glufosinate-Ammonium (Phosphinothricin)-Tolerant Transgenic Plants No. 25, 2002, ENV/JM/MONO(2002)14
- Consensus Document on General Information Concerning the Genes and Their Enzymes that Confer Tolerance to Phosphinothricin Herbicid No. 11, 1999, ENV/JM/MONO(99)13

- Consensus Document on General Information Concerning the Genes and Their Enzymes that Confer Tolerance to Glyphosate Herbicide No. 10, 1999, ENV/JM/MONO(99)9
- Consensus Document on General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection No. 5, 1996, OCDE/GD(96)162
- Nelson, K.C.; Banker, M.J. Problem formulation and options assessment handbook. 2007, A publication of the GMO ERA Project
- Cellini et al. (2004): Unintended effects and their detection in genetically modified crops. Food and Chemical Toxicology 42, 1089-1125

Guidance on specific types of GMOs.

- Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events by the Scientific Panel on Genetically Modified Organisms (GMO) EFSA Journal (2007) 512,1-5.
- Consensus Document on Safety Information on Transgenic Plants Expressing *Bacillus thuringiensis* - Derived Insect Control Protein No. 42, 2007, ENV/JM/MONO(2007)14
- Consensus Document on the Biology of the Native North American Larches: Subalpine Larch (*Larix Lyalli*), Western Larch (*Larix occidentalis*) and Tamarack (*Larix laricina*) No. 41, 2007, ENV/JM/MONO(2007)7
- Consensus Document on Biology of *Pinus banksiana* (Jack Pine) No 40, 2006, ENV/JM/MONO(2006)28
- Consensus Document on Information Used in the Assessment of Environmental Applications Involving *Acidithiobacillus.* No 37, 2006, ENV/JM/MONO(2006)3
- Consensus Document on the Biology of *Capsicum annuum* Complex (Chili Peppers, Hot Peppers and Sweet Peppers) No 36, 2006, ENV/JM/MONO(2006)2
- Consensus Document on the Biology of *Pleurotus* spp. (Oyster Mushroom) No. 34, 2005, ENV/JM/MONO(2005)17
- Consensus Document on the Biology of Papaya (*Carica papaya*) No. 33, 2005, ENV/JM/MONO(2005)16
- Consensus Document on the Biology of *Helianthus annuus* L. (Sunflower) No. 31, 2004, ENV/JM/MONO(2004)30

- Consensus Document on the Biology of European White Birch (*Betula pendula* Roth) No. 28, 2003, ENV/JM/MONO(2003)12
- Consensus Document on the Biology of *Zea mays* (Maize) No. 27, 2003, ENV/JM/MONO(2003)11
- Consensus Document on the Biology of *Prunus* spp. (Stone Fruits) No. 24, 2002, EN/JM/MONO(2002)13
- Consensus Document on the Biology of *Pinus strobus* L. (Eastern White Pine) No. 22, 2002, ENV/JM/MONO(2002)3
- Consensus Document on the Biology of *Picea sitchensis* (Bong.) Carr. (Sitka Spruce) No. 21, 2002, ENV/JM/MONO(2002)2
- Consensus Document on Information Used in the Assessment of Environmental Applications Involving Baculoviruses No. 20, 2002, ENV/JM/MONO(2002)1
- Consensus Document on the Biology of *Beta vulgaris* L. (Sugar Beet) No. 18, 2001, ENV/JM/MONO(2001)11
- Consensus Document on the Biology of *Populus* L. (Poplars) No. 16, 2000, ENV/JM/MONO(2000)10
- Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean) No. 15, 2000, ENV/JM/MONO(2000)9
- Consensus Document on the Biology of *Oryza sativa* (Rice) No. 14, 1999, ENV/JM/MONO(99)26
- Consensus Document on the Biology of *Picea glauca* (Moench) Voss (White Spruce) No. 13, 1999, ENV/JM/MONO(99)25
- Consensus Document on the Biology of *Picea abies* (L) Karst (Noway Spruce) No. 12, 1999, ENV/JM/MONO(99)14
- Consensus Document on the Biology of *Triticum aestivum* (Bread Wheat) No. 9, 1999, ENV/JM/MONO(99)8
- Consensus Document on the Biology of *Solanum tuberosum* subsp. *tuberosum* (Potato) No. 8, 1997, OCDE/GD(97)143
- Consensus Document on the Biology of *Brassica napus* L. (Oilseed rape) No. 7, 1997, OCDE/GD(97)63
- Consensus Document on information Used in the Assessment of Environmental Applications Involving *Pseudomonas* No. 6, 1997, OCDE/GD(97)22

B. JAPAN

-----Original Message-----
Sent: Monday, January 19, 2009 8:18 AM
Subject: Japanese guidance documents on risk assessment
SCBD/BS/MPDM/ABw/65409

SCBD Madam/Sir,

In response to the SCBD notification Ref: SCBD/BS/MPDM/ABw/65409 which invited Parties to submit information on existing guidance documents on risk assessment as one of the inputs for the deliberations of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management, I send Japanese guidance documents on risk assessment.
Please find attached files;

(1)The Guidance of Implementation of Assessment of Adverse Effect on Biological Diversity of Type 1 Use of Living Modified Organisms

(2) Document Concerning the Application for Approval of Type 1 Use Regulations with regard to the genetically modified plants, the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries

(3) Document Concerning the Application for Approval of Type 1 Use Regulations with regard to the genetically modified live vaccines, the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries

- More information about Japanese law and regulations;
http://www.bch.biodic.go.jp/english/law.html

- List of approved LMOs and assessment documents;
http://www.bch.biodic.go.jp/english/lmo.html

Best Regards,
Kyoko NODA

.....
Ms. Kyoko NODA

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The Guidance of Implementation of Assessment of Adverse Effect on Biological Diversity of Type 1 Use of Living Modified Organisms

(Tentative Translation)

[1] Object

This guidance stipulates necessary matters to ensure that the assessment of Adverse Effect on Biological Diversity to be performed by a person who wishes to obtain approval under the provisions of Article 4 paragraph 2 of the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (hereinafter "the Law") can be performed scientifically and correctly and that the Biological Diversity Risk Assessment Report showing the result can be prepared correctly.

This guidance shall be reviewed as occasion demands, with future amplification of scientific knowledge on the adverse effects of living modified organisms on biological diversity and international trends concerning the assessment of the adverse effects of living modified organisms on biological diversity taken into account.

[2] Information Necessary for Assessment of Adverse Effect on Biological Diversity

The assessment of Adverse Effect on Biological Diversity shall be carried out by collecting information shown in Table 1 and using the collected information. Nevertheless, if there is a rational reason for not using part of the information shown in Table 1, such information need not be collected.

In case the need to collect other information than that shown in Table 1 arises during the process of the assessment in accordance with the procedure of assessment of Adverse Effect on Biological Diversity shown in Table 3, the assessment shall be carried only after additional collection of said information.

[3] Items and Procedure of Assessment of Adverse Effect on Biological Diversity

The assessment of Adverse Effect on Biological Diversity shall be carried out for each of the items listed in the left-hand column in Table 2 for the appropriate category of living modified organisms listed in the right-hand column in Table 2, in accordance with the procedure of assessment of Adverse Effect on Biological Diversity shown in Table 3, and it shall be judged comprehensively on the basis of the assessment of each item whether Adverse Effect on Biological Diversity could arise or not..

[4] Description in the Biological Diversity Risk Assessment Report

The Biological Diversity Risk Assessment Report shall be written in the order of items provided in Table 4.

Table 1 (Related to [2])

1. Information concerning a recipient organism (that is, a living organism in which nucleic acid, or its replicated product, obtained by using technology provided in Article 2 paragraph 2 subparagraph 1 of the Law is transferred; the same applies to the rest of this guidance) or the species to which the recipient organism belongs:

(1) Taxonomical position and state of distribution in natural environment

(2) History and present state of Use

(3) Physiological and ecological properties

A. Basic properties

B. Environmental conditions allowing inhabiting or growth

C. Predacity or parasitism

D. Mode of propagation or reproduction

E. Pathogenicity

F. Productivity of harmful substances

G. Other information

2. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid (that is, nucleic acid or replicated product thereof, obtained by using technology provided in Article 2 paragraph 2 subparagraph 1 of the Law, excluding nucleic acid and its replicated products which replicate, in the recipient organism transferred, the whole or a part thereof (hereinafter "vector"); the same applies to the rest of this guidance).

A. Composition and origins of component elements

B. Functions of component elements

(2) Information concerning vector

A. Name and origin

B. Properties

(3) Method of preparing living modified organisms

A. Structure of the entire nucleic acid transferred in recipient organism

B. Method of transferring nucleic acid transferred in recipient organism

C. Processes of rearing of living modified organisms

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

(6) Difference from the recipient organism or the species to which the recipient organism belongs

3. Information concerning the Use of living modified organisms

(1) Content of the Use

(2) Method of the Use

(3) Method of collecting information by person who wishes to obtain approval after the start of Type 1 Use

(4) Emergency measures which should be taken to prevent Adverse Effect on Biological Diversity in case Adverse Effect on Biological Diversity could arise

(5) The results of Use in laboratory or Use in similar environment to the environment in which Type 1 Use is intended (in principle, to be carried out for an appropriate period that is commensurate with its life-cycle or generation time of the living modified organisms)

6. Information obtained from Use abroad

Table 2 (Related to [3])

Category of Living Modified Organisms	Assessment Items (Property of living modified organisms which might cause Adverse Effect on Biological Diversity)
Plants (living organisms belonging to Plantae and mushroom belonging to Fungi)	Competitiveness (Property of competing against wild plants for resources such as nutrients, sunshine, habitat, etc. and interfering with their growth)
	Productivity of harmful substances (Property of producing substances interfering with the living and growth of wild plants or animals, or microorganisms (hereinafter "wildlife")
	Crossability (Property of hybridizing with related wild plants and transmitting nucleic acid transferred by the technologies regulated by the Law to them)
	Other properties (Properties other than those mentioned above, such as one which indirectly affects wildlife by changing the base of the ecosystem, which are considered to require an assessment of Adverse Effect on Biological Diversity)
Animals (living organisms belonging to Animalia)	Competitiveness (Property of competing against wild animals for resources such as food, nesting places and habitats, etc. and interfering with their living)
	Predacity or parasitism (Property of interfering with living or growth of wildlife by preying upon them or by being parasitic on them)
	Productivity of harmful substances (Property of producing substances interfering with living or growth of wildlife)
	Crossability (Property of hybridizing with related wild animals and transmitting nucleic acid transferred by the technologies regulated by the Law to them)
	Other properties (Properties other than those mentioned above, such as one which indirectly affects wildlife by changing the base of the ecosystem, which are considered to require an assessment of Adverse Effect on Biological Diversity)
Microorganisms (living organisms belonging to Fungi [excluding mushroom], those belonging to the Protista, viruses and viroids)	Property of reducing other microorganisms (Property of reducing other microorganisms by competition, productivity of harmful substances, etc.)
	Pathogenicity (Property of interfering with living or growth of wild plants or animals by infecting them)
	Productivity of harmful substances (Property of producing substances interfering with living or growth of wild plants or animals)
	Property of transmitting nucleic acid horizontally (Property of transmitting nucleic acid being transferred by the technologies regulated by the Law to wild plants and animals and other microorganisms)

	Other properties (Properties other than those mentioned above, such as one which indirectly affects wildlife by changing the base of the ecosystem, which are considered to require an assessment of Adverse Effect on Biological Diversity)

Table 3 (Related to [3])

Procedure of Assessment of Adverse Effect on Biological Diversity	Method of Implementing the Assessment
1. Identification of wildlife likely to be affected	Types of wildlife assumed to be affected by the properties of living modified organisms mentioned under assessment items in the right-hand column of Table 2 shall be identified by taxonomical categories and other genetic characters. If the species of pertinent wildlife are large in number, some species of wildlife deemed to be appropriate as the subject in carrying out the assessment shown in Procedure 2-4 may be selected in consideration of the growth and living environment of those species, their sensitivity to harmful substances produced by living modified organisms for Type 1 Use, relatedness to living modified organisms, etc. Nevertheless, if Japan has experience in the long-term use of the recipient organism of the living modified organism or the species to which the recipient organism belongs, and if there is no difference between the properties of the living modified organism mentioned under the assessment item in the right-hand column of Table 1 and those of the host or the species to which the host belongs, the wildlife likely to be affected need not be specified.
2. Evaluation of concrete details of adverse effect	Concrete details of adverse effect of living modified organism on wildlife identified or selected in Procedure 1 shall be evaluated, for example, by conducting experiments on reaction of individuals of the wildlife and collecting relevant information.
3. Evaluation of likelihood of adverse effect	The likelihood of adverse effect on wildlife identified or selected in Procedure 1 caused by living modified organism in carrying out Type 1 Use in accordance with Type 1 Use regulations shall be evaluated while collecting information on the places or periods of time of living or growth of said wildlife and other pertinent matters.
4. Judgment of existence of Adverse Effect on Biological Diversity	Whether the preservations of the species or population of the wildlife might be impaired or not shall be judged. If Japan has experience in the long-term use of the recipient organism of living modified organisms or the species to which the recipient organism belongs, judgment may be based on whether the degree of adverse effect is higher compared to that of the recipient organism or the species to which the recipient organism belongs.

Table 4 (Related to [4])

1. Information collected prior to assessing Adverse Effect on Biological Diversity

Information collected under the provisions of [2] shall be mentioned according to the items shown in Table 1. When this is done, sources of the information shall be indicated clearly (if the information is based on knowledge or experience of experts or persons performing assessment, such fact should be mentioned).

2. Item-by-item Assessment of Adverse Effect on Biological Diversity

The content of assessment carried out according to the procedure of assessment of Adverse Effect on Biological Diversity as set forth in Table 3 shall be described for each assessment item shown in Table 2. When this is done, sources of information used in carrying out the assessment shall be indicated clearly (if the information is based on knowledge or experience of experts or persons performing assessment, such fact should be mentioned clearly). For any judgment made by a person who performs assessment, the grounds for said judgment should be clarified.

3. Comprehensive assessment of Adverse Effect on Biological Diversity

An outline of the result of assessment for each item of Table 2 and the result of comprehensive judgment that takes such assessment results into account shall be mentioned.

Concerning the Application for Approval of Type 1 Use Regulations with regard to the genetically modified plants, the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries

I. Objectives

Among genetically modified organisms, with regard to the ones belonging to Plantae (excluding algae; hereinafter referred to as “genetically modified plants”), the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries, Application for Approval of Type 1 Use Regulations under the provisions of Article 4 paragraph 2 of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Genetically Modified Organisms (Act No.97 of 2003; hereinafter referred to as the “Act”) shall be in accordance with the matters and items specified in this notification in addition to those stipulated in the Regulations related to the Enforcement of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Genetically Modified Organisms (Ministerial Ordinance No.1 of 2003 from the Ministry of Finance; Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labour and Welfare; Ministry of Agriculture, Forestry and Fisheries; Ministry of Economy, Trade and Industry; and Ministry of the Environment; hereinafter referred to as "Regulations related to the Enforcement of the Act"), the Ministerial Notification No.1 from the Ministry of Finance; Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labour and Welfare; Ministry of Agriculture, Forestry and Fisheries; Ministry of Economy, Trade and Industry; and Ministry of the Environment, dated November 21, 2003 (Basic Matters under the Provisions of Article 3 of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Genetically Modified Organisms; hereinafter referred to as "Basic Matters") and the Ministerial Notification No.2 from the Ministry of Finance; Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labour and Welfare; Ministry of Agriculture, Forestry and Fisheries; Ministry of Economy, Trade and Industry; and Ministry of the Environment, dated November 21, 2003 (Implementation Guidance for Assessment of Adverse Effect on Biological Diversity of Type 1 Use of Genetically Modified Organisms; hereinafter referred to as “Implementation Guidance”).

The matters and items mentioned herein shall be reviewed as occasion demands, with future amplification of scientific knowledge on the adverse effects on biological diversity caused by Type 1 Use of genetically modified organisms and/or international trends concerning the assessment or control of the adverse effects of genetically modified organisms on biological diversity taken into account.

II. Matters and items concerning the procedures for Application for Approval of Type 1 Use Regulations

1. Destination of submission of an application and other documents

The destination of an application and other documents stipulated in the provisions of Article 41 paragraph 1 of the Regulations related to the Enforcement of the Act shall be Plant Products Safety Division, Food Safety and Consumer Affairs Bureau, the Ministry of Agriculture, Forestry and Fisheries. In addition, any electromagnetic records holding the

contents of the application and other documents, if available, shall be submitted together with the written application and other documents.

2. Hearing by academic experts

When hearing the opinions pursuant to Article 4 paragraph 4 of the Act about the documents submitted, a review board (hereinafter referred to as the "review board") shall be set up, comprising persons with special knowledge and experience who are listed in the register of names of experts prepared and announced under the provisions of Article 10 of the Regulations related to the Enforcement of the Act (hereinafter referred to as "Experts").
This review board shall be held under the direction of the Director-General of Agriculture, Forestry and Fisheries Research Council, the Ministry of Agriculture, Forestry and Fisheries and the Director-General of Nature Conservation Bureau, the Ministry of the Environment.

3. Explanation by an applicant of an application and other documents

The review board mentioned in 2 above can ask a person who wishes to file an Application for Approval of Type 1 Use Regulation (hereinafter referred to as "Applicant") as necessary to explain his/her application and other documents and answer any questions from Experts.

4. Normal processing period

The normal processing period from submission of an application and other documents to the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment through to the approval granted under the provisions of Article 4 paragraph 5 of the Act, the instruction provided under the provisions of Article 5 paragraph 1 of the Act, or the rejection given under the provisions of Article 5 paragraph 3 of the Act (including the mutatis mutandis application stipulated in Article 9 paragraph 4 of the Act) shall be six (6) months. However, this normal processing period precludes the period of time required for Applicant to amend any inadequacy in the submitted application and other documents and/or the period of time required for Applicant to submit any additional information or documents instructed based on the consultation with Experts.

5. Inquiry about the scope of living organism and technology covered by the Act

If Applicant is hard to determine whether or not the genetically modified plants pertaining to Application for Approval of Type 1 Use Regulation fall under the cells stipulated in Article 1 of the Regulations related to the Enforcement of the Act and whether or not the technologies used to obtain the genetically modified plants fall under the technologies stipulated in Article 2 and 3 of the Regulations related to the Enforcement of the Act, and/or he/she wishes to inquire about some matters regarding Application, he/she shall consult with the Plant Products Safety Division, Food Safety and Consumer Affairs Bureau, the Ministry of Agriculture, Forestry and Fisheries.

III. Matters concerning the contents of an application and other documents

1. Common items

(1) Unit of Application

For the genetically modified plants among those derived from the genetically modified plants that contain the nucleic acid obtained with use of the technologies stipulated in Article 2 of the Regulations related to Enforcement of the Act, which are difficult to distinguish from the original genetically modified plants even by identification of copies of the nucleic acid and the neighboring nucleic acids but are able to be subjected to Assessment of Adverse Effect on Biological Diversity jointly in consideration of the extent of variations in physiological and ecological characteristics, Application for Approval of Type 1 Use Regulations shall be

submitted at once as single unit [excluding the isolated filed tests stipulated in III-2-(2) and III-3-(2)]. The person obtaining approval shall endeavor to collect information concerning any genetically modified plants which exhibit the physiological or ecological characteristics that exceed the extent of variation in physiological or ecological characteristics taken into account prior to the approval among the genetically organisms raised with use of the genetically organisms having obtained approval for Type 1 Use Regulation [excluding those, in the process of breeding of which the technologies stipulated in Article 2 and Article 3 of the Regulations related to the Enforcement of the Act have been used: Referred to as progeny in III-1-(2)], and he/she shall report the Plant Products Safety Division, Food Safety and Consumer Affairs Bureau, the Ministry of Agriculture, Forestry and Fisheries of the information, if obtained, that the said genetically modified plants have been raised.

(2) Application of stack lines

For every stack line (referring to a line to be raised by crossing different genetically modified plants) which is raised by cross-breeding only the genetically modified plants that have obtained approval of Type 1 Use Regulation, every inter-specific cross line (referring to a line to be raised by crossing the plants which belong to different taxonomical species from each other) which is raised by crossing any genetically modified plants that have obtained approval of Type 1 Use Regulation and any plants other than genetically modified plants, and other such progeny line except those which have been approved as a single unit of application under the provisions of III-1-(1), Approval of Type 1 Use Regulation shall be obtained.

(3) Information required to be further collected in the process of Assessment of Adverse Effect on Biological Diversity

If any wildlife deemed likely to be affected is identified in the process of implementing the assessment in accordance with the Procedure of Assessment of Adverse Effect on Biological Diversity stipulated in Table 3 attached to the Implementation Guidance, then scientific information concerning the adverse effects shall be collected by conducting experiments on reaction of the individuals of the wildlife and by collecting information on the places or periods of time of living or growth of said wildlife, in addition to the information stipulated in Attached Table 1 of the Implementation Guidance. Based on the information, assessment shall be conducted and the result of the assessment shall be submitted in conjunction (for example, where assessment of details of adverse effect regarding the productivity of harmful substances is to be conducted, bioassay and/or other tests shall be implemented as required using the wildlife identified as likely to be affected).

(4) Plan of Emergency Measure

Applicant shall have established proper measures in advance, which are useful for efficient prevention of Adverse Effect on Biological Diversity, which he/she could take within his/her ability in cases when Adverse Effect on Biological Diversity is feared to arise due to Type 1 Use concerning the application (hereinafter referred to as “Emergency Measure”). Then Applicant shall draw up a plan including the matters and items (hereinafter referred to as “Plan of Emergency Measure”) and attach it to the application form.

(i) Implementation system and a responsible person
(ii) Methods for identifying the status of Type 1 Use pertaining to the Application [excluding the isolated field tests stipulated in III-2-(2) and III-3-(2)]
(iii) Methods to ensure that a person who makes Type 1 Use pertaining to the Application is informed well of the contents of Emergency Measure to be taken
(vi) Concrete details of measures for inactivation of genetically modified plants pertaining to the Application (artificially transforming the genetically modified plants to any organisms other than the cell stipulated in Article 1 of the Regulations related to the Enforcement of the Act; the same applies to the rest of this Notification) or taking the containment measures and continuing the use of the genetically modified plants

pertaining to the Application (only when the containment measures to be taken have been established in advance in accordance with the Act)

(v) Methods for contact with the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment

(vi) Other necessary information

(5) Monitoring Plan

(i) Cases requiring the Monitoring Plan

In any of the cases that fall under the following (a) or (b), Applicant shall draw up a plan for monitoring (referring to the investigations on the presence or absence of adverse effects of Type 1 Use pertaining to the Application on wildlife and concrete details of adverse effects, if present; the same applies to the rest of this Notification) (hereinafter referred to as "Monitoring Plan") and attach it to the application form.

Even in cases that do not fall under the following (a) or (b), if experts, together with specific assessment items, advise the necessity of monitoring in the process of application reviews, the Applicant shall draw up a monitoring plan and attach it to the application form.

(a) Cases where Adverse Effect on Biological Diversity is to be prevented by regulating the methods of Type 1 Use pertaining to the Application [excluding the isolated field tests which do not fall within the cases in 2-(7) in Attached Table 3 among those stipulated in III-2-(2) and which do not fall within the cases in 2-(8) in Attached Table 6 among those stipulated in III-3-(2)].

(b) Cases where Applicant has decided to implement Monitoring by himself/herself to prevent Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the Application.

(ii) Matters and items to be mentioned in Monitoring Plan

Monitoring Plan shall include the matters and items listed below.

(a) Implementation system and a responsible person

(b) The name of the type of wild animals and wild plants subject to Monitoring

(c) Places subject to Monitoring and the living or growth conditions of the wild animals and wild plants concerned in the places

(d) Periods of time of Monitoring

(e) Monitoring methods, eg. time of implementation, frequency, and etc.

(f) Methods of analysis of the result of Monitoring

(g) Methods for reporting the result to the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment

(h) Other necessary matters and items

(6) Information collection in isolated fields

In the cases of Type 1 Use of those genetically modified plants, for which a good deal of findings have been acquired on the characteristics based on the results of use in laboratory or use under natural conditions in foreign countries but the characteristics in the growth under natural conditions in Japan have not yet been clarified from the scientific point of view, information shall be collected pertaining to use in a similar environment to the one in which Type 1 Use stipulated in Basic Matters 1-1-(1)-(a)-iv is intended and the characteristics when those genetically modified plants grown under natural conditions in Japan shall be clarified.

Said collection of information shall be carried out in isolated fields [referring to the facilities meeting the requirements listed in Attached Table 3 for genetically modified crops (genetically modified plants for agricultural crops; the same shall apply hereinafter) and the facilities meeting the requirements listed in Attached Table 6 for genetically modified trees (genetically modified woody plants excluding agricultural crops)].

2. Matters and items concerning the application for approval of genetically modified crops

(1) Matters and items concerning the Report on the Assessment of Adverse Effects on Biological Diversity

(i) Information collection and items to be included in the Assessment Report

When formulating the Biological Diversity Risk Assessment Report stipulated in Article 4 paragraph 2 of the Act (hereinafter referred to as the "Assessment Report"), specific details of information in Attached Table 1 of the Implementation Guidance and the specific method of collecting information in Attached Table 4-1 of the Implementation Guidance shall be as shown in the right column content of Attached Table 1 specified by individual type in the left column of the table. However, where there is a rational reason for not using part of the information in the right column of the table, such information need not be collected.

(ii) Method for collecting information

Those concrete details of the information listed in the left column of Attached Table 2 among those stipulated in the right column of Attached Table 1 shall be collected based on the analysis or investigation listed in the right column of Attached Table 2. However, more appropriate methods than those stipulated in the right column of Attached Table 2, if found to exist, may be utilized. For each analysis or investigation carried out based on corresponding methods, reference materials including involved test samples, procedures, results, discussion and other information shall be attached to the Assessment Report.

(2) Application for tests in isolated fields

With respect to application for tests in isolated fields (referring to Type 1 Use in isolated fields listed in III-1-(6); the same shall apply hereinafter), the statement "cultivation, storage, transportation, disposal and acts incidental to them in isolated fields" shall be entered in the section on Content of Type 1 Use of Living Modified Organisms in the Application Form for Approval of Type 1 Use (hereinafter referred to as "Content of Type 1 Use") as stipulated in Article 7 of the Regulations related to the Enforcement of the Act, and concrete details of the facilities concerned and the working procedures shall be described in the section on Method of Type 1 Use of Living Modified Organisms (hereinafter referred to as "Method of Type 1 Use") in the said Application Form.

(3) Application for approval of any genetically modified crops which are not intended for cultivation

A person who wishes to make an application for approval of any genetically modified crops, which are not intended for cultivation, shall estimate the degree of possible mixing with seeds for cultivation, when the mixing is expected unavoidable, in the Assessment of Adverse Effect on Biological Diversity prior to the application, taking into account the actual conditions of production and distribution of the genetically modified crops, and also evaluate Adverse Effect on Biological Diversity arising from the mixing. In addition, in this case, the block of Content of the Type 1 Use of Genetically Modified Organism shall include the statement of including cultivation caused by mixing to the extent of the estimated degree of mixing (If any genetically modified crops, which have obtained approval of Type 1 Use Regulation not involving cultivation in the Content of the Type 1 Use, become mixed with seeds for cultivation and the seeds are cultivated, it means the Type 1 Use not stipulated in the Type 1 Use Regulation is to be implemented and then it is subject to the order of recall).

3. Matters and items concerning the application for approval of genetically modified trees

(1) Matters and items concerning the Statement of the Assessment of Adverse Effect on Biological Diversity

(i) Information collection and items to be included in the Assessment Report

When formulating the Assessment Report, specific details of information in Attached Table 1 of the Implementation Guidance and the specific method of collecting information in Attached Table 4-1 of the Implementation Guidance shall be as shown in the right column content of Attached Table 4 specified by individual type in the left column of the table. However, where there is a rational reason for not using part of the information in the right column of the table, such information need not be collected.

(ii) Method for collecting information

Those concrete details of the information listed in the left column of Attached Table 5 among those stipulated in the right column of Attached Table 4 shall be collected based on the analysis or investigation listed in the right column of Attached Table 5. However, more appropriate methods than those stipulated in the right column of Attached Table 5, if found to exist, may be utilized. For each analysis or investigation carried out based on corresponding methods, reference materials including involved test samples, procedures, results, discussion and other information shall be attached to the Assessment Report.

(2) Application for tests in isolated fields

With respect to application for tests in isolated fields, in the section of Content of Type 1 Use, the statement "cultivation, storage, transportation, disposal and acts incidental to them in isolated fields" shall be entered, and in the section of Method of Type 1 Use, concrete details of the facilities concerned and the working procedures shall be mentioned.

When there are rational reasons for not taking measures prescribed in the Attached Table 6-2-(1), documents listing the reasons shall be attached to the application.

IV. Matters concerning Organization of the Implementation System for Type 1 Use

1. Setup of a committee

A person, who intends to obtain Approval of Type 1 Use Regulation with any limited methods of Type 1 Use, shall endeavor to set up a committee to discuss the matters concerning prevention of Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the Application in accordance with the provisions of II-2 of Basic Matters (hereinafter referred to as "Committee"), and he/she shall submit the register of names of committee members in conjunction with the application and other documents when the Committee is set up.

2. Composition of Committee

The Committee described in the above 1 shall endeavor to select the members among those listed below. In addition, when Applicant is a corporation, it is preferably whenever possible that members are selected from other than those who belong to the corporation.

(1) Persons with special knowledge and expertise concerning the characteristics of the genetically modified plants pertaining to the application

(2) Persons with special knowledge and expertise concerning the actual conditions of Type 1 Use including use, breeding, and transportation of the genetically modified plants pertaining to the application

(3) Persons with special knowledge and expertise concerning the wild animals and wild plants likely to be affected by Type 1 Use pertaining to the application and the ecological systems

(4) Persons in charge of management of the places where Type 1 Use pertaining to the application is implemented

3. Matters to be considered at Committee

Committee considers the matters listed below.

(1) Method of Type 1 Use pertaining to the application
(2) Content of Monitoring Plan
(3) Content of Plan of Emergency Measure
(4) Judgment whether or not there is the likelihood of Adverse Effect on Biological Diversity
(5) Method of educational training for persons making Type 1 Use pertaining to the application
(6) Other matters concerning prevention of Adverse Effect on Biological Diversity due to Type 1 Use pertaining to the application

4. Designation of a management representative and a management supervisor

A person, who wishes to obtain Approval of Type 1 Use Regulation under limited method of Type 1 Use shall act to perform the roles listed below; he/she shall have a thorough knowledge of the laws and regulations concerning the use of genetically modified plants, he (she) shall designate a management representative and a management supervisor who assists the management representative from those who have experience in Type 1 Use of genetically modified plants, and he/she shall assign these positions to the every establishment performing isolated field tests in the case of isolated field tests and the major business establishment in the cases other than Type 1 Use mentioned on the above.

(1) Implement educational training for persons who are engaged in Type 1 Use pertaining to the application.
(2) Conduct monitoring in accordance with a Monitoring Plan, if established.
(3) Take Emergency Response Measures in accordance with The Plan of Emergency Measure if Adverse Effect on Biological Diversity is feared to arise.
(4) Perform the maintenance on the facilities, if any, for prevention of Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the application.
(5) Record the progress of Type 1 Use in the case of isolated field tests and maintain the records.
(6) Verify that Information on Correct Use is correctly provided to any person who receives transfer or supply of genetically modified plants pertaining to the application or who receives entrustment to make Type 1 Use in cases of Type 1 Use other than that at isolated field tests and where Information on Correct Use is stipulated.

Attached Table 1 (Related to III-2-(1)-(i) Matters and items concerning the details of collecting information pertaining to genetically modified crops and filling of the Assessment Report)

Items included in Table 1 attached to the Implementation Guidance	Concrete details of the information and concrete method for describing information in Assessment Report
1. Information concerning a recipient organism or the species to which the recipient organism belongs	For the items (1) through (3) in the left column [excluding (1)-(ii) in the right column], information concerning the taxonomical species to which the recipient organism belongs (the taxonomic rank when the scope of application unit covers any sub-species or any taxonomic rank lower than the species to which the recipient organism belongs; the same applies to the rest of this Table) shall be collected. When Consensus Documents for the Work on Harmonization of Regulatory Oversight in Biotechnology developed by the Environment Directorate of Organization for Economic Co-operation and Development (OECD) exists for the information (Visit the web site at http://www.oecd.org/document/51/0,2340,en_2649_34385_1889395_1_1_1_1,00.html), the information shall be written in Assessment Report of Adverse Effect on Biological Diversity based on the contents of that information in the Consensus Documents.
(1) Taxonomical position and state of distribution in natural environment	(i) Japanese name, English name, and Scientific name [Names in Current Use for Extant Plant Genera, The International Plant Names Index (http://www.ipni.org/index.html), "Wild plants in Japan" (edited by Yoshisuke Satake et. al) and/or other such widely used classification system shall be used and the sources used shall be noted.] (ii) Name of varieties of the recipient organism [including Registration No. and Date of Registration if the varieties has been registered in accordance with the Seeds and Seedlings Act (Act No.83 of 1998)] or name of the line concerned (iii) Wild habitat under natural environment (including the information regarding the center of the origin and/or the center of genetic diversity, if they have been identified, and the information regarding any area where the recipient organism have had an effect on the biological diversity as introduced species and the degree of the effect)
(2) History and present state of Use	(i) History of Type 1 Use both at home in Japan and abroad (ii) Main cultivation areas, cultivation methods, state of circulation and uses
(3) Physiological and ecological properties	For the following items 3- (a) through (g) in the left column, physiological and ecological characteristics shall be mentioned whenever possible under similar natural conditions as those in Japan.

(a) Basic properties	Morphological characteristics, classification as annual, biennial or perennial and other basic properties if there is no prolonged experience of Type 1 Use in Japan
(b) Environmental conditions allowing inhabiting or growth	Temperature range, moisture condition and soil condition allowing growth
(c) Predacity or parasitism	-
(d) Mode of propagation or reproduction	(i) Shedding habit, mode of dispersion, dormancy and longevity of the seed (ii) Mode of vegetative propagation (sucker, tuber, tuberous root, runner, etc.) and the property of budding from any tissue or organ which could regenerate the plant body under natural conditions (iii) The degree of autogamy and allogamy, presence or absence of self-incompatibility, crossability with wild relative, and the degree of apomixes causing characteristics, if present (iv) Production, fertility, shape, method of pollination, dispersal distance and longevity of pollen
(e) Pathogenicity	-
(f) Production of harmful substances	Information concerning the type, toxicity, production, and exposure route of substances, if known to be produced under natural conditions and to affect the inhabiting or growth of wild animals and wild plants in the surroundings
(g) Other information	Those which should be taken into consideration in accordance with the right column, other than listed in (a) through (f) in the left column (3)
2. Information concerning preparation of genetically modified organisms	-
(1) Information concerning donor nucleic acid	-
(a) Composition and origins of component elements	Origins, the number of base pairs, and nucleotide sequences of component elements of donor nucleic acid including target gene, gene regulatory region, localization signal, and selectable marker [shall be described in the order of sequence by expression cassette (referring to a combination of one target gene or one selectable marker and promoter, terminator, and/or localization signal which regulates the target gene or selectable marker). Shall be described as Others if not belonging to any expression cassette. The nucleotide sequence may be substituted by registration number or other access method if registered in open databases such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database.]

(b) Functions of component elements	(i) Functions of component elements of donor nucleic acid, including target gene, gene regulatory region, localization signal, and selectable marker (ii) Functions of proteins produced by the expression of target gene and selectable markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity (excluding the allergenicity as food) (iii) Contents of any change caused to the metabolic system of recipient organism
(2) Information concerning vector	-
(a) Name and origin	Name and taxnomical position of vector shall be provided.
(b) Properties	(i) The number of base pairs and nucleotide sequence of vector (ii) Presence or absence of nucleotide sequence having specific functions, and the functions (which may be substituted by the registration number and/or other access method if registered in open database such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database) (iii) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present
(3) Method of preparing genetically modified organisms	-
(a) Structure of the entire nucleic acid transferred in the recipient organism	Location and direction of component elements of donor nucleic acid in vector, and the diagram of restriction sites
(b) Method of transferring nucleic acid transferred in the recipient organism	Name of method of transferring nucleic acid such as *Agrobacterium* method, electroporation, and particle gun bombardment
(c) Processes of breeding of genetically modified organisms	(i) Mode of selection of the cell in which nucleic acid is transferred (ii) Presence or absence of remaining *Agrobacterium* when the method of transferring nucleic acid is based on *Agrobacterium* method (iii) Process of breeding of the lines used to confirm the existence of copies of transferred nucleic acid in the cells to which nucleic acid was transferred, to perform isolated field tests, and to collect information necessary for the Assessment of Adverse Effect on Biological Diversity, and the pedigree tress

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid	(i) Place where the replication product of transferred nucleic acid exists (on chromosome, in cellular organelles, or in protoplasm) (ii) The number of copies of transferred nucleic acid and stability of its inheritance through multiple generations (iii) Nearby or separate location of multiple copies, if present, on chromosome (iv) With respect to the characteristics described specifically in the right column (i) corresponding to the left column (6), the stability of the expression among individuals and generations under natural conditions (v) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants
(5) Methods of detection and identification of genetically modified organisms and their sensitivity and reliability	Qualitative methods for detection and identification of genetically modified crops, such as identification of replication product of transferred nucleic acid and neighboring nucleic acids, and their sensitivity and reliability [including quantitative methods for genetically modified crops in the case of III-2-(3)]
(6) Difference from the recipient organism or the taxonomical species to which the recipient organism belongs	(i) Details of physiological or ecological properties conferred as a result of the expression of copies of the introduced nucleic acid (including the contents, if expressed in specific tissue or at specific growth stage) (ii) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified crops and taxonomical species to which the recipient organism belongs, and the degree of difference, if present [excluding the cases where these characteristics have been clarified in (i)] a) Morphological and growth characteristics b) Cold-tolerance or heat-tolerance at early stage of growth c) Overwintering ability and summer survival of matured plant (excluding the cases where application is intended for isolated field tests) d) Fertility and size of pollen e) Production, shedding habit, dormancy, and germination rate of seed f) Crossability (applicable in only the case when there exist wild relatives that can be crossed grow in Japan) g) Productivity of harmful substances (secreted substances from roots to affect the other plants, secreted substances from roots to affect microorganisms in soil, substances in the plant body to affect the other plants after dying out, and any other types of harmful substances known to be produced by any taxonomical species to which the recipient organism belongs)

3. Information concerning the Use of genetically modified organisms	-
(1) Content of the Use	The same matters shall be described as written in the section of Content of the Type 1 Use of Genetically Modified Organism in the Application for Approval of Type 1 Use Regulation.
(2) Method of the Use	The same matters shall be described as written in the section of Methods of Type 1 Use in the Application for Approval of Type 1 Use Regulation, and in the application for isolated field tests, the map showing the location of the isolated field concerned, the layout of the test plots in the isolated field and the plan of tests in isolated fields shall be attached.
(3) Method of collecting information by person who wishes to obtain approval after the start of Type 1 Use	When Monitoring Plan has been drawn up, the statement "Refer to Monitoring Plan" shall be included.
(4) Emergency Measure which should be taken to prevent Adverse Effects on Biological Diversity in case Adverse Effects on Biological Diversity could arise	The statement "Refer to Plan of Emergency Measure" shall be included.
(5) The results of Use in laboratory or Use in similar environment to the environment in which Type 1 Use is intended	Information, if any, other than the information required to include in the left column 2-(6) “Difference from the recipient organism or the taxonomical species to which the recipient organism belongs”, and to be considered as referenced in the Assessment of Adverse Effect on Biological Diversity, shall be additionally described.
(6) Information obtained from Use abroad	Scientific information, if any, used in the Assessment of Adverse Effect on Biological Diversity in foreign countries, the result of Assessment, and any measures available for prevention of adverse effects shall be described, and the documents submitted for the Assessment shall be attached as necessary. In addition, the state of Type 1 Use in the foreign countries shall be described, and also the literature, if any, which evaluates the results of Type 1 Use in foreign countries from the viewpoint of Adverse Effect on Biological Diversity shall be attached.

Attached Table 2 (Related to III-2-(1)-(ii) (Methods of collecting information on genetically modified crops))

Concrete details of information	Methods of collecting information
The number of copies of transferred nucleic acid and stability of its inheritance through multiple generations, and nearby or separate location of multiple copies, if present, on the chromosome [Related to 2-(4)-(ii) and (iii) in Attached Table 1]	The replication product of transferred nucleic acid shall be analyzed based on the Southern hybridization method or PCR method.
The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acid [Related to 2-(4)-(iv) in Attached Table 1]	Observation of phenotype, Analysis of RNA transcribed from the transferred target gene and selectable markers with use of either method, Northern hybridization or RT-PCR, otherwise analysis of protein which is produced or inhibited by the expression of transferred target gene and selectable markers with use of either method, Immuno-blotting technique or ELISA.
Morphological characteristics [2-(6)-(ii)-a) in Attached Table 1]	Culm length, ear length, plant type, tiller number and other characteristics shall be examined sequentially with time. In the examination, items examined shall be selected by making reference to the test guideline for plant varieties registration, if present, for the taxonomical species to which the recipient organism belongs in accordance with the Seeds and Seedlings Act [The lines of genetically modified crops and control agricultural crops used in the examination shall be those cultivated in the same conditions. Whenever possible, the control agricultural crops shall be comparable to the lines of the genetically modified crops used in the examination in physiological and ecological characteristics (excluding those conferred by the expression of copies of transferred nucleic acid) and they shall be other than genetically modified crops. In addition, where the Application refers to Type 1 Use other than isolated field tests, the genetically modified crops and the control agricultural crops which have been cultivated in the isolated field shall be used; the same applies to the rest of this Table.]

Growth characteristics [Related to 2-(6)-(ii)-a) in Attached Table 1]	Time of initiation of germination, time of uniforming of germination, time of heading, anthesis, time of flower completion and other timing of growth stages shall be examined. In the examination, items examined shall be selected by making reference to the test guideline for plant varieties registration, if present, for the taxonomical species to which the recipient organism belongs in accordance with the Seeds and Seedlings Act.
Cold-tolerance and heat-tolerance at the early stage of growth [Related to 2-(6)-(ii)-b) in Attached Table 1]	After raising the seeds in an incubator or other proper means, the conditions of growth shall be observed in the temperature condition assuming the winter season in Japan for summer crops or summer season in Japan for winter crops.
Overwintering ability and summer survival of the matured plant (excluding the case of application for isolated field tests) [Related to 2-(6)-(ii)-c) in Attached Table 1]	In winter for the agricultural crops planted in summer or in summer for the agricultural crops planted in winter, the conditions of growth shall be observed, and the regenerating ability of plant body shall be examined as necessary after passing the winter or summer, respectively.
Fertility of the pollen [Related to 2-(6)-(ii)-d) in Attached Table 1]	Fertility of pollen shall be examined by collecting pollen grains during the flowering period and staining them with iodine potassium iodine solution, acetocarmine or other such staining solutions for examination of fertility of pollen.
Size of the pollen [Related to 2-(6)-(ii)–d) in Attached Table 1]	Size of pollen grains during the flowering period shall be examined.
Production of the seed [Related to 2-(6)-(ii)-e) in Attached Table 1]	The number of seeds produced by one individual shall be counted.
Shedding habit of the seed [Related to 2-(6)-(ii)-e) in Attached Table 1]	Shedding habit of seed shall be examined by counting the number of seeds shed when the ear is held with hands during the maturation period or using other proper methods for individual agricultural crops.
Dormancy and germination rate of the seed [Related to 2-(6)-(ii)-e) in Attached Table 1]	Germination rate and the germination speed shall be examined over time by maintaining the seeds in the conditions considered appropriate for examination of dormancy and using the method of generally recognized germination tests for individual agricultural crops.
Crossing rate [Related to 2-(6)-(ii)-f) in Attached Table 1]	Any of the following methods shall be used. However, in the case of application for Type 1 Use other than that in isolated field tests, the method in (i) shall be used whenever possible. Information concerning the wind velocity, temperature, humidity and other environmental conditions in the places where the examination is conducted based on any of the following methods shall be included in the reference material attached to the Assessment Report in accordance with III-2-(1)-(ii).

	(i) In a field, around the genetically modified crops and the control agricultural crops, relative plants are arranged by specific adjoining distance. Then the crossability of the relative plants with pollen grains derived from the genetically modified crops and the control agricultural crops shall be examined. With regard to the entomophily of the genetically modified crops, Type and number of pollinating insects shall be examined when necessary. (ii) In indoor environment, the genetically modified crops and the control agricultural crops during the flowering period are exposed to an artificial airflow at a wind velocity of 3 to 4 m/s. Then the crossability with relative plants arranged in the leeward side at regular pitches shall be examined (only applicable to anemophilae crops). (iii) In indoor environment, pollinating insects are released, and the crossability of relative plants arranged at different distances from the genetically modified crops and the control agricultural crops shall be examined (only applicable to entomophilous crops).
Productivity of harmful substances (secretion from roots to affect the other plants) [Related to 2-(6)-(ii)-g) in Attached Table 1]	Any of the following methods shall be used. (i) Plant box method [The plants under examination and test plants are planted in conjunction on an agar media in a plant box for tissue culture, and the growth condition of the test plants is observed. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 8:31-32 (1991).] (ii) Rhizosphere soil method [Soil is collected from the roots of plants examined, added with agar to form media, on which test plants are cultivated, and the growth condition of the test plants is observed. For detail, refer to the Journal of Weed Science and Technology 48 (Extra Issue): 142-143 (2003).] (iii) Succeeding crop test [Soil is collected from the field where plants examined were cultivated until they became matured. In the soil, test plants are cultivated, and the growth condition of the test plants is observed. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 8:31-32 (1991).]
Productivity of harmful substances (secretion from roots to affect microorganisms in soil) [Related to 2-(6)-(ii)-g) in Attached Table 1]	Examination shall be conduced based on the dilution plate method by collecting the soil in which the agricultural crops examined were cultivated until they became matured.

Productivity of harmful substances (substances in the plant body to affect the other plants after dying out) [Related to 2-(6)-(ii)-g) in Attached Table 1]	Any of the following methods shall be used. In any of the methods, care must be taken so that the part with trans-gene expression is included in the sample material. (i) Plow-in method (The growth condition shall be observed by drying and crushing the above-ground part of matured plants, mixing it with soil, and cultivating test plants in the soil). (ii) Sandwich method [The growth condition shall be observed by embedding leaf or stem in agar media in the form of sandwich and cultivating test plants on the media. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 14:35-36 (1997).]

Attached Table 3 (Related to III-1-(6) (Requirements for an isolated field))

1. Facilities having the following equipments

(1) Fence or other enclosure to prevent the entry of any unauthorized persons

(2) A notice board showing that the facility is an isolated field, unauthorized persons are prohibited to enter the facility, and the name of management representative, when designated in accordance with IV-4, posted in visible places

(3) Equipments intended to clear any genetically modified crops away from the machinery or equipment used in the isolated field, or the shoes of the persons engaged in the work in the isolated field, and another equipments to prevent unintended outflow of any genetically modified crops to the outside of the isolated field

(4) Shelterbelt, windbreak net, and other equipments to minimize dispersion of pollens (only applicable when those genetically modified crops are cultivated that could cause extensive dispersion of pollens)

2. Working procedures for compliance of the following matters

(1) Minimize possible growth of any plants in the isolated filed other than the genetically modified crops and the control agricultural crops

(2) Prevent possible escape of the genetically modified crops (including the plants other than the genetically modified crops cultivated in the isolated field, which are difficult to distinguish from the genetically modified crops; the same applies to (3) and (4) below) in cases when the genetically modified crops are transferred to outside of the isolated field otherwise they are stored outside of the isolated field

(3) Inactivate the genetically modified crops in the isolated field after cultivation of the genetically modified crops excluding the cases described in (2)

(4) Prevent unintended escape of genetically modified crops to outside of the isolated field while adhering to the machinery or equipment used in the isolated field or the shoes of persons engaged in the work in the isolated field

(5) Ensure that the equipments function properly as intended

(6) Ensure that the persons undertake Type 1 Use in conformity with the matters listed in (1) through (5)

(7) Implement the monitoring in the areas including the area exposed to dispersion of pollen where any wild animals and wild plants likely to be affected are inhabiting or growing

(8) Securely take the measures established in accordance with III-1-(4) when Adverse Effect on Biological Diversity could arise

Attached Table 4 (Related to III-3-(1)-(i) (Matters and items concerning the details of collecting information pertaining to genetically modified trees and filling of the Assessment Report

Items included in Table 1 attached to the Implementation Guidance	Concrete details of the information and concrete method for describing information in Assessment Report
1. Information concerning a recipient organism or the species to which the recipient organism belongs	For each item (1) through (3) in the left column [excluding (1)-(ii)], information concerning the taxonomical species to which the recipient organism belongs (the taxonomic rank when the scope of application unit covers any sub-species or any taxonomic rank lower than the species to which the recipient organism belongs; the same applies to the rest of this Table) shall be collected. When Consensus Documents for the Work on Harmonization of Regulatory Oversight in Biotechnology developed by the Environment Directorate of Organization for Economic Co-operation and Development (OECD) exists for the information (Visit the web site at http://www.oecd.org/document/51/0,2340,en_2649_34385_1889395_1_1_1_1,00.html), the information shall be written in Assessment Report of Adverse Effect on Biological Diversity based on the contents of that information in the Consensus Documents.
(1) Taxonomical position and state of distribution in natural environment	(i) Japanese name, English name, and Scientific name [Names in Current Use for Extant Plant Genera, The International Plant Names Index (http://www.ipni.org/index.html), "Wild plants in Japan" (edited by Yoshisuke Satake et. al) ,"Illustrated book of tree"(writed by Keiji Uehara) and/or other such widely used classification system shall be used and the sources used shall be noted.] (ii) Name of varieties of the recipient organism [including Registration No. and Date of Registration if the varieties has been registered in accordance with the Seeds and Seedlings Act (Act No.83 of 1998)] or name of the line concerned (iii) Wild habitat under natural environment (including the information regarding the center of the origin and/or the center of genetic diversity, if they have been identified, and the information regarding any area where the recipient organism have had an effect on the biological diversity of genetically organisms as the introduced species and the degree of the effect)
(2) History and present state of Use	(i) History of Type 1 Use both at home in Japan and abroad (ii) Main cultivation areas, cultivation methods, tree age in use, state of circulation and uses
(3) Physiological and ecological properties	For the items in the left column 3-(a) through (g), physiological and ecological characteristics shall be mentioned whenever possible under similar natural conditions as those in Japan.

(a) Basic properties	Morphological characteristics, characteristics of growth and other basic properties if there is no prolonged experience of Type 1 Use in Japan
(b) Environmental conditions allowing inhabiting or growth	Temperature range, moisture condition and soil condition allowing growth
(c) Predacity or parasitism	-
(d) Mode of propagation or reproduction	(i) Mode of dispersion dormancy and longevity, shape of the seed, reproductive age, distance of dispersion, shapes of male and female organs (ii) Mode of vegetative propagation (runner and bud, etc.) and the property of budding from any tissue or organ which could regenerate the plant body under natural conditions (iii) The degree of autogamy and allogamy, presence or absence of self-incompatibility, crossability with wild relative, and the degree of apomixes causing characteristics, if present (iv) Production, fertility, shape, method of pollination, age at which reproduction begins, dispersal distance and longevity of pollen
(e) Pathogenicity	-
(f) Production of harmful substances	Information concerning the type, toxicity, production, and exposure route of substances, if known to be produced under natural conditions and to affect the inhabiting or growth of wild animals and wild plants in the surroundings
(g) Other information	Any physiological or ecological characteristics other than listed in the left column (a) through (f), which should be taken into consideration
2. Information concerning preparation of genetically modified organisms	-
(1) Information concerning donor nucleic acid	-
(a) Composition and origins of component elements	Origins, the number of base pairs, and nucleotide sequences of component elements of donor nucleic acid including target gene, gene regulatory region, localization signal, and selectable marker [shall be described in the order of sequence by expression cassette (referring to a combination of one target gene or one selectable marker and promoter, terminator, and/or localization signal which regulates the target gene or selectable marker). Shall be described as Others if not belonging to any expression cassette. The nucleotide sequence may be substituted by registration number or other access method if registered in open databases such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database.]

(b) Functions of component elements	(i) Functions of component elements of donor nucleic acid, including target gene, gene regulatory region, localization signal, and selectable marker (ii) Functions of proteins produced by the expression of target gene and selectable markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity (excluding the allergenicity as food) (iii) Contents of any change caused to the metabolic system of recipient organism
(2) Information concerning vector	-
(a) Name and origin	Name of vector and taxonomic status of the original organism shall be entered.
(b) Properties	(i) The number of base pairs and nucleotide sequence of vector (ii) Presence or absence of nucleotide sequence having specific functions, and the functions (which may be substituted by the registration number and/or other access method if registered in open database such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database) (iii) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present
(3) Method of preparing genetically modified organisms	-
(a) Structure of the entire nucleic acid transferred in the recipient organism	Location and direction of component elements of donor nucleic acid in vector, and the diagram of restriction sites
(b) Method of transferring nucleic acid transferred in the recipient organism	Name of method of transferring nucleic acid such as *Agrobacterium* method, electroporation, and particle gun bombardment
(c) Processes of breeding of genetically modified organisms	(i) Mode of selection of the cell in which nucleic acid is transferred (ii) Presence or absence of remaining *Agrobacterium* when the method of transferring nucleic acid is based on *Agrobacterium* method (iii) Process of breeding of the lines used to confirm the existence of copies of transferred nucleic acid in the cells to which nucleic acid was transferred, to perform isolated field tests, and to collect information necessary for the Assessment of Adverse Effect on Biological Diversity, and the pedigree tress

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid	(i) Place where the replication product of transferred nucleic acid exists (on chromosome, in cellular organelles, or in protoplasm) (ii) The number of copies of transferred nucleic acid and stability of its inheritance through multiple generations (iii) Nearby or separate location of multiple copies, if present, on chromosome (iv) With respect to the characteristics described specifically in the left column (6)-(i), the stability of the expression among individuals and generations under natural conditions (v) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants
(5) Methods of detection and identification of genetically modified organisms and their sensitivity and reliability	Qualitative methods for detection and identification of genetically modified trees, such as identification of replication product of transferred nucleic acid and neighboring nucleic acids, and their sensitivity and reliability
(6) Difference from the recipient organism or the taxonomical species to which the recipient organism belongs	(i) Details of physiological or ecological properties conferred as a result of the expression of copies of the introduced nucleic acid (including the contents, if expressed in specific tissue or at specific growth stage) (ii) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified trees and taxonomical species to which the recipient organism belongs, and the degree of difference, if present [excluding the cases where these characteristics have been clarified in (i)] a) Morphological and growth characteristics b) Cold-tolerance or heat-tolerance at early stage of growth c) Fertility, size and longevity of pollen and age at which reproduction begins d) Production, dormancy, and germination rate of seed and age at which reproduction begins e) Crossability (applicable in only the case when there exist relative plants that can be crossed grow in Japan) f) Productivity of harmful substances (secreted substances from roots to affect the other plants, secreted substances from roots to affect microorganisms in soil, substances in the plant body to affect the other plants after dying out, and any other types of harmful substances known to be produced by any taxonomical species to which the recipient organism belongs)

3. Information concerning the Use of genetically modified organisms	-
(1) Content of the Use	The same matters shall be described as written in the section of Content of the Type 1 Use of Genetically Modified Organism in the Application for Approval of Type 1 Use Regulation.
(2) Method of the Use	The same matters shall be described as written in the section of Methods of Type 1 Use in the Application for Approval of Type 1 Use Regulation, and in the application for isolated field tests, the map showing the location of the isolated field concerned and the layout of the test plots in the isolated field shall be attached.
(3) Method of collecting information by person who wishes to obtain approval after the start of Type 1 Use	When Monitoring Plan has been drawn up, the statement "Refer to Monitoring Plan" shall be included.
(4) Emergency Measure which should be taken to prevent Adverse Effects on Biological Diversity in case Adverse Effects on Biological Diversity could arise	The statement "Refer to Plan of Emergency Measure" shall be included.
(5) The results of Use in laboratory or Use in similar environment to the environment in which Type 1 Use is intended	Information, if any, other than the information required to include in the left column 2-(6) “Difference from the recipient organism or the taxonomical species to which the recipient organism belongs”, and to be considered as referenced in the Assessment of Adverse Effect on Biological Diversity, shall be additionally described.
(6) Information obtained from Use abroad	Scientific information, if any, used in the Assessment of Adverse Effect on Biological Diversity in foreign countries, the result of Assessment, and any measures available for prevention of adverse effects shall be described, and the documents submitted for the Assessment shall be attached as necessary. In addition, the state of Type 1 Use in the foreign countries shall be described, and also the literature, if any, which evaluates the results of Type 1 Use in foreign countries from the viewpoint of Adverse Effect on Biological Diversity shall be attached.

Attached Table 5 (Related to III-3-(1)-(ii) (Methods of collecting information on genetically modified trees))

Concrete details of information	Methods of collecting information
The number of copies of transferred nucleic acid and stability of its inheritance through multiple generations, and nearby or separate location of multiple copies, if present, on the chromosome [Related to 2-(4)-(ii) and (iii) in Attached Table 4]	The replication product of transferred nucleic acid shall be analyzed based on the Southern hybridization method or PCR method.
The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acid [Related to 2-(4)-(iv) in Attached Table 4]	Observation of phenotype, Analysis of RNA transcribed from the transferred target gene and selectable markers with use of either method, Northern hybridization or RT-PCR, otherwise analysis of protein which is produced or inhibited by the expression of transferred target gene and selectable markers with use of either method, Immuno-blotting technique or ELISA.
Morphological characteristics [2-(6)-(ii)-a] in Attached Table 4]	Shapes of tree, trunk, branch, and leaf and other characteristics shall be examined sequentially with time. In the examination, items examined shall be selected by making reference to the test guideline for plant varieties registration, if present, for the taxonomical species to which the recipient organism belongs in accordance with the Seeds and Seedlings Act [The lines of genetically modified crops and control agricultural crops used in the examination shall be those cultivated in the same conditions. Whenever possible, the control agricultural trees shall be comparable to the lines of the genetically modified trees used in the examination in physiological and ecological characteristics (excluding those conferred by the expression of copies of transferred nucleic acid) and they shall be other than genetically modified trees. In addition, where the Application refers to Type 1 Use other than isolated field tests, the genetically modified trees and the control agricultural trees which have been cultivated in the isolated field shall be used; the same applies to the rest of this Table.]

Growth characteristics [Related to 2-(6)-(ii)-a) in Attached Table 4]	Growth, blooming and others shall be examined sequentially with time. In the examination, items examined shall be selected by making reference to the test guideline for plant varieties registration, if present, for the taxonomical species to which the recipient organism belongs in accordance with the Seeds and Seedlings Act.
Cold-tolerance and heat-tolerance at the early stage of growth [Related to 2-(6)-(ii)-b) in Attached Table 4]	After raising the seeds in an incubator or other proper means, the conditions of growth shall be observed in the temperature condition assuming the northern limit and southern limit in Japan of the distribution of the host plant.
Fertility of the pollen [Related to 2-(6)-(ii)-c) in Attached Table 4]	Fertility of pollen shall be examined by staining with iodine potassium iodine solution, acetocarmine or other such staining solutions for examination of fertility of pollen.
Size of the pollen [Related to 2-(6)-(ii)–c) in Attached Table 4]	Size of the fertile pollen grains shall be examined.
Longevity of pollen [Related to 2-(6)-(ii)–c) in Attached Table 4]	While keeping the fertile pollen in the similar environment to the one in which Type 1 Use is intended, the germination rate shall be examined sequentially with time.
Age at which trees start producing pollen [Related to 2-(6)-(ii)–c) in Attached Table 4]	The age at which trees start producing pollen shall be examined in the natural condition.
Production of the seed [Related to 2-(6)-(ii)-d) in Attached Table 4]	The number of seeds produced by one individual shall be counted.
Dormancy and germination rate of the seed [Related to 2-(6)-(ii)-d) in Attached Table 4]	Germination rate and the germination speed shall be examined over time by maintaining the seeds in the conditions considered appropriate for examination of dormancy and using the method of generally recognized germination tests for individual agricultural crops.
Age at which trees start producing seeds [Related to 2-(6)-(ii)–d) in Attached Table 4]	The age at which trees start producing seeds shall be examined in the natural condition.
Crossing rate [Related to 2-(6)-(ii)-e) in Attached Table 4]	Any of the following methods shall be used. Information concerning the temperature, humidity and other environmental conditions in the places where the examination is conducted based on any of the following methods shall be included in the reference material attached to the Assessment Report in accordance with III-3-(1)-(ii) (i) In field or indoor environment, the crossabiltiy shall be examined between the genetically modified trees and the relative plants and between the control trees and the relative plants. (ii) In indoor environment, pollinating insects are released, and the crossability of relative plants arranged at different distances from the genetically modified trees

	and the control agricultural trees shall be examined (only applicable to entomophilous crops).
Productivity of harmful substances (secretion from roots to affect the other plants) [Related to 2-(6)-(ii)-f) in Attached Table 4]	Any of the following methods shall be used. (i) Plant box method [The plants under examination and test plants are planted in conjunction on an agar media in a plant box for tissue culture, and the growth condition of the test plants is observed. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 8:31-32 (1991).] (ii) Rhizosphere soil method [Soil is collected from the roots of plants examined, added with agar to form media, on which test plants are cultivated, and the growth condition of the test plants is observed. For detail, refer to the Journal of Weed Science and Technology 48 (Extra Issue): 142-143 (2003).] (iii) Succeeding crop tests [soil is collected from the field where subject plants were cultivated. Using the soil, test plants are cultivated, and the growth condition of the test plants is observed. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 8:31-32 (1991).]
Productivity of harmful substances (secretion from roots to affect microorganisms in soil) [Related to 2-(6)-(ii)-f) in Attached Table 4]	Examination shall be conduced based on the dilution plate method by collecting the soil in which the examined trees were cultivated and grew.
Productivity of harmful substances (substances in the plant body to affect the other plants after dying out) [Related to 2-(6)-(ii)-f) in Attached Table 4]	Any of the following methods shall be used. In any of the methods, care must be taken so that the part with trans-gene expression is included in the sample material. (i) Plow-in method (The growth condition shall be observed by drying and crushing the above-ground part of matured plants, mixing it with soil, and cultivating test plants in the soil). (ii) Sandwich method [The growth condition shall be observed by embedding leaf or stem in agar media in the form of sandwich and cultivating test plants on the media. For detail, refer to the Bulletin of National Institute for Agro-Environmental Sciences 14:35-36 (1997).]

Attached Table 6 (Related to III-1-(6) (Requirements for an isolated field))

1. Facilities having the following equipments

(1) Fence or other enclosure to prevent the entry of any unauthorized persons

(2) A notice board showing that the facility is an isolated field, unauthorized persons are prohibited to enter the facility, and the name of management representative, when designated in accordance with IV-4, posted in visible places

(3) Equipments intended to clear any genetically modified trees away from the machinery or equipment used in the isolated field, or the shoes of the persons engaged in the work in the isolated field, and another equipments to prevent unintended outflow of any genetically modified trees to the outside of the isolated field

(4) Shelterbelt, windbreak net, and other equipments to minimize dispersion of pollen and seeds (only applicable when those genetically modified trees are cultivated that could cause extensive dispersion of pollen and seeds)

2. Working procedures for compliance of the following matters

(1) Take measure to prevent dispersion of pollens and seeds of genetically modified trees, including emasculation, fruit thinning, and bagging.

(2) Minimize possible growth of any plants in the isolated filed other than the genetically modified trees and the control agricultural trees

(3) Prevent possible escape of the genetically modified crops (including the plants other than the genetically modified crops cultivated in the isolated field, which are difficult to distinguish from the genetically modified crops; the same applies to (4) and (5) below) in cases when the genetically modified crops are transferred to outside of the isolated field otherwise they are stored outside of the isolated field

(4) Inactivate the genetically modified trees in the isolated field after cultivation of the genetically modified trees excluding the cases described in (3)

(5) Prevent unintended escape of genetically modified trees to outside of the isolated field while adhering to the machinery or equipment used in the isolated field or the shoes of persons engaged in the work in the isolated field

(6) Ensure that the equipments function properly as intended

(7) Ensure that the persons undertake Type 1 Use in conformity with the matters listed in (1) through (6)

(8) Implement the monitoring in the areas including the area exposed to dispersion of pollen and seeds where any wild animals and wild plants likely to be affected are inhabiting or growing

(9) Securely take the measures established in accordance with III-1-(4) when Adverse Effect on Biological Diversity could arise

Concerning the Application for Approval of Type 1 Use Regulations with regard to the genetically modified live vaccines, the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries

I. Objectives

With regard to microorganisms [referring to living organisms belonging to Fungi (except mushrooms), and living organisms belonging to Protista and Prokaryote, virus and viroid classifications; hereinafter the same shall apply] among living modified organisms, the production or circulation of which falls within the jurisdiction of the Ministry of Agriculture, Forestry and Fisheries, as well as with regard to veterinary drugs derived from these microorganisms and injected into animals' bodies for the purpose of preventing infections in the animals (hereinafter referred to as "genetically modified live vaccine"), application for Approval of Type 1 Use Regulations under the provisions of Article 4 paragraph 2 of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No.97 of 2003; hereinafter referred to as the "Act") shall be in accordance with the matters and items specified in this notification in addition to those stipulated in the Regulations related to the Enforcement of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Ministerial Ordinance No.1 of 2003 from the Ministry of Finance; the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; the Ministry of Agriculture, Forestry and Fisheries; the Ministry of Economy, Trade and Industry; and the Ministry of the Environment; hereinafter referred to as "Regulations related to the Enforcement of the Act"), the Ministerial Notification No.1 from the Ministry of Finance; the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; the Ministry of Agriculture, Forestry and Fisheries; the Ministry of Economy, Trade and Industry; and the Ministry of the Environment, dated November 21, 2003 (Basic Matters under the Provisions of Article 3 of the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms; hereinafter referred to as "Basic Matters") and the Ministerial Notification No.2 from the Ministry of Finance; the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare; the Ministry of Agriculture, Forestry and Fisheries; the Ministry of Economy, Trade and Industry; and the Ministry of the Environment, dated November 21, 2003 (The Guidance of Implementation of Assessment of Adverse Effect on Biological Diversity of Type 1 Use of Living Modified Organisms; hereinafter referred to as "Implementation Guidance").

Since use of genetically modified live vaccines should be in compliance with the Pharmaceutical Affairs Act (Act No.145 of 1960), some documents required pursuant to the said Act shall also be submitted.

The matters and items mentioned herein shall be reviewed as occasion demands, with future amplification of scientific knowledge on the adverse effects on biological diversity caused by Type 1 Use of living modified organisms and/or international trends concerning the assessment or control of the adverse effects of living modified organisms on biological diversity taken into account.

II. Matters and items concerning the procedures for Application for Approval of Type 1 Use Regulations

1. Destination of submission of an application and other documents

The destination of an application and other documents stipulated in the provisions of Article 41 paragraph 1 of the Regulations related to the Enforcement of the Act shall be Plant Products Safety Division, Food Safety and Consumer Affairs Bureau, the Ministry of Agriculture, Forestry and Fisheries. In addition, any electromagnetic records holding the contents of the application and other documents, if available, shall be submitted together with the written application and other documents.

2. Hearing by academic experts

When hearing the opinions pursuant to Article 4 paragraph 4 of the Act about the documents submitted, a review board (hereinafter referred to as the "review board") shall be set up, comprising persons with special knowledge and experience who are listed in the register of names of experts prepared and announced under the provisions of Article 10 of the Regulations related to the Enforcement of the Act (hereinafter referred to as "Experts").
This review board shall be convened by the Chairperson of the Pharmaceutical Affairs and Food Sanitation Council's Subcommittee of Pharmaceutical Affairs.

3 Explanation by an applicant of an application and other documents

The review board described in 2 above shall ask a person who wishes to file an Application for Approval of Type 1 Use Regulation (hereinafter referred to as Applicant) as necessary to explain his/her application and other documents and answer any questions from Experts.

4. Normal processing period

The normal processing period from submission of an application and other documents to the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment through to the approval granted under the provisions of Article 4 paragraph 5 of the Act, the instruction provided under the provisions of Article 5 paragraph 1 of the Act, or the rejection given under the provisions of Article 5 paragraph 3 of the Act (including the mutatis mutandis application stipulated in Article 9 paragraph 4 of the Act) shall be six (6) months. However, this normal processing period precludes the period of time required for Applicant to amend any inadequacy in the submitted application and other documents and/or the period of time required for Applicant to submit any additional information or documents instructed based on the consultation with Experts.

5 Inquiry about the scope of living organism and technology covered by the Act

If applicant is hard to determine whether or not the genetically modified live vaccines pertaining to Application for Approval of Type 1 Use Regulation fall under the cells or viruses stipulated in Article 1 of the Regulations related to the Enforcement of the Act and whether or not the technologies used to obtain the genetically modified live vaccines fall under the technologies stipulated in Article 2 and 3 of the Regulations related to the Enforcement of the Act, and/or he/she wishes to inquire about some matters regarding Application, he/she shall consult with the Plant Products Safety Division, Food Safety and Consumer Affairs Bureau, the Ministry of Agriculture, Forestry and Fisheries.

III. Matters concerning the contents of an application and other documents

1 Matters and items concerning the Report on the Assessment of Adverse Effects on Biological Diversity

(1) Information collection and items to be included in the Assessment Report

When formulating the Report on the Assessment of Adverse Effects on Biological Diversity stipulated in Article 4 paragraph 2 of the Act (hereinafter referred to as the "Assessment Report"), the specific details of information in Attached Table 1 and the specific method of information collection in Attached Table 4-1 shall be entered in the right column of Attached Table 1 which corresponds to the section in the left column. However, where there is a rational reason for not using part of the information in the right column of the Table, such information need not be collected.

Information collection under Attached Table 1 of the Implementation Guidance and III-1-(2) of this document shall be conducted appropriately based on scientific knowledge. Documents listing sample materials, procedures, results and interpretations of individual analyses and studies shall be attached to the Assessment Report.

(2) Information required to be further collected and the method of listing such information

If any wildlife deemed likely to be affected is identified in the process of implementing the assessment in accordance with the Procedures for Assessment of Adverse Effects on Biological Diversity stipulated in Attached Table 3 of the Implementation Guidance, scientific information concerning the adverse effects shall be collected by conducting experiments on reaction of the individuals of the wildlife and by collecting information on the places or periods of time of living or growth of said wildlife, in addition to the information stipulated in Table 1 of the Implementation Guidance. Based on the information, assessment shall be conducted and the result of the assessment shall be submitted in conjunction (for example, where assessment of details of adverse effect regarding the productivity of harmful substances is to be conducted, bioassay and/or other tests shall be implemented as required using the wildlife identified as likely to be affected).

Furthermore, in cases where genetically modified live vaccines may be passed into the environment via inoculated animals (referring to the animals injected with genetically modified live vaccines; hereinafter the same shall apply), the following information concerning the behavior of genetically modified vaccines is necessary for the assessment of adverse effects on biological diversity. Thus, information under the conditions that are similar to natural conditions in Japan shall be collected and if necessary, information obtained from simulated environment tests (referring to Type 1 Use in facilities meeting the requirements listed in Attached Table 2; hereinafter the same shall apply) shall be collected.

(i) Information concerning the withdrawal of genetically modified live vaccines in the body of inoculated animals;
(ii) Information concerning the dispersion of genetically modified live vaccines into the environment from the body, excretory substances, blood/body fluids or eggs of inoculated animals;
(iii) Information concerning the possibility of vertical infection of genetically modified live vaccines in inoculated animals;
(iv) Information concerning the possibility of transmission to wild plants and animals; and
(v) Other necessary information.

With respect to specific method of entering the information specified from (i) through (v), the section "(7) Information concerning the behavior of genetically modified vaccines" shall be added to Implementation Guideline I-(3) and the information from (i) through (v) shall be entered into the relevant section.

2. Matters and items to be included in the application form for the Approval of Type 1 Use Regulations

(1) Name of the type of living modified organisms, etc.

When giving the "name of the type of living modified organisms, etc.", an applicant shall decide a proper name which can be clearly discerned from other genetically modified live vaccines by including information on the donor organism(s), donor nucleic acid and modified microorganism, as well as the internationally recognized name of the introduced gene and the scientific name of the recipient organism, etc. If there is an identification number given by the developer or an internationally standardized identification number, such number shall be added. The name shall be listed in the relevant section of the application form for the Approval of Type I Use stipulated in Article 7 of the Regulations related to the Enforcement of the Act (hereinafter referred to as the "Application Form").

The aforementioned scientific names of the transferred gene and recipient organism, and identification numbers of living modified organisms shall be listed in the brackets; for instance, "Derived from … virus, …gene transfer, ….virus, ….strain (scientific names of the transferred gene and recipient organism, etc.) (identification number)."

Documents published by the International Committee on Taxonomy of Viruses (ICTV) shall be referred to list the name of the concerned gene and scientific name of recipient organism.

(2) Content of Type I Use of Living Modified Organisms

In the case of Type I Use of genetically modified live vaccines under routine vaccination conditions, the use of the concerned genetically modified live vaccine shall be solely for the purpose of preventing infections in animals and it is necessary to consider compliance with the laws and regulations related to the Pharmaceutical Affairs Act, etc. Thus, an applicant shall list in the relevant sections of the Application Form the actions corresponding to any of following items from (i) to (vii), as the "Content of Type I Use of Living Modified Organisms".

(i) Transportation and storage (including transportation and storage of animals inoculated with the viable, modified live vaccine);

(ii) In cases falling under the category of a study to collect data on clinical research, which must be submitted pursuant to Article 14 paragraph 3 of the Pharmaceutical Affairs Act (hereinafter referred to as the "clinical trial"), such use shall be in accordance with a Clinical Trial Plan Notification to be submitted pursuant to Article 80-2, paragraph 2 of the said Act and a Clinical Trial Protocol prepared according to Article 7 of the Ministerial Ordinance for good clinical practices in new veterinary drugs (No. 75 of 1997 Ordinance of the Ministry of Agriculture, Forestry and Fishery);

(iii) Any use shall be in accordance with the application for approval as specified by Article 14, paragraph 1 of the Pharmaceutical Affairs Act [except operations corresponding to (iv)];

(iv) Vaccination;

(v) Disposal of devices and residues after inoculation in accordance with the standards for disposal of infectious industrial waste provided for in Article 12-2 of the Waste Disposal and Public Cleansing Act (Act No. 137 of 1970);

(vi) Disposal excluding those in (v) (including cases that accompany the disposal of inoculated animals carrying the viable modified live vaccine); and

(vii) Acts incidental to (i) to (vi).

Furthermore, if there is a possibility that meat, milk and other products of inoculated animals will be consumed as food, the inoculated animals shall be specified. Any case with no possibility of human consumption shall be mentioned.

3 Matters concerning attached documents

(1) Plan of Emergency Measure

Applicant shall have established proper measures in advance, which are useful for efficient prevention of Adverse Effect on Biological Diversity, which he/she could take within his/her

ability in cases when Adverse Effect on Biological Diversity is feared to arise due to Type 1 Use concerning the application (hereinafter referred to as "Emergency Measure"). Then Applicant shall draw up a plan including the matters and items (hereinafter referred to as "Plan of Emergency Measure") and attach it to the application form.

(i) Implementation system and a responsible person
(ii) Methods for identifying the status of Type 1 Use pertaining to the Application [excluding the simulated environmental tests stipulated in III-4-(2)]
(iii) Methods to inform a person who makes Type 1 Use pertaining to the Application about concrete details of Emergency Measure to be taken
(iv) Concrete details of measures for inactivation of genetically modified live vaccines pertaining to the Application or taking the containment measures and continuing the use of the living modified organisms pertaining to the Application (only when the containment measures to be taken have been established in advance in accordance with the Act)
(v) Methods for contact with the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment
(vi) Other necessary information

With respect to (iv), specific measures for inactivation taken according to the condition of the genetically modified live vaccine shall be described.

(2) Monitoring Plan

(i) Cases requiring the Monitoring Plan

In any of (a) or (b) cases listed below, Applicant shall draw up a plan for monitoring (referring to the investigations on the presence or absence of adverse effects of Type 1 Use pertaining to the Application on wildlife and concrete details of adverse effects, if present; the same applies to the rest of this Notification) (hereinafter referred to as "Monitoring Plan") and attach it to the Application Form

Even in cases that do not fall under the following (a) or (b), if experts, together with specific assessment items, advise the necessity of monitoring in the process of application reviews, the Applicant shall draw up a monitoring plan and attach it to the Application Form.

(a) Cases where Adverse Effect on Biological Diversity is to be prevented by regulating the methods of Type 1 Use pertaining to the Application
(b) Cases where Applicant has decided to implement Monitoring by himself/herself to prevent Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the Application.

(ii) Matters and items to be mentioned in Monitoring Plan

Monitoring Plan shall include the matters and items listed below.

(a) Implementation system and a responsible person
(b) The name of the type of wild animals and wild plants subject to Monitoring
(c) Places subject to Monitoring and the living or growth conditions of the wild animals and wild plants concerned in the places
(d) Periods of time of Monitoring
(e) Monitoring methods, eg. time of implementation, frequency, and etc.
(f) Methods of analysis of the result of Monitoring
(g) Methods for reporting the result to the Minister of Agriculture, Forestry and Fisheries and the Minister of the Environment
(h) Other necessary matters and items

4. Matters related to the application for simulated environment test

(1) Use in a similar environment to the one in which Type 1 Use is intended

In the case of Type 1 Use of genetically modified live vaccines, for which a good deal of findings have been acquired on the characteristics based on the results of use in laboratories or use under natural conditions in foreign countries but for which the characteristics when used in ordinary inoculation conditions in Japan have not yet been clarified from the scientific point of view, information shall be collected pertaining to use in a similar environment to that in which Type 1 Use stipulated in Basic Matters 1-1-(1)-(a)-iv is intended and the characteristics shall be clarified when those genetically modified live vaccines are used under ordinary conditions in Japan.
The said information collection shall be carried out by simulated environment tests.

(2) Application for simulated environment test

With respect to application for simulated environment test, "inoculation to animals in simulated environment test (with respect to "animals," all animals subject to inoculation shall be listed), storage, transportation, disposal and acts incidental to these" shall be entered in the section of "Content of the Type 1 Use of Living Modified Organism" in the Application Form, and concrete details of the facilities concerned and the working procedures shall be mentioned in the section of "Method of Type 1 Use of Living Modified Organism" in the said form.

IV. Matters concerning Organization of the Implementation System for Type 1 Use

1 Setup of a committee

A person, who intends to obtain Approval of Type 1 Use Regulation with any limited methods of Type 1 Use, shall endeavor to set up a committee to discuss the matters concerning prevention of Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the Application in accordance with the provisions of II-2 of Basic Matters (hereinafter referred to as "Committee"), and he/she shall submit the register of names of committee members in conjunction with the application and other documents when the Committee is set up.

2. Composition of Committee

The Committee described in the above 1 shall endeavor to select the members among those listed below. In addition, when Applicant is a corporation, it is preferably whenever possible that members are selected from other than those who belong to the corporation.

(1) Persons with special knowledge and expertise concerning the characteristics of the genetically modified live vaccines pertaining to the application
(2) Persons with special knowledge and expertise concerning the actual conditions of Type 1 Use including use, breeding, and transportation of the genetically modified live vaccines pertaining to the application
(3) Persons with special knowledge and expertise concerning the wild animals and wild plants likely to be affected by Type 1 Use pertaining to the application and concerning the ecological systems
(4) Persons in charge of management of the places where Type 1 Use pertaining to the application is implemented

3. Matters to be considered at Committee

Committee considers the matters listed below.

(1) Method of Type 1 Use pertaining to the application
(2) Content of Monitoring Plan
(3) Content of Plan of Emergency Measure
(4) Judgment whether or not there is the likelihood of Adverse Effect on Biological Diversity

(5) Method of educational training for persons making Type 1 Use pertaining to the application
(6) Other matters concerning prevention of Adverse Effect on Biological Diversity due to Type 1 Use pertaining to the application

4. Designation of a management representative and a management supervisor

A person, who wishes to obtain Approval of Type 1 Use Regulation under limited method of Type 1 Use shall act to perform the roles listed below; he/she shall have a thorough knowledge of the laws and regulations concerning the use of genetically modified live vaccines, he/she shall designate a management representative and a management supervisor who assists the management representative from those who have experience in Type 1 Use of genetically modified live vaccines, and he/she shall assign these positions to every establishment performing simulated environmental tests and the major business establishment in the cases other than Type 1 Use mentioned on the above.

(1) Implement educational training for persons who are engaged in Type 1 Use pertaining to the application.
(2) Conduct monitoring in accordance with a Monitoring Plan, if established.
(3) Take Emergency Response Measures in accordance with The Plan of Emergency Measure if Adverse Effect on Biological Diversity is feared to arise.
(4) Perform the maintenance on the facilities, if any, for prevention of Adverse Effect on Biological Diversity caused by Type 1 Use pertaining to the application.
(5) Record the progress of Type 1 Use in the case of simulated environmental tests and maintain the records.
(6) Verify that Information on Correct Use is correctly provided to any person who receives transfer or supply of genetically modified live vaccines pertaining to the application or who receives entrustment to make Type 1 Use in cases of Type 1 Use other than that in simulated environmental tests and where Information on Correct Use is stipulated.

Attached Table 1 (Related to III-1-(1) (Information Collection and Items to be Included in Assessment Report)

Items included in Table 1 attached to the Implementation Guidance	Concrete details of the information and concrete method for describing information in Assessment Report
1 Information concerning a recipient organism or the species to which the recipient organism belongs	
(1) Taxonomical position and state of distribution in natural environment	(i) Taxonomic status, scientific name (genus and species), and name of strain [documents issued by the International Committee on Taxonomy of Viruses:(ICTV) shall be referred to for scientific name] (ii) If obtained from a public microorganism collection center, the name of the center, strain number and date of receipt; (iii) In cases other than (ii), items verifying the identification (points that are the same as or different from species already recognized by a scientific name and the reasons, place of deposit and storage number of the strain for separation and type of strain produced from it, etc. (vi) Details of the genetic modification used to induce the recipient organism [Lineage from wild strain to recipient strain intended for use, as well as added characteristics and operations taken to transfer such characteristics (for example, induced mutation by ultraviolet irradiation, zygotic effects,etc.) shall be listed. However, with respect to those already listed in other documents, etc, lineage may be replaced by the relevant document and only the modified genetic characteristics need be entered. With respect to those newly induced from a strain already listed in other documents, their derivation from the strain shall be described.] (v) If a wild strain is used as a recipient organism, distribution in the natural environment shall be described and related documents shall be attached if necessary.
(2) History and present state of Use	If a strain used as a recipient organism has a history of industrial use, the details and duration of such use shall be given and related documents shall be attached if necessary. In particular, if there is a history of use as a live vaccine, the problems associated with such use shall be listed.
(3) Physiological and ecological (biological) properties	For the following items (a) through (g) in (3) of the left column, physiological and biological characteristics shall be mentioned whenever possible under similar natural conditions as those in Japan.
(a) Basic properties	Describe the biological properties of the recipient organism and infectiveness of recipient to animals, including humans.

(b) Environmental conditions allowing inhabiting or growth (reproduction)	Environmental conditions that allow reproduction (temperature, anaerobic factors, aerobic factors, nutrition conditions, fertile tissues of animals, etc.)
(c) Predacity or parasitism	-
(d) Mode of propagation or reproduction	The following points shall be entered with respect to the mode of propagation and genetic properties of the recipient organism: (i) Details of the genetic modification used to induce the recipient organism [Lineage from wild strain to recipient strain intended for use, as well as added characteristics and operations taken to transfer such characteristics (for example, induced mutation by ultraviolet irradiation, zygotic effects, etc.) shall be listed. However, with respect to those already listed in other documents, etc, lineage may be replaced by the relevant document and only the modified genetic characteristics need be entered. With respect to those newly induced from a strain already listed in other documents, their derivation from the strain shall be described.] (ii) Viability or reproductive capacity (with respect to viability or reproductive capacity, reproductive temperature range, reproductive speed, auxotrophy, drug susceptibility, etc. shall be entered and related documents shall be attached when necessary). (iii) Mode of reproduction and crossability (mode of reproduction such as reproduction cycle shall be entered as best as known, as well as crossability between related or homogeneous strains. If there is crossability, the scope and frequency of crossability shall be described as best as known).
(e) Pathogenicity	The following points shall be entered with respect to the pathogenicity of the recipient organism (including carcinogenicity; hereinafter the same shall apply to (i) and (iii) in this column): (i) Pathogenicity (if there are records or reports on pathogenicity in wild animals and plants, related documents shall be attached. If pathogenic tests have been conducted, the results shall be given.) (ii) Existence of a virus or plasmid associated with pathogenicity. (iii)Content of pathogenicity and methods of prevention and treatment (if there is pathogenicity in wild animals and plants, the name of the disease, an outline of the symptoms and the presence and details of the method of diagnosis, preventive measures and treatment shall be described. Anything that survives as a latent infection without causing disease shall be mentioned. If any organism that may be infected is identified, the range of such organism shall be provided.)

(f) Production of harmful substances	Existence/nonexistence of production, such as biologically active substances that may adversely affect wild animals and plants, shall be mentioned. If the existence of the relevant substances is known, the names, activity and the levels of toxicity of such substances shall also be listed. In addition, major physiological properties, such as productivity of antibiotics, shall be entered, and related documents shall be attached when necessary.
(g) Other information	Type of animals known to be infected or contagious and the infectiveness or contagiousness (mode of infection, level of infectiveness, etc.) shall be entered, as well as other matters that need to be considered other than those listed in the right column corresponding to the left column (3)-(a) through (f) (such as excretion, infectiveness by cohabitation, etc.)
2 Information concerning preparation of living modified organisms	
(1) Information concerning donor nucleic acid	With respect to items (1)-(a) and (b) in the left column shall be listed and organized in accordance with the right column. Here,donor nucleic acid means that the sequences of elements inserted into vectors.
(a) Composition and origins of component elements	(i) Origins, the number of base pairs, and nucleotide sequences of component elements of donor nucleic acid including target gene, gene regulatory region, localization signal, and selectable marker [shall be described in the order of sequence by expression cassette (referring to a combination of one target gene or one selectable marker and promoter, terminator, and/or localization signal which regulates the target gene or selectable marker). Shall be described as Others if not belonging to any expression cassette. The nucleotide sequence may be substituted by registration number or other access method if registered in open databases such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database. (ii) With respect to composition, the restriction enzyme map and the number of base pairs shall be entered when necessary. Procedures taken before obtaining the said composition shall also be described (method of preparation, introduction methods for mutation, such as defection, replacement, etc.)
(b) Functions of component elements	(i) Functions of component elements of donor nucleic acid, including target gene, gene regulatory region, localization signal, and selectable marker (ii) Functions of proteins produced by the expression of target gene and selectable markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity (excluding the allergenicity as food)

	(iii) Whether nor not the metabolic system of recipient organism is changed. If a change is made, details of such a change.
(2) Information concerning vector	With respect to items (2)-(a) and (b) in the left column, shall be listed and organized in accordance with the right column. Here,vectors means that vectors without donar nucleic acid.
(a) Name and origin	The name of the vector and the taxonomic status of the original organism shall be listed.
(b) Properties	(i) The number of base pairs and nucleotide sequence of vector (ii) Presence or absence of nucleotide sequence having specific functions, and the functions (which may be substituted by the registration number and/or other access method if registered in open database such as GenBank, DNA Data Bank of Japan, and European Molecular Biology Laboratory Nucleotide Sequence Database) (iii) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present (iv) If a new vector is developed through modification or refinement of a known vector, documents concerning the vector without modification or refinement shall be attached, and provide a specific explanation on the method of modification and refinement. (v) Properties of the original organism of a vector shall also be listed when necessary.
(3) Method of preparing living modified organisms	With regard to the process from the insertion of a donor nucleic acid into a vector to the production of living modified organisms, items from (a) to (c) of the left column (3) shall be listed and organized in accordance with the right column.
(a) Structure of the entire nucleic acid transferred in the recipient organism	(i) Location and direction of component elements of donor nucleic acid in vector, and the diagram of restriction sites (ii) The method of insertion of the donor nucleic acid into the vector shall be described, and its main points shall be illustrated.
(b) Method of transferring nucleic acid transferred in the recipient organism	Name of method of transferring nucleic acid such as *Agrobacterium* method, electroporation, and particle gun bombardment, and its main points shall be illustrated.
(c) Processes of breeding of living modified organisms	The outline of the process of preparing a raw strain of genetically modified live vaccine (method of selecting the living modified organism and the subsequent breeding process and establishment of a cell bank) shall be described and its main points shall be illustrated when necessary.

(4) State of existence of nucleic acid transferred in cells (within the recipient's body) and stability of expression of traits caused by the nucleic acid	i) Whether the target gene has been integrated into the chromosome or into the plasmid of a recipient. ii) Stability of integrated nucleic acid through multiple generations.. iii) With regard to the expression of target genes in animals, the difference among individuals and stability of expression against changes in culture condition. iv) If there is a risk of transfer of the target gene to organisms other than a recipient organism due, for example, to an infection of a specific phage, test results concerning the transfer shall be provided, as well as the mode of expression of the target gene as best as known.
(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability	Qualitative methods for detection and identification of living modified organisms, such as identification of replication product of transferred nucleic acid and neighboring nucleic acids, and their sensitivity and reliability
(6) Difference from the recipient organism or the taxonomical species to which the recipient organism belongs	i) Differences in the following properties between a living modified organism and the recipient organism used for preparing the modified organism or the species to which the recipient organism belongs shall be described. a) Mode of reproduction (including the possibility of generation of viremia and new infectious viruses) and genetic properties b) Pathogenicity (including carcinogenicity) c) Infectivity (including tissue tropism and sustained infectiveness) d) Possibility of activation of endogenous viruses and endowment of pathogenicity e) Amount of dispersion into the natural environment when extracted from inoculated animals f) Survival capacity g) Cohabitation infection h) Productivity of toxic substances i) Other physiological differences ii) If there are characteristics that help discern the differences between living modified organisms and the recipient organism, such as colony formation and chromogenicity, they shall be added.
3 Information concerning the Use of living modified organisms	
(1) Content of the Use	The same matters shall be described as written in the section of Content of the Type 1 Use of Living Modified Organism in the Application for Approval of Type 1 Use Regulation.
(2) Method of the Use	The same matters shall be described as written in the section of Methods of Type 1 Use in the Application for Approval of

	Type 1 Use Regulation, and in the application for confined field tests, the map showing the location of the isolated field and facilities concerned and the layout of the test plots in the isolated field, equipments and facilities shall be attached.

(3) Method of collecting information by person who wishes to obtain approval after the start of Type 1 Use	When Monitoring Plan has been drawn up, the statement "Refer to Monitoring Plan" shall be included.
(4) Emergency Measure which should be taken to prevent Adverse Effects on Biological Diversity in case Adverse Effects on Biological Diversity could arise	The statement "Refer to Plan of Emergency Measure" shall be included.
(5) The results of Use in laboratory or Use in similar environment to the environment in which Type 1 Use is intended	Information, if any, other than the information required to include in the section 2-(6) of the left column, “Difference from the recipient organism or the taxonomical species to which the recipient organism belongs”, and to be considered as referenced in the Assessment of Adverse Effect on Biological Diversity, shall be additionally described.
(6) Information obtained from Use abroad	Scientific information, if any, used in the Assessment of Adverse Effect on Biological Diversity in foreign countries, the result of Assessment, and any measures available for prevention of adverse effects shall be described, and the documents submitted for the Assessment shall be attached as necessary. In addition, the state of Type 1 Use in the foreign countries shall be described, and also the literature, if any, which evaluates the results of Type 1 Use in foreign countries from the viewpoint of Adverse Effect on Biological Diversity shall be attached.

Attached Table 2 (Related to III-4-(2) Requirements for facilities related to simulated environment test)

1. The following equipment and facilities shall be installed:
   (1) Facilities to prevent escape, in accordance with the inherent habits of inoculated animals, facilities to stop entry of wild animals and facilities to prevent trespassing of unauthorized persons shall be installed in the zone where inoculated animals are kept and raised (hereinafter referred to as “controlled zone”).
   (2) A sign shall be posted in a visible site in the controlled zone. Such a sign shall contain the information that a simulated environment test is under way, that genetically modified live vaccines are in use, that the place is off limits to unauthorized persons, that the names of the management supervisors who have been appointed pursuant to IV-4 of this document.
   (3) Facilities to clean the machines, the equipment used in the controlled zone and the shoes worn by the staff engaged in work in the controlled zone and facilities to prevent genetically modified live vaccines from being accidentally taken off the premises of the controlled zone.
   (4) Facilities to appropriately store genetically modified live vaccines. A sign posted in a visible site with the message “Genetically Modified Live Vaccines (simulated environment test) in Storage”
   (5) Facilities to inactivate, burn and disinfect genetically modified live vaccines or their remains and wastes associated with inoculated animals (including dead inoculated animals and products)

2. Work guidelines shall be developed to comply with the following items:
   (1) Measures to prevent genetically modified live vaccines from coming into direct contact with humans;
   (2) Measures to clean the machines, the equipment used in the controlled zone and the shoes used in the controlled zone, to wear a specific work uniform in the controlled zone, to disinfect work uniforms, to prevent genetically modified live vaccines from being accidentally taken off the premises of the controlled zone;
   (3) Measures to deny unauthorized persons access to the controlled zone;
   (4) Measures to inactivate remains associated with genetically modified live vaccines. Wastes related to inoculated animals shall be treated by, for example, disinfecting or burning them whenever necessary;
   (5) Genetically modified live vaccines shall be stored in a storage facility with a message indicating the presence of genetically modified live vaccines. A catalogue of stored items, including genetically modified live vaccines, shall be prepared and stored;
   (6) When transporting a genetically modified live vaccine or its remains, measures shall be taken to prevent leakage, for example, by putting it in a container with sufficient strength and sealing it off. A “Handle with Care” message in red shall be prominently displayed on the container. When transporting inoculated animals out of the controlled zone, measures shall be taken to prevent runaways in accordance with the inherent habits of the animals;
   (7) Measures to prevent inoculated animals from running away;
   (8) Keep the controlled zone clean, minimize the raising and management of animals that are not related to use of the concerned genetically modified vaccines in the controlled zone and its vicinity. It is desirable to discern individual inoculated animals. If it is difficult to do so, animals shall be kept grouped;
   (9) With respect to facilities/equipment related to the use of genetically modified live vaccines and raising facilities/equipment related to caring for inoculated animals,

performance shall be checked immediately after the installation and periodically to confirm that the intrinsic performance potential of the said facilities/equipment is fully realized.

(10) When conducting monitoring, state that “monitoring will be conducted in compliance with the attached monitoring protocol”

(11) Secure measures established in accordance with III-3-(1) to be taken when there is a risk of adverse effects on biological diversity.

C. NORWAY

Input to the AHTEG on risk assessment and risk management

Submission of views and/or information in preparation for the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management.

Looking through the BIRC it is clear that the majority of existing guidance documents, both general and specific, may be found there. Guidance documents from the Cartagena Protocol, Codex, EU, EFSA, OECD and more is available through the BIRC and is therefore already stated as a basis of the deliberations of the AHTEG according to point 2d in the annex of decision BS-IV/11.

2. The deliberations of the Ad Hoc Technical Expert Group shall be based primarily on:

(d) Guidance materials available in the Biosafety Information Resource Centre of the Biosafety Clearing House;

Several of the elements in the following list may be found in the BIRC.

Guidance documents – general

- The Norwegian Regulations relating to impact assessment pursuant to the Gene Technology Act (http://www.regjeringen.no/nb/dep/md/dok/lover_regler/forskrifter/2005/regulations-relating-to-impact-assessmen.html?id=440455). As stated in §2 of the regulation "*The impact assessment is intended to provide a basis for assessing the risk of adverse effects on the environment or human or animal health and other consequences of projects for which approval is mandatory*". The regulations and appendixes outline the content of risk assessments and give a general description of the information necessary to make a decision regarding release. The regulations do not give detailed guidance on how to collect the necessary data.

- EFSA guidance documents:

  Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events by the Scientific Panel on Genetically Modified Organisms (GMO)
  Published: 25 July 2007 Adopted: 16 May 2007
  Guidance document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use by the Scientific Panel on Genetically Modified Organisms (GMO)[1]
  Published: 6 July 2006 Adopted: 17 May 2006
  Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO) - including draft document updated in 2008
  Published: 28 April 2006 Adopted: 24 September 2004

- Biosafety assessment tool (BAT). Work in progress.

This web based risk assessment tool is mentioned in para 37 (b) of the information document UNEP/CBD/BS/COP-MOP/4/INF/9. The project is funded by NORAD with

substantial support in-kind provided by the University of Canterbury, New Zealand and is a joint project of the Centre for Integrated Research in Biosafety (INBI) and GenØk – Centre for Biosafety, Norway.

The purpose of BAT is to function as guidance for risk assessments. For more information see http://english.genok.org/biosafety_assessment_tool/cms/83. According to the information on the GenØk website the BAT will be launched in February 2009. Contact http://english.genok.org/ for further information.

Guidance documents – specific

- Point 2b in the annex of Decision BS-IV/11 includes the report from the Canada-Norway Workshop, Montreal 2007 (UNEP/CBD/BS/COP-MOP/4/INF/13). The report points to existing guidance, gaps in guidance and knowledge gaps for GM-fish, GM-trees, Pharma plants and GM-virus vaccines for management of animal populations. The following recommendations were adopted by the workshop:

    - The general principles and methodologies for risk assessment contained in Annex III to the Cartagena Protocol also apply to transgenic fish, trees, viruses and pharmaplants.
    - There is insufficient guidance on how to perform risk assessment for GM fish and viruses.
    - There may be a need to develop specific methodologies and specific protocols for generating data necessary to conduct risk assessments for the future applications of modern biotechnology, especially for transgenic fish, trees and viruses.
    - All risk assessments of living modified organisms should be conducted on a case-by-case basis as the impacts depend upon the trait inserted, the recipient organism and the environment into which it is released.
    - There is a need for additional data on several elements necessary to conduct risk assessments for all four types of transgenic organisms (fish, trees, viruses and pharmaplants). Further research is recommended to fill the knowledge gaps, inter alia the specific gaps identified during the workshop.
    - Field trials may be a useful tool to generate data on the impacts of living modified organisms, but may give raise to particular concerns. Alternative models for generating data, as well as containment and confinement measures should be considered when appropriate. Baseline information on the specific organism in question is very important for risk assessments.
    - There is value in considering the differences between highly managed systems such as cultivated fruit trees and the more variable cases such as some forest systems and animal wildlife, and whether the recipient organisms are domesticated, semi-domesticated or non-domesticated species.
    - Existing guidelines, methodologies, baseline information and risk assessments should be made readily available through the Biosafety Clearing House and other relevant international databases.

To find the more detailed discussions for each topic see Part B of the report.

- CABI (www.cabi.org)

Environmental Risk Assessment of Genetically Modified Organisms, Volume 1-4. Specific case studies (Bt-maize in Kenya, Bt-cotton in Brazil, transgenic fish and Bt-cotton in Vietnam) give guidelines for Biosafety testing. The publications give both specific and general guidelines.

- OECD (2006). Safety Assessment of Transgenic Organisms: OECD Consensus Documents Vols. 1 and 2. ISBN Number:92-64-02258-9.

Specific for organisms and for traits. The list of consensus documents has been updated since 2006.

- OECD Consensus Document on Atlantic Salmon. Work in progress.

The first draft was presented to the OECD WGHROB in 2008. The document is most likely not available until completed, but perhaps the OECD Secretariat may be contacted for further details.

Two Expert Workshops on the Biology of Atlantic salmon have been arranged. Abstracts from the first workshop in Russia (2004) may be found as record 47263 in the BIRC. The summary record of the second Expert Workshop on the Biology of Atlantic salmon, held in Oct 2005 in Trondheim, Norway, exists as document ENV/JM/BIO(2005)14. To my knowledge the document is official, but as I was not able to locate the document in the OECD homepage I feel it is necessary to contact the OECD secretariat to verify the status of the document. I will do this and send the document to the CBD Secretariat when possible.

II. SUBMISSIONS FROM OTHER GOVERNMENTS

D. AUSTRALIA

AUSTRALIAN GOVERNMENT SUBMISSION – JANUARY 2009

Notification SCBD/BS/MPDM/ABw/65409

Invitation for Parties, other Governments and relevant organisations to submit to the Executive Secretariat information relevant to the work of the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management, particularly on existing guidance documents on risk assessment.

Existing Guidance Documents On Risk Assessment

Australian guidance documents for Risk Assessment

Annex III to the Protocol sets out the general principles for the conduct of risk assessment. The legislative and regulatory requirements of Australia are consistent with these principles. In Australia, the Gene Technology Regulator (the Regulator) is responsible for protecting human health and safety and the environment, by identifying and managing risks posed by or as a result of gene technology. Risk assessments are science-based and are carried out on a case-by-case basis and involve extensive consultation with experts.

Australia wishes to draw to the attention of the AHTEG a number of documents which provide guidance relevant to the risk assessment and risk management of LMOs, including documents produced by Australia's Office of the Gene Technology Regulator: Risk Analysis Framework; Risk Assessment and Risk Management Plans (RARMPs); Biology documents; and detailed Application Forms.

Risk Analysis Framework

The *Risk Analysis Framework* (RAF) is a key explanatory document that provides guidance on how the Regulator, and staff under the Regulator's direction in the Office of the Gene Technology Regulator (OGTR) approach the risk analyses of living modified organisms (LMOs). The RAF incorporates risk assessment, risk management and risk communication and provides guidance on how to characterise and deal with uncertainty. The RAF may provide guidance to other countries establishing and implementing risk assessment processes for LMOs. The current version of the RAF published in November 2007 is available on the OGTR website at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1. The RAF is currently under review and the revised version of the RAF may be available at the time of the next AHTEG meeting.

Risk Assessment and Risk Management Plans (RARMPs)

The Regulator's assessment of each application to release a LMO into the environment involves the preparation of a Risk Assessment and Risk Management Plan (RARMP), which includes a critical assessment of data provided by the applicant together with a thorough review of other relevant national and international scientific literature. The risk assessment takes account of risks to human health and safety and the environment posed by the dealing and the risk management plan determines how those risks can be managed. The principles and approach set out in the RAF are put into practice in the RARMP.

The RARMP sets out in detail the data and information considered in the assessment, the analysis and the conclusions. RARMPs are substantial documents of approximately 80 – 100 pages and are extensively referenced. They also outline how issues raised during consultations are taken into account in the risk assessment process. RARMPs can provide an example of how a logical, stepwise consideration of risks posed by a LMO is conducted.

Copies of RARMPs and licence conditions are publicly available through the Record of GMO and GM Product dealings (the GMO Record) on the Office of the Gene Technology Regulator (OGTR) website at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1.

The AHTEG may wish to refer to the commercial release RARMPs undertaken by the Regulator as illustrative of the conduct and documentation of risk analysis of LMOs. The Regulator has approved for unrestricted commercial release of a number of lines of GM cotton, canola and carnations and a live and viable GM cholera vaccine.

Field trials of various GM crops with a variety of introduced traits have also been licensed and conducted under limited and controlled conditions in Australia and the RARMPs are available from the OGTR website.

Specific example – Bt cotton

The MOP4 agenda paper UNEP/CBD/BS/COP-MOP/4/10 and corrigendum UNEP/CBD/BS/COP-MOP/4/10/Corr2 referred to the Australian assessments for the commercial release of Bt cotton.

GM cotton is the most cultivated GM crop in Australia to date. Australia considers that the RARMPs demonstrates how a cautious, step by step approach to risk assessment of GMOs, including addressing areas of uncertainty, can be undertaken

The initial assessment in 2002 identified concerns regarding a potential risk of weediness in northern Australia and the commercial release of Bt cotton was not approved in this region at that time (see DIR 012 RARMP at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir012-2002).

However in 2006, on the basis of consideration of additional information, the Regulator subsequently approved the same GM cotton for Australia-wide release (see DIR 066 RARMP at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir066-2006).

Application forms

Information relevant to guidance on risk assessment is contained in application forms for environmental release of LMOs in Australia.

The detailed application form provides guidance to applicants and outlines the type of information considered necessary to prepare a RARMP for each application to release an LMO into the Australian environment. The application form has specific sections including for plants, animals, bacteria and therapeutics and specific questions to elicit

information necessary to address important considerations relevant to each LMO application.

Applicants must provide comprehensive information about the proposed dealings with the LMO including possible risks posed by the dealings and proposed ways each risk could be managed. All responses must be supported by appropriate data and literature citations. Additional data relevant to the application is also often sought during the risk assessment process. A copy of the OGTR application form is available from the OGTR website at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/forms-1.

Biology documents

Risk assessments identify risks attributable to gene technology by considering the risks posed by a particular LMO in the context of the risks posed by the unmodified parental organism in the receiving environment. The OGTR has prepared biology documents for a number of species that provide an overview of baseline biology information to support comparative risk assessments. The biology documents may be of use to other countries conducting risk assessments on relevant GM species and are available on the OGTR website at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1

Other work being undertaken or completed by international organisations

We note that several international organisations including the Organisation for the Economic Cooperation and Development (OECD), World Organisation for Animal Health (OIE), Codex Alimentarius (CODEX) and the International Plant Protection Convention (IPPC) and institutes such as the International Life Sciences Institute (ILSI) have developed guidance documents on risk assessment and risk management that could be applicable to Annex III (this includes on issues such as long term effects and persistence and dissemination of LMOs in the environment). Many of these international documents were referred to in the 2005 Risk Assessment and Risk Management Paper UNEP/CBD/BS/COP-MOP/2/INF/2. To avoid duplication, we recommend the AHTEG/Secretariat consider these organisation's previous and current activities.

Guidance on Specific Aspects of Risk Assessment

Australia considers capacity-building and a focus on practical steps for the implementation of the Protocol to be critical for the development and operation of robust institutional and legislative frameworks, the conduct of science-based risk assessment and effective decision-making at a national level. This could include collaborations that promote action and exchange of information, especially 'risk assessment reports' for LMOs authorised by particular countries. This could be conducted through the Biosafety Clearing House and could assist countries in undertaking risk assessment.

Further guidance for particular types of LMOs and introduced traits

Risk assessment involves identifying risks from plausible sets of circumstances that result in harm to desirable entities, estimating the level of risk on the basis of the seriousness and likelihood of harm, and evaluating whether or not these risks should

be managed. Risk management involves selecting and implementing plans or actions to ensure that identified risks are appropriately managed. Risk communication involves the exchange of information, ideas and opinions between the Regulator and stakeholders. Risk communication also articulates the reasoning for decisions made by the Regulator.

Decision BS-IV/11, recorded at the 2008 COP-MOP 4 took note of the:

- report of the Norway-Canada Workshop on Risk Assessment for Emerging Applications of LMOs; and
- conclusions and recommendations of the regional and subregional workshops on capacity building and exchange of experiences on risk assessment and risk management regarding the need to develop additional guidance on specific aspects of risk assessment.

The Norway-Canada workshop concluded that there are issues that are unique to GM trees, viruses, fish and pharma plants which would require the development of specific risk assessment methodology and generation of specific data for risk assessment.

Australia considers that many of the issues raised at the workshop are not unique and that experience from other assessments, such as for GM crop plants, can provide helpful and adequate guidance for other risk assessments. For example a number of issues that are identified in the Norway-Canada report as 'unique to fish' could equally apply to outcrossing grass species that have high reproductive rates and highly effective seed dispersal mechanisms. The sections titled 'Whole ecosystem effects' and 'Invasion, establishment and spread' could apply, with very little modification, to weedy plants as well as to fish.

Australia considers that different organisms and traits do not need different risk assessment methodologies to be developed. The assessment method should remain the same, although the data used will differ depending on the context (trait, organism and environment). Risk management measures will be devised to manage risks identified in the risk assessment and should be proportionate to the level of risk identified.

For example, the OGTR *Risk Analysis Framework* has proved adequate to inform the approach and considerations for the conduct of assessments of organisms such as GM viruses for veterinary therapeutic use and field trials of GM oilseed poppies modified for altered opiate content for pharmaceutical production.

Many of the issues raised in the regional and subregional workshops were concerned with identifying the relevant risks to be considered for different types of LMOs. This is not unique to the cases discussed but illustrates the importance of ensuring an appropriate emphasis on risk identification at the start of a risk assessment. Risk identification includes consideration of credible pathways to harm, and the likelihood that harm will occur for risk scenarios. The process identifies risks that may require management and assists decision making.

Further guidance on receiving environments

Australia agrees that the receiving environment is an important aspect in conducting LMO risk assessment. However receiving environments vary between countries, including in regard to the physical environment, flora and fauna. Because of this

variability, detailed consideration of the receiving environment *per se* must be undertaken on a case by case basis and consequently the development of general guidance on the receiving environment may be problematic.

Australia notes that the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology has undertaken a project on *Environmental Consideration of Risk/Safety Assessment of the Release of Transgenic Plants*. The project intends to provide a comprehensive package of information elements to be used in considerations during risk/safety assessment of transgenic plants.

Further guidance on the monitoring of long term effects of LMOs released into the environment

In the Australian regulatory context, the long and the short term must be considered for LMO risk assessments and a Post-Release Review Framework (PRR) for general/commercial releases of GM crops in Australia has been developed. The PRR component has been integrated into the OGTR's *Risk Analysis Framework* which is available on the OGTR website at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1.

The PRR framework provides for ongoing oversight of LMO releases. It focuses on monitoring specific indicators of harm(s) in relation to potential risks identified in the risk assessment. For example licence conditions may require the licence holder to supply, or enable the Regulator to collect, specific information on the progress of the release to verify findings of the RARMP.

To date, the Regulator has concluded that LMOs approved for general/commercial release in Australia, are as safe as conventional varieties and are able to be used in the same manner as their conventional counterparts. However, licence holders must inform the OGTR if they become aware of any additional information indicating a risk to the health and safety of people or the environment, or of any unintended effects associated with the LMOs authorised by the licence. Information may also be supplied by other persons covered by a licence or by any other organisation or individual. The Regulator may vary, suspend or cancel licences, in order to be satisfied that risks are managed to protect human health and safety and the environment.

Insectide and herbicide use and resistance management

Australia also notes that issues associated with insecticide\herbicide use related to LMOs (including resistance development) may be managed under regulatory frameworks not specific to LMOs. For example, in Australia the Regulator gives consideration to risks to human health and safety and the environment with regard to the genetic modification while the regulation of pesticide and herbicide use in Australia is conducted by the Australian Pesticides and Veterinary Medicines Authority.

E. UNITED STATES OF AMERICA

Information from the United States Regarding Decision BS-IV/11 on Risk Assessment and Risk Management

Prepared 30 January 2009

The United States appreciates this opportunity to provide information relevant to Decision BS-IV/11 and the Ad Hoc Technical Experts Group (AHTEG) on risk assessment and risk management. The purpose of this paper is to inform the discussions related to existing guidance materials used in the risk assessment and risk management of living modified organisms (LMOs) including Annex III of the Protocol. This paper offers the following points for consideration:

1. Current guidance in Annex III is adequate to ensure a common approach in risk assessment and risk management for the transboundary movement of all LMOs. The Annex lays out a thorough and structured approach that is still flexible enough to be used in assessing a wide range of LMOs and their potential impacts on the sustainable use of biodiversity. The structured yet flexible approach of Annex III enables countries to use the Annex to meet the needs of a range of domestic environmental laws as well as international obligations related to environmental safety. Further, the approach of Annex III is consistent with the approaches described in a number of guidance documents developed among countries. (see Table 1 below for examples). The methodology described in Annex III follows the conventional risk assessment paradigm, beginning with identification of a potential hazard, such as characteristics of an LMO, which may have an adverse effect on biodiversity. Risks are then characterized based on combined evaluation of the likelihood of adverse effects, and the consequences should those effects be realized.

2. Guidance documents are intended to be structured yet flexible in order to be broadly applicable. Annex III, like other guidance documents, is not intended to have as much detail as one would find in a full risk assessment that would be done in specific cases. This illustrates the structured yet flexible nature of the approach of Annex III. The United States offers some representative examples of individual risk assessments that have been performed for specific LMOs in the United States in a manner consistent with the provisions of Annex III. The two specific examples cited in Table 2 below serve to illustrate the general applicability of guidance documents such as Annex III and the use of the best available science as part of the risk assessment process. Both U.S. agencies that conduct environmental risk assessments for LMOs in the United States (EPA and USDA-APHIS) follow the Annex III approach, even though the assessments are carried out under different laws and address different formal requirements. Examples of additional specific risk assessments undertaken by United States regulatory agencies are available through the United States government's unified web page for biotechnology (http://usbiotechreg.nbii.gov/).

Based upon experience in the United States and other countries, it is clear that additional **guidance** is not needed to supplement Annex III, but rather what is needed is better sharing of information about how the guidance is actually used in individual cases of risk

assessments. This is one of main objectives of the Biosafety Clearing House (BCH), and it provides a constructive, systematic way to share how risk assessments were done in individual cases. The BCH and several national web sites provide both the risk assessments and information about how these risk assessments were used to make decisions under that country's legal system.

If the AHTEG were to decide that some additional guidance is needed, the United States strongly urges that any additional guidance developed continue to have broad applicability for many types of LMOs, adaptability across the wide range of legal systems in different countries, and facilitate rather than replace the accepted approach of case-by-case assessments.

3. Decisions about the suitability of an LMO should take into account a case-specific risk assessment. In decision BS-IV/11, the Parties asked the AHTEG to "consider possible modalities for cooperation in identifying living modified organisms or specific traits that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health."

The United States believes that undertaking this task as an a priori exercise would violate the established principle set out in Annex III that case-by-case analyses should be used tomake decisions about an LMO. The United States notes that Annex III sets out general principles, methodological steps, and points to consider in the conduct of risk assessment. The general principles include, among others, the concepts that:

- Risk assessment should be carried out in a scientifically sound and transparent manner;
- 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;
- Risks should be considered in the context of risks posed by the non-modified recipients or parental organisms; and that
- Risks should be assessed on a case-by-base basis.

As noted above, the methodology described in Annex III of the Protocol follows the conventional risk assessment paradigm, beginning with identification of a potential hazard, such as characteristics of an LMO, which may have an adverse effect on biodiversity. Risks are then characterized based on combined evaluation of the likelihood of adverse effects, and the consequences should those effects be realized.

Therefore, the task of identifying such LMOs (i.e. "that may have adverse effects on the conservation and sustainable use of biological diversity…") in fact contradicts the very foundation that risk assessment plays in providing scientifically sound assessments to decision makers, regardless of whether the decisions are being made under the Protocol or under a national biosafety legal system. It is not possible to reach valid conclusions on hypothetical LMOs, because there is no specific real information to analyze, and this analysis would not take into account the particularities of different receiving environments as well as differences in how a particular LMO might be used. Furthermore, it is unclear how such a list of LMOs would relate to Parties' obligations

under the Protocol. In addition, such a list would not remove the obligation to make decisions on transboundary movements.

4. The United States supports an alternative approach for the AHTEG's work on makings lists of LMOs. Under this alternative approach, the AHTEG would consider modalities for developing a process to examine existing case-specific risk assessments of LMOs in order to extract any consensus conclusions that have been broadly validated by many countries in risk assessments that have been undertaken in a manner consistent with Annex III. Such reviews may be able to identify broad-based consensus on LMOs whose transboundary movement are unlikely to have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. There are now quite a few LMOs that have been subjected to multiple assessments by different countries, and it may be useful for other countries to be aware of the extent of agreement across these risk assessments.

5. The AHTEG should focus its consideration of risk assessment and risk management relevant to the objectives of the Protocol, not food safety. The United States urges the AHTEG to focus on the objectives of the Protocol, namely to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements. Food safety assessments are not within the purview of the Protocol, but the Codex Alimentarius has adopted appropriate guidance in the documents *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* and the *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology*. The United States strongly supports the work of Codex in the field of food safety.

***Table 1**. The following table provides a listing and brief description of currently existing international guidance documents pertaining to risk assessment and risk management of LMOs. This information is offered as an illustration of the well established practicality of the approach laid out in Annex III of the Protocol, as well as the adaptability of this approach in evaluating environmental safety.*

Organization	Links to Documents	Description
OECD (Organization for Economic Cooperation and Development)	OECD "Blue Book" – Recombinant DNA Safety Considerations http://www.oecd.org/LongAbstract/0,3425,en_2649_34537_40986856_1_1_1_1,00.html Other Relevant OECD Biosafety Documents (BioTrack): http://www.oecd.org/findDocument/0,3354,en_2649_34385_1_1_1_1_1,00.html	The OECD Working Group on the Harmonization of Regulatory Oversight in Biotechnology has prepared a number of documents to assist in performing environmental risk assessment for products of biotechnology. These include a number of consensus documents on the biology of crops and traits, as well as guidance documents for safety considerations for working with recombinant organisms.
U.S. National Academies National Research Council Reports:	http://www.nationalacademies.org/publications/ NRC. 1989. Field testing genetically modified organisms: Framework for decisions. Edited by National Research Council. Washington, DC: National Academy Press. 1996. Understanding Risk: Informing Decisions in a Democratic Society. Washington, DC: National Academy Press. 2002. Animal Biotechnology: Science Based Concerns. Washington, DC: National Academy Press. 2000. Genetically Modified Pest-Protected Plants: Science and Regulation (2000)	The National Research Council (NRC) functions under the auspices of the National Academy of Sciences (NAS), the National Academy of Engineering (NAE), and the Institute of Medicine (IOM). The NAS, NAE, IOM, and NRC are part of a private, nonprofit institution that provides science, technology and health policy advice under a congressional charter signed by President Abraham Lincoln that was originally granted to the NAS in 1863. The mission of the NRC is to improve government decision making and public policy, increase public education and understanding, and promote the acquisition and dissemination of knowledge in matters involving science, engineering, technology, and health. The NRC has published numerous reports that are relevant to biosafety. Some highlights have been listed here.
UNEP (United Nations Environment Program)	http://www.unep.org/Documents.Multilingual/?Default.asp?DocumentID+96&ArticleID=1473&1=en	International Technical Guidelines for Safety in Biotechnology (1995) provide assistance for risk assessment and risk management of products of biotechnology.
IPPC (International Plant Protection	ISPM 11 https://www.ippc.int/servlet/BinaryDownloaderServlet/ISPM_11_200	A standard for pest risk analysis of LMOs was developed as a supplement to an existing IPPC standard, ISPM-11, and adopted at the meeting

Convention)	4_En.pdf?filename=1086078360577_ISPM_11_2004.pdf	of the Interim Commission on Phytosanitary Measures in April 2004. The document contains an annex providing guidance on determining if an LMO poses a risk as a potential plant pest. This guidance is consistent with the risk assessment guidance provided in Annex III of the Protocol. The scope of the guidance under the IPPC includes risks to plant health, and includes risks to both managed and unmanaged ecosystems posed by plants or other organisms, including risks to plant biodiversity.
WTO SPS Agreement	http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm	The SPS agreement serves as a model for how an international agreement can reference and encourage the use of guidance for science based assessment
U.S. Regulatory Agencies Unified Biotechnology Website	http://usbiotechreg.nbii.gov/	The Unified Biotechnology Website serves as a portal to the primary U.S. government agencies that regulate biotechnology – EPA, FDA, and USDA
Organization	**Links to Documents**	**Description**
UNEP (United Nations Environment Program)	http://www.unep.org/Documents.Multilingual/?Default.asp?DocumentID+96&ArticleID=1473&1=en	International Technical Guidelines for Safety in Biotechnology (1995) provide assistance for risk assessment and risk management of products of biotechnology.
IPPC (International Plant Protection Convention)	https://www.ippc.int/servlet/BinaryDownloaderServlet/ISPM_11_2004_En.pdf?filename=1086078360577_ISPM_11_2004.pdf	A standard for pest risk analysis of LMOs was developed as a supplement to an existing IPPC standard, ISPM-11, and adopted at the meeting of the Interim Commission on Phytosanitary Measures in April 2004. The document contains an annex providing guidance on determining if an LMO poses a risk as a potential plant pest. This guidance is consistent with the risk assessment guidance provided in Annex III of the Protocol. The scope of the guidance under the IPPC includes risks to plant health, and includes risks to both managed and unmanaged ecosystems posed by plants or other organisms, including risks to plant biodiversity.
UNEP	http://www.biosafetyprotocol.be/UNEPGuid/UNEP_03.html	Annex III of the UNEP International Technical Guidelines for Safety in Biotechnology (1995)
OECD (Organization for Economic Cooperation and Development)	http://www.oecd.org/findDocument/0,2350,en_2649_34387_1_119829_1_1_1,00.html http://www.oecd.org/searchResult/0,2665,en_2649_37437_1_1_1_1_37437,00.html	The OECD Working Group on the Harmonization of Regulatory Oversight in Biotechnology has prepared a number of documents to assist in performing environmental risk assessment for products of biotechnology. These include a number of consensus documents on the biology of crops and traits, as well as guidance documents for safety considerations for working with recombinant organisms.

Table 2. *Examples of case-specific risk assessments for LMOs*

Organization	**Links to documents**	**Description**

USDA-APHIS	http://www.aphis.usda.gov/brs/aphisdocs2/06_27101p_com.pdf	Determination of nonregulated status under APHIS regulations 7 CFR Part 340 for soybean 356043 (DP-356Ø43-5) engineered for tolerance to the herbicide glyphosate and herbicides that inhibit the enzyme acetolactate synthase
US-EPA	http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006484.htm	Biopesticide Registration Action Document (BRAD) for Bacillus thuringiensis Cry3Bb1 Protein and the Genetic Material Necessary for its Production (Vector ZMIR13L) in Event MON863 Corn (006484)

Selected resources for additional information:

Safety Considerations for Biotechnology: Scale-up of Crop Plants, OECD, 1993a.

Traditional Crop Breeding Practices: An Historical Review to serve as a Baseline for Assessing the Role of Modern Biotechnology, OECD 1993b.

Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results, OECD 1995a.

Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology, OECD 1995b.

Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology, OECD 1995c.

Safety Assessment of Transgenic Organisms: OECD Consensus Documents Vols. 1 and 2. ISBN Number:92-64-02258-9. Publication Date:24 July 2006. Pages: 823 in two volumes, or individual consensus documents online at: http://www.oecd.org/document/51/0,3343,en_2649_34387_1889395_1_1_1_1,00.html

Codex Alimentarius Commission. 2003. *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants*. CAC/GL 45-2003.

Codex Alimentarius Commission. 2003. *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology.* CAC/GL 45-2003.

NRC. 2002. Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation. Edited by National Research Council. Washington, DC: National Academy Press.

NRC. 2004. Safety of Genetically Engineered Foods - Approaches to Assessing Unintended Health Effects. Edited by Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health. National Research Council. Washington, DC: National Academies Press.

III. SUBMISSIONS FROM RELEVANT ORGANIZATIONS

F. CODEX ALIMENTARIUS COMMISSION

Information on the Work of the Codex Alimentarius Commission pertaining to Risk Assessment and Risk Management of Living Modified Organisms (LMOs)

Secretariat of the Codex Alimentarius Commission
Joint FAO/WHO Food Standards Programme

(January 2009)

There are several documents already adopted by the Codex Alimentarius Commission and ongoing discussions pertaining to risk assessment and risk management of LMOs. However, it should be noted that any existing or future Codex texts cover only foods, which may or may not be LMOs and, in principle, focus only on the aspects of food safety and quality and not on the environment, because the primary objectives of the Codex Alimentarius Commission are to protect the health of the consumers and to ensure fair practices in the food trade.

1. Principles and Guidelines for Risk Assessment and Risk Management of LMOs

There are currently four Codex texts providing guidance on risk assessment and risk management of LMOs as follows:

i) *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology* (CAC/GL 44-2003)

ii) *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (CAC/GL 45-2003);

iii) *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals* (CAC/GL 68-2008); and

iv) *Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms* (CAC/GL 46-2003)

Principles for the Risk Analysis of Foods Derived from Modern Biotechnology provides an overarching framework for undertaking risk analysis on the safety and nutritional aspect of foods derived from biotechnology, namely, risk assessment, risk management and risk communication. Most importantly, under "risk assessment", it introduces the concept of "safety assessment", which is based on the comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences. Only when a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

The other three texts respectively provide guidelines for conducting safety assessments specifically for foods derived from recombinant-DNA plants, animals and microorganisms, in accordance with the framework given in the above Principles.

The *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* contains an Annex on Food Safety Assessment in Situations of Low-level Presence of Recombinant-DNA Plant Material in Food, which was adopted by the Commission in 2008. An increasing number of recombinant-DNA plants are being authorized for commercialization in many countries. However, they are not necessarily authorized for use in trade partner countries at the same time. As a consequence of asymmetric authorizations, low levels of recombinant DNA plant materials that have passed a food safety assessment according to the Codex Plant Guideline in one or more countries may, on occasion, be present in food in importing countries in which the food safety of the relevant recombinant-DNA plants has not been determined. This Annex describes the recommended approach to the food safety assessment in such situations of low-level presence of recombinant-DNA plant material or in-advance preparation for such potential circumstances. In order to support the provisions on information exchange contained in this Annex, an international database has been developed, with a view to facilitating the exchange of information on official food safety assessments of recombinant-DNA plants conducted by governments. This database will be maintained by FAO, in cooperation with the OECD (*see* Section 3 below for details).

2. Consideration of other issues pertaining to Risk Management and Risk Management of LMOs

Labelling of foods derived from biotechnology

The Codex Committee on Food Labelling (CCFL) has been considering, since 1996, appropriate food labelling provisions for foods derived from biotechnology. This work aims at establishing "Definitions and Guidelines for the Labelling of Foods obtained through Certain Techniques of Genetic Modification/Genetic Engineering".

However, these draft texts are still under discussion due to lack of consensus. The most controversial point is whether or not mandatory labelling provisions should be established for the case where the difference between original products and genetically modified products is solely the production method.

The 36th Session of CCFL (28 April - 2 May 2008 in Ottawa, Canada), following up on its previous discussions, had an exchange of views on labelling requirements as related to "Foods Obtained Through Certain Techniques of Genetic Modification/Genetic Engineering". The Committee recognised that there was large support for proceeding with the work and agreed to circulate the Proposed Draft Recommendations for the Labelling of Foods and Food Ingredients Obtained Through Certain Techniques of Genetic Modification/Genetic Engineering at Step 3 for comments and consideration at the 37th Session of the CCFL in May 2009.

Methods of detection and analysis for the foods derived from biotechnology

The Codex Committee on Methods of Analysis and Sampling (CCMAS) has been discussing appropriate methods of detection and analysis for the GM foods since 2002. In view of the absence of specific labelling provisions for GMOs in Codex and of difficulties with the practical application of methodology in this area, the CCMAS proposed to develop recommendations with respect to criteria for methods of analysis and for quality control measures that should be introduced in laboratories offering GM analysis (Guidelines for the Validations and Quality Control Requirements for the Analysis of Foods derived from Biotechnology).

The 28th Session of the CCMAS held in March 2007 considered a new revised document on the criteria for the detection and identification of foods derived from biotechnology, including: i) the information required for the validation of quantitative and qualitative methods, ii) the characteristics that could be used to consider existing validated methods; iii) issues related to measurement uncertainty and interpretation of the results; and iv) proficiency testing. The 29th Session of CCMAS in March 2008 further discussed the matter and agreed to start new work for developing Guidelines on Criteria for Methods for the Detection and Identification of Foods Derived from Biotechnology, which was subsequently approved by the 31st Session of the Commission.

3. Relevant works of the FAO and WHO that complement Codex work

Scientific advice on the safety assessment of foods derived from biotechnology

In order to provide scientific advice to the Codex as a basis for its consideration of guidelines on the assessment of foods derived from biotechnology, FAO and WHO had held several expert meeting as follows (details are available at http://www.fao.org/ag/agn/agns/biotechnology_expert_en.asp):

- *Safety aspects of genetically modified foods of plant origin* (Joint FAO/WHO Expert Consultation on Food Derived from Biotechnology, 29 May–2 June 2000, Geneva, Switzerland)
- *Evaluation of allergenicity of genetically modified foods* (Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22–25 January, 2001, Rome, Italy)
- *Safety assessment of foods derived from genetically modified micro-organisms* (Joint FAO/WHO Expert Consultation on Food Derived from Biotechnology, 24-28 September 2001, Geneva, Switzerland)
- *Safety assessment of foods derived from genetically modified animals, including fish* (Joint FAO/WHO Expert Consultation on Food Derived from Biotechnology, 17-21 November 2003, Rome, Italy)
- Joint FAO/WHO expert consultation on safety assessment of food derived from biotechnology (26 February - 2 March 2007, WHO headquarters, Geneva, Switzerland)

Relevant publications

FAO developed a standardized training package to assist countries in implementing relevant Codex texts related to the food safety assessment of foods derived from recombinant-DNA plants. This training package, entitled "GM food safety assessment: tools for trainers", contains both theory and practical examples of risk assessments of foods derived from modern biotechnology and a guide for training regulators. The publication is expected to be out in early 2009.

WHO has published several informative documents on modern food technology by itself or in cooperation with other organizations, including *Modern food biotechnology, human health and development: an evidence-based study* (WHO, 2005). Full list of WHO publications on genetically modified foods are available at http://www.who.int/foodsafety/publications/biotech/en/index.html.

International Portal on Food Safety, Animal and Plant Health (IPFSAPH)

The International Portal on Food Safety, Animal and Plant Health (IPFSAPH) is a portal site managed by FAO in cooperation with Codex, CBD, IPPC, OIE, WHO and WTO, which provides links to SPS-related regulatory information with powerful search function. (http://www.ipfsaph.org)

Recently, a data set for food safety assessment of recombinant-DNA plants authorized in accordance with the Codex Guideline for the Conduct of Food Safety Assessments of Foods Derived from Recombinant-DNA Plants was created. In cooperation with the OECD BioTrack database, an interoperable central database, publicly accessible from www.ipfsaph.org, containing food safety assessments relating to foods derived from recombinant-DNA plants has been developed and populated. The data set pools food safety assessment records for approved transformation events from various official online sources including OECD BioTrack, the Biosafety Clearing House and the EC Register of Genetically Modified Food and Feed, amongst others.

G. GLOBAL INDUSTRY COALITION

21 January 2009

COMPILATION OF ENVIRONMENTAL RISK ASSESSMENT GUIDANCE

GLOBAL INDUSTRY COALITION

The Global Industry Coalition (GIC)[1] submits the following information in response to Notification 2008-10-23 from Secretariat to the Convention on Biological Diversity dated 23 October 2008, inviting Parties, other Governments and relevant international organizations to provide to the Executive Secretary relevant existing guidance materials regarding risk assessment and risk management of living modified organisms (LMOs). These materials serve as important background for future ad hoc technical expert groups (AHTEGs) on Risk Assessment and Risk Management. Furthermore, the information being provided in Annexes I-III updates a submission of information made in December 2004 on existing guidance materials for emerging technologies, which is still relevant for use by the future AHTEGs.

Research on biotech crops has been ongoing since the early days of genetic modification of plants. After over 12 years of the successful and safe deployment of approved biotech crops, a wealth of published information continues to corroborate the key points that have been made in decisions published following meetings of the Parties (MOPs). The first point is that sufficient guidance exists on risk assessment for Parties to use in decision-making processes, and there is no need for additional guidance on risk assessment for biotech plants. Secondly, current needs are effective capacity building on risk assessment, better information sharing among Parties, and organization of existing information on the Biosafety Clearing-House, that together will help Parties conduct efficient and effective risk assessments. The GIC would like to highlight for the Secretariat and Parties to the Cartagena Protocol on Biosafety the following literature that has been published since December 2004.

I. GUIDANCE MATERIALS DIRECTLY APPLICABLE TO RISK ASSESSMENT AND RISK MANAGEMENT OF LMOS UNDER THE PROTOCOL

A. Australian Government, Department of Health and Aging, Office of the Gene Technology Regulator. Risk Analysis Framework. January 2005. 106 pages. Available through: www.ogtr.gov.au .

B. Brazil, Comissão Técnica Nacional de Biossegurança. Normative Resolution # 5, of March 12, 2008. Rules on the commercial release of genetically-modified organisms and derivatives thereof. Available at: http://www.ctnbio.gov.br/index.php/content/view/11444.html (in Portuguese).

C. European Union, Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed, *the EFSA Journal* (2006) 99, 1-100.

D. Comisión Federal para la Protección contra Riesgos Sanitarios. Procedimiento de Evaluación de Inocuidad de Organismos Genéticamente Modificados Destinados al Uso

[1] The Global Industry Coalition (GIC) for the Cartagena Protocol on Biosafety receives input and direction from trade associations representing thousands of companies from all over the world. Participants include associations representing and companies engaged in a variety of industrial sectors such as plant science, seeds, agricultural biotechnology, food production, animal agriculture, human and animal health care, and the environment.

o Consumo Humano, Procesamiento de Alimentos, Biorremediación y Salud Pública (Procedure for Safety Evaluation of Genetically Modified Organisms Intended for Use or Human Consumption, Food Processing, Bioremediation and Public Health). Fecha de Revisión: **14/04/2005** (http://cofepris.salud.gob.mx/pyp/biotec/Proc_eval_OGMs.pdf)

E. United States of America, US EPA. The US EPA publishes Biopesticides Registration Action Documents on all plant incorporated protectants (PIPs) after they have been registered by the agency. These documents are extensive and provide useful information on the GM crops (typically Bt crops) and the risk assessments conducted by the US EPA. The documents are publicly available by searching at: www.epa.gov/pesticides/pips/index.htm . In addition, detailed risk assessment information is available by searching a the US data base of Completed Regulatory Agency Reviews: http://usbiotechreg.nbii.gov/database_pub.asp .

II. RECENT PUBLICATIONS ON THE BASIC CONCEPTS UNDERPINNING ENVIRONMENTAL RISK ASSESSMENT

Craig, W., et al. 2008. An Overview of General Features of Risk Assessments of Genetically Modified Crops. *Euphytica* 164: 853-880

Garcia-Alonzo, M., et al. 2006. A Tiered System for Assessing the Risk of Genetically Modified Plants to Non-target Organisms. *Environ. Biosafety Res.* 5: 57-65.

Johnson, K., et al. 2007. How Does Scientific Risk Assessment of GM Crops Fit Within the Wider Risk Analysis. *Trends in Plant Sciences* 12: 1-5.

Nickson, T., Horak, M. 2006. Assessing Familiarity: the Role of Plant Characterization. IN: Proceedings of the Ninth International symposium on the Biosafety of Genetically Modified Organisms. September 24-29, 2006, Jeju Island, Korea. International Society for Biosafety Research, pp 76-80.

Nickson, T. 2008. Planning Environmental Risk Assessment for Genetically Modified Crops: Problem Formulation for Stress-tolerant Crops. *Plant Physiol.* 147: 494-502.

Raybould, A. 2007. Ecological versus Ecotoxicological Methods for Assessing the Environmental Risks of Transgenic Crops. *Plant Science* 173: 589-602.

Raybould, A. 2006. Problem Formulation and Hypothesis Testing for Environmental Risk Assessments of Genetically Modified Crops. *Environ. Biosafety Res.* 5: 119-125.

Romeis, J., et al. 2008 Assessment of Risk of Insect Resistant Crops to Nontarget Arthropods. *Nature Biotechnology* 26(2): 203-208.

III. RECENT PUBLICATIONS DESCRIBING SPECIFIC EXAMPLES OF ENVIRONMENTAL RISK ASSESSMENTS

Chandler, S., Dunwell, J. 2008. Gene Flow, Risk Assessment and the Environmental Release of Transgenic Plants. *Critical Reviews in Plant Sciences* 27(1): 25-49.

Espinoza-Esquivel, A.M. & Arrieta-Espinoza1, G. 2007. A multidisciplinary approach directed towards the commercial release of transgenic herbicide-tolerant rice in Costa Rica. *Transgenic Research* 16: 541-555.

Fuchs, M., Cambra, M., Capote, N., Jelkmann, W., Kundu, J., Laval, V., Martelli, G.P., Minafra, A., Petrovic, N., Pfeiffer, P., Pompe-Novak, M., Ravelonandro, M., Saldarelli, P., Stussi-Garaud, C., Vigne, E. & Zagrai, I. 2007. Safety assessment of transgenic plums and grapevines expressing viral coat protein genes: new insights into real environmental impact of perennial plants engineered for virus resistance. *Journal of Plant Pathology* 89: 5-12.

Horak, M., et al. 2007. Characterization of Roundup Ready Flex Cotton – MON 88913 – for Use in Ecological Risk Assessment Evaluation of Seed Germination, Vegetative and Reproductive Growth and Ecological Interactions. *Crop Science* 47: 268-277.

Koga-Ban, Y.; Tabei, Y., Ishimoto, M., Nishizawa, Y., Tsuchiya, K., Imaizumi N., Nakamura, H., Kayano, T., Tanaka, H. 2006. Biosafety assessment of transgenic plants in the greenhouse and the field: A case study of transgenic cucumber. *Japan Agricultural Research Quarterly* 38: 167-174.

Peterson, R., Sharma, L. 2005. A Comparative Risk Assessment of Genetically Engineered, Mutagenic and Conventional Wheat Production Systems. *Transgenic Res.* 14: 859-875.

Peterson, R., et al. 2006. Genetically Engineered Plants, Endangered Species, and Risk: A Temporal and Spatial Exposure Assessment for Karner Blue Butterfly Larvae and Bt Maize Pollen. *Risk Analysis* 26(3): 845-858.

Raybould, A. 2005. Assessing the Environmental Risks of Transgenic Volunteer Weeds. IN: Crop Ferality and Volunteerism. Ed. Gressel, J. Taylor and Francis, Boca Raton, FL. Chapter 23: 389-402.

Raybould, A., et al. 2007. Non-target Organism Risk Assessment of MIR604 Maize Expressing mCry3A for Control of Corn Rootworm. *J. Applied Entomol.* 131(6): 391-399.

Raybould, A., Cooper, I. 2006. Tiered Tests to Assess the Environmental Risks of Fitness Changes in Hybrids between Transgenic Crops and Wild Relatives: The Example of Virus Resistant *Brassica napus*. *Environ. Biosafety Res.* 4:127-140.

Turturo, C., Friscina, A., Gaubert, S., Jacquemond, M., Thompson, J.R. & Tepfer, M. 2008. Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences. *Journal of General Virology* 89: 327-335.

Wolt, J., et al. 2006. An Ecological Risk Assessment of Cry1F Maize Pollen Impact to Pale Grass Blue Butterfly. *Environ. Biosafety Res.* 4: 243-251.

IV. RECENTLY PUBLISHED REVIEWS RELEVANT TO RISK ASSESSMENT

Cerdeira, A., Duke, S. 2006. The Current Status and Environmental Impacts of Glyphosate-resistant Crops: A review. *J. Environ. Qual.* 35: 1633-1658.

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21 January 2009

ANNEX I
COMPILATION OF ENVIRONMENTAL RISK ASSESSMENT GUIDANCE: TRANSGENIC TREES

Research on transgenic trees has been ongoing since the early days of genetic modification of plants. While only a few countries have undertaken transgenic tree environmental risk assessments, those countries in which companies and research organizations are conducting work on transgenic trees – including the United States, Canada, and Brazil – have successfully applied their existing regulatory frameworks and guidance for transgenic plants to transgenic trees as well. The fundamental principles of environmental risk assessment are the same and existing frameworks within these countries are sufficiently flexible to meet the needs of a wide range of transgenic plant species, including trees.

The following three areas point to the successful application of existing environmental risk assessment principles to the application of transgenic trees:

1) An established track record of risk assessment and supporting documentation for assessment of transgenic trees;
2) History of safe use of transgenic trees in over 800 trials around the world; and
3) An established dialogue on appropriate data and safeguards for transgenic trees.

I. EXISTING ENVIRONMENTAL RISK ASSESSMENTS AND SUPPORTING DOCUMENTATION FOR ENVIRONMENTAL RISK ASSESSMENT

Through their review and approval of field test permit applications, at least 20 countries have completed some form of risk assessment on transgenic trees, representing over 20 different species (Table 1). The following information points to specific examples of published risk assessments in different countries.

I.1. United States

A number of in-depth environmental risk assessments for transgenic trees have been published by the United States through the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA/APHIS) and are available on-line at: http://www.aphis.usda.gov/regulations/biotech/brs_environmental_assessments.shtml.

USDA/APHIS is required to evaluate scientific and other data when the agency receives an application for permission to introduce a "regulated article". The agency undertakes a comprehensive assessment if the regulated article is new or unfamiliar and in these specific cases sought public input into their risk assessments and decision making. Risk assessments have been made and published as part of APHIS review for deregulation for virus resistant papaya and plum (a second virus resistant papaya is currently pending a final decision). In addition APHIS has published 13 Environmental Assessments for permits for field release of transgenic trees. Species assessed include: Poplar (4), Spruce (1), walnut (2) Apple (3), Allegheny Serviceberry (1), Papaya (1), Plum (1), and Eucalyptus (1). Traits include: marker genes, insect resistance, virus resistance, bacterial resistance, reproductive sterility, cold tolerance.

The environmental consequences of the field test were considered by USDA/APHIS under the following principles:

- The risks associated with the introduction of transgenic organisms are "the same in kind" as those associated with introducing unmodified organisms and those modified by other techniques.
- Risk assessments for introducing transgenic organisms "should be based on the nature of the organism and the environment into which it is to be introduced." (1987 NAS report)

Table 1. Summary of risk assessments/approved field trials and supporting documentation for transgenic trees

Risk Assessments completed – Field Trials Approved	**Species Assessed**	**Traits Assessed**	**OECD Consensus Documents**
Argentina Australia Brazil Canada China Czech Republic Belgium France Finland Germany Italy Japan Netherlands New Zealand Norway Portugal Romania Spain Sweden United Kingdom United States	✓ poplar ✓ apple ✓ papaya ✓ pine ✓ aspen ✓ chestnut ✓ cherry ✓ elm ✓ banana ✓ coffee ✓ plum ✓ citrus (various) ✓ birch ✓ walnut ✓ cherry ✓ kiwifruit ✓ eucalyptus ✓ spruce ✓ walnut ✓ avocado ✓ pear ✓ persimmon ✓ sweetgum ✓ Variety of other woody perennials such as grape, blueberry , cranberry, raspberry and sugarcane	✓ Marker genes ✓ Insect resistance ✓ Virus resistance ✓ Bacterial resistance ✓ Drought resistance ✓ Reproductive sterility ✓ Cold tolerance ✓ Reduced or altered lignin ✓ Improved wood quality ✓ Increased biomass or yield	<u>Completed:</u> ✓ poplars ✓ *Prunus* ✓ papaya ✓ Jack pine ✓ Eastern white pine ✓ Sitka spruce ✓ white spruce ✓ Norway spruce ✓ *Larix* ✓ Birch ✓ Western white pine ✓ Douglas-fir ✓ lodgepole pine
Large Scale Production Approved			**Genome sequencing:**
<u>China:</u> ▪ Insect resistant poplars <u>United States</u>: ▪ Virus resistant papaya ▪ Virus resistant plum			<u>Completed:</u> ✓ Poplar ✓ Papaya <u>In advanced stages</u>: ▪ eucalyptus

I.2. <u>EU</u>

Risk assessments for several tree species have been performed and published by the European Union (http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx). These include plum, apple, pear, a variety of citrus species, birch and poplar. Each report includes details of an assessment of environmental impact and risk management. Previous field trials approvals (prior to 1991) include additional species: kiwi, eucalyptus, aspen, pine, cherry and spruce (http://bgmo.jrc.ec.europa.eu/deliberate/doc/snifs.pdf .

I.3. Organisation for Economic Co-operation and Development (OECD)

The OECD's Working Group for Harmonisation of Regulatory Oversight in Biotechnology publishes Biosafety Consensus Documents to ensure that countries use common methods to collect data and consistent information for risk/safety assessments in their development of biotechnology regulations and guidance. An important underlying concept to these efforts is that all national governments share the need to undertake environmental assessments and that any information relating to the biology of an organism would be the same regardless of what country's regulatory system was involved. The Consensus Documents are intended to share information on key components that member countries believe are relevant to an environmental safety review. Thirteen consensus documents have already been published including poplars, *Prunus*, papaya, *Larix*, and several pine and spruce species (Table 1).

II. HISTORY OF SAFE USE IN FIELD TRIALS

The Food and Agriculture Organization's (FAO) 2004 Report "Preliminary Review of Biotechnology in Forestry, Including Genetic Modification" provides a summary of the status of field tests with transgenic forest trees species up to that time. An updated summary for some countries is provided below.

II.1. Argentina

A search of Argentina's biotech regulatory authority website (http://www.sagpya.mecon.gov.ar/new/0-/programas/conabia/liberaciones_ogm_2005.php) and similar pages indicates that there have been 22 approved field trials of woody perennial crops in Argentina, the majority of which have been with papaya.

II.2. Australia/New Zealand

Researchers supported by the Australian government are seeking to improve the quality of wood fibers from native eucalyptus trees. A search of Australia's biotech regulatory authority website (http://www.ogtr.gov.au/gmorec/ir.htm) indicates that there have been six approved field trials of woody perennial crops in Australia (including papaya, grape, sugarcane and roses).

Environmental Risk Management Authority (ERMA) in New Zealand oversees experiments (both in the laboratory and field trials) of new organisms. ERMA's searchable database (http://www.ermanz.govt.nz/search/registers.html) indicates that they have reviewed and approved numerous experiments in a variety of tree species including: apple, Pinus, Eucalyptus, kiwifruit, grapes and roses. Field trials have been conducted with Pinus radiata.

II.3. Brazil

CTNBio (National Technical Commission on Biosafety) has regulatory authority over field trials and commercial approvals of transgenic organisms. There have been 17 applications for field trials of transgenic woody perennial species. These include: Eucalyptus (14), citrus (2) and papaya (1). Traits include: virus resistance, reduced or altered lignin, improved wood quality, increased biomass or yield, drought resistant. Several of these applications are currently pending. CTNBio does not have specific regulations for transgenic trees but uses their existing

framework to consider each application on a case-by-case basis with consideration of the biology of the species.

II.4. Canada

Under their existing risk assessment scheme, Canada has reviewed applications for and issued 50 approvals for field trials of perennial woody species. In Canada these trials are reviewed and renewed on an annual basis so this number is an overestimate. Species have included poplars and spruces as well as fruit bearing woody perennials such as cherry and grape.

II.5. European Union

A search of the EU's biotech field trial databases (http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx and http://bgmo.jrc.ec.europa.eu/deliberate/gmo.asp) indicate that almost 70 field trials have been approved for perennial woody species. These include forest tree species (various *Populus* species and hybrids, Eucalyptus, pines, birch and spruce) as well as grapes and fruit trees (apple, plum, pear, cherry, various citrus fruits, olive and kiwi).

II.6. United States

USDA/APHIS has issued several release permits for transgenic trees and several risk assessments were published (see above) in conjunction with the field test applications. To date two transgenic tree species have been deregulated by the agency. As with other deregulation decisions in other species, USDA/APHIS conducted a broad environmental risk assessment as part of their decision making process. Over the past 20 years, USDA/APHIS has approved over 700 field trials of woody perennial species under their existing risk assessment process (http://www.isb.vt.edu/cfdocs/fieldtests1.cfm).

It is noteworthy that for this ~800+ record of approved field trials these were assessed under existing criteria for transgenic plants and that none of these tests have reported any harm to biodiversity, human health or the environment. This indicates that the current regulatory framework within these countries is sufficiently broad and flexible to manage plants with a wide range of biological characteristics, including trees.

III. ESTABLISHED WITHIN COUNTRY AND INTERNATIONAL DIALOGUE ON APPROPRIATE OVERSIGHT FOR TRANSGENIC TREES

There have been multiple workshops held in different countries to discuss and evaluate criteria for risk assessment of transgenic trees. Several of these have been held jointly between the United States and Canada. In these countries, as well as others, there is already an established and ongoing dialogue on how the basic principles of environmental risk assessment and regulatory oversight are applicable to trees. Just a few examples of this dialogue are given below.

The GIC believes that, based on the content of these dialogues, the principles for environmental risk assessment are the same for transgenic trees as for other transgenic plants. Further, assessing risk on a case-by-case basis using these principles is appropriate.

III.1. Brazil

Numerous workshops and discussion groups have been held in Brazil on biotechnology applications, including the annual Congresso Brasileiro de Biotecnologia organized by ANBio (National Association of Biosafety) and tree species have been included in many of these meetings. In addition there have been meetings that specifically address Biosafety for transgenic trees including two organized by IPEF (Institute for Forestry Research and Studies):

- *Workshop Biossegurança Florestal: Diretrizes para Avaliação de Impactos de Árvores Geneticamente Modificadas* (Forest Biosafety Workshop: Lines of direction for Evaluation of Impacts of Genetically Modified Trees. July 5 and 6, 2004
- *I Curso Intensivo de Biossegurança com Organismos Geneticamente Modificados - Avaliação de Riscos de Impactos Ambientais* (Intensive Course on Biosafety with Genetically Modified Organisms - Evaluation of Risks of Environmental Impacts. April 8-11, 2003
- There is an ongoing discussion and research group on Forest Biosafety more specifically with Eucalyptus at Agriculture College of São Paulo University. Scientific researchers from several public universities and the main Brazilian forest companies participate actively in this group. http://www.bioflor.esalq.usp.br.

III.2. Canada

a. Workshop on Finding the Balance: What is the Least Intrusive and Most Effective Way to Regulate the Environmental Release of Fruit Trees and Ornamental Trees with Novel Traits? British Columbia, Feb. 20-21, 2006. http://www.inspection.gc.ca/english/plaveg/bio/consult/ornamen/2006/2006repe.pdf.
b. From Sept. 26, 2006 to Nov. 17, 2006, the Canadian Food Inspection Agency (CFIA) undertook a web-based consultation on Draft Updates to Directive 2000-07 for Plants with Novel Traits that Are Forest Trees.
c. Ongoing Research Projects at the Canadian Forest Service are actively addressing appropriate oversight for trees. Projects include investigating the persistence of DNA from transgenic trees in the environment, and assessing risks associated with the dispersion of genes.

III.3. United States

a. USDA APHIS Public Meeting: Genetically Engineered Forest and Fruit Trees. July 8-9, 2003. Meeting considered the list of 17 traits used by USDA/APHIS and the Canadian Food Inspection Agency for assessment of transgenic plants and concluded that these were an adequate rubric (http://www.aphis.usda.gov/brs/tree_discuss.html)
b. IFB (Institute of Forest Biotechnology) has sponsored several meetings on genetically engineered trees. Summaries of some of these are available at http://www.forestbiotech.org/ifb-publications.php.

III.4. The International Union of Forest Research Organizations (IUFRO)

IUFRO created a Task Force on Forests and Genetically Modified Trees (http://www.iufro.org/science/task-forces/genetics/?L=3print%2Fprint%2F). In 1999 IUFRO issued a Position Statement on the Benefits and Risks of Transgenic Plantations (see Strauss et al. 1999 *Nature Biotechnology 17, 1145*). IUFRO has also sponsored international meetings

gathering leading experts from around the world to discuss issues focusing on genetically modified trees:

- Tree Biotechnology in the New Millennium. July 22-27, 2001. Stevenson, WA, USA
- IUFRO Tree Biotechnology 2005. November 6-11, 2005. Pretoria, South Africa.

III.5. Organization for Economic Cooperation and Development (OECD)

OECD's Working Group on Harmonization of Regulatory Oversight in Biotechnology organized a workshop in September 1999 to clarify issues in long-lived plant species including trees and to identify a mechanism to address those issues: "Workshop on the Environmental Considerations of Genetically Modified Trees". Norway, September 1999. *http://www.olis.oecd.org/olis/2001doc.nsf/43bb6130e5e86e5fc12569fa005d004c/dbcd36c857435717c1256b27005d5d7a/$FILE/JT00118742.PDF*.

III.6. United Nations/Food and Agriculture Organization (FAO)

In 2004, the FAO published a report: "Preliminary Review of Biotechnology in Forestry, Including Genetic Modification" which provides statistical information on the extent and patterns of transgenic research and applications to forest trees globally (*ftp://ftp.fao.org/docrep/fao/008/ae574e/ae574e00.pdf*). It also includes responses to a questionnaire on the potential risks and benefits from GM forest trees. Sixty-five percent of respondents indicated that the regulatory framework in their country was adequate to assess benefits and risks.

From April 25 to June 30, 2000, FAO also organized an electronic discussion forum on "How appropriate are currently available biotechnologies for the forestry sector in developing countries?" A summary of the conference and key points is available at http://www.fao.org/Biotech/Conf2.htm. Other discussion fora in this same series addressed forestry alongside crops, livestock and fish in a variety of issue areas.

IV. CONCLUDING REMARKS

The GIC is of the view that, due to the vast experience with and understanding of the safety of transgenic trees, already defined environmental risk assessment principles that are sufficiently broad and flexible enough to deal with the range of characteristics of other plants have been, and can continue to successfully be, applied to transgenic trees.

ANNEX II
COMPILATION OF ENVIRONMENTAL RISK ASSESSMENT GUIDANCE: PLANT-MADE PHARMACEUTICALS (PMPS)

Living modified organisms (LMOs) expressing plant-made pharmaceuticals (PMPs) represent a special class of genetically modified plants in that they have been specifically modified to express therapeutic agents (pharmaceutical proteins) and are not generally intended for widespread adoption in agriculture or use in food or animal feeds. Crops such as corn, tobacco, rice and soybean are genetically altered to yield proteins with purity and activity equivalent to those produced by other manufacturing systems, with advantages that include large volume production capacity, reduced capital requirements, and freedom from potential viral and animal protein contamination.

A number of countries are in the process of developing, or adapting, their regulatory frameworks to deal with the commercial production of PMPs, and some of these have established risk management processes to permit the evaluation and production of these substances under controlled conditions. Generally, the principles of risk management as applied to confined field trials of LMOs expressing agronomic or plant quality traits are also applicable to the production of PMPs under confined conditions.

The purpose of this Annex is provide a brief overview of how some selected countries have adapted existing risk management practices for the conduct of confined field trials to enable the safe production of PMPs under confined, or closed-loop, production systems. The approaches described below illustrate how the risk management objectives of preventing establishment and persistence in the environment, preventing spread in the environment, and preventing introduction into the human food and animal feed pathways, can be practically achieved for LMOs expressing PMPs.

I. COUNTRY EXAMPLES

I.1 UNITED STATES

The Biotechnology Regulatory Services (BRS) of the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) is responsible for regulating the environmental introduction of all genetically engineered plants, including those expressing PMPs, under the federal *Plant Protection Act*. APHIS requires that an applicant describe the modified plant in detail and provide information on the processes and procedures that will be used to confine the regulated article to a field test site, or while it is in transport.

APHIS has published specific guidance on the field testing and/or movement of organisms intended for the production of PMPs or industrial proteins. In assessing applications, BRS requires information on the following:

- Detailed description of the organism/and or product
- Location of the intended release

- Anticipated or actual expression of the alter genetic material in the regulated article
- Detailed description of molecular characterization
- Source of the regulated article
- Purpose for the regulated article
- Description of processes to prevent dissemination
- Description of processes to prevent dissemination at each destination

Using this information, BRS first determines whether the proposed action to be taken with the PMP will pose a plant pest risk, or a risk to the environment, including humans and animals. The focus of this analysis is to determine whether the potential, or actual, risks can be managed and whether the applicant demonstrates in the permit application that the PMP will not escape into and persist in the environment. In BRS' experience, PMPs are no more likely to escape and persist than plants expressing other traits, so BRS' analysis focuses on the answers to two questions:

1) What is the likelihood that a particular confinement approach will prevent unanticipated environmental exposure? And–
2) What is the likelihood that unanticipated environmental exposure will result in significant impacts?

Typically, BRS will consult with other agencies, such as FDA, EPA, and the Fish and Wildlife Service regarding the impacts from unanticipated exposure on humans, the environment, and wildlife.

When BRS has little or no previous experience with a particular PMP, it may, as required by the National Environmental Policy Act, undertake the preparation of an Environmental Assessment (EA). The EA will undergo largely the same analyses, (i.e., plant pest risk and environmental risk) as described above; however, the EA is submitted to the public for comment. BRS will finalize the EA, addressing public comments as appropriate, before the PMP permit can be issued.

I.1.1 Bibliography

APHIS (2008). Guidance for APHIS permits for field testing or movement of organisms intended for pharmaceutical or industrial use. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Biotechnology Regulatory Services. July 2008. Available online at: http://www.aphis.usda.gov/brs/pdf/Pharma_Guidance.pdf

I.2 CANADA

In Canada, the environmental release of plants with novel traits (PNTs)[2] is governed under Part V of the Seeds Regulations, which are administered by the Canadian Food Inspection Agency

[2] Because the scope of Canada's regulatory approach is broader than just genetically engineered plants and foods, Canadian regulators have adopted unique terminology and definitions. Rather than referring to "GM plants" or

(CFIA). Within the CFIA, the Plant Biosafety Office (PBO) is responsible for coordinating risk assessments of PNTs and ensuring the implementation of appropriate risk management practices for PNTs, including those expressing PMPs, in confined field trials. The additional information and risk management requirements for PMPs are contained within CFIA's Directive 2000-07: Conducting Confined Research Field Trials of Plant with Novel Traits in Canada.

In addition to the standard terms and conditions applicable to the release of PNTs in confined field trials, CFIA imposes certain conditions specific to PNTs used for plant molecular farming, including:

- Increased isolation distances for PMPs produced in traditional food or feed crop species
- Restrictions on the use of surrounding land (i.e., within 50 m of the trial site) for food or feed production, including grazing of livestock
- Restrictions on the cultivation of crops for food or feed use, or on grazing of livestock, on trial sites during the post-harvest period
- Enhanced reporting and inspection requirements

As well, CFIA has advised applicants to consider the following when applying for confined field trial release of PMPs:

- The use of major food or feed crop species for plant molecular farming is not recommended
- The use of crop species that are pollinated by bees that contribute to commercial honey production is not recommended
- Developers are encouraged to consider fibre crops, crops with only minor food or feed use, small-acreage specialty food or feed crops, or new crops as production platforms
- The host species should be as amenable to confinement as possible, i.e. developers should consider level of outcrossing, mode of pollination, weediness, seed dormancy, seed dispersal, harvest efficiency, tendency to volunteer, and available reproductive control mechanisms in choosing a production platform
- Genetic mechanisms such as tissue-specific or post-harvest inducible expression of the compound may be useful in mitigating environmental exposures.

Applicants must also provide contingency plans for responding to accidental breaches of confinement. These plans must include immediate notification of regulatory authorities and commodity handlers, and provide for monitoring, tracking, recall and destruction of accidentally-released plant material from the environment and/or food or feed supply chains.

Applications for confined releases of PNTs expressing PMPs are also subject to additional reviews by Health Canada and the CFIA Animal Feed Division.

"GM foods", the guidelines and regulations refer to "plants with novel traits" (PNTs) and "novel foods", respectively.

The CFIA is currently in the process of developing separate guidance dealing with 'commercial confined environmental release' (CCER), which would be applicable to the commercial production of PMPs. It is anticipated that applications for CCER would be required to include release management strategies outlining how the developer intends to ensure that these plants would remain segregated from the food/feed chains and how dispersal into the environment would be minimized. Additionally, it is expected that plants authorized for CCER would be subject to ongoing regulatory oversight, including on-site inspections during planting, growing, seed production and harvest, and any post-harvest periods of land use restriction.

I.2.1 Bibliography

CFIA (2005). Developing a regulatory framework for the environmental release of plants with novel traits intended for commercial plant molecular farming in Canada. A discussion document developed by the Canadian Food Inspection Agency's Plant Biosafety Office. Available online at:
http://www.inspection.gc.ca/english/plaveg/bio/mf/fracad/commere.shtml

CFIA (2008). Directive Dir2000-07: Conducting Confined Research Field Trials of Plant with Novel Traits in Canada. Canadian Food Inspection Agency. Available online at: http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml

I.3 EUROPEAN UNION

Under Directive 2001/18/EC, EU Member States are individually responsible for the safety of all "genetically modified organisms," including PMPs, released into the environment.[3] The Directive requires a "competent authority" to be empowered by each Member State to evaluate proposals for GMO releases, to determine whether each proposal complies with the Directive's requirements, and to organize appropriate inspections and other control measures to ensure compliance with the Directive. Each deliberate release of a PMP within an EU Member State is evaluated on a case-by-case basis, but there is currently no EU legislation specifically for PMPs. To date, all field releases of PMPs have been carried out under Part B of Directive 2001/18/EC (i.e., for research purposes), ruling out their commercialization. Pathways for commercialization have yet to be addressed by the European Commission.

Before an applicant may approach a Member State with a notification of a deliberate release of a PMP,[4] Article 4 of the Directive requires the applicant to prepare an environmental risk assessment (ERA), as described in Annex II of the Directive. The ERA must consider direct and both immediate and delayed indirect impacts, comparing the release of the PMP with the release of a non-transgenic comparator plant. The analysis must: 1) assume adverse effects will occur; 2)

[3] The Directive requires Member States to act in accordance with "the precautionary principle," but this translates to a standard of care no different from that used by the United States and Canada, namely ensuring "that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release" of a GMO.

[4] There is a separate Directive, 90/219/EEC, for PMPs to be grown under containment. (This Directive has been amended as 98/81/EC.)

evaluate the likelihood of each adverse effect; 3) estimate the risks posed by the release; 4) analyze available risk management strategies and their effectiveness; and 5) determine the overall risk resulting from the release.

In Annex III B, the Directive lists the required information to be included in the ERA. This information is similar in scope and detail to the types of information required by the United States and Canada, and includes information about the biology of the plant and the engineered trait, a description of the release site and the nature of the release, and a description of confinement measures designed to minimize dispersal and persistence of the PMP.

Article 6 of the Directive describes the process for notifying the competent authority of the deliberate release. The applicant supplies the competent authority with the appropriate information and with the completed ERA. The authority responds, saying that either the application is in compliance with the Directive and the release may occur, or the proposed release does not meet the Directive's requirements and may not occur. Article 9 of the Directive provides for consultation with the public prior to authorizing each deliberate release.

I.3.1 Bibliography

EU (1998). Council Directive 98/81/EC amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms. Available online at: http://eur-lex.europa.eu/LexUriServ/site/en/oj/1998/l_330/l_33019981205en00130031.pdf

ANNEX III
COMPILATION OF ENVIRONMENTAL RISK ASSESSMENT GUIDANCE: TRANSGENIC ANIMALS, INCLUDING FISH

At the present time, there are no internationally-recognized guidelines developed specifically for the environmental risk assessment of transgenic animals. Rather, existing guidance on the risk assessment of transgenic plants is widely used, as the underlying principles are equally applicable. In addition, a number of international organizations and governments are working on guidance directly applicable to environmental risk assessment of transgenic animals. Annex III of the Protocol, which sets out principles and methodologies on how to conduct an environmental risk assessment applies to all potential LMOs (plants, animals, trees, insects and micro-organisms). As such, in many cases, the existing environmental risk assessment guidance may also be applied to environmental risk assessment of transgenic animals and should be considered in conjunction with the information provided in this document.

The purpose of this Annex is to provide an overview of the regulatory and review procedures in selected countries as they apply to the environmental risk assessment of transgenic animals, including fish. The examples provided illustrate how some countries have adapted existing regulatory frameworks and risk assessment procedures to deal with these new products of modern biotechnology. Furthermore, these examples demonstrate that the risk assessment framework described in Annex III of the Protocol is directly applicable to the risk assessment of transgenic animals and that other approaches are not required.

I. COUNTRY EXAMPLES

I.1 JAPAN

I.1.1 Regulatory Framework

After ratifying the Cartagena Protocol on Biosafety in November 2003, Japan enforced the Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms, "Cartagena Law"[5] in February 2004. The "Cartagena Law" applies to all LMOs that are governed under the Protocol, including transgenic animals. Four key ministries are involved in the regulation of agricultural biotechnology (Table 2): the Ministry of Agriculture, Forestry and Fisheries (MAFF); the Ministry of Health, Labour and Welfare (MHLW); the Ministry of Environment (MoE); and the Ministry of Education, Culture, Sports, Science and Technology (MEXT). These ministries act as a secretariat in the regulatory framework to obtain appropriate ministers' approvals and each ministry has separate advisory committees and scientific expert panels to perform risk assessments and safety evaluations.

The risk assessment evaluates the likelihood of an adverse environmental effect by assessing the differences between transgenic animals and recipient animals on the basis of information about the recipient, transformation vectors, and introduced genes. It also takes into account the intended use (i.e., receiving environment) of the transgenic animals.

[5] http://www.biodic.go.jp/cbd/biosafety/pdf/LawVer.1.1.PDF

Table 2: Overview of agencies, laws and regulations applicable to LMOs in Japan

	Environmental Release/ Domestic Cultivation	Food	Feed
Agency	Ministry of Agriculture, Forestry and Fisheries (MAFF) Ministry of Environment (MoE)	Ministry of Health, Labour and Welfare (MHLW)	Ministry of Agriculture, Forestry and Fisheries (MAFF)
Law	Law 97 0f 2003 – "Cartagena Law"	Law 55 of 2003 - Food Sanitation Law	Feed Safety law
Regulations	Enforcement Regulations for the "Cartagena Law"	Food Sanitation Law Enforcement Regulations	
Guidelines	Guidance for Assessment of Adverse Impact on Biodiversity of Type 1 use of LMO; Guidelines for Application of Recombinant DNA Organisms in Agriculture, Forestry, Fisheries, The Food Industry and Other Related Industries	Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)	Guidelines for Safety assessments of Application of rDNA Organisms in Feed

I.1.2 *Regulatory and Risk Assessment Processes*

- Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) controls the field use of transgenic animals.
- The MAFF Agriculture, Forestry and Fisheries Research Council working group on genetically modified animals considers the following factors for the risk assessment of transgenic animals where the host animal is neither insect nor aquatic:
    - Methods of preparing and distinguishing genetically modified animals:
        - Structure and construction methods of recombinant molecules
        - Methods of introduction recombinant molecules into recipient cells
        - Developmental and maintenance processes of genetically modified animals
        - Methods of detecting and identifying recombinant molecules
    - Location of recombinant molecules in recipient cells, stability of expression
    - Differences between transgenic animals and host animals or animals of the same biological species from which the transgenic animals are derived:
        - Reproductive and propagative properties; genetic characteristics
        - Survival and reproductive abilities in the natural environment

- Production of infectious viruses
- Other major physiological characteristics

I.1.3 Conclusions

Generally, Japan's risk assessment framework for transgenic animals is modeled after the risk assessment guidance developed for plant LMOs, and there is great similarity in the types of information required.

I.1.4 Bibliography

Government of Japan (2004). Law Concerning the Conservation and Sustainable Use of Biological Diversity on the Use of Living Modified Organisms (Law no. 97 of 2003). Official Gazette Special Issue No. 134, Ministry of Finance Printing Office, Tokyo, 78-84. Available online at: http://www.biodic.go.jp/cbd/biosafety/pdf/LawVer.1.1.PDF

Yamanouchi, K. (2007). Regulatory considerations on transgenic livestock in Japan in relation to the Cartagena protocol. *Theriogenology* **67**(1): 185–187.

I.2 CANADA

I.2.1 Regulatory Framework

Under the *Canadian Environmental Protection Act 1999* (CEPA 1999), Environment Canada has overall responsibility for ensuring that environmental risk assessments of new substances manufactured or imported into Canada, including organisms produced through biotechnology, are performed prior to release. The requirement, under CEPA 1999, for an environmental assessment by Environment Canada does not apply if the product is regulated pursuant to another Act requiring equivalent environmental assessment, as determined by the Governor General in Council. Thus, the Canadian Food Inspection Agency (CFIA) conducts all environmental assessments for plants with novel traits because the applicable statutory instruments have been deemed to be equivalent (i.e., listed under Schedule 4 of CEPA 1999).[6]

Currently in Canada, biotechnology-derived animals, such as transgenic and other biotechnology-derived livestock animals are regulated under CEPA 1999 and its New Substances Notification Regulations (NSNR). CEPA 1999 is co-administered by Environment Canada and by Health Canada. Part II.1 of the NSNR implements provisions of Part 6 of CEPA 1999 by prescribing the information as well as the timelines for notifying Environment Canada

[6] *Pest Control Products Act* and Pest Control Products Regulations; *Feeds Act* and Feeds Regulation; *Seeds Act* and Seeds Regulation; *Fertilizers Act* and Fertilizers Regulations; and *Health of Animals Act* and Health of Animals Regulations (veterinary biologics). Assessment of the following substances would be pursuant to the New Substances Notification Regulations of CEPA 1999: new industrial chemicals, biochemicals, polymers and biopolymers, (e.g. pigments, plasticizers, additives, etc.) and organisms (e.g. used in bioremediation, industrial enzyme production, etc.); imports of plant material with novel (new) traits (PNT) intended for direct use as food, non-livestock feed, or for processing into food or industrial products and not covered by either the *Seeds Act* or the *Feeds Act* and Regulations; genetically modified microorganisms not covered by a CEPA 1999 listed Act and Regulation; novel feeds for non-livestock animals (e.g. new substances in pet foods); transgenic animals and fish; new substances used as intermediates to manufacture pest control products; and new substances in drugs (human and veterinary), human biologics, cosmetics, or medical devices.

of the intent to manufacture or import such animal or its derived products. Environment Canada and Health Canada then assess for whether or not the animal is toxic or has potential of being "toxic".

I.2.2 Regulatory and Risk Assessment Processes

The process of risk assessment under the NSNR is initiated by a request from the notifier if they are going to manufacture, import, or sell the animal, or release the animal into the environment. Potential risks for human consumption of any transgenic livestock are assessed under Division 28, Part B, of the Food and Drug Regulations (Novel Food Regulations) by Health Canada, while potential livestock feed risks are assessed under the Feeds Regulations, by CFIA. Assessments for food and feed use are not discussed here.

The environmental risk assessment of transgenic animals includes consideration of the following information items:

- The identification, or current taxonomic name to the species or sub-species level, strain, common names, trade name
- A description of any modifications of the organism
    - the purpose of the modifications
    - the methods and steps taken to make the modifications
    - the phenotypic and genotypic changes that resulted from the modifications
    - the genetic stability of the changes that resulted from the modifications
    - the nature, source and function of any introduced genetic material
- A description of methods that can be used to distinguish and detect the modified organism
- A description of the biological and ecological characteristics of the organism, including:
    - Its life cycle
    - Its reproductive biology, including species with which the organism could interbreed in Canada
    - Its involvement in adverse ecological effects, including pathogenicity, toxicity and invasiveness
    - A description of the geographic distribution and habitat of the organism
    - The potential for dispersal of its traits by gene transfer
    - The locations and situations where the organism has caused adverse ecological effects
    - Its interactions with other organisms in the environment
    - The conditions required for its survival, growth, and reproduction
    - Its capability to act as a vector for agents involved in adverse effects
    - The mechanisms of its dispersal and the modes of interaction with any dispersal agents
- The identification of any patent or other rights, or any application for a patent or other rights, as the case may be

- the intended and potential uses of the organism, and the potential locations of introduction, including:
    - A description of the procedures for the introduction of the organism
    - Any activities associated with its introduction
    - Any recommended procedures for the storage and handling of any surplus organism
    - Any contingency plans in the event of an accidental release and any reproductive isolation measures
    - A description of any recommended procedures for terminating the introduction of the organism
    - A description of the procedures for the disposal of remaining biomass and residues of the organism

I.2.3 Conclusions

Although not a Party to the Protocol, Canada's assessment of the potential risks associated with the environmental release of transgenic animals is entirely consistent with the risk assessment framework described in Annex III of the Protocol, and with Canada's approach to environmental risk assessment of plants with novel traits (e.g., transgenic plants).

I.2.4 Bibliography

CFIA (2004a). Animal Health Risk Analysis Framework for Biotechnology-Derived Animals. Canadian Food Inspection Agency.
Available online at: http://www.inspection.gc.ca/english/sci/ahra/bioanima/bioanimae.shtml

CFIA (2004b). Notification Guidelines for the Environmental Assessment of the Use of Animal Biotechnology in Livestock. Animal Biotechnology Unit, Veterinary Biologics Section, Terrestrial Animal Health Division, Animal Products Directorate, Canadian Food Inspection Agency.
Available online at: http://www.inspection.gc.ca/english/anima/biotech/guidedirecte.shtml

Kochar, H.P.S. and Evans, B.R. (2007). Current status of regulating biotechnology-derived animals in Canada: animal health and food safety considerations. *Theriogenology* **67**: 188–197.

I.3 UNITED STATES

I.3.1 Regulatory Framework

The US Food and Drug Administration (FDA) regulates the environmental release of transgenic animals under the National Environmental Policy Act (NEPA) of 1970, which requires an environmental assessment for all agricultural products of biotechnology, including plants and animals. NEPA establishes a consistent process by which federal agencies must consider the consequences of their proposed actions on the human environment prior to a decision. NEPA requires federal agencies to prepare a detailed "environmental impact statement" (EIS) for all major Federal actions significantly affecting the quality of the human environment. The Council on Environmental Quality (CEQ), an agency established by Congress in NEPA, has promulgated

regulations that are applicable to federal agencies in their compliance with NEPA. The CEQ regulations provide that federal agencies may prepare an environmental assessment (EA) to determine whether a proposed action is likely to have a significant impact on the environment, thus triggering the need to prepare an EIS. CEQ regulations also provide that certain types of federal activities may be categorically excluded from NEPA review.

I.3.2 Risk Assessment

Under NEPA, FDA is obligated to cooperate with other involved federal agencies, and in the case of transgenic fish this includes working with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service, in development of a scientifically based environmental risk assessment.

Although considered on a case-by-case basis, risk assessment of a transgenic (genetically engineered) animal considers at least:

- Information on the genetically engineered (GE) animal
  - Ploidy of the GE animal
  - Zygosity of the GE animal
  - Description of the animal (e.g., common name/breed/line; genus and species)
  - Number of copies of the recombinant-DNA (r-DNA) construct
  - Construct name
  - Characterization of the insertion site(s)
  - Name of the GE animal line
  - The intended use or claim being made for the GE animal
- Molecular characterization of the construct, including:
  - A description of the source(s) of the various functional components of the construct
  - The sequence of the r-DNA construct
  - The purpose of the modification
  - Details of how the r-DNA construct was assembled
  - The intended function(s) of the introduced DNA
  - The purity of the preparation containing the r-DNA construct prior to introduction into recipient animals or cells
- Molecular characterization of the GE animal lineage (i.e., information on the production of the GE animal(s) intended to be used in commerce and any potential hazards that may be introduced into those animals as part of their production)
- A method of detection that can be used to identify the r-DNA construct in the GE animal
- Phenotypic characterization of the GE animal
- Genotypic and phenotypic durability (i.e., stability of the genetic modification over a number of generations)
- Food and feed safety
- Environmental and other factors – specific considerations are developed on a case-by-case basis during consultation with FDA, but could include:

- Whether there is anything about the GE animal that poses a human, animal, or environmental risk (e.g., does the construct contain sequences that can cause human or animal disease either intrinsically or by recombination?)
- Whether, in the event of an environmental release, the GE animal poses any more of an environmental risk than its non-GE counterpart
- Whether there are concerns over the disposition of GE animals that could pose human, animal, or environmental risks (e.g., would disposal of large numbers of dead GE ferrets containing a construct that makes them resistant to rabies pose a particular risk?)
- Whether there are any other safety questions that have not been adequately addressed by the sponsor

I.3.3 Conclusions

Consistent with the risk assessment framework described in Annex III of the Protocol, risk assessment of transgenic animals in the United States considers: the recipient organism, the intended use and potential receiving environment, the transformation vector, the inserted DNA and characteristics of the modification, any differences between the transgenic animal and the recipient animal, and methods of detection.

I.3.4 Bibliography

FDA (2008). Guidance for Industry 187, Regulation of Genetically Engineered Animals Containing Heritable r-DNA Constructs. US Food and Drug Administration, Center for Veterinary Medicine.
Available online at: http://www.fda.gov/OHRMS/DOCKETS/98fr/FDA-2008-D-0394-gdl.pdf

CEQ-OSTP (2001). CEQ/OSTP Assessment: Case Studies of Environmental Regulation for Biotechnology. Office of Science and Technology Policy. Available online at: http://www.ostp.gov/cs/issues/CEQ_OSTP_Environmental_Regulation.html

II. OTHER GUIDANCE

II.1 OECD

The Organization for Economic Cooperation and Development's (OECD's) Working Group on Harmonization of Regulatory Oversight in Biotechnology publishes Biosafety Consensus Documents as part of its core mission. The goal of the Working Group is to ensure that countries use common methods to collect data and consistent baseline information for risk/safety assessments in their development of biotechnology regulations and guidance. An important underlying concept to these efforts is that all national governments share the need to undertake environmental assessments and that any information relating to the biology of an organism would be the same regardless of what country's regulatory system was involved. The Consensus Documents are intended to prevent duplication of efforts and to share information on key components that member countries believe are relevant to an environmental safety review. The OECD envisions the documents being used as a tool by (1) scientists preparing applications for

regulatory authorizations; (2) regulators conducting environmental risk assessments for biotech organisms; and (3) governments promoting public education, research, and information-sharing.

The Working Group is currently developing a Consensus Document on the Biology of Atlantic salmon. Efforts are underway to identify and review the kinds, and availability, of baseline information of the non-transgenic variety of Atlantic salmon to determine what information is relevant to risk/safety assessment, and what kind of information might be needed for the development of a biology consensus document. This is the first review of environmental risk/safety issues associated with an animal species undertaken by this workgroup. The Working Group member will receive an update on the status of this document at their next meeting, occurring 9–11 February 2009 in Paris.

The Working Group agreed to develop this Consensus Document following an Expert Workshop on the Biology of Atlantic salmon organized by the OECD in Moscow from 29 November to 1 December 2004. This was the first occasion for the Working Group to address environmental safety issues related to animals. The objectives of the workshop were: to identify and review the kinds of (and availability of) baseline information of (non-transgenic) traditional fish farming or breeding and to determine which of this information might be relevant to risk/ safety assessment; and to identify what information might be needed for the development of a biology document. The purpose of the workshop was to take the first steps in considering whether the general approaches which have been used by the Working Group in the past to address risk assessment of transgenic plants could be applicable to risk assessment of transgenic fish.

II.1.1 Bibliography

OECD (2006). Abstracts of the OECD workshop on the biology of Atlantic salmon (Moscow, 29 November–1 December, 2004). Organization for Economic Cooperation and Development, Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 39.
Available online at: http://www.oecd.org/dataoecd/46/13/37369550.pdf

II.2 UNITED STATES NAS

The National Academy of Science (NAS), whose mandate is to advise the federal government of the United States on scientific and technical matters, through the National Research Council convened a Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology. This committee generated a report that identified concerns that should be considered when creating a regulatory policy for animals derived from biotechnology. Chapter Five of the report focuses on the general principles of an environmental risk assessment.

Among the conclusions of the report, were:

- The process of prioritizing concerns will vary from case to case because of the uniqueness of each genetically-engineered construct, transgenic founder individual from which a line is derived and receiving ecosystem.
- Concerns should be prioritized by considering the following variables:

- Effect of the transgene on the "fitness" of the animal within the ecosystem into which it is released
- Ability of the biotech animals to escape and disperse into diverse communities, and
- Stability and resiliency of the receiving community

II.2.1 Bibliography

NRC (2002). Animal Biotechnology: Science-based Concerns. The National Academies Press, Washington, DC. pp. 73–92.

III. OTHER LITERATURE

Kapuscinski, A.R., Hayes, K.R., Li, S. and Dana, G. (2007). Environmental Risk Assessment of Genetically Modified Organisms, Vol 3: Methodologies for Transgenic Fish. pp. 1–305. Eds: Kapuscinski AR; Hayes KR; Li S; Dana G; Oxford University Press, USA/CAB International.

Main Contents - 1: Introduction to environmental risk assessment for transgenic fish 2: Problem formulation and options assessment: science-guided deliberation in risk assessment of transgenic fish 3: Development of transgenic fish: scientific background 4: Gene construct and expression: information relevant for risk assessment and management 5: Approaches to assessing gene flow 6: Assessing ecological effects of transgenic fish prior to entry into nature 7: Introduction to the concepts and methods of uncertainty analysis 8: Risk management: Reducing risk through confinement of transgenic fish 9: Risk management: Post-approval monitoring and remediation 10: Summary and synthesis. Main Description - The decline of many individual and wild fish stocks has commanded an increase in aquaculture production to meet the protein demands of a growing population. Alongside selective breeding schemes and expanding facilities, transgenic methods have received increasing attention as a potential factor in meeting these demands. With a focus on developing countries, this third text in the series provides detailed information on environmental biosafety policy and regulation and presents methodologies for assessing ecological risks associated with transgenic fish.

H. INTERNATIONAL MARITIME ORGANIZATION

IMO GUIDANCE ON RISK ASSESSMENT IN RELATION TO BIOINVASIONS

In response to a request from the CBD Secretariat, this document provides information regarding existing guidance on risk assessment developed by the International Maritime Organization (IMO). The information is meant as input to the deliberations of the AHTEG meeting, which will be held later next year.

1 Risk Assessment Methodology to assess the risk of marine bioinvasions mediated through ballast water

In 2003-2004, the GEF/UNDP/IMO GloBallast project (http://globallast.imo.org) developed a Risk Assessment Methodology to assist the Member States to assess the risk of marine bioinvasions mediated through ballast water. The user-guide for this tool developed by the Globallast project are attached in Annex 1. The entire package contains databases of over 300 ports. The GloBallast Ballast Water Risk Assessment methodology has been used by 9 countries already, including e.g. Canada, Australia, Brazil, China, India, South Africa and Ukraine. Based on the feedbacks we will be updating the database soon.

Risk assessment is an essential approach for invasive species management and the Ballast Water Risk Assessment tool development has contributed significantly to the efforts of the member states in assessing the risks associated with ballast water and in identifying appropriate management strategies.

Further information regarding these guidelines can be provided by the GloBallast Project Coordination Unit at IMO (http://globallast.imo.org).

2 Guidelines for risk assessment under regulation A-4 of the BWM Convention (G7)

Whilst the IMO's Ballast Water Management (BWM) Convention requires that the discharge of ballast water shall only be conducted through Ballast Water Management to meet the Ballast Water Exchange Standard (Regulation D-1) or Ballast Water Performance Standard (regulation D-2) no later than 2016, Regulation A-4 of the Convention provides that Parties may grant exemptions to ships on specific voyages based on the Guidelines on risk assessment. The Guidelines (G7) adopted by the Marine Environmental Protection Committee, MEPC (Resolution MEPC.162(56)), outline three risk assessment methods (Environmental matching risk assessment, Species' biogeographical risk assessment and Species-specific risk assessment) that will enable Parties to ensure that there is no unacceptable environmental risks when granting exemptions to ships. The guidelines are attached to this document as Annex 2.

For further information on these guidelines please do not hesitate to contact Mr. Dandu Pughic or Mr. Tian-Bing Huang, Marine Biosafety Section, Marine Environment Division, IMO.

3 Formal Safety Assessment (FSA)

The IMO Formal Safety Assessment (FSA) approach should be considered as "work in progress". FSA was introduced by the IMO as a rational and systematic process for assessing the risk related to maritime safety and the protection of the marine environment and for evaluating the costs and benefits of introducing options to reduce risks with the ultimate aim that FSA would be of use and support the IMO rule-making process. In this context IMO, through its MEPC, is currently developing environmental risk evaluation criteria as part of the FSA

methodology. Presently these are limited to oil pollution from oil tankers but in future as experience is gained with the methodology, it is envisaged that a broad spectrum of environmental consequences such as those from ship recycling residues, ballast water, gas emissions etc. will be dealt with. As mentioned, this is still work in progress and we will be able to inform the CBD Secretariat accordingly once the work on the methodology is finalised.

***

ANNEX 1

GEF/UNDP/IMO
Global Ballast Water Management Programme
Ballast Water Risk Assessment

User Guide for the BWRA Database/GIS System

GEF/UNDP/IMO

GLOBAL BALLAST WATER MANAGEMENT PROGRAMME

BALLAST WATER RISK ASSESSMENT

(Activity 3.1)

USER GUIDE

(v1.4)

for the

BWRA Database/GIS System

PREFACE

SOFTWARE LICENCE REQUIREMENTS

The Global Ballast Water Management Programme (GloBallast Programme) is a cooperative international initiative of the Global Environmental Facility (GEF), United Nations Development Programme (UNDP) and International Maritime Organization (IMO). Legal use of the Ballast Water Risk Assessment (BWRA) Database and associated software requires, at a minimum, single-station licensed applications provided by licensed distributors of:

- Microsoft's Windows 2000, MS Access 2000 and MS Excel 7 (or higher versions).
- ESRI's ArcView 3.2
- Primer-E Pty Ltd's PRIMER v5.

Such licences have been obtained for Pilot Country participants of Activity 3.1 of the GloBallast Programme, and have been licensed to either the IMO or specific Country Focal Points (CFPs) upon written instructions of the IMO to URS Australia Pty Ltd and Meridian-GIS Pty Ltd.

COPYRIGHT AND PROPRIETARY RIGHTS

The Ballast Water Risk Assessment (BWRA) Database and associated interfaces, GIS maps and this User Guide have been developed by URS Australia Pty Ltd and Meridian-GIS Pty Ltd for the specific purpose of BWRA training and evaluation by pilot country participants of Activity 3.1 of the GloBallast Programme, as administered by the IMO.

The proprietary rights to modify, make copies or otherwise distribute all or parts of the BWRA Database or its associated interfaces, GIS maps and User Guide are vested with the IMO and its Programme Coordination Unit (PCU) under international copyright law and written contract.

INTERPRETATION AND APPLICATION OF BWRA OUTPUTS

The BWRA Database software has been developed to provide a 'first-pass' risk assessment for training, demonstration and evaluation purposes. The accuracy and reliability of its output depends heavily on the quality of data entered from ship's Ballast Water Reporting Forms, published and unpublished reports, and inputs from marine scientists. Responsibility for applying the BWRA database and/or its results for any ballast water management purpose by a Pilot Country rests with the Country Focal Point (CFP) of that country.

Table of Contents

LIST OF APPENDICES

1. INTRODUCTION

1.1 BWRA SYSTEM OVERVIEW

URS Australia Pty Ltd and Meridian-GIS Pty Ltd have produced this User Guide to help data-entry operators and managers use the computer system developed for the Ballast Water Risk Assessment (**BWRA**) of the GEF-UNDP-IMO Global Ballast Water Programme (GloBallast Activity 3.1; see http://globallast.imo.org/ for details and Pilot Country BWRA reports).

A major objective of Activity 3.1 was to provide, for each Demonstration Site, a user-friendly Database-GIS system that can manage and integrate key shipping, environmental and risk species data for undertaking 'first-pass' risk assessments.

The BWRA Database is operated by MS Access 2000 and allows users to:

(1) Store, edit and manage all data recorded on IMO BW Reporting Forms (Task 5 of BWRA Activity 3.1).

(2) List all source ports from which BW is imported by the demonstration site and tabulate the frequencies and amounts of ballast water discharged from each these ports (Tasks 2 and 3 of BWRA Activity);

(3) Interact with Excel and PRIMER applications for calculating, storing and using results from multivariate environmental similarity analysis (Task 6 of BWRA Activity).

(4) Store and edit the types, regional locations and status information of risk species which have been transferred between ports by ships' ballast water and other vectors (Task 8 of BWRA Activity).

(5) Undertake 'first-pass' risk assessments using a semi-quantitative method that minimises subjective input[1] (Task 9 of BWRA Activity).

The graphical information system (GIS) is operated by ArcView 3.2, and allows users to:

(6) Map and display all coastal and marine resources (biological, social/cultural and commercial) in and around the demonstration port that might be impacted by introduced marine species (Task 1 of BWRA Activity).

(7) Map and display the de-ballasting and ballasting patterns in and around the demonstration port including locations, frequencies and volumes of ballast water discharges and uptakes (Task 2 of BWRA Activity).

(8) Display, in a convenient colour-graphic style, the:

  (a) location of all source ports from which BW is imported (Task 3 of BWRA Activity);

  (b) location and frequency of departures to all potential 'destination ports' (= next ports of call) to which ballast water is exported (Tasks 4, 11 of BWRA Activity);

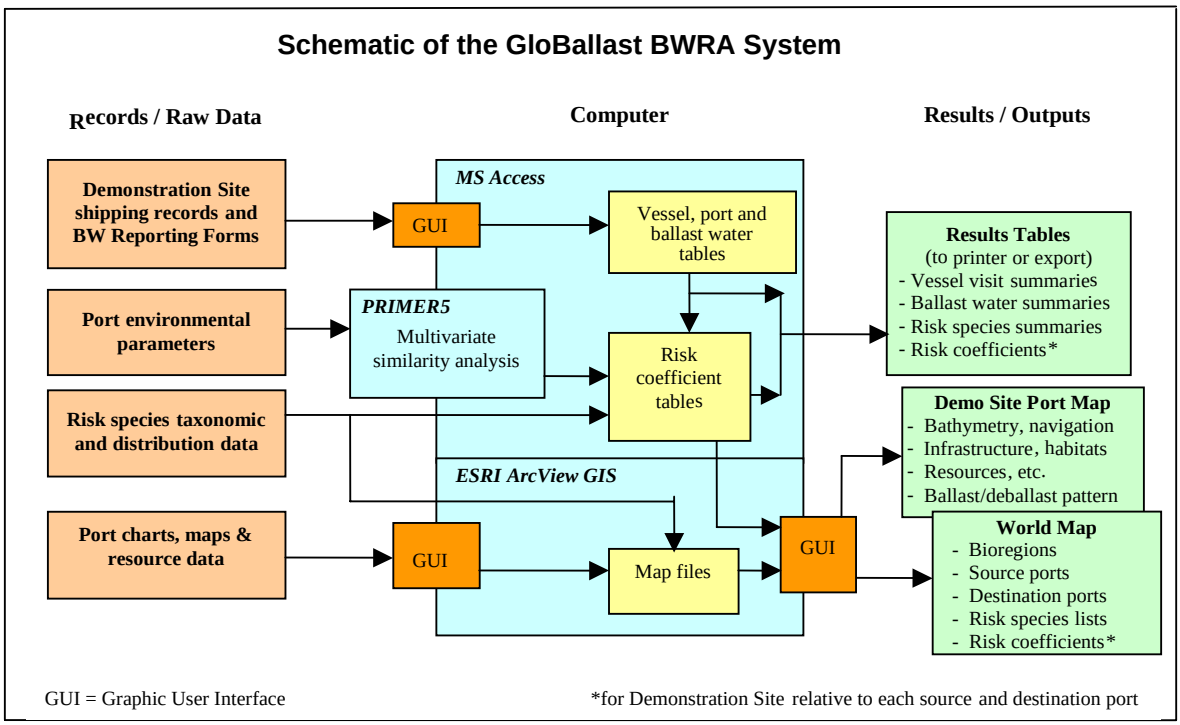
  (c) environmental similarity between the demonstration port and its various source and destination ports (Task 7 of BWRA Activity);

[1] see Appendix 1 for a summary description of qualitative, semi-quantitative and quantitative methods of risk assessment.

(d) the types of risk species present at the source ports and demonstration port that might be introduced either to the demonstration site or exported to its destination ports, respectively (Task 8 of BWRA Activity); and

(e) the five levels of relative risk (= very low, low, moderate, high, very high) posed by each source port for the introduction of unwanted species to the demonstration site, based on its present pattern of trade (Tasks 9 and 11 of BWRA Activity).

This User Guide also explains how to perform the multivariate environmental similarity analysis (Task 6 of the BWRA Activity) using the PRIMER statistical package. The following graphic shows the complete BWRA computer system described in this User Guide:



1.2 RATIONALE FOR 'FIRST-PASS' RISK ASSESSMENTS

Current IMO BW management guidelines provide port states significant flexibility in determining the nature and extent of their national BW management regimes. This flexibility is warranted given that nations are still experimenting with different approaches. Thus a port state may wish to apply its management regime uniformly to all vessels arriving with ballast water, or it may wish to apply a more selective regime by attempting to identify those trading routes and/or vessels which have a significant chance of introducing unwanted aquatic species.

The uniform approach offers the advantage of a simple programme administration, in that there are no judgements to be made (or justified) by the port state regarding which vessels must participate. It also requires minimal information management and offers more protection from unexpected, novel invaders. The primary disadvantages of the uniform approach are:

(1) it causes considerable costs to vessels which otherwise might not need to take action;

(2) because all vessels are involved in undertaking the required BW control measures, compliance monitoring costs by the port state will be high and inordinate berthing and cargo loading delays may be experienced.

A selective (risk management) approach can reduce the number of ships subject to ballast water controls and compliance monitoring, and is also attractive to nations that wish to avoid introductions of particular harmful species, such as toxic dinoflagellates. If fewer restrictions are placed on 'low risk' vessels, then more time and funds will be available for applying more rigorous measures on ships deemed as 'high risk'. The disadvantages of the selective approach are:

(1) it requires the port state to implement an information management system whose effectiveness depends on the quality of the supporting information, computer systems and management infrastructure;

(2) it requires an organized, defensible means of evaluating the risk posed by each route or vessel.

(3) it may leave the port state more vulnerable to introduction risks from non-targeted or unknown species.

To help the pilot countries evaluate the choice of adopting either a uniform or selective approach, the GloBallast Programme included a 'first-past' BWRA for their demonstration sites (Activity 3.1). The assessment undertaken for each of these sites used the same Access-ArcView system described in this User Guide. The system allows operators to identify shipping arrival patterns and sources of 'imported' ballast water (Section 4), and gives an indication of the relative risk posed by each trading route by integrating this information with source port/demonstration site environmental similarity (Section 5) and the regional locations of risk species (Section 6).

1.3 IMPORTANCE OF GIS COMPONENT

The GIS component is invaluable for graphically displaying key information and results, in a user-friendly and interactive fashion, using its Port and World maps. It is possible to collate all the shipping, environmental and species data and calculate the first-pass risk assessments without installing or using the ArcView GIS. However, checking and interpreting the results in the large output tables produced by the Access database is difficult and time-consuming. Displaying results with ArcView GIS helps results checking and evaluation, and it provides convenient graphic outputs for BWRA demonstrations and training.

It is also possible to link the outputs from the Access database to other GIS packages, such as ArcInfo or MapInfo. However, the ESRI *ArcView 3.2* has been used for the following reasons:

- it is an affordable, widely used, well-tested and stable platform for the Windows operating system.
- it remains a *de facto* industry standard for GIS tasks that is well supported by ESRI and the global user community (various types of geographic files and data produced by ERSRI and ArcView users are easy to acquire from the web and share).
- there is excellent product and 'distance' support, including a wide variety of help and training resources on the Internet.
- it is easy to customise its user interface and tailor its functionality, therefore permitting an uncluttered application that facilitates BWRA training, use and demonstration.

1.4 BWRA SYSTEM DEVELOPMENT AND ACKNOWLEDGEMENTS

The BWRA system and this User Guide were developed by the following team at URS Australia Pty Ltd and Meridian-GIS Pty Ltd:

Dr Rob Hilliard (Team leader, and calculation and treatment of risk coefficients)

Mr Chris Clarke (BWRA Database/GIS integration and GIS interfaces)

Mr Christopher Stevens (BWRA Database customisation and Graphic User Interfaces)

Mr John Polglaze and Cmdr Terry Hayes (BW Reporting Form – BWRA Database compatibility)

The following people assisted with 'hands-on' evaluations of various components of the developmental versions, and are thanked for their useful suggestions and information inputs:

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Dr AC Anil, National Institute of Oceanography, Dona Paula, Goa, India.

Mr Roman Bashtannyy, Shipping Safety Inspectorate, Ministry of Transport, Odessa, Ukraine.

Ms Leticia Greyling, National Ports Authority, Johannesburg, South Africa.

Mr Rob Healy, Meridian-GIS Pty Ltd, Perth, Australia.

Dr Andréa de O.R. Junqueira, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil.

Mr Victor Khmelevskiy, Sea Commercial Port of Odessa, Odessa, Ukraine.

Mr Daniel Lins Menucci, Agência Nacional de Vigilância Sanitária, Gerência Geral de Portos e Fronteiras, Brasilia.

Mr Alexandre de C. L. Neto, Programa GloBallast, Diretoria Portas e Costas, Rio de Janeiro, Brazil.

Dr Nikolay E. Pavlenko, Institute of Transport Medicine, Odessa, Ukraine.

Mr Naseer Kayvan Rad, Ports & Shipping Organisation, Tehran, IR Iran.

Mr Liu Yan, Maritime Safety Administration [Liaoning Province], Dalian, PR China.

The base layer of the World Bioregion Map and advice on risk coefficient options was kindly provided by Dr Chad Hewitt, CSIRO Marine Research (now at Marine Biosecurity, Ministry of Fisheries, Wellington, New Zealand). The following are also thanked for their review and improvements to the World Bioregion Map and/or provision of risk species information:

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Dr Flavio Fernandes, Instituto de Estudos do Mar Almirante P-Moreira, Arraial do Cabo, Brazil.

Dr Luciano Felício Fernandes, Universidade Federal do Paraná, Brazil.

Dr Gustaaf Hallegraeff, University of Tasmania, Hobart, Australia.

Dr Pat Hutchings, Australian Museum, Sydney, Australia.

Dr Di Jones, Western Australian Museum, Perth, Australia.

Dr John Lewis, Defence Science and Technology Organisation, Melbourne, Australia.

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Dr Barry Russell, Museum of the Northern Territory, Darwin, Australia.

Dr S Sawant, National Institute of Oceanography, Dona Paula, Goa, India.

Dr Fred Wells, Western Australian Museum, Perth, Australia.

Dr Vahid Yavari, University of Ahvaz, Ahvaz, IR Iran.

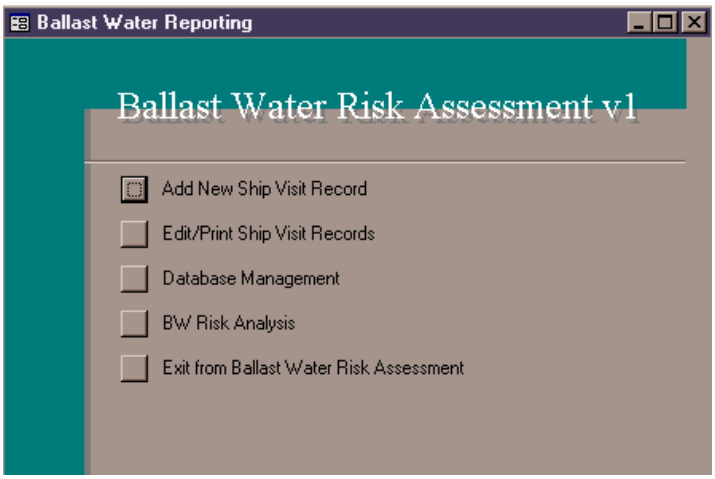
Professor Y. Zaitsev, Institute of Biology of Southern Seas [Odessa Branch], Ukraine National Academy of Science, Odessa, Ukraine.

2. BW REPORTING FORMS – DATA ENTRY AND MANAGEMENT

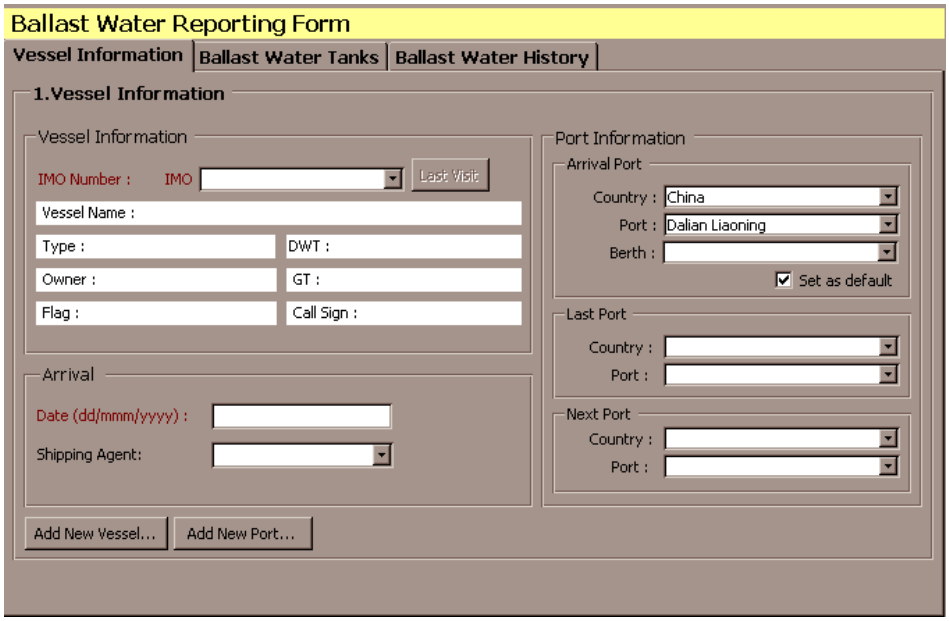
2.1 DATABASE OVERVIEW

The Database can store all information provided in all cells of the standard IMO Ballast Water Reporting Form (**BWRF**; Appendix 6). Entering and saving the data from a single BWRF creates a new '**Ship Visit Record**' in the Database. The Database also allows the Deadweight tonnage (DWT) and berth of the visiting ship to be part of this record (this information is not requested in the present IMO BWRF, although the '*Discharge Location*' cell of this form can be used to record which terminal or berth the ship intends discharging BW; Appendix 6).

The graphic user interfaces (GUIs or 'windows') for adding and for editing or printing the ship visit data are similar. The data input window is opened by clicking the '**Add New Ship Visit Record'** button on the main menu. To locate a previously saved Ship Visit Record (for editing or printing), click on the '**Edit/Print Ship Visit Records**' button in the main menu.



Both the 'Add' and the 'Edit/Print' Ship Visit Record windows have three 'tabs' which open sub-windows (the tabs are labelled '**Vessel Information**', '**Ballast Water Tanks**' and '**Ballast Water History**'). The three sub-windows follow the same structure of the IMO standard BWRF (Appendix 6). This allows the BWRF data to be entered and/or edited conveniently.



It is important to recognise that some BWRFs issued by Port States or used by shipping companies may not be exact replicas of the IMO BWRF. These forms may contain a different number of questions, and/or the questions may be located on different places on the form. Extra care is required when entering data from different types of BWRFs, to ensure the information is entered into the correct data entry-field of the Input interface.

2.2 CHECKING AND AVOIDING ERRORS

BWRFs handed to Port Officers without any checking system are very likely to be incomplete and/or contain errors. These errors usually represent mistakes or misunderstandings by the ship's officer or agent when filling out the form. Data-Entry Operators need to use their experience and judgement when entering BWRF records, and must not enter information into the Database if it is clearly wrong or illogical. There are four types of errors:

(a) Missing information (empty cells on the BWRF);

(b) Illegible (unreadable) and misspelled information (e.g. from a poor photocopy, fax or bad writing, misspelled port or ship names);

(c) Factual mistakes in the BWRF data (including illogical dates, conflicting BW volumes, BW tank numbers, etc); and

(d) Transcription errors made by the Data-Entry Operator (when entering or editing the BWRF data).

(a, b) Missing and Unreadable Information

These errors are common and will always occur unless adequate BWRF checking procedures have been implemented at the Receiving Port. Much of the critical information about a particular ship can be found or checked if the Data-Entry Operator has access to a recent *Lloyds Ship-Finder* CD (as provided by the GloBallast PCU to the Demonstration Sites). International Port Guides (e.g. the *Fairplay Ports Guide* CD or hard cover directory) are also invaluable for checking the correct spelling of source port and destination port names, their UN port codes and geographic coordinates (all of which are used by the Database).

Where the BW discharge volume is missing, unreadable or illogical, an Excel file is available in the **Utilities Folder** (**Estimating BW discharges from port records.xls**) for estimating typical discharge volumes for different vessel types, based on their deadweight tonnage (DWT) and cargo-loading/unloading behaviour. DWTs can be found in the *Lloyds Ship-Finder* CD for all listed ships.

(c) Factual and Logical Errors

These BWRF errors typically comprise conflicting information with respect to the:

- Logical sequence of *BW Source*, *Exchange*, *Ship Arrival* and *Intended Discharge* dates;
- *Number of Ballast tanks containing BW* (versus the ship's *Total Number of BW Tanks*);
- Ballast Tank volumes declared for intended discharge, versus the ship's Total BW Capacity, its DWT and its cargo-loading requirement at the Receiving Port.

Care is needed to interpret and enter all dates correctly. For example, "01-02-03" could be 01-Feb-2003, or 02-Jan-2003, or 03-Feb-2001, depending on the date format system (i.e. typical European, American and Asian respectively). It is therefore important to check if all dates written on any BWRF conform to the Database's fixed **dd/mmm/yyyy** format.

Apart from checking the dates on a BWRF, Data-Entry Operators must also check the logical sequence of the BW source date, BW exchange date, the ship arrival date and the intended (or known) BW discharge date. It is obviously impossible for a ship to arrive or discharge its BW before it has taken it up, or made an exchange. For example, if the BW tank exchange date/s are recorded on the BWRF, these must be after the BW source date (date of uptake) and before the ship's arrival date and BW discharge date.

Data-Entry Operators must be alert for these surprisingly common errors, which are often caused by haste, unfamiliarity and confusion when a ship's officer is completing the BWRF.

Dates recorded on the BWRF for the BW uptake (and subsequent possible BW exchange at sea) must also be consistent with the duration of the ship's voyage from the BW source port to the Arrival Port, and the size and number of filled BW tanks. Data-Entry Operators should remember that even small ships require 18 hours or more to complete a BW exchange of all tanks. For large bulk carriers and crude carriers that use the x3 'flow-through' method, a complete BW exchange can take 4-6 days or more, depending on weather and sea state.

(d) Transcription Errors

These errors can be made by the Date-Entry Operator when entering the data, including accidental miss-typing ('typos'). Transcription errors include the Data-Entry Operator misunderstanding the 'day-month-year' sequence (date format) in the dates on the BWRF.

The date format used by a ship's officer may not be same as the fixed format used by the BWRA Database (**dd/mmm/yyyy**; see Section 2.4). Date formats on BWRFs will vary according to the origin and training of ship's officers, and may be ambiguous. For example, a date may be written as "*mm/dd/yy*" (e.g. by North American officers), "*yy/mm/dd*" (e.g. by Chinese, Korean or Japanese officers), or "*dd/mm/yy*" (e.g. by officers from European and Commonwealth countries).

If any piece of BWRF information is illogical or highly questionable, but cannot be checked or corrected from other data sources, it is better not to enter it (especially if the information is not a **Mandatory** item; see Section 2.3).

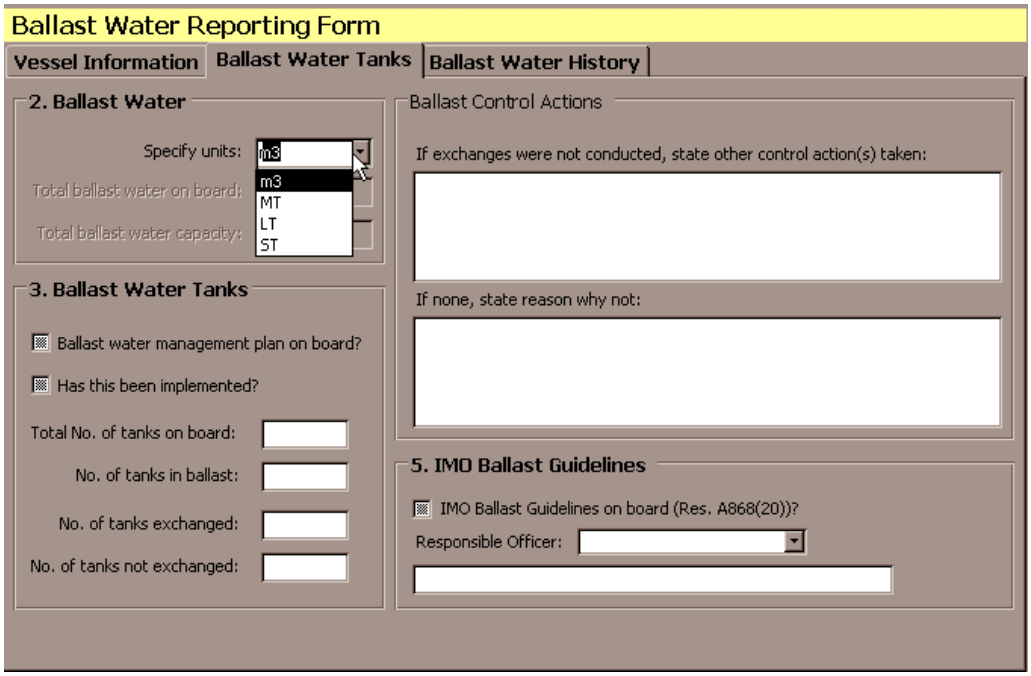
Most GloBallast Pilot Countries began implementing a BWRF checking system to reduce the incidence of incomplete/incorrect forms during Activity 3.1. While future BWRFs should be more complete and have fewer mistakes, Data-Entry Operators should maintain the habit of carefully examining every BWRF before starting to enter its data into the Database. To help Data-Entry Operators understand English phrases commonly used in shipping and ballasting requirements, Appendix 5 contains a glossary of 'Maritime Jargon', including the factors which influence the timing and volumes of BW discharges.

2.3 MANDATORY DATA REQUIRED BY DATABASE

Not all of the data-entry fields need be completed in order for the Risk Assessment to function. However some data-entry fields are **Mandatory**, and the Database will not allow a new Visit Record to be saved until these fields are entered. The following mandatory fields are labelled in **red text** on the 'Add' and 'Edit/Print' Visit Record windows:

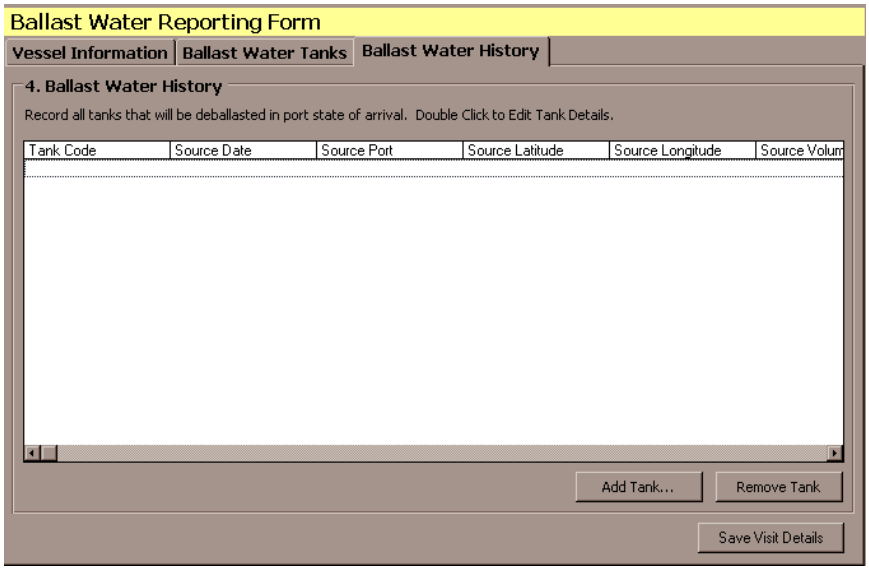
- **IMO Number**
- **Arrival Date**
- **Tank Code**
- **Tank Volume**

The IMO number is the unique 7-digit identifier of the vessel. This ID number remains the same for the whole life of the ship, irrespective of any changes to its name. Other fields which should be completed are the 'Arrival Port' (in the '**Vessel Information**' sub-window) and the unit of discharged BW (in 'Specify units' in the '**Ballast Water Tanks**' sub-window):

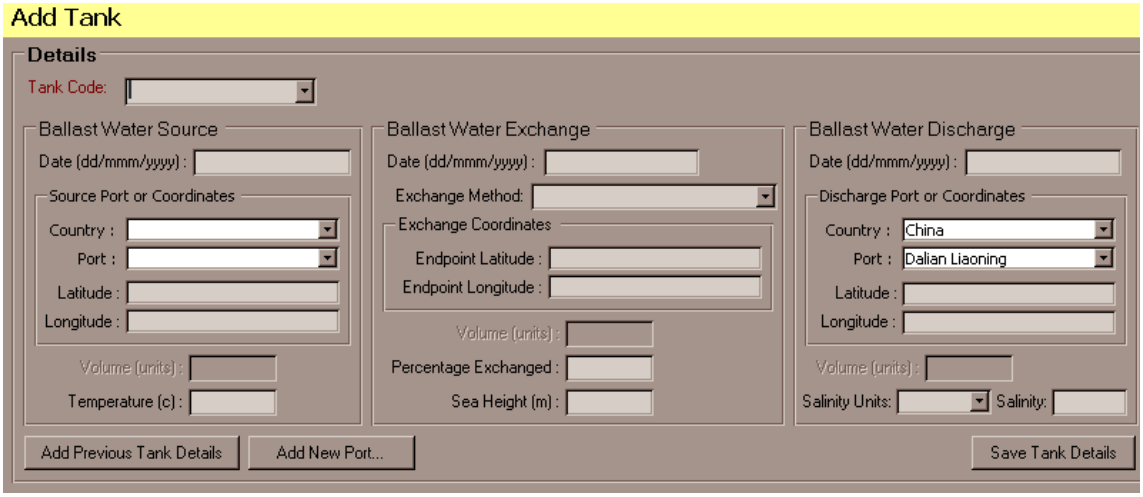


The unit of BW discharge is recorded on the IMO BWRF, and is specified for each ship visit record by selecting from the drop-down list either **m3** (cubic metres), **MT** (metric tonnes), **LT** (long tons) or **ST** (short tons; see Section 2.4.6 and Appendix 5). Until a unit is selected, the other data-entry fields for 'Total Ballast Water On Board' and 'Total Ballast Water Capacity' will remain unavailable in the '**Ballast Water Tanks**' sub-window. If no unit is present on the BWRF, select **MT** as the default unit (this is the most common unit used by ships).

The Database will not save a Ship Visit Record until all **Mandatory** fields have been completed and the '**Save Visit Details**' button is clicked (at bottom right of the '**Ballast Water History**' subwindow):



Similarly, no Ballast Tank details will be saved unless the '**Save Tank Details**' button is clicked. This button is located in the bottom right corner of the '**Add Tank**' sub-window):

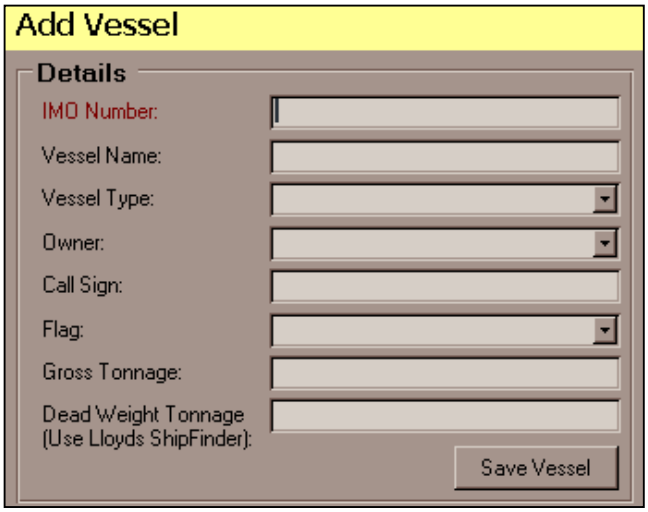


2.4 ENTERING THE VESSEL VISIT AND TANK INFORMATION

2.4.1 IMO Number and Vessel Name

- Always check and enter the ship's unique IMO Number first. This **mandatory** ship identifier is used by the Database to identify all Visit Records. The ship's IMO Number may already be present in the drop-down list.
- If the ship is already on the Database, its IMO Number should be selected from the drop-down list. This will cause the ship's Name, Type, DWT, Owner, GT, Flag and Call Sign to automatically appear in the respective data-entry fields.
- If the IMO Number is not present or appears incorrect on the BWRF (e.g. not 7 digits), use the Vessel Name or its Call Sign to obtain or confirm the IMO Number from the *Lloyd's Ship-Finder* CD. The *Lloyd's Ship-Finder* also lists other valuable information including ship Type, GT and DWT.

If the IMO number is not in the drop-down list, then click on the '**Add New Vessel...**' button (in the '**Vessel Information**' subwindow) to enter the new ship information in the cells of the '**Add Vessel**' subwindow. Use the *Lloyd's Ship-Finder* to check and fill any data gaps:



Be careful with the vessel's name spelling, especially on hastily written BWRFs. For example, the correct, official name of '*Tokyo Maru II*' may be '*Tokyo Maru 11*', '*Tokyo Maru No. 11*', or '*Tokyo Maru No. 2*'). Similarly, the correct name of the tanker '*MT Tokyo Maru*' is probably '*Tokyo Maru*'. This is because 'MT' means 'motor tanker' and is not part of the ship's registered name. Similar abbreviations include 'MV' (motor vessel), 'SS' (steam ship), 'RV' (research vessel), etc. In the case of company names or initials (e.g. *OCL Pacific Trader*, *NYK Pacific Trader, Evergreen Pacific Trader* etc), sometimes they are part of the official name in *Lloyds' Ship-Finder*, sometimes they are not.

Be aware that ships often change their name, radio call sign, flag, owner or local agent. This does not matter for the Database, provided the IMO Number matches the basic ship details in the *Lloyd's Ship-Finder* (i.e. Vessel Type, GT and DWT). If a ship changes its name after a previous visit and Database entry, there is an '**Edit Vessel**' feature for updating its details such as a new Name, Call Sign, Flag, Owner, Agent, etc (see Section 2.5).

2.4.2 Vessel Type

- To allow the Database to generate accurate reports for particular Vessel Types, the exact spelling of Vessel Type must conform to one of the following labels used by the Database:

Bulk Carrier	**Oil/Bulk Ore Carrier** ('OBO' and 'O/O')
Bulk/Container carrier	**Chemical Tanker**
Container Ship	**Chemical/Products Tanker**
General Cargo Ship	**Crude Oil Tanker**
Reefer (Refrigerated Cargo Ship)	**Products Tanker**
Ro-Ro Cargo Ship	**Gas Tanker (incl. LPG, LNG, etc)**
Vehicles Carrier	**Passenger Ship** (includes ferries, liners)
Other (for miscellaneous types)	**Passenger/Ro-Ro Cargo Ship** (includes
Tanker (for multipurpose/unclassed types)	many roll-on/roll-off ferries)

- Spelling mistakes will accidentally create a 'new' and unnecessary Vessel Type (e.g. typing in 'Product Tanker' will generate a new vessel type, as it is different from 'Product**s** Tanker'). To avoid this error, always use the drop-down list to select the Vessel Type, unless it is actually a new vessel type which is making frequent visits to the Receiving Port (infrequent and miscellaneous vessel types such as a Research Ship or a Dredge should be classified as '**Other**').

This tip applies to all data-fields which have drop-down lists, such as Country, Port, IMO Number, Vessel Name, Owner, Agent etc.

When using the Database to review Vessel Types or allocate them to particular berths and terminals within the Arrival Port, be aware that many of the smaller 'general cargo ships' and 'bulk carriers' (<15,000 GT) can trade both general cargo and bulk cargo. Many general cargo ships also carry containers which can be worked by their own deck gear or by wharf cranes.

2.4.3 Other Vessel Information

- The ship's Name, Flag, Owner, Port and Country should be entered with initial Capital Letter, followed by lower case.
- All of the Call Sign should be entered with UPPERCASE letters.
- As with all dates in the Database, the mandatory arrival '**Date (dd/mmm/yyyy)**' has to be entered in this format (i.e. '21-01-03' must be entered as "**21/Jan/2003**").

- Remember that date formats used by ship's officers may be different, and these require careful interpretation (see Section 2.2 for details).

The **'Last Visit**' button (in the **Vessel Information** sub-window) accelerates the data entry task. The '**Last Visit**' will display all information concerning the last visit made by the selected ship to the Arrival Port. This is very useful for ships which make regular port visits on scheduled services (for example Odessa - Bourgas, Khark Island - Ain Sukhna, Dalian - Pusan, Mumbai - Colombo, Saldanha Bay - Rotterdam, Sepetiba - Taranto, etc).

2.4.4 Port Information

- Ensure that the '**Arrival Port**' name is correct and is ticked in the '**Set as default'** tick box. This saves time as well as ensuring the Database will use the Visit Record. If this box is not ticked, the Visit Record will not be included in the Database risk calculations.
- Similarly, the name of the **Berth** should be selected only from the berth or terminal names already used by the ArcView GIS Port Map. These names will be available for quick selection in the drop-down box.

> NOTE: Although 'Berth' is not specifically requested in the IMO BWRF, it asks for the location of the BW discharge. A Receiving Port can request all ships to enter the number or name of their planned berth, terminal or anchorage as the Discharge Location (plus a 'Lat-Long' position if a discharge is to be made in the port's approach channel or in an emergency anchorage, etc).

2.4.5 Last Port, Next Port and Add New Port

- A large number of ports and associated details are already present in the Database, and these can be quickly selected by using the 'Country' then 'Port' drop-down lists.
- If the name of the Last Port or Next Port was not written on the BWRF, but the next or last Country is present, then only Country name may be entered by going to the Port drop-down box for that Country and selecting **'Not Reported'** (at top of Port list).
- If the Country of the missing Last Port or Next Port is also absent on the BWRF, then '**Not Reported**' needs to be selected from the Country drop-down menu (at top of Country list). Visit Records that have a '**Not Reported**' Last Port will not be listed in the risk calculation outputs, but they will be used by the Database for compiling accurate statistics and reports about the Receiving Port's vessel visits, etc.
- Some Country and Port names may appear to be absent from the Drop-down lists because of different spellings. Data-Entry Operators need to understand that some Country names may be spelled in English or in the language of that country (e.g. 'Spain' = 'Espagna'; 'Ivory Coast' = 'Cote D'Ivoire').
- Similarly, the port of 'Vlissingen' in the Netherlands may be written on a BWRF as 'Flushing' (which is not the official name). Therefore Data-Entry Operators should refer to an international Port Guide before entering the name and details of any new port.
- Another problem is that a port name hastily written on a BWRF may actually refer to two or more different ports, sometimes within the same country! For example, 'Tenerife' on a BWRF may refer to the famous port of 'Tenerife' in Spain's Canary Islands, or to the busy bulk import port of 'Santa Cruz de Tenerife', which is also in Spain.
- Many Middle East ports have an official prefix (typically 'Al' or 'El') which is not added to the BWRF, while others are provided an unofficial prefix on the BWRF. Therefore the names of some Middle East ports may appear to be 'missing' from the Database. To

confirm if the port is already present on the Database, Data-Entry Operators should quickly check for the 'Al', 'El' or no prefix spelling in the Port drop-down list.

- Port name errors are caused by haste, laziness or ignorance of ships' officers when writing the BWRF. The vessel type, size and last/next ports of call (plus its history of previous visits on the Database) should alert the Data-Entry Operator to this problem.
- Before adding a new port to the Database, it is important to use a recent international Port Guide publication (e.g. *Fairplay Ports Guide* CD) to check the 'new' port is not:
  - the name of a terminal or 'sub-port' within a larger port (e.g. *Hansaport* is a sub-port of Hamburg, *Europoort* is the big container terminal at Rotterdam);
  - the name of a town or city beside an existing port which has a different name;
  - lacking an official prefix such as 'Al' (or was given an unofficial prefix); and/or
  - is misspelled (this is common for many Japanese, Korean and Chinese ports).

  In China, 'Zhousan' and 'Dinghai' refer to the same port, as do 'Yantai' and 'Muping', and 'Xiamen' and 'Weitou'. The City State of Singapore has several ports, one of which is called Singapore (i.e. Singapore Singapore, as well as Singapore Jurong, Singapore Keppel, Singapore Semawang, etc). For confusing Japanese or Chinese port names, use the port's Prefecture Name (the second name after the port name in the Database) and the UN Port Codes to help confirm the correct port name and spelling.
- All ports in the Database have a unique, five letter **UN Port Code**. The first two letters denote the Country (prefix codes of most countries are in Appendix 2). The last three letters identify the port in that country. For example, Saldanha Bay in South Africa is ZASDB and Cape Town is ZACPT. For the port of Mumbai in India (previously known as Bombay), its code is INBOM, while Dalian is CNDLC and Sepetiba is BRSPB.
- All ports in the Database already have their five letter code. As with the IMO Number, the Database uses the unique UN Port Codes to ensure all data are managed correctly for the risk calculations.
- To Add and Save a New Port, the Data-Entry Operator must confirm the correct spelling of its Country, Name, UN Port Code and obtain its Latitude/Longitude and Bioregion, before clicking on the '**Add New Port**…' button to add these details.
- Apart from a port's Bioregion, all port details can be obtained from publications such as the *Fairplay Port Guide* CD.
- All Bioregions in the GIS World Map (Section 3) are already listed in the drop-down list inside the 'Add New Port' subwindow. Use the World Map to find the correct Bioregion. A copy of this map is in a zoomable Adobe file of the **Utilities Folder** (**Bioregions.pdf**), as well as on the ArcView GIS (Section 3).
- Not every port in the world has been assigned a UN Port Code, including many domestic ports in China. For ports without a UN Port Code, the first three letters of the port's name may be added behind the two letters of its Country code prefix (Appendix 2) to form a unique port code. If the grouping of letters is already used for another port, the Database will not accept it. In this case, the 3rd, 4th or 5th letter of the new code can be altered to make a unique combination that the Database will accept. Alternatively, numbers can be used (e.g. CN008 for a small port in China).
- As with all latitude/longitude entries to the Database, the port's latitude and longitude should be entered in the **Decimal Degree** format (i.e. not as Degrees, Minutes, Seconds). Latitudes South and Longitudes West are entered as negatives (e.g. -30.25).

- Lat/Long minutes (') and seconds (") need to be converted to Decimal Degrees before adding to the whole degree number. The full conversion can be done automatically using the Excel PED File (see Section 5.3), or by referring to a simple conversion table such as:

Minutes	Decimal	Minutes	Decimal	Minutes	Decimal	Minutes	Decimal
01	**0.02**	16	**0.27**	31	**0.52**	46	**0.77**
02	**0.03**	17	**0.28**	32	**0.53**	47	**0.78**
03	**0.05**	18	**0.30**	33	**0.55**	48	**0.80**
04	**0.07**	19	**0.32**	34	**0.57**	49	**0.82**
05	**0.08**	20	**0.33**	35	**0.58**	50	**0.83**
06	**0.10**	21	**0.35**	36	**0.60**	51	**0.85**
07	**0.12**	22	**0.37**	37	**0.62**	52	**0.87**
08	**0.13**	23	**0.38**	38	**0.63**	53	**0.88**
09	**0.15**	24	**0.40**	39	**0.65**	54	**0.90**
10	**0.17**	25	**0.42**	40	**0.67**	55	**0.92**
11	**0.18**	26	**0.43**	41	**0.68**	56	**0.93**
12	**0.20**	27	**0.45**	42	**0.70**	57	**0.95**
13	**0.22**	28	**0.47**	43	**0.72**	58	**0.97**
14	**0.23**	29	**0.48**	44	**0.73**	59	**0.98**
15	**0.25**	30	**0.50**	45	**0.75**	60	**1.00**

Coordinates South of Equator and West of the 0^{o} Meridian require the minus sign (-) prefix

2.4.6 Entering data into the 'Ballast Water Tanks' sub-window

- Specifying the Units of BW volume must be completed for each ship Visit Record (select **MT** as the default value if the Unit is missing from the BWRF).
- Units of BW volume are typically **MT** (metric tonnes), **m3** (cubic metres), Long Tons (2200 pounds) or **Short Tons** (2000 pounds), all of which are available in the drop-down list. They are roughly equivalent (see Appendix 5).
- Always check for inconsistencies between the 'Total Number of BW Tanks', the 'Number of Tanks in ballast', the 'Number of Tanks not in ballast' and the 'Total Number of Tanks Exchanged'. These numbers should form a logical relationship.
- Similarly, 'Total BW On Board' should never exceed 'Total BW Capacity'.
- If the BW exchange data have been completed incorrectly, it is not necessary to obtain the correct information because the BW exchange details are not used by the 'first-pass' BWRA calculations (Section 4.1).
- Other data-entry fields in the '**Ballast Water Tanks**' window are not critical to the Risk Assessment calculations, but can be entered to provide a complete database to permit other reviews, data export/analysis and reporting requirements.

2.4.7 Entering data into the 'Ballast Water History' sub-window

The reported BW discharge volume, source port/s, and the source, arrival and discharge dates must all be logical with respect to the duration of the voyage, the vessel's type and cargo loading pattern, its size (DWT) and its ballast water capacity (see Section 2.2). Common errors on BWRFs include:

- Inconsistencies between the dates of ballast water uptake, exchange and discharge.

It is impossible for a ship to discharge ballast water before it has taken it up. However Data-Entry Operators must alert for this surprisingly common error, probably caused by haste or confusion by ship's officer with the form. Also, if a BW exchange has been reported, then the date/s of this exchange must logically be after the date of uptake, and before the dates of arrival and subsequent discharge.

- The date/s recorded on the BWRF for the BW uptake (and possible BW exchange) must be consistent with the voyage time from the BW source location to the arrival port.
- Latitude/longitude positions for the BW source and discharge locations are only required if these operations were not conducted in the usual port locations (the Database and GIS use and display only pre-determined discharge locations such as berths, terminals, anchorages; see Section 3).
- Latitude/longitude positions are usually provided for the voyage sector where BW was reported to be exchanged. Data-Entry Operators need to remember that even small ships may require 24 hours to complete a ~95% BW exchange for all tanks. For large bulk carriers and crude carriers which use the x3 'flow-through' method, a complete exchange often requires 4-6 days (or more if bad weather and seas interrupt the exchange).
- The reported method of BW exchange should be recorded in the Database. If an exchange is reported but no method was recorded on the BWRF, use the **'Not Specified'** option (in the drop-down window for BW Exchange Method).
- 'Sea height' refers to the height of waves and swells at the time of the reported BW exchange. 'Sea height' is typically between 0 and 6 metres (maybe up to 20 metres for a severe winter storm or cyclone, which ships always try to avoid). Anything greater than 10 metres on the BWRF very probably represents a mistake or misunderstanding, and large values should not be entered. High seas and rough weather provide a genuine reason for a ship not exchanging ballast en route. However it is common for a Ship's officer to think that 'Sea height' refers to water depth at the time of the exchange, and not to the wave height.
- Sea temperature values, if present, should lie between –1ºC (minimum) to about 32ºC (maximum).
- Check that all BW discharges recorded on the BWRF are actually for the correct arrival port (i.e. the port where the ship submits the BWRF). For example, if a ship arrives at Sepetiba and submits a BWRF that includes tank/s discharges at the port of Santos, these discharge volumes should not be entered into the Sepetiba Database.
- Salinity units are usually recorded as 'SG' (Specific Gravity; often 1.025 for coastal port waters). The units may be 'g/L' (about 35 g/L for typical ocean water), or 'µS/cm'. After a salinity unit has been entered to the database the first time, it will always appear in the drop-down list. The 'first-pass' BWRA does not use salinity in the calculations (Section 4.1).

After the BW History details for the first Tank have been entered for a particular Visit, entering data for the subsequent tanks can be accelerated by clicking on the '**Add Previous Tank Details**' button. This will populate the new tank sheet with the same details, and the operator can quickly change minor details (such as tank name, BW source, source date, BW volume, exchange details). This removes the need to re-enter the same information for every tank.

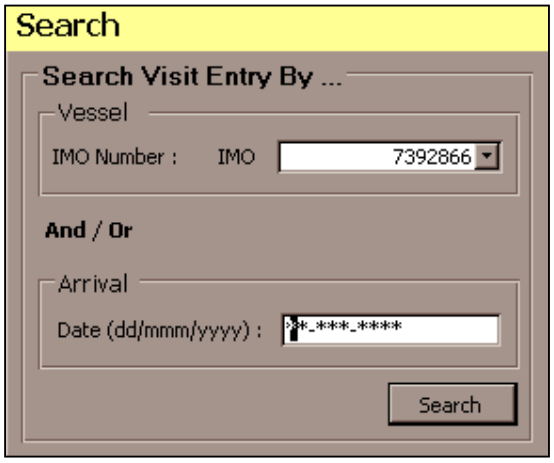
2.4.8 Bunkering and Part-Loading Cargos

When interpreting BWRFs, it is important to remember that port and BW details may be influenced by previous 'temporary' port calls to obtain fuel, change crews or part-load a cargo.

- BWRFs for crude oil tankers operating to the ROPME Sea Area commonly present this 'last port of call' ≠ 'BW source port' problem. Many tankers, including VLCCs and ULCCs (Appendix 5) often stop at Fujairah or Khor Fakkan for bunkering, stores and/or a crew change, usually on their inbound voyage but sometimes on their outbound voyage. The BWRF entries for "last port" and "next port" can therefore be these ports, although they may not be a true BW source or destination port. While the true source port should normally be identified from "BW Source" field (Part 4 of the BWRF), the 'Next Port' for a true BW destination can only be deduced for tankers involved in a "liner trade" (such as Ain Sukhna - Khark Island - Ain Sukhna), or by checking the oil terminal's trading records and cargo manifests. However this may be difficult for commercial confidentiality reasons.
- Large tankers and bulk carriers may also visit one or more terminals to part-load cargo before arriving at the Receiving Port, having discharged some or all of their BW at the previous stops. If only a 'top-up' cargo is loaded at the Receiving Port, this operation may involve a small or zero discharge of BW. If the BWRF has been entered clearly and correctly, any BW discharge associated with a part-loading operation can be interpreted without a problem. If there are doubts, Data-Entry Operators should refer to port trading records and cargo loading lists, or try contacting the local Shipping Agent for advice.

2.5 EDITING AND PRINTING THE SHIP VISIT RECORDS

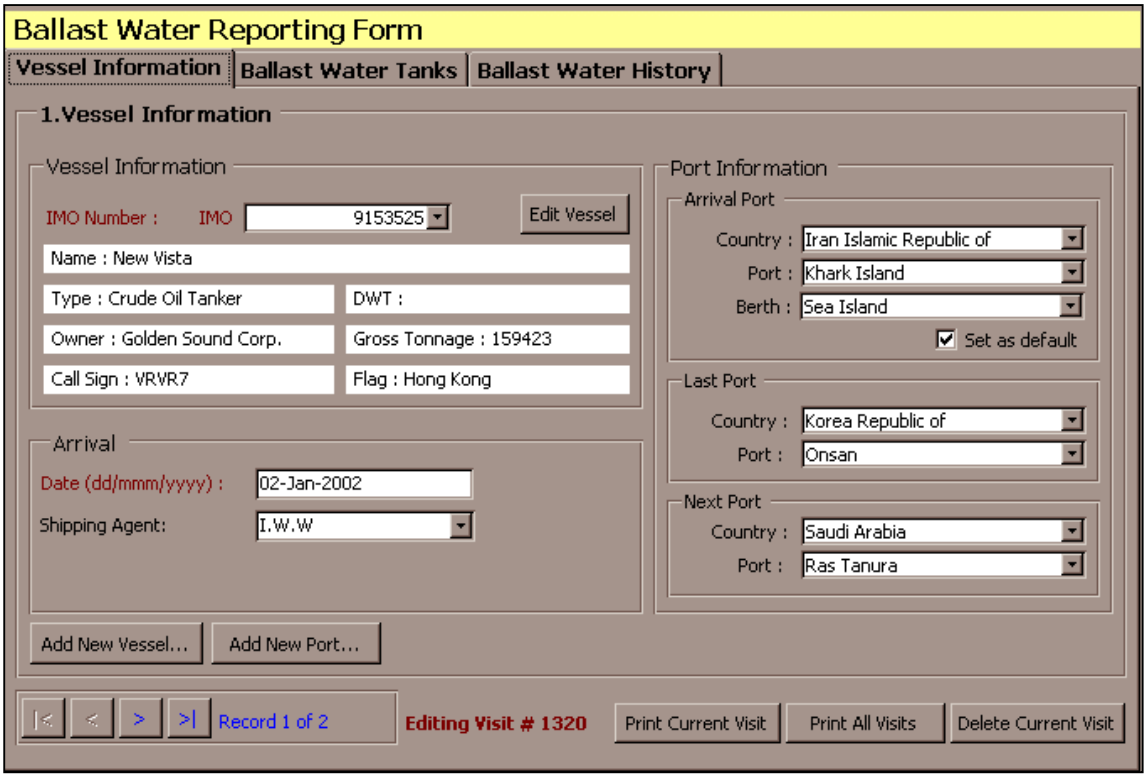
After a Ship Visit Record has been added to the Database, it can be edited or printed by going to '**Edit/Print Ship Visit Records**' from the main menu. Find the Record by selecting the ship's IMO Number and/or its Arrival Date from the Search Window:



If the particular Arrival Date is not known, leave the Date field empty (**_***_****), and click the **Search** button. If the Arrival Date is known but not the IMO Number, leave the IMO Number field blank, enter the Date and click the **Search** button.

Both methods take you to the Edit/Print window, which has blue 'scroll' buttons to locate the specific Arrival Date (for a selected ship), or the specific ship (for a selected Arrival Date). These forward/reverse buttons are at the bottom left corner of the Edit/Print window (this window is shown at the top of the next page).

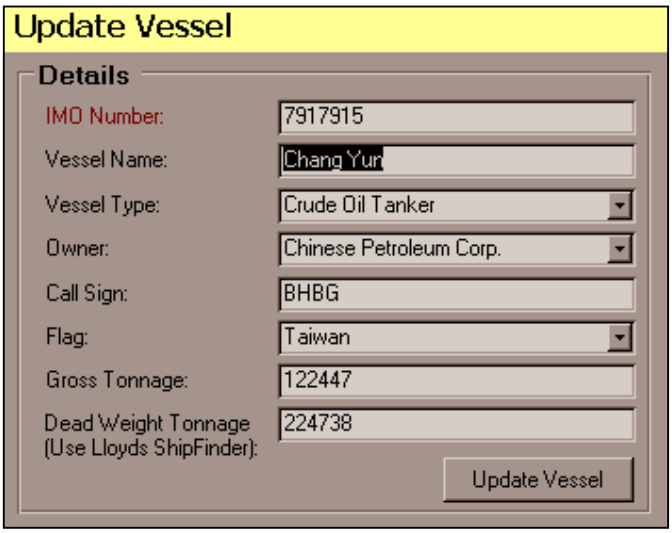
After locating the Visit Record, details in the three tab sub-windows can be changed, including individual BW tanks, which can be edited, deleted or added in the '**Ballast Water History**' sub-window. To remove a complete BW tank and all of its details, select the tank then click the 'Remove Tank' button.



After making the edits, save the amended Visit Record by clicking the 'Save Visit Details' button in the bottom right of the '**Ballast Water History**' sub-window.

To make an A4-size printout of the selected Visit Record, click on the '**Print Current Visit**' button (in the '**Vessel Information**' subwindow). The page layout ('landscape') and format closely resembles the standard IMO-BWRF. It may be necessary to adjust your Printer *Properties* settings to prevent the printed record using two 'portrait' pages (go to 'Print…' in the Access 'File' menu, then click 'Properties' and check the paper size, layout etc).

To update the Vessel Details, click the 'Edit Vessel' button on the '**Vessel Information'** sub-window, which opens the smaller '**Update Vessel**' subwindow:



Any update to the Vessel details will apply to all of its previous and future Ship Visit Records. Note that the Vessel's unique **IMO Number** **cannot** be changed in this window. If a Vessel has been entered with an incorrect IMO number, it is necessary to:

(1) Add a New Vessel, using the correct IMO Number and vessel details (click the '**Add New Vessel...**' button, near the bottom left of the Edit/Print Records window).

(2) Re-assign all Visit Records which are linked to the incorrect IMO Number, to the 'new vessel' which has the correct IMO Number. Do this re-assignation by:
- locating all Visits assigned to the incorrect IMO Number (i.e. select the incorrect IMO Number in the '**Search**' entry window to 'Edit/Print Visit Records').
- using the blue **FWD/REV** scroll buttons, go to each Visit Record and select the correct IMO Number from the drop-down list, then Save each new Visit Record by clicking the 'Save Visit Details' button in the '**Ballast Water History**' sub-window.

(3) After all Visits have been saved to the correct IMO Number, return to the incorrect IMO Number and delete the unwanted Visits (use the 'Delete Current Visit' button in the bottom right of the '**Vessel Information**' sub-window).

(4) The Vessel with the incorrect IMO Number can now be deleted from the Database (the vessel deletion is made in the '**Database Management**' window; see Section 2.6).

If deletion or edit of incorrect visits and tank discharges causes one more BW Source Ports to be no longer associated with any discharge, the Database will continue to store these port/s in its previous risk calculation tables, but they will have '0' BW frequency and volume risk coefficients.

To remove any Source Ports or Next Ports with zero BW discharges from the risk calculations, the list of source ports (or next ports) with BW tank discharge records must be updated. This is achieved by re-generating the list of source (or next) ports which require an Environmental Matching Coefficient (see Section 7.3.1).

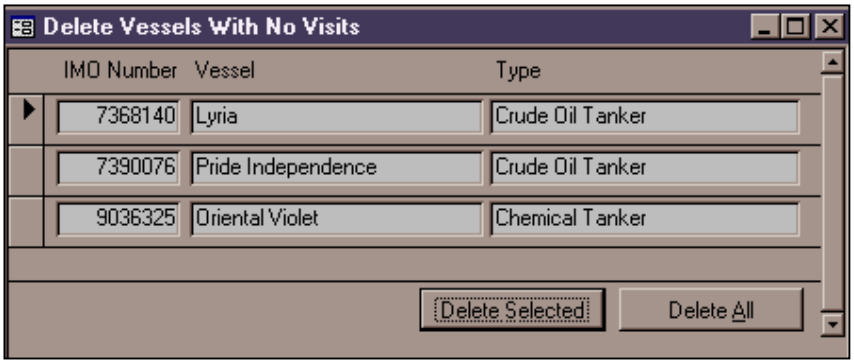
2.6 DATABASE MANAGEMENT

The Database Management menu is accessed from the Main Menu. There are three features in the Database Management menu:

- **Delete Ships With No Visits**
- **Edit Port Details**
- **Export Ship Visit Details to Excel**

'Delete Ships With No Visits'

This house-keeping item displays a window that lists all Vessels in the Database which are **not** linked to any Ship Visit Record:

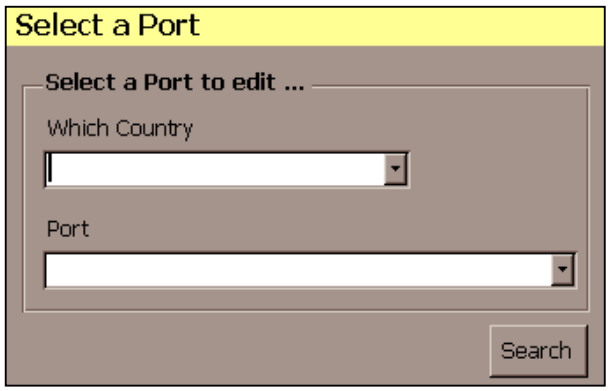


If you are trying to delete a vessel with an incorrect IMO Number and it is **not** present in this list, this means it still has one (or more) Ship Visit Records which need to be unlinked (and perhaps reassigned to the ship with the correct IMO Number; see Section 2.5).

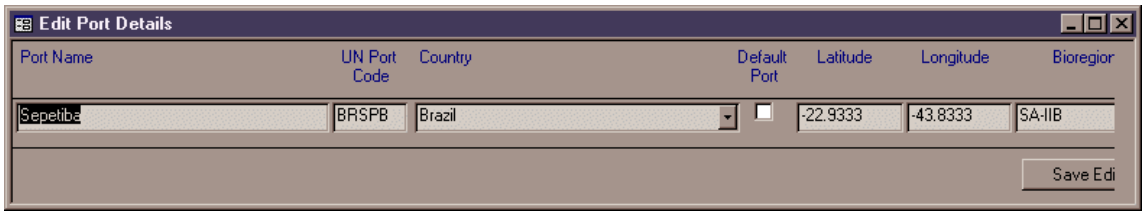
The list may also show ships which have correct IMO Numbers etc, but have made no visit (or provided no BWRF) to the Arrival Port. These vessels may be safely left in the Database for possible future visits and BWRFs.

'Edit Port Details'

This feature provides a small port 'Search' window, using Country and Port drop-down lists:



After selecting the port, an **Edit Port Details** sub-window is provided for correcting the port details, including its name, unique UN Port Code, Lat/Long position and Bioregion:



'Export Visit Details to Excel'

This feature permits a range of visit record information (for all or specified vessel types, visit dates, berthing areas, BW source ports, last ports of call, next ports of call and BW tank discharge details) to be automatically exported into Excel spreadsheet files, which can be used for other types of analysis, evaluation, formatting, copying or printing outside of the Database.

Select the data to be exported by following the prompts that allow particular combinations of arrival dates, vessels or vessel types, berthing areas and BW source ports, last ports of call or next ports of call. Then generate the Excel file by clicking the 'Export' button, and use the standard Save window to name and save the new .xls file in a directory/folder.

Using MS Access for other data manipulation tasks

Database managers may wish to use the Query facility of MS Access to achieve other data management tasks, or file management tools such as '*Compact and Repair…*' (go to '*Database Utilities*' in the MS Access 'Tool' menu). Using the various Design and Query functions of MS Access to produce customised tables and report files of the various vessel, visit, /port, BW tank and risk species data is beyond the scope of this User Guide. Database managers should refer to the MS Access 2000 Handbook, and are highly recommended to seek 'hands-on' demonstrations and guidance from experienced MS Access users.

3. USING THE ARCVIEW GIS

3.1 GIS SCOPE AND TECHNICAL ASSISTANCE

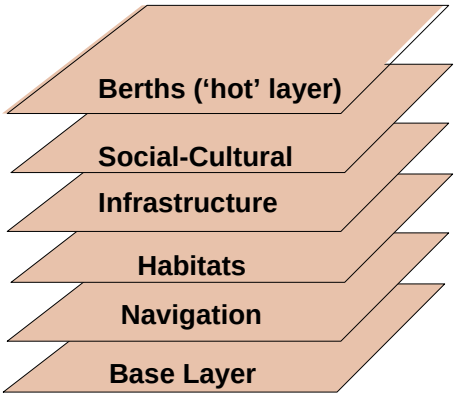
The GIS (Geographical Information System) component of the BWRA system is ESRI's ArcView 3.2. This is used to display the Port Map, the World Bioregions Map and the results of the risk assessment which are generated in the output table of the BWRA Database.

Technical knowledge and detailed guidance with GIS housekeeping tasks are not required for operating the BWRA and are beyond the scope of this User Guide. However GIS managers and operators should work through the Self-Help Tutorial that is supplied with the ArcView 3.2 application. They can also access the public (free) knowledge base at the ESRI website (www.esri.com). This website also contains links to various Self-Help GIS tutorial courses at ESRI's 'Virtual Campus'.

3.2 DEVELOPING THE PORT MAP

3.2.1 Structure

The Port Map displays the navigational and infrastructure features of the demonstration site, plus its coastal habitats and resources. A useful Port Map requires several types of information to be collated and managed, including spatial data on pertinent navigational, geophysical, environmental, socio-cultural, commercial and planimetric features. For clarity and convenience of data management and display, each type of data ('theme') deserves a separate layer which can be compiled using copies of the 'base layer'. The layers used for the Port Maps are as follows:



The base layer can be constructed from nautical hydrographic charts that are available in electronic format (preferable) or paper form from the nation's maritime authority or local chart suppliers respectively. It is often the case that useful data are already available in electronic formats which can be readily converted to the ESRI ArcView file format.

Some regional topographic data is supplied with ArcView and more can be obtained by web searches. Electronic versions of many nautical charts are available from such companies as C-Maps (www.c-map.com). Government agencies, universities, survey companies and local GIS specialists can hold significant quantities of useful data. The trick is to find the people who are keeping it or know where to find it. The protocols for each thematic layer are outlined in the following subsections.

3.2.2 Base Layer

The base layer can be derived from nautical charts of the port and its approaches. It should include the bathymetry (seafloor contours) and other key *planimetric* features, such as lighthouses, important channel markers and other permanent or near-permanent features. Planimetric data include 'ground reference features' which are unlikely to change or move frequently, such as main roads, railway lines, large chimneys, mountain peaks, hill tops, bridges, TV/Radio towers, bridges and other prominent structures or buildings. All the other geophysical, environmental, cultural and commercial features of the port and its surrounding area (= the port 'catchment') can be layered over this base.

Some pilot countries already have access to electronic data suitable for the base layer, such as CAD (*Computer Aided Drawing*) bathymetry maps. If electronic charts are not available, up-to-date vertical colour or black/white aerial photographs (e.g. 1:10000 – 1:50000) can be scanned at high resolution (>300 dpi) and geo-referenced. These can provide an alternative base layer that provides a useful frame of reference. If photographs are used for the base layer and no electronic bathymetry data are available, the latter can be added by digitising from scans or using a digitiser board.

NOTE: If no 'in-house' GIS/data building capacity exists at the port, local companies or other government agencies which have GIS specialists are often pleased to negotiate a data-capture and/or GIS mapping service for the port authority.

The key features of the base layer should comprise:

- The entire coastline - including harbour walls, breakwaters and jetties. The coastline is the same as the high tide mark, as shown on all nautical charts.
- The lowest tide mark (= the 0 m bathymetric contour [*Isobath*]). The 'Chart Datum' of nautical maps is typically very close to sea level at the Lowest Astronomical Tide. It may also refer to the 'Port Datum' or a National or Regional 'Height Datum'.
- 5 metre isobath (often the first continuous contour line below the low tide mark)
- 10 metre isobath
- 20 metre isobath
- 30 or 50 metre isobath
- Edges of dredged shipping channels (often as blue or purple lines which also show the boundary of the channel depths which are maintained by port dredging programs).

Important land contours can also be added to the base layer, particularly for ports edged by distinctive hills or mountains. The colours and colour shades used to highlight the land, the intertidal zone and the various subtidal depth zones, should follow those of the local nautical chart as closely as possible. Otherwise the map will look unfamiliar and difficult to interpret.

3.2.3 Navigational Layer

The navigation features of this layer should try to follow the standard symbols used by nautical charts. The prime source for the symbology has been the Admiralty Standard Reference for Nautical Charts (Chart No. 5011, which is in booklet form).

Unfortunately the symbol libraries (for point and pattern symbols) that are supplied with ArcView do not contain international navigation symbols, and third-party symbols that follow the Admiralty standard could not be found. Therefore a 'closest match' has been used.

3.2.4 Habitat Layer

This layer needs to show the main intertidal and subtidal habitats using a logical colour scheme which helps users to recognise the type and depth of the habitat. Subtidal habitats occupy all areas below the 0 m isobath (low tide mark), while intertidal habitats must occupy all areas between the 0 m isobath and the high tide mark (Section 3.2.2). The intertidal areas should extend along the sides of tidal creeks and estuaries.

The subtidal habitats displayed by a typical Port Map are:

- muddy seafloor (= muds, sandy muds, shelly muds; middle brown colour).
- sandy seafloor (= sands, muddy, sands, shelly sands; pale brown colour).
- rocky seafloor (= rocky pavement and subtidal rock reefs; dark grey colour).
- coral reef (= true carbonate reef built by corals and coralline algae; pink or red colour).
- significant seagrass or seaweed beds (e.g. *Zostera, Laminaria, Dictyotis*; mid-green colour).

The intertidal habitats displayed by a typical Port Map are:

- high intertidal salt flats / sabkahs / marsh areas (may extend to supratidal zone; white).
- sand beaches and sand spits (often occur along the back edges of bays and beside a sandy seafloor in the shallow subtidal area; strong yellow colour).
- mangrove forests (these usually occupy middle to high tide area; dark green colour).
- low tide mud flats (= become fully exposed only during low spring tides). Often wide with a gentle slope, and along the edges of estuaries, lagoons, river channels and/or a shallow muddy seafloor of the subtidal zone; olive-brown colour).
- cliffs / rocky coastline (where the gap between the low and high tide marks does not exist, or is very small, because of the almost vertical slope into the subtidal zone; black lines for vertical cliffs, medium-grey colour for rocky platforms and terraces).
- stony beaches (predominantly boulders, cobbles, stones or gravel, with any sand forming narrow, thin strips or small patches in middle or upper beach; cream or pale grey colour).
- exposed coral reef platform (true carbonate reef built by corals, coralline algae; pink/red).
- artificial seawalls and rocky breakwaters (includes corniches, public promenades and the seaward edges of land reclamation projects and some flood defences; purple/black lines).

The habitat layer should not be developed until the base Layer has been completed, printed and checked. It also requires assistance from local marine scientists who are familiar with the port, particularly if they have undertaken fieldwork for Baseline Biological Surveys.

Coastal scientists and marine biologists with reasonable local knowledge can identify the location of most habitats by interpreting the various isobaths, seafloor symbols, coastal aspects and gradients which are displayed on quality nautical charts. A half-day field excursion and some aerial photographs are invaluable for confirming habitat boundaries and filling 'gaps'. Checking with University marine researchers, local fishermen and/or members of local SCUBA diving clubs can provide valuable knowledge sources for the subtidal zones.

Habitat features are often displayed on small-scale, quality nautical charts by special symbols and colours. These include:

- High tidal marshes and sabkahs.
- Sand beaches, rocky shorelines and cliff lines.
- Seaward edge of mangrove fringes.

- Intertidal edges of coral reef (different symbol compared to rock reef).
- Intertidal and subtidal exposures of rocky reef (different to coral reef).
- Intertidal mud flats.
- Several types of artificial rocky shoreline, including breakwaters.
- Presence of shelly, sandy, muddy or rocky seafloors, denoted by letters such as Sh, S, M and R respectively. For example, MS is muddy sand (which means sand is dominant), while ShM is shelly mud (i.e. the silty mud is dominant).

A key reference for the standard symbology and colours used by nautical charts to show marine habitat features is the Admiralty Standard Reference for Nautical Charts (Booklet Chart 5011). Some nations produce their own versions of this guide which are very similar.

3.2.5 Port Infrastructure Layer

The Infrastructure Layer should show the key 'working' components of the port and its surrounding urban area, including any large and prominent oil tank farm, coastal power station, refinery, desalination plant, subsea petroleum pipeline, railway line, port control tower and municipal airport, plus visually-dominant structures such as a large bridge, landfill site or TV/communications tower. These items improve site orientation and familiarisation by Port Map users. Some of these items may be used in the base layer to provide key planimetric features (Section 3.2.2).

The GIS Port Map may be used for other port environmental management purposes, including Oil Spill Contingency Plans (OSCPs). In these cases the Infrastructure Layer should include the sites of storage sheds for oil combat equipment and command nodes. 'Pop-up' tables providing telephone numbers, emergency radio channels and communication/command procedures can also be added, as may available for copying from a local OSCP.

3.2.6 Social-Cultural Layer

Significant social-cultural resources at risk from introduced species include recreational, artesanal and/or commercial fishing areas, plus aquaculture and mariculture facilities (i.e. trawling grounds, shrimp ponds, fish traps, fish cages, seaweed growing areas and popular angling sites etc). They also include declared or known fish nursery areas, popular recreational or swimming beaches, boating areas and culturally significant sites (e.g. historic shipwrecks, archaeological sites, coastal memorials, places of worship, etc).

A specific layer may be created to display all these resources, or they may be added to the Infrastructure layer (e.g. mosques, shoreline memorials), the Habitat Layer (e.g. fishery and aquaculture resources) or the Navigation Layer (e.g. historic shipwrecks), as appropriate.

The social-cultural layer should also show the boundaries of conservation sites, including Wildlife Reserves, Fish Nursery Areas, Marine Protected Areas (MPAs), Coastal Nature Reserves, Special Conservation Zones or Marine Parks. These sensitive areas should include any locally or nationally recognised site for seabirds, turtles or sea lions (e.g. breeding or nesting sites, feeding sites, loafing areas, haul-out sites).

Declared wildlife reserves, nature sanctuaries and fish nursery areas are often prohibited to trawling, netting or boating, so their boundaries are shown on nautical charts. Boundaries of other wildlife conservation reserves, MPAs and parks are usually shown on maps produced by Environment, Natural Resource and/or Fishery departments (and sometimes by the local office of a national or international NGO such as WWF).

3.2.7 Active Berth Layer

This layer shows all the berthing, mooring and anchoring areas at the Arrival port. The names or numbers of the berths and berthing areas can be obtained from the Harbour Master or a detailed port chart. It is important that the names used by the GIS for the different berthing areas (ArcView's *Attributes*) are identical to the names of the same berthing areas used in the Access Database, so that summary tables of the BW discharge data can be automatically taken from the Database and displayed in the correct locations via the ODBC (Section 3.4).

3.2.8 Other Geophysical and Biological Data

This data includes topographic (land contours) as well as hydrographical information such as local streams, tidal creeks, rivers and canals.

Where available, animated or video files (.gif/.avi/ etc) can be linked to ArcView to show prevailing tidal currents etc. These movie files can display the directions of water movement to and from the various ballasting/deballasting locations, and can help determine potential time-slots that would reduce BW and suspended sediment discharge/uptake risks for particular sites. Seasonal extremes in turbidity (e.g. suspended solids; mg/L) and/or phytoplankton blooms are also very useful for BW risk assessment work, and these can be added to the Port Map as spatial information or in 'pop-up' tables.

There is no limit concerning the types of data that can be linked or added to the Port Map. Pertinent tables or graphics include the direction of prevailing seasonal winds, plus the maxima, minima and averages for salinities, water temperatures, turbidities and/or red tides of dinoflagellates for particular areas for each season, month or other convenient timescale.

3.3 STRUCTURE OF THE WORLD BIOREGIONS MAP

The World Bioregion Map is used as a backdrop for displaying the BWRA results. It has been compiled from a base map provided by CSIRO-CRIMP and modified according to advice provided by marine scientists in the Pilot Countries (Section 1.4; see Appendix 3). Bioregions for several large river systems have also been added to accommodate some river ports.

The present map displays 204 discrete bioregions. Each region is labelled with a unique code (e.g. **NWP-6** is Northwest Pacific 6). A zoomable Adobe file of the World Bioregion Map (**Bioregions.pdf**) is available in the **Utilities Folder** of the BWRA directory.

The same bioregions are also stored in the Database, where each bioregion has a particular set of risk species linked to it (Section 6.2). Except for the Receiving Port, any port located within a particular bioregion is considered likely to contain the risk species of that bioregion, (= precautionary approach; see Appendices 1 and 3).

3.4 LINKING THE ARCVIEW GIS TO THE ACCESS DATABASE

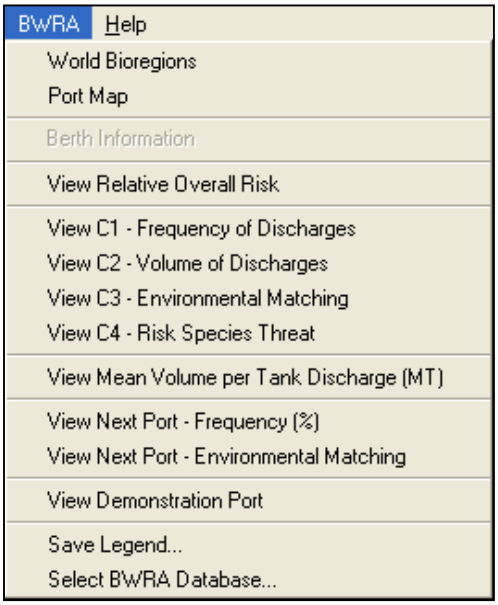
To display the results of the BWRA, ArcView needs to access the Database using ODBC (Open Database Connectivity). Details for establishing this connection are given in Appendix 4.

3.5 DISPLAYING THE BWRA RESULTS

The ArcView *User Interface* has been customised to allow convenient interpretation of the BWRA results. Customised button tools allow the various risk coefficient results as well as the Relative Overall Risk (ROR) and summary statistics for each BW source port to be displayed on the World Map, while statistics for each berthing area can be accessed from the Port Map. The display features provided in the ArcView GIS are described in the following sub-sections.

3.5.1 ArcView's BWRA Menu

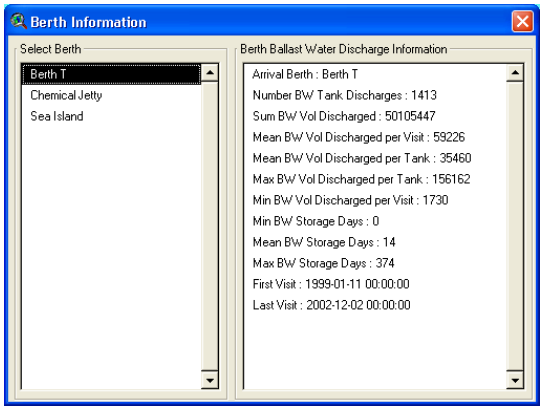
The BWRA menu is a customised 'pull-down' menu that gives easy access to graphically displaying the various BWRA results and statistics on the World and Port Maps:



World Bioregions: Toggles from the Port Map to the World Bioregions Map.

Port Map: Toggles from the World Bioregions Map to the Port Map.

Berth Information: Launches the Berth Information dialog window, which shows summary BWRA statistics for each berthing area that is used by the Access Database:



NOTE: This 'Berth Information' option is active only when the Port Map is being displayed.

View Relative Overall Risk: Displays the Relative Overall Risk, as calculated by the BWRA Database (Section 6). This option is active only when the World Bioregions Map is displayed.

View C1 – Frequency of Discharges: Displays the Frequency of BW Discharges from each source port (= Risk Coefficient C1). Active only when the World Bioregions Map is displayed.

View C2 – Volume of Discharges: Displays the Volume of Discharges from each source port (= Risk Coefficient C2). Active only when the World Bioregions Map is displayed.

View C3 – Environmental Matching: Displays the Environmental Matching Coefficient (= Risk Coefficient C3). Active only when the World Bioregions Map is displayed.

View C4 – Risk Species Threat: Displays the Risk Species Threat from each source port (= Risk Coefficient C4). Active only when the World Bioregions Map is displayed.

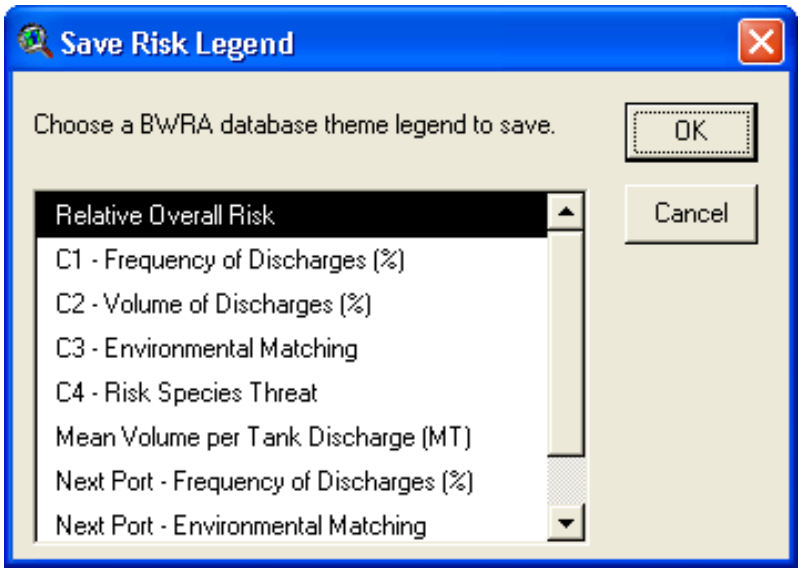
View Mean Volume per Tank Discharge (MT): Displays the mean volume per Tank Discharge (as converted to metric tonnes) from each source port. Active only when the World Bioregions Map is displayed.

View Next Port – Frequency (%): Displays each Next Port of Call and the vessel departure frequency to each of them. Active only when World Bioregions Map is displayed.

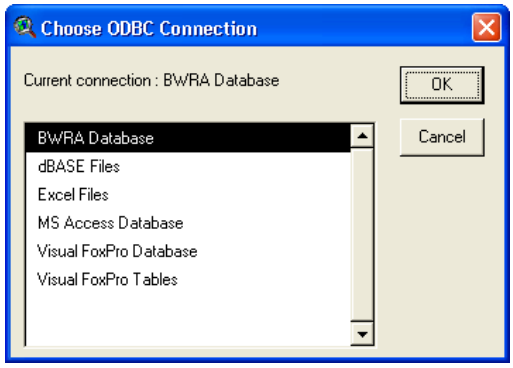
View Next Port – Environmental Matching: Displays the Environmental Matching (Risk Coefficient C3) for each Next Port of Call. Active only when World Bioregions Map is displayed.

View Demonstration Port: Highlights the location of Demonstration Port (= Arrival Port). Active only when the World Bioregions Map is displayed.

Save BWRA Legend... Users can change the 'Default Legend' to customise the display of the BWRA results (i.e. colours, sizes and fonts of symbols and text labels, etc). If the Default Legend is changed, the customised legend can be saved as the new Default. This subwindow option is active only when the World Bioregion Map is displayed:



Select BWRA Database... Provides a list of available ODBC connections (see Appendix 4). If an ODBC connection to the Database has not been previously made, this dialog box will open automatically when ArcView is first launched.

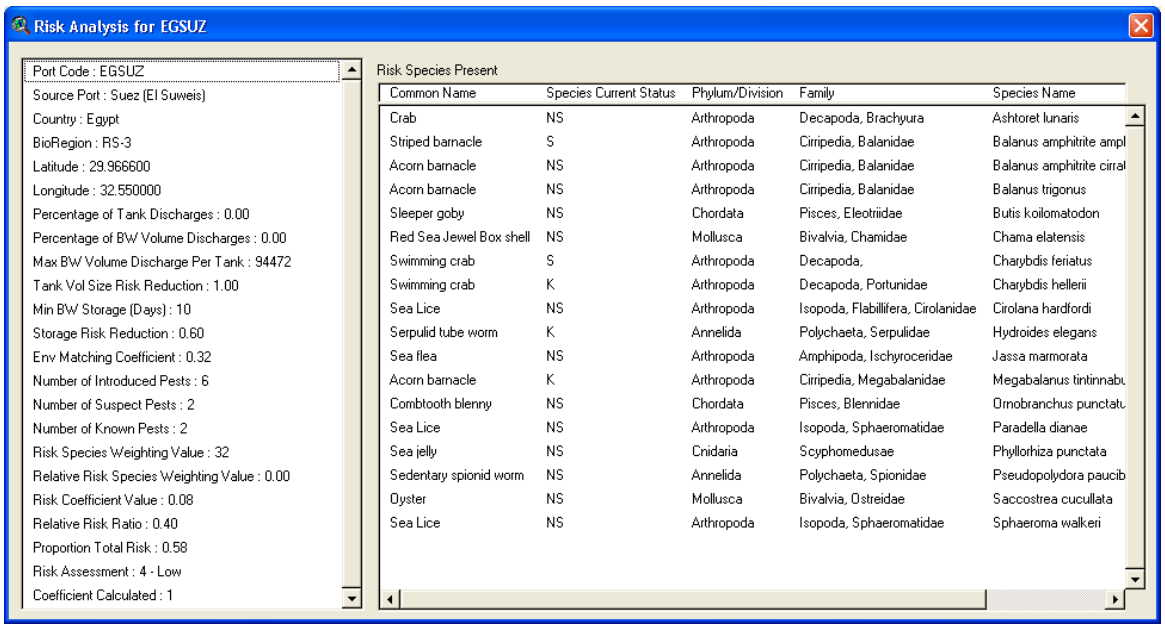


3.5.2 ArcView's BWRA Tools

Two tools have been added to the ArcView toolbar to enable easy querying of the BWRA Database. The buttons to these special tools are located on the right side of the lower bar:

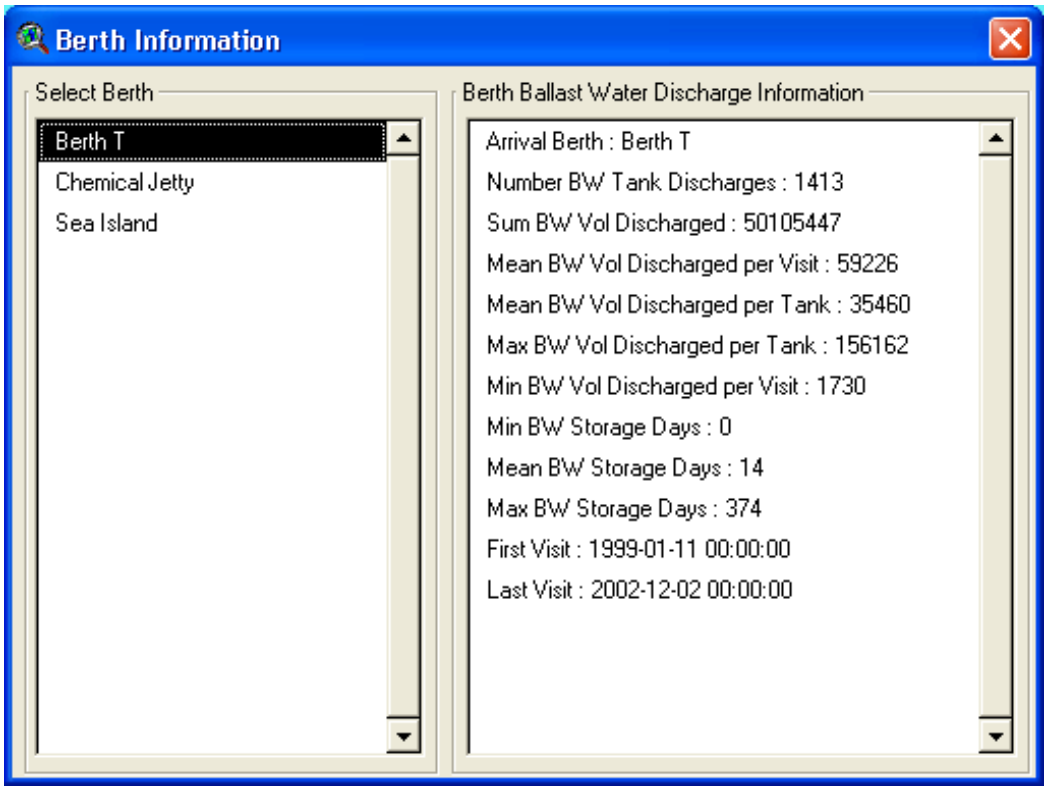
Risk Assessment Information

This tool is available only when the World Bioregions Map is displayed. Selecting this tool allows a User to click on any port displayed in any layer of the World Map, to launch a summary results table for that port. The table includes a list of the risk species present in the bioregion of that port:



Berth Summary Information

This tool is available only when the Port Map is displayed. Selecting this tool allows the User to click on any berthing areas in the Arrival Port to launch a summary results table for that berthing area. The table presents a summary of the BWRA Database statistics for the berthing area:



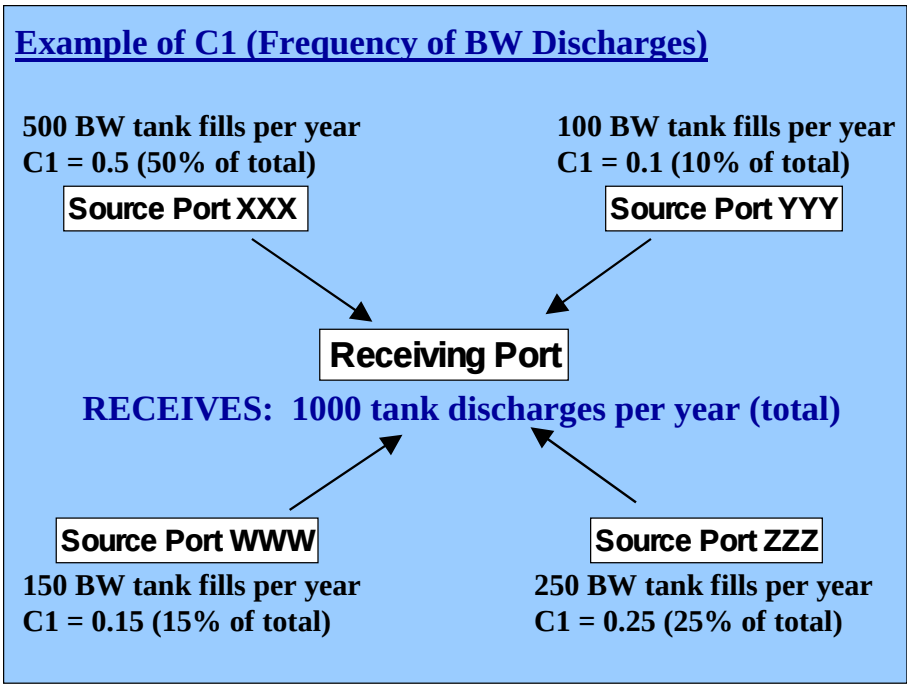
The next Sections of the User Guide (Sections 4-6) provide more details about the various results and Risk Coefficients that can be displayed by the ArcView GIS.

4. RISK COEFFICIENTS AND FACTORS FROM BWRF DATA

4.1 BW DISCHARGE FREQUENCY (C1 RISK COEFFICIENT)

'Packets' of source port water which are ballasted, transferred to a receiving port and then deballasted represent a series of discrete 'injections', each containing a mixture of surviving organisms (= 'inoculations'). The more times these injections occur, the higher the chance that conditions promoting the uptake of organisms and their survival both during and after the voyage may coincide to permit a 'successful' inoculation (i.e. the surviving organisms grow and reproduce to establish a new population at the receiving port; Appendix 1).

In other words, the larger the number of vessel visits involving BW tank discharges from a particular source port, the greater the chance of successful transfers and subsequent introduction of at least one Non-Indigenous Species (NIS). The proportional frequency of such voyages from each source port is automatically calculated by the Database for use and display by the GIS (Section 3.3 and Section 7). Calculating this risk coefficient (C1) for each source port is shown as follows:



The sum of the coefficients for all source ports amounts to 1 (= total hazard due to BW discharge frequency), with the size of the individual C1 coefficients in the above example between 0.5 and 0.1. In terms of discharge frequency (= number of inoculations), tank discharges from Port XXX (0.5) provide five times more chance of causing an introduction than tank discharges from Port YYY (0.1), and twice as many as tank discharges from Port ZZZ (0.25). This assumes the BWRF data provide a reliable picture of the receiving port's current shipping trade (e.g. all vessel arrivals for at least one and preferably two or three years).

Since the BWRF Database also contains the records of BW Exchanges, these could be added to the risk assessment calculation to provide a 'hazard-reduction' function. However, the

current BW exchange data are not particularly reliable for the 'first-pass' risk assessments for various reasons, including:

- not all ships can undertake ideal 'blue water' exchanges due to constraints imposed by their route, weather, vessel design, pump condition, etc.
- systematic compliance monitoring is required to check the truth of claimed BW exchanges (not yet commenced);
- the success of exchanges is highly variable and their ability to eliminate organisms depends on many factors (the best reported removal rates are in the 50-90% range);
- many organisms can avoid removal by various behaviours (including sediment burial, swimming up-current and/or seeking quiescent areas within the tank);
- the objective of the 'first-pass' risk assessment is to identify the most hazardous trading routes and associated vessel types, irrespective of the proportion of ships which regularly achieve effective, risk-reduction exchanges on each route.

The Database also stores the reported Salinity of the discharged BW source water, and this could similarly be used for a risk-reduction function (e.g. discharges of BW from a freshwater river port present lower risk to an open coastal port). Again, there are several reasons why the Database does not attempt to use the salinity values of the BWRFs for the 'first-pass' BWRAs:

- not all ships are reporting the salinity value, or measuring it reliably.
- there is a need to standardise the meaning and use of this value (its original intent was to check if ships are performing an adequate 'blue-water' ocean exchange, which would reduce the risk of invasions for BW discharges into permanently fresh lakes and waterways such as the Great Lakes system).
- some euryhaline species can survive in both freshwater and brackish water ports (e.g. the golden Asian mussel, *Limnoperna fortunei*);
- some ports are highly freshwater during their local wet season but become salty during their dry season (e.g. Montevideo, Buenos Aires, Karumba, Rotterdam etc).

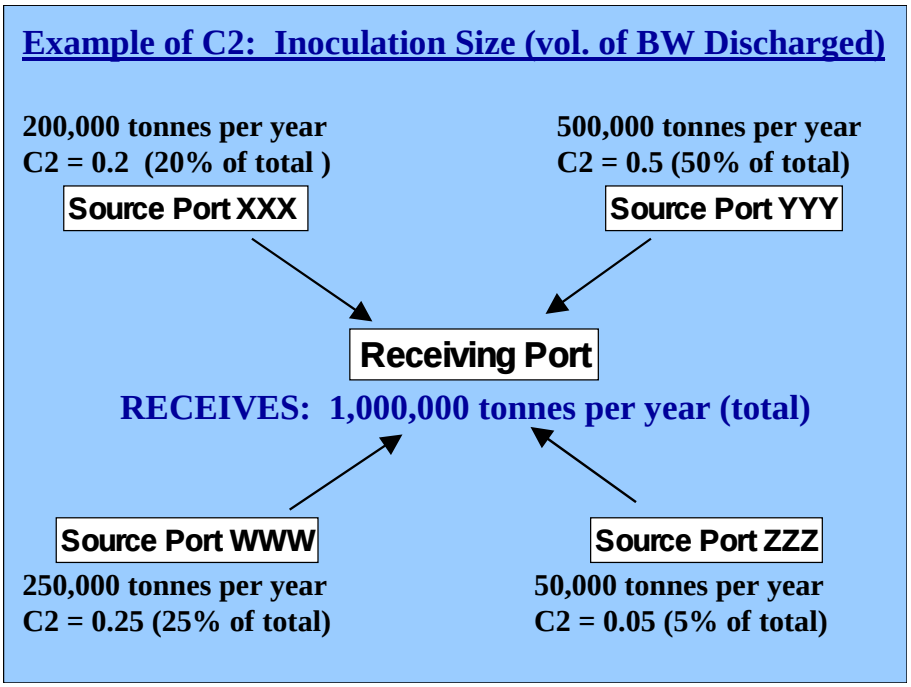
To use salinity, the Database would therefore need a risk-reduction function that is 'customised' to fit the salinity regime of the Arrival Port/Demonstration Site. The port would also need to arrange a ship education programme and compliance monitoring to ensure all visiting ships were reliably reporting the BW salinity after uptake at the source port, and after any BW exchange that was attempted during the voyage.

4.2 BW DISCHARGE VOLUME (C2 RISK COEFFICIENT)

The C2 coefficient assumes there is a simple linear relationship between the amount of BW imported from a source port and the number of organisms transferred with this water (= 'inoculation size'; Appendix 1). For example, if overall average plankton/nekton densities at two source ports are similar, then inoculations from the port which annually exports, say, ten times more BW tonnage, may be expected to supply ten times more organisms.

Water quality in larger tanks usually declines at slower rates than in small tanks owing to the surface area/volume ratio, meaning that more organisms tend to survive (see Section 4.2.3). Finally, the volume of BW imported from a source port is more closely linked to the type, size and trade of the ships than to the frequency of visits involving a BW discharge (C1). Thus the amount of BW imported from each source port deserves separate treatment by the BWRA.

The proportion of BW volume received from each source port is treated as a measure of inoculation size by the BWRA, and is automatically calculated by the Database. Calculating this simple risk coefficient (C2) is shown graphically as follows:



As with C1, each source port contributes a proportion of the total inoculation 'size'. The example also shows that arrivals from Port XXX may provide only 20% of the total inoculation size (i.e. 200,000 of the 1 million tonnes annually imported), but pose a higher risk in terms of inoculation frequency (i.e. 50% of C1). This would occur when ships arriving from Port XXX with ballast water are a different type, smaller and/or partly-loaded with cargo, compared to the fewer ships from Port YYY that are discharging 50% of the total received volume (C2).

Whether or not the inoculation frequency (C1) should be treated by the BWRA calculation with more importance ('weight') than inoculation size (C2) is addressed in Section 7.4.

4.3 RISK REDUCTION FACTORS (R1 AND R2)

In addition to C1 and C2, the BWRA Database uses data from the BWRFs to calculate two risk reduction factors, namely the maximum BW tank size (R1) and the minimum BW storage time (R1) of the ships which bring BW from each source port.

4.3.1 Maximum BW Tank Size (R1)

There is good field and laboratory evidence indicating that oxygen levels and water quality in large ballast tanks (which includes cargo holds for some classes of bulk carriers) decline at slower rates (and experience smaller temperature variations) than in small tanks (e.g. Oemcke 1999[2]). This is mostly due to the 'surface area : volume ratio effect' ($a^2 : v^3$). For voyages of similar length, large bulk carriers or tankers with big, segregated BW tanks can therefore be expected to provide better water quality (= improved organism survival) than vessel types

[2] Oemcke D, 1999. The treatment of ship's ballast water. *EcoPorts Monograph Series* 18, Ports Corporation of Queensland, Brisbane, Australia.

with relatively small BW tanks (e.g. Container Ships, Ro-Ro Ships and General Cargo Ships less than 20,000 DWT).
The BWRA Database automatically records the largest (MAX) tank discharge volume recorded for vessels arriving from each BW source port, as a second measure of voyage survivability. Using the MAX discharge is precautionary, since average (AVG) tank discharge could yield an unreliable picture where various vessel types and sizes are discharging significant quantities of BW from a particular source port.

The BWRA Database automatically assigns a risk reduction factor (R1) to each MAX number, using the following default set of inverse logarithmic categories:

MAX tank discharge volume:	<100	100-500	500-1000	>1000 tonnes
Risk reduction factor (R1):	0.4	0.6	0.8	1

These default values can be readily altered (Section 7.4). However R1 comes into play only if ships correctly record their individual tank discharge on the BWRFs, and these are entered into the database. If combined or total tank discharges have been recorded, then the value of R1 will most probably remain at 1 (i.e. providing no influence to the BWRA calculation and results).

4.3.2 Minimum BW Tank Storage Time (R2)

The longer BW is stored in a tank, the smaller will be the number of surviving plants and animals as a consequence of the lack of light and decline in water quality, especially from falling oxygen and pH levels due to biological respiration and rusting (iron oxidation)[3]. Even relatively short storage periods (5 days) cause noticeable declines in the density of many planktonic plant and animals (e.g. Oemcke 1999). Long storage periods (>20 days) typically result in large mortalities of the planktonic life-cycle stages of most kinds of aerobic species, but not for the hardy spore and cyst stages of some species (especially toxic dinoflagellates), nor for anaerobes including pathogens and viruses. Whether or not the BW tank storage time should be used by the BWRA calculation therefore depends on which types of species are being considered (Section 7.4).

The BWRA Database automatically calculates, for each tank discharge from a particular source port, the number of days between the BW uptake date at the source port and the BW discharge date at the Arrival Port, then finds the minimum number of days (MIN) that it calculated for each source port (= precautionary approach). The BWRA Database automatically assigns a risk reduction factor (R2) to each MIN, using the following default set of inverse logarithmic categories:

MIN number of storage days:	<5	5-10	10-20	20-50	>50 days
Risk Reduction Factor (R2):	1	0.8	0.6	0.4	0.2

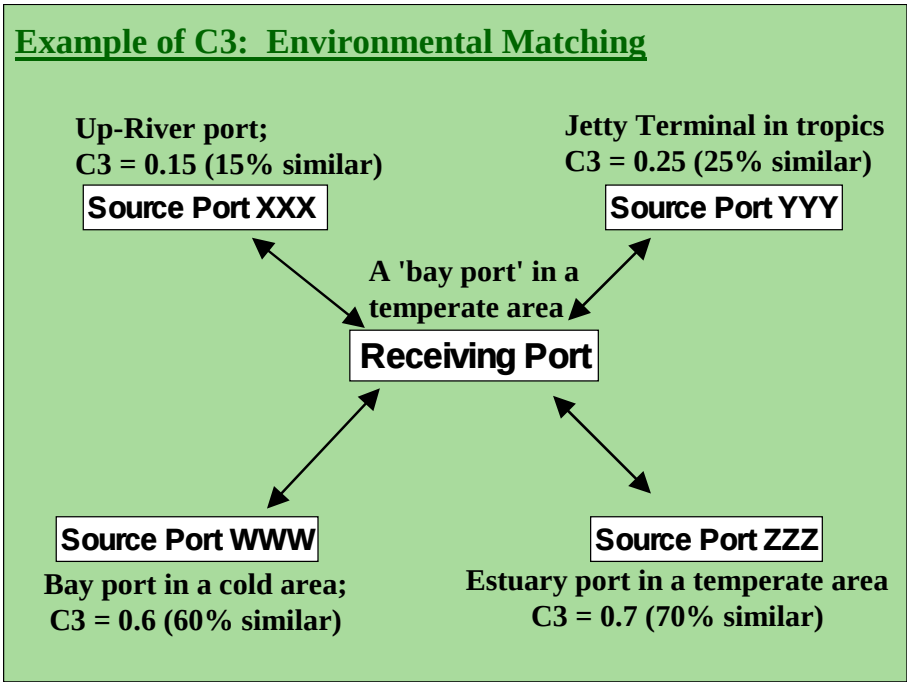
The above R2 values can be readily changed if the BWRA is to be focussed on pathogens, toxic dinoflagellate cysts and other anaerobic life forms and life-cycle stages (Section 7.4). The minimum (MIN) rather than average (AVG) number of storage days is used for the default calculation, since AVG may not provide a reliable picture for a source port with relatively few voyages to the receiving port, especially if various routes have been taken (e.g. some vessels using an intermediate port of call would produce a bimodal distribution of tank storage time). If inspection of the database shows that the majority of source ports are represented by >15 voyages with a similar voyage time, the default 'MIN' can be readily substituted with 'AVG' (Section 7.4) to determine how much it influences the results.

[3] The air pocket between the water level and ballast tank ceilings (= 'head-space') is usually minimised ('pressed up') to prevent dangerous water 'slamming' and 'slop' effects.

5. ENVIRONMENTAL MATCHING COEFFICIENT

5.1 OVERVIEW OF THE C3 COEFFICIENT

The Environmental Matching coefficient (C3) provides an indirect measure of the likely survivorship of organisms following their discharge into the receiving port, plus their potential to establish a new population (Appendix 1). The following graphic portrays a simple example:



Essentially, the more the receiving port is environmentally similar to a BW source port, the greater the chance that an introduction from the source port will survive and reproduce to establish a viable population.

The C3 coefficients are derived from the results of a multivariate Environmental Similarity Analysis (ESA), which is conducted outside the BWRA Database using the PRIMER package. These coefficients are brought into the Database via automatic import of an Excel spreadsheet (Section 5.5). Each coefficient lies between 1.0 (perfect environmental matching) and 0.05 (least environmental matching). Unlike C1 and C2, each C3 does not represent a specific proportion of the sum total risk, and essentially acts as a risk reduction measure (Section 7.4).

The ESA is undertaken by using the PRIMER package to calculate, from an Excel data file containing 34 environmental variables, the **normalised Euclidean distances** between the receiving port and its BW source ports and next ports of call (the latter can be used for a 'forward' BW risk assessment; Section 7.3). The Cluster and Ordination modules of PRIMER are used to review and check the distance results before their conversion into C3 coefficients and importation to the Database.

Users requiring technical information about ESA (including optional data transformations not used for the standard 'first-pass' method) should consult the PRIMER manuals and other statistical guides. Note that the Access Database can import any type of C3 coefficient calculated by any statistics program, by simply copying the C3 values into the Excel file which the Database produces for its C3 'import' feature (see Section 5.5).

5.2 COLLATING THE PORT ENVIRONMENTAL DATA (PED)

The following 34 variables were selected from review of similar analysis, plus suggestions from IBSS scientists at Odessa, for performing the ESA and producing the C3 coefficients used for the BW source ports and destination ports.

No	Name	Variable Type
1.	Port Type[4]	Categorical
2.	Mean water temperature during warmest season (°C)	Scalable
3.	Maximum water temperature at warmest time of year (°C)	"
4.	Mean water temperature during coolest season (°C)	"
5.	Minimum water temperature at coolest time of year (°C)	"
6.	Mean day-time air temperature recorded in warmest season (°C)	"
7.	Maximum day-time air temperature recorded in warmest season (°C)	"
8.	Mean night-time air temperature recorded in coolest season (°C)	"
9.	Minimum night-time air temperature recorded in coolest season (°C)	"
10.	Mean water salinity during wettest period of the year (ppt)	"
11.	Lowest water salinity at wettest time of the year (ppt)	"
12.	Mean water salinity during driest period of year (ppt).	"
13.	Maximum water salinity at driest time of year (ppt).	"
14.	Mean spring tidal range (metres)	"
15.	Mean neap tidal Range (metres)	"
16.	Total rainfall during driest 6 months (millimetres)	"
17.	Total rainfall during wettest 6 months (millimetres)	"
18.	Fewest months accounting for 75% of total annual rainfall	Integer
19.	Distance to nearest river mouth (kilometres) - negative value if upstream	Scalable
20.	Catchment size of nearest river with significant flow (square kilometres)	"
	Logarithmic habitat-distance category - from closest BW discharge site to nearest:	
21.	Smooth artificial wall	Categorical
22.	Rocky artificial wall	"
23.	Wooden pilings	"
24.	High tide salt marsh, saline flats or sabkah	"
25.	Sand beach	"
26.	Stony beach	"
27.	Low tide mud flat	"
28.	Mangroves	"
29.	Natural rocky shore or cliff	"
30.	Subtidal firm sands	"
31.	Subtidal soft mud	"
32.	Seagrass meadow[5]	"
33.	Rocky reef or pavement	"
34.	Coral reef (carbonate framework)	"

The values for each parameter were obtained or derived from data and information culled from scientific, government and port publications, web sites, sampling records, survey reports, SST charts, salinity charts, climate databases, atlases, national tide-tables, nautical charts, coastal habitat maps, national habitat databases, OSCPs, aerial photographs and local expert advice. Details are provided in the Pilot Country BWRA Reports (http://globallast.imo.org/).

[4] Offshore jetty/ mooring; Natural bay; Breakwater harbour; Tidal creek; Estuary; River.

[5] Kelp forest/macroalgae bank was not included but is recommended for future calculations of C3

5.3 MANAGING THE PORT ENVIRONMENTAL DATA WITH EXCEL

Collating and analysing the Port Environmental Data (PED) is typically undertaken by marine scientists who are located at institutions away from the port office where BWRFs are added to the Database. By using the Excel PED file in the **Utilities Folder** (**Port Environmental Data for PRIMER.xls**), the environmental data collating and ESA work can proceed independently, to avoid clashing with Database activities such as BWRF data entry/editing. Using the Excel files allows convenient analysis and review of the PED/ESA work without the need to install MS Access 2000. The Excel PED file contains five interconnected **Worksheets**, used for the following tasks:

1. '**Input Port Enviro Data here...**' : This sheet is formatted to enable convenient entry, review and editing of the 34 PED variables. The variables are stored in a 'port samples by rows' format. The Port name, UN Port Code and location (inputted as degrees, minutes, seconds) occupy the first 8 columns of each row.
2. '**Lat-Long Decimal data**' : This sheet automatically calculates and displays the Decimal Degree positions for each port (Section 2.4.5), and is formatted to provide an optional export to PRIMER (e.g. for 'geographically' plotting the port positions by 2D Ordination).
3. '**WkSht3 PRIMER Input**' : This sheet automatically presents the values placed in Worksheet 1 in the format that permits their direct import by PRIMER, for the ESA.
4. '**WkSht4 Transform Input**' : This sheet provides a location to investigate the distributions of the variables, and the effect of variable transformations on the ESA results. The format permits the direct import of the transformed variables by PRIMER.
5. '**PRIMER Output**' : This sheet is organised for receiving the ESA results from PRIMER (into the **White Cells**). The sheet automatically converts the White Cell values into the C3 matching coefficients, which are displayed in the bright **Green Cells**.

Note: If the order and number of ports in Worksheet 1 is altered by a Row insertion, deletion or Copy/Replication, this will affect the order of port rows in the other worksheets. To quickly re-order the Rows in Worksheets 2-5, use a simple **'Select Row then Drag-Copy Down'** mouse operation inside each sheet will 'refresh' its Rows, so that they follow the new order of ports developed in Worksheet 1. In each sheet, start this operation at a Row located **above** any change/s that were made in Worksheet 1. If the first port (in Row 4 of Worksheet 1) had been changed,

It is important to understand how PRIMER reads the PED data in an Excel worksheet (full details are in the PRIMER manual). PRIMER expects the following:

- The FIRST CELL in the FIRST ROW of the worksheet provides comment that PRIMER will add to its PED data file as a simple **descriptive label**. Any text comment, port name or calendar date can be placed in this cell.
- The SECOND ROW must contain **unique labels** for each Variable. The environmental labels are already provided in Row 2 of Worksheets 2-5 of the Excel PED file.
- The FIRST COLUMN must contain **unique labels** for each 'Port Sample'. The UN Port Codes are obviously the most convenient labels and are present in the Excel PED file.

The **Arrival Port** (BW Receiving Port; e.g. Dalian, Odessa, etc) must be the first port sample (top row). Otherwise there will be no quick and convenient way to review and copy the results from PRIMER's *Similarity Distance Matrix* output.

The similarity measures produced by PRIMER are **normalised Euclidean distances** which give a direct but inverse measure of environmental matching (a zero distance (0) represents a 'perfect match' of 1 (100%; Section 5.1). These distance measures are converted into C3 matching coefficients by inverting them into proportional values between 1 and 0.05 (not 0 because no environment should be infinitely distant from another on a single planet).

5.4 USING PRIMER FOR THE ENVIRONMENTAL SIMILARITY ANALYSIS (ESA)

5.4.1 ESA Process Overview

The environmental similarity analysis (ESA) cannot be undertaken until all the environment data cells for each port have been given a value in Worksheet 1 of the Excel PED file, and the port row order in Worksheets 3 and 5 has been checked (see Note in Section 5.3).

PRIMER will detect any 'empty' cells within a port's row of variables, provide a 'Missing Value' warning message, and stop the analysis. However there may be sufficient information to permit a reliable estimate for the missing value, so this can be inserted in Worksheet 1. If there is no reliable estimate and/or there are several missing values, the incomplete port row should be removed from the Excel worksheets. To keep any incomplete port/s in the ESA, every incomplete column of values must be removed by deleting these from the Excel worksheets (i.e. reducing the total number of variables read by PRIMER).

The ESA is undertaken in four steps:

1: Import the PED data into PRIMER (from Worksheet 3 of the Excel file).

2: Use PRIMER to produce the Similarity Distance Matrix.

3: Check the Similarity Distance results by generating Cluster and Ordination plots.

4: Export the Similarity Distance results to Worksheet 5 of the Excel PED file for automatic conversion into the C3 Environmental Matching coefficients (Section 5.3).

The C3 coefficients in Worksheet 5 of the Excel PED file can then be imported into the BWRA Database, using the BWRA Database's C3 import facility (Section 7).

5.4.2 Importing the Excel Port Environmental Data into PRIMER

(a) Save and Close the Excel PED.xls file, and note its Directory/Folder location.

(b) Open PRIMER (e.g. using the PRIMER shortcut on the Desktop)

(c) Click the 'Open file' icon on Primer's toolbar (or go to the 'File' drop-down menu and select 'Open…'). This displays the **Open - File Selection** window of Primer.

(d) Go to '**Files of Type**' and select '**Excel (*.xls)**' from the drop-down list.

(e) Browse to the Directory/Folder containing the Excel PED.xls and open it in the usual way.

(f) PRIMER now presents two boxes with options, which you select as follows:

In the '**Excel File Properties**' box, select:
Worksheet '**3**'
'Sample data'
'Includes title'
'Includes row labels'
'**OK**'

If the Excel file has the correct structure, PRIMER will now read it successfully. If there is an **Error Message**, or the next box does not appear after 1-2 minutes, close PRIMER and go to Excel to re-check the structure of the PED data in Worksheets 1 and 3 (Section 5.3).

In the '**Sample Data Properties**' box, select:
Samples as '**Rows**'
'**OK**'

PRIMER will now display the imported data using its own data file format (*.pri). The PRIMER layout is more similar to a data table in Access. For example, the port names and environment labels are now 'outside' the rows and columns of data. It may be useful but not necessary to save the data table as a PRIMER file (*.pri) before performing the ESA.

5.4.3 Producing the Similarity Distance Matrix

(a) Make sure the PRIMER data table window is the active window ('highlighted')

(b) Go to the 'Data' drop-down menu and select 'Similarity…'

(c) In the **Similarity** options box, select:
Analyse between: **Samples**
Transformation: **None**
Standardise: [ ] (leave blank)
Measure: **Normalised Euclidean Distance**
'OK'

[see Appendix 1 and PRIMER manual for information on *normalised Euclidean distance*]

(d) PRIMER will now undertake the similarity analysis and provide two new windows:

The first window shows a saveable text file called '**Results1'**. This contains a log of your calculations and option selections, in rich text format (*.rtf) which can be read by MS Word. The log provides a record of the options selected for the analysis. It is not necessary to save this file unless you are exploring different options and methods.

The second window is called **'Sheet1 (Similarity Matrix)**'. This is the matrix which shows the environmental similarity distances between all of the ports. This matrix has the same structure as a 'City-to-City' distance table in a Road Map/Tourist Guide Book.

The first column lists the environmental distances between the Arrival Port and all of the source ports, provided Excel's Worksheet 3 had the correct structure (see Section 5.3).

(e) Save the Similarity Matrix, firstly as a PRIMER file (Sheet1.pri), then again as an Excel file (Sheet1.xls). For example, select '**Save As...**' from the 'File' drop-down menu, then choose '**Excel (*.xls)**' in the '**Save as Type**' drop-down list, then the pathway to your BWRA Directory Folder.

5.4.4 Checking the ESA results by Clustering and Ordination plots

A simple mistake in the Port Environmental Data can produce a big difference in the ESA results, particularly a decimal point error (e.g. 2.50 instead of 25.0 for summer water temperature, or 150.000 instead of 150,000 for river catchment size). It is therefore important to check the similarity distance results before they are converted to Environmental Matching Coefficients (C3) and placed in the BWRA Database. PRIMER allows the following:

CLUSTERING

(a) Make sure the PRIMER 'Similarity Matrix' window is selected (= 'highlighted')

(b) Go to the 'Analyse' drop-down menu and select 'CLUSTER…'

(c) In the **Cluster** options box, select:
Cluster mode: **Group average**
Use ranked similarities: [ ] (no)
Plot dendrogram: [✓] (yes)
'OK'

PRIMER will now perform group-averaged hierarchical clustering of the ESA results and produce a dendrogram. Print to A4 or A3 paper and check the groupings of the ports.

If the Port Labels on the dendrogram are too small to read, go to the 'Options' drop-down menu, select the 'Graphs' tab, and change the 'Print Scale' value (e.g. from 0.5 to 1, or from 1 to 1.5, etc). Repeat the Cluster analysis and print the improved dendrogram.

(d) Check the dendrogram for any unexpected port groupings or patterns such as:

- a single Black Sea port grouped with a bunch of warm-water ports;
- a River port grouped with open-water coastal ports; and/or
- any solitary Port (grouped 'by itself', because it is very distant from all other ports).

Environmental Distance on the dendrogram is measured on the Vertical Axis only – there is no 'Horizontal' axis. The dendrogram is like a mobile hanging from the ceiling of a child's bedroom: the vertical 'strings' holding each group can rotate around each other.

If there is any unexpected or peculiar grouping, you should go back to the Excel PED file and carefully check the port's environment data in Worksheets 1,3 for suspicious values.

ORDINATION

(a) Make sure the PRIMER 'Similarity Matrix' Window is selected (= 'highlighted')

(b) Go to the 'Analyse' drop-down menu and select 'MDS…'

(c) In the **MDS** options box, select:

Number of restarts:	**6** (*minimum*)
Plot graph:	[✓] (yes)
'OK'	

(d) PRIMER will now perform a 2D ordination of the 'non-metric dimensionless scaling' of the similarity results. The position of each port is an estimate, in 2 dimensions, of the actual distance between each port, as measured in the 34 dimensions representing each environmental parameter.

The MDS ordination plot can be rotated (by 'click-and-drag'). This allows you to check for any peculiar feature in the general pattern. Look for ports that are very isolated (all by themselves), or inside a group ports which you do not believe to be very similar.

Remember that the "Stress" of trying to show true positions of all ports in only 2D may be high. The blue 'Stress Number' is on the upper right corner of the 2D plot, and it is also listed in the log file. The Stress should be below 0.2 (preferably below 0.15). You can select the 3D ordination option to reduce the stress value (go to 'Graph' drop-down menu, select 'Properties…' and click the '3D' button). The stress number for 3D plots is provided only in the log file (the text file in the other window).

The 3D plot can also be rotated ('click-and-drag') but it cannot show the port labels. Therefore you will have to identify the different ports using colours and symbol shapes for each group (level) you make inside a FACTOR (see the PRIMER manual for details).

If there is an unusual pattern in 2D or 3D, or a strange group of ports within the pattern, go back to the Excel PED file and carefully check the environment data of the suspect port/s in Worksheets 1 and 3.

If the port labels on the Ordination Plot are too small to read, go to:

The 'Options' drop-down menu, select the 'Graphs' tab, and change the 'Display Scale' and 'Print Scale' values (e.g. from 0.5 to 1, or from 1 to 1.5, etc).

Now repeat the MDS and print the new plot (see the PRIMER manuals for details). Generally, any unusual pattern in the MDS Ordination will also show in CLUSTER, but sometimes a peculiar feature may not be easily seen in one of them. It is therefore wise to check the outputs of both methods. To gain an insight of how the ESA and Ordination methods work, follow the same ESA and Ordination procedures described above using Worksheet 2 (the ports latitudes and longitudes) instead of Worksheet 3. The 2D Ordination plot will, with some rotation, reveal a familiar geographical 'world plot' of the ports with very low stress (only 2 variables were used). The Euclidean distance can also be used instead of the normalised Euclidean distance as the two variables use the same units, and the similarity matrix lists the port distances in degrees.

5.4.5 Exporting the Similarity Distance Results from PRIMER

A similarity distance of '0' between two ports means a 'perfect' environmental matching of 1. This can only happen if all their environmental parameters have exactly the same values. As discussed in Sections 5.1 and 5.3, the Similarity Distances need to be converted into the C3 Environmental Matching coefficients (for example, converting distance values in a 0 - 12.678 range to the 1 - 0.05 range).

The conversion is done automatically in Worksheet 5 of the Excel file (Section 5.3). It is therefore necessary to take a copy of the first two columns of the Similarity Distance Matrix (i.e. the results for the Demonstration Port only) and paste these into Worksheet. Because the number of columns in PRIMER's similarity table is more than the maximum permitted by an Excel file, it is not possible to simply save the table as a new Excel file (corruptions occur).

The following method uses an intermediate text file (.txt) to avoid the problem, and allows the correct similarity values to be quickly placed into Worksheet 5 of the Excel PED file:

(a) Check the PRIMER Similarity Matrix window is active (its top border is 'highlighted')

(b) Go to the 'File' drop-down menu and select 'Save As…' to display the **Save** file box.

(c) Go to 'Save as type' and select 'Text Files (*.txt)' from the drop-down list.

(d) Browse to your Directory/Folder containing the port environment data Excel file, and save the Similarity Matrix as a Text file called '**Sheet1.txt**', in the usual manner.

(e) Close PRIMER and open the Excel PED file, select Worksheet 5, then open the new '**Sheet1.txt**' text file (select '***.* All files**' in Excel's 'File Open' window to see the file).

(i) When you open **Sheet1.txt**, Excel will display a '**Text Import Wizard**' box to help you import text into a new Excel spreadsheet.

(ii) In **Step 1** of the Wizard Box, check the 'Delimited' radio button, go to the 'Start Import Row' and replace the 1 with a 2, then press the 'Next >' button.

(iii) In **Step 2**, the 'Tab' delimiter should be checked, and 'Text qualifier' should be **"**.

(iv) In **Step 3**, select all columns in the Wizard Box after the 2nd column. Do this quickly by selecting the 3rd column (it will become dark shaded). Then go to the

end of the columns (using the Slide Bar under the window of columns), then 'Shift Key-Select' this last column (all unwanted columns will become dark shaded). Now click the '**Do Not Import Column (Skip)**' radio button. The word '**Skip**' will now show in the header of the unwanted columns.

(v) Now 'Finish' the Wizard Box. A simple spreadsheet will appear, with the UN Port Codes in column A and the distance values in column B. Save this as 'Sheet1.xls'.

(f) From the 'Sheet1.xls' file, select all Port codes and distance values, starting with the first port name in the first column (the Arrival Port) and its blank distance value (in second column). Copy this selection using 'Control-C' or clicking on the 'Copy' button () if this is present in your toolbar.

(g) Open the Excel PED file, go to Worksheet 5 (called 'PRIMER Output') and select (highlight) the first **White cell** in Column F, Row 2 (i.e. the start of the Port code list).

(h) Go to the 'Paste Special…' command in the 'Edit' drop-down menu (**Note**: This command may not be visible if your Excel customisation has been set to show '**Recently used commands only**'). To show the full list of commands in the 'Edit' menu, click on its double vertical arrows: 

(i) In the 'Paste Special' box, select the '**Values**' button then hit **'OK'**. The 'paste values' option maintains the User-Friendly colours and formats in Worksheet 5.

(j) After pasting the port label and distance values into the White Cells, you will see that the **Green Cells** in Column I will automatically show the C3 Environmental Matching Coefficients. You should re-save the Excel PED file at this stage.

The C3 values generated and saved in Worksheet 5 of the Excel PED file are now ready for importing into the ACCESS Database, using its Export/Import File feature (Section 7.3).

6. RISK SPECIES COEFFICIENT (C4)

6.1 CALCULATION OF C4

The C4 risk species coefficient provides a measure of the relative threat of harmful introductions, as posed by each BW source port, due to the number and status of the risk species present in its bioregion (Appendix 3). The taxonomic description, bioregional distribution, native status and threat status of each risk species are stored in the BWRA Database, and these can be displayed for review, update and edits.

The Database automatically calculates the C4 value for each BW source port. Each value represents a proportion of the 'total risk species threat'. The total threat is the sum of all risk species in the bioregions of all source ports which export BW to the Receiving Port. Risk species which occur in more than one bioregion are summed only once. The sum includes all non-native species (NIS) in the bioregion of the Receiving Port, because the calculation assumes the Receiving Port has not been 'infected' by any risk species (= the precautionary approach). The calculation does not include any risk species which have a native status in the bioregion of the Receival Port.

NOTE: After a Port Baseline Survey and its taxonomic identifications have been completed for a Receiving Port, a copy of the Database can be made, in which all risk species found at the Receiving Port can be re-assigned as 'Native' for the bioregion of the Receiving Port. This customised copy of the Database cannot be used to undertake BWRAs for other Receiving Ports.

The Database allows each risk species to be assigned to one of three levels of threat. The Database assigns a default weighting value to each level of threat, as follows:

- Lowest threat level (weight value = 1): This is for NIS with no special status, other than its known introduction to at least one bioregion (i.e. the non-native population has a proven ability to be transferred and establish in a new bioregion). The Lowest level is also the default level assigned to any species when first added to the BWRA Database.
- Second level (weight value = 3): This is for potential or '**S**uspected' pests. Risk species assigned to this level have a default weighting [**w1**] of 3 in both their native and introduced bioregions.
- Top level (weight value = 10): This is for **K**nown harmful species (i.e. species already listed, reported or declared as a nuisance or pest in a scientific, government agency or NGO report or web database).

The Database also allows users to change (a) the threat status level assigned to each species, and (b) the size of the default weighting values given to Suspected (**w1)** and Known pests (**w2**).

The Database calculates the C4 value for each BW source port by firstly summing:

- the number of NIS in its bioregion which have no suspected or known harmful status. This number is a measure of the species which have no recognised harmful status but proven 'transfer credentials' (e.g. 'ready-to-go' genes enabling their transfer to another port or bioregion with unknown/unpredictable consequences).
- the number of Suspected harmful species, either introduced or native, in its bioregion. Each suspected species is tripled in importance by the weighting factor **w1** (the default value of **w1** is 3 for the project standard BWRA).

- the number of Known harmful species, either introduced or native, in its bioregion. These species are multiplied by **w2** (its default value is 10 for the project standard BWRA, in accordance with the log principle used for measuring increases in biological risk).

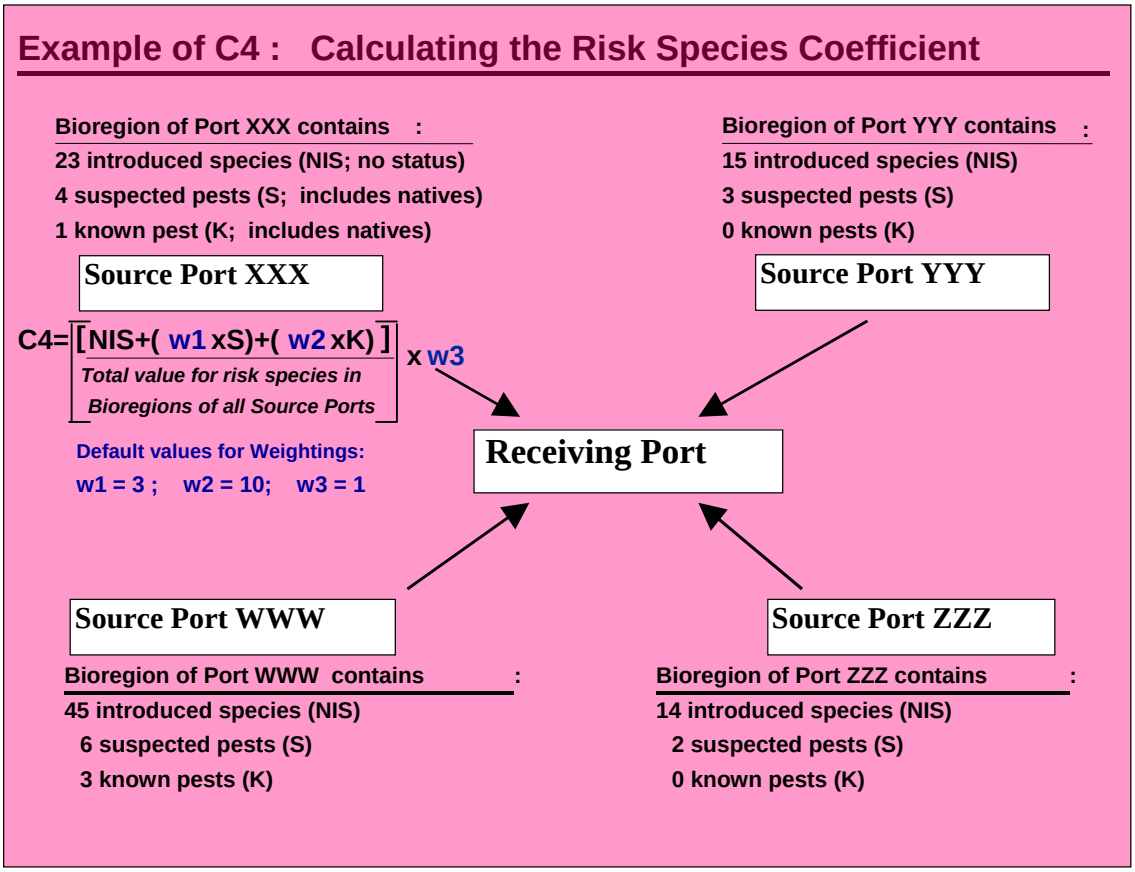
In the second step of the C4 calculation, the total sum for each BW source port is divided by the overall sum of the risk species values (i.e. from all of the BW source port bioregions):

C4 = (NIS + [Suspected Harmfuls x **w1**] + [Known Harmfuls x **w2**]) / Total Value all source port Bioregions

Dividing by the Total Value (does not include every bioregion in the world) produces a relative measure of the total risk (C4 values are between 0-1). Thus each C4 value represents a proportion of all risk species that could be transported and discharged into the Receiving Port via its contemporary BW importation routes. Note that the C4 values of BW source ports located in the same bioregion are identical.

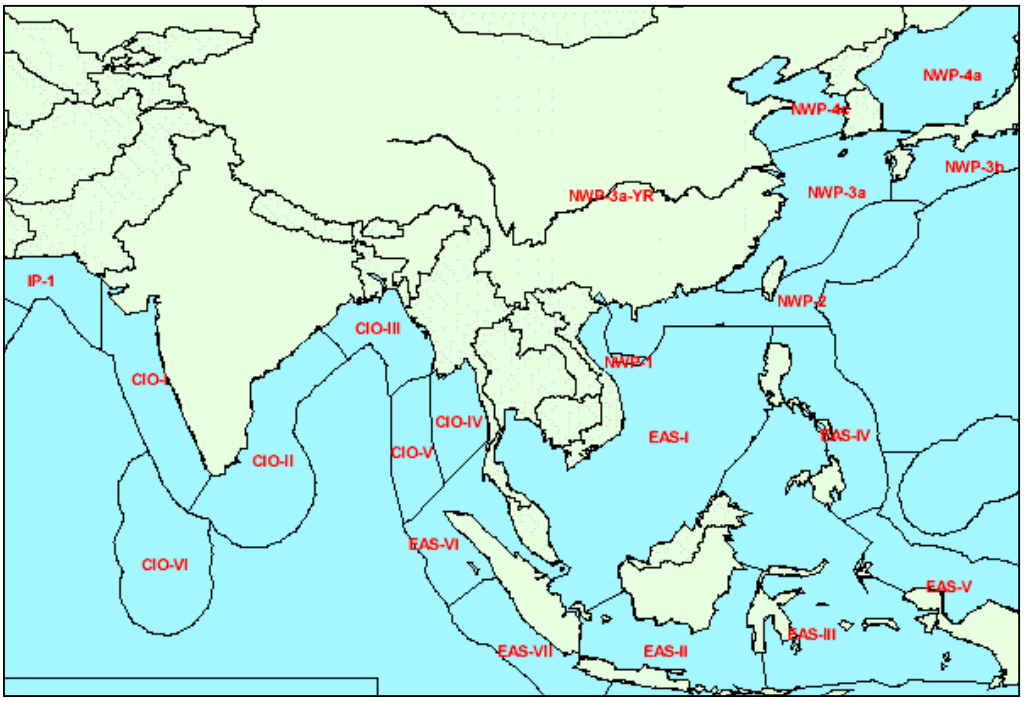
The C4 graphic box shows how the Database calculates the relative risk species threat for the example which has four BW source ports.

Note there is third weighting value (**w3**). This can be used to uniformly increase all C4 values by altering its default setting of 1. Using **w3** to increase or decrease the influence of C4 in the calculation relative overall risk is addressed in Section 7.4.



6.2 USING THE WORLD BIOREGIONS

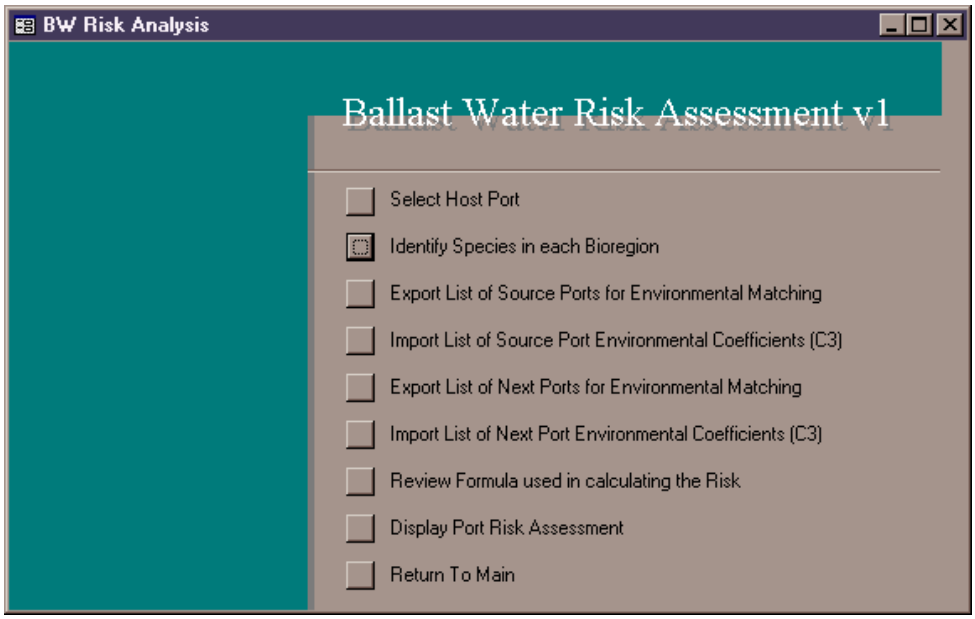
The distribution and status of each risk species in each bioregion is managed by the BWRA Database, which uses the same 204 bioregions that are displayed on the GIS World Map (Section 3.3; Appendices 3, 4). Part of the world map is shown below:



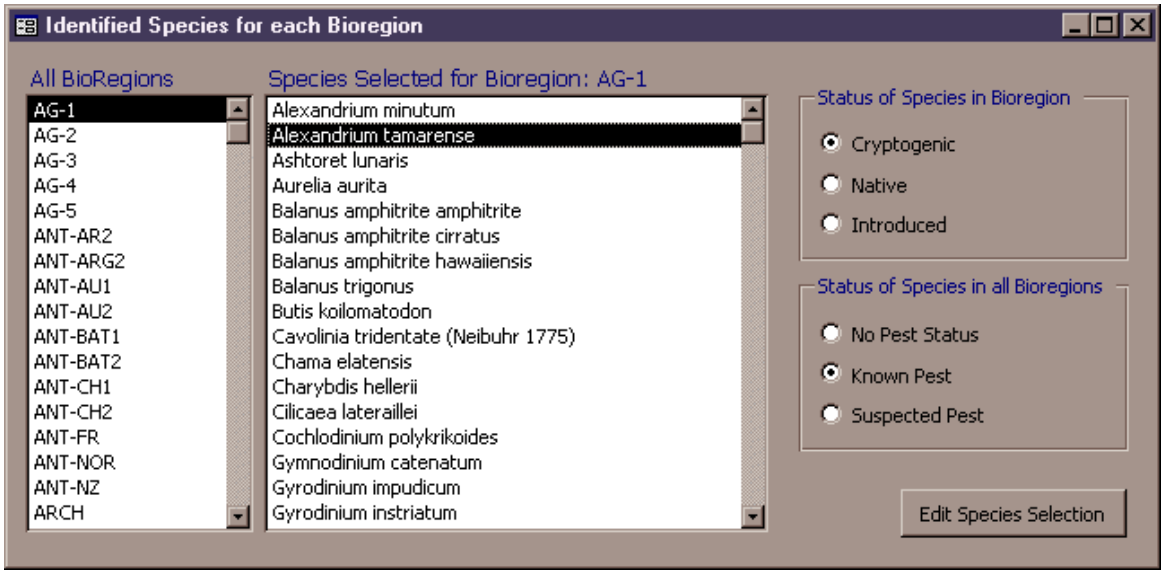
A zoomable Adobe (.pdf) file of the full map is in the **Utilities Folder** (**Bioregions.pdf**).

6.3 ADDING/EDITING THE RISK SPECIES DATA

To add, review or edit a risk species, go to the '**BW Risk Analysis**' sub-menu from the main menu and click on '**Identify Species in each Bioregion**':



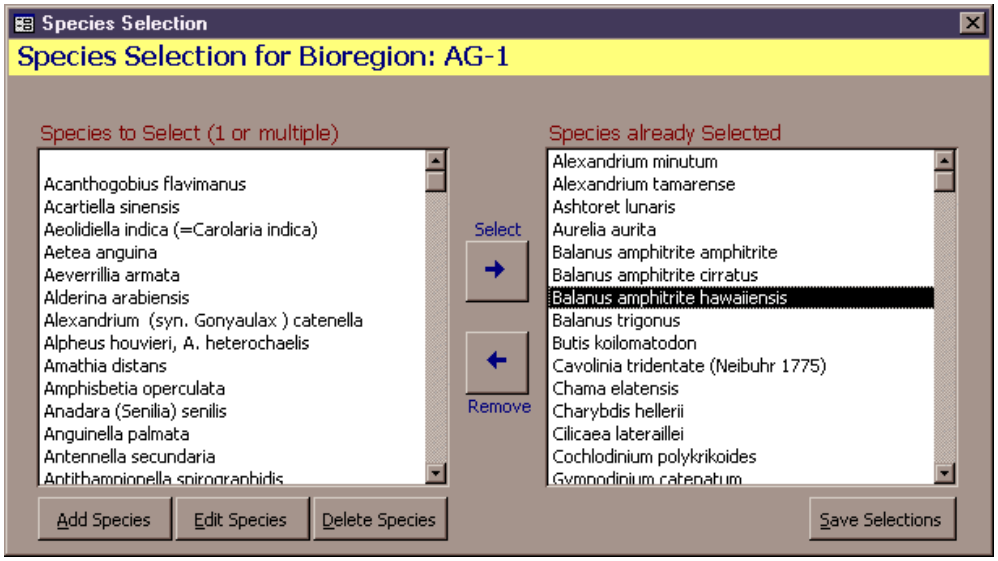
This takes you to the bioregion/risk species review window, which lists the species that have been assigned to particular bioregions (**AG-1** in the following example).



When a Bioregion is selected in the left table, the various species assigned to that region are listed in the centre table. When a particular species is selected (*Alexandrium tamarense* in above example), the radio buttons in the upper-right box show its origin **Status** in the AG-1 bioregion (i.e. if it is a cryptogenic, native or introduced species in AG-1). The radio button in the lower-right box (◉) shows its threat **Status** as a pest, which remains the same for all bioregions unless it is native in the bioregion of the Receiving Port (Section 6.1).

Note that the Cryptogenic/Native/Introduced **Status** of a species changes according to the particular bioregion (and these differences are stored in the Database), but its pest status remains the same for all bioregions. Both the 'bioregion origin' and 'global pest status' of a species selected in this window can be changed by clicking a different radio button in the boxes. Clicking on a different button and closing the window will automatically save the status change. No separate 'Save' command is needed since the radio buttons which are on (◉) when the window is closed, will store both statuses in the Database.

To add or remove a species from the selected Bioregion, click on '**Edit Species Selection**' (bottom right button). This takes you to the '**Species Selection**' editing window:

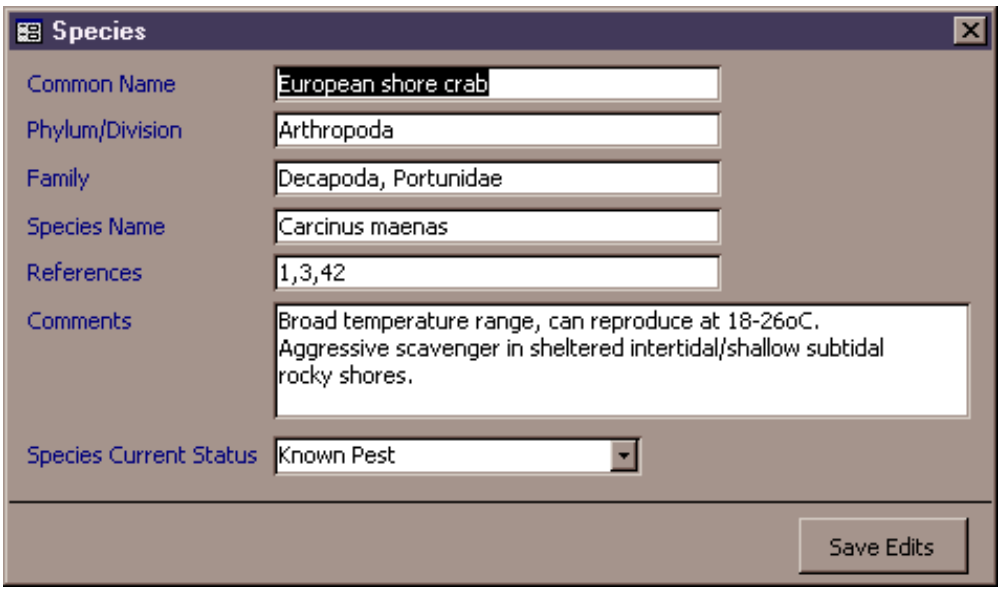


The left table lists all species not assigned to bioregion AG-1, and the right table lists the species which have been assigned to this bioregion. In the example above, the barnacle *Balanus amphitrite hawaiiensis* has been selected for removal from AG-1.

If any species are removed or added to a bioregion from the general list, the change needs to be saved by clicking the '**Save Selections**' button in the lower-right corner. This also returns you to the previous window where other bioregions can be selected for editing.

A new species can be added to the general list of the Database by clicking the '**Add Species**' button (which takes you to the **Species** window). Clicking the '**Edit Species'** button will launch the same **Species** window, but this will display the details of the species selected in the general list for editing. The '**Delete**' button allows a species that has been entered twice or more (e.g. because of a species name synonym or misspelling) to be deleted.

The following graphic shows the **Species** window displaying information previously entered for the European shore crab:



The numbers in the **References** box refer to the list of references stored in an Excel spreadsheet file, which is kept in the **Utilities Folder** ('**Risk Species Master List v1.xls**').

This Excel file contains a list of the risk species in the Database, including their taxonomy, status, comments and references. Using an Excel file allows convenient sharing and updates of risk species information between marine scientists involved in Port Baseline Surveys and BWRAs.

The Excel file has no 'automatic' link to the Database. Therefore any updates made to the Excel file should be highlighted to facilitate making additions or edits to the BWRA Database.

7. RUNNING THE BALLAST WATER RISK ASSESSMENT

7.1 OVERVIEW

The BWRA is set up and run using features which are accessed via the '**BW Risk Analysis**' sub-menu (Section 6.3). As noted in the previous sections, the BWRA will not work or provide consistent results unless:

- sufficient BWRF data have been checked and added to the BW Database (Section 4);
- C3 matching coefficients have been obtained for all source and/or next ports (Section 5);
- Risk species assigned to bioregions have been given an appropriate origin status and global pest status (Section 6).

The first step of the BWRA is to confirm the identity of the Receiving Port to the Database. Go to '**Select Host Port**' in the **BW Risk Analysis** sub-menu, and select its name from the drop-down list.

The next step is to import the C3 matching coefficients for both the source ports and the next ports, and this is described in Section 7.3. C3 values are required for all Next Ports of Call if a 'Forward Risk Assessment' is to be undertaken (Sections 7.2 and 7.6).

Finally, before running the BWRA, the formulae and weightings used by the BW Risk Analysis can be checked (and if necessary changed) by clicking on the '**Review Formulae for Calculating the Risk**' in the '**BW Risk Analysis**' sub-menu. Changing the formulae and/or their default values is described in Section 7.4.

If the Database is ready, the BWRA can be run by clicking on the '**Display Port Risk Assessment**' button in the '**BW Risk Analysis'** sub-menu. This causes the Database to perform all calculations and generate a large results table. This table will include all BW Source Ports that have been provided a C3 environmental matching coefficient (source ports with a missing C3 value will not be listed, nor contribute to the C1, C2 or C4 calculations).

In each source port row, the table displays its overall **Relative Overall Risk** value (ROR), as well as the C1-C4 coefficient and R1-R2 risk reduction values (Section 7.4). Results from this table can also displayed on the ArcView GIS, provided the Database is connected to ArcView by ODBC (Appendix 4). Additional tables showing statistical results for each berthing area in the Receiving Port are also shown by the GIS Port Map.

7.2 DATA REQUIREMENTS FOR A RELIABLE ASSESSMENT

The BWRA Database should contain at least 12 months (preferably 24 months) of reliable BWRF data collected from all vessels visiting the port. There is no advantage to collate +3 years because international trade patterns have been changing rapidly throughout the 1990s following independence of the former Soviet states and satellite countries, and the continuing rise in the importance of the major South and East Asian economies. The value of adding discharge data from old port records is also limited compared to the steady collection and input of reliable BWRFs. After 3 years of BWRF data have been added to a Database, it is advisable to remove and archive any +3 year data, so as to maintain an up-to-date, contemporary picture.

If the collection of BWRFs involves 'targeting' only vessels that intend to discharge BW, the results will not provide a true picture of the risk associated with all trading routes. Limiting the collection or data entry of BWRFs to vessels discharging BW will also prevent a 'Forward' risk assessment, since this must focus on ships which uplift and export BW from the Receiving Port to its trading ports.

For unchecked and incomplete BWRFs, critical gaps can be filled for a percentage of these forms by careful analysis of previous visits made by repeat-arrival vessels, plus their type, size and pattern of trade as determined from port records. The value of reliable, computerised port records, the *Lloyds Register of Ships*, and international port and terminal guides such as *Fairplay* cannot be overestimated when undertaking a gap-filling analysis (see Section 2.2).

Gap-filling exercises are likely to be most reliable and fruitful for crude oil tankers, product tankers, gas tankers and bulk carriers which are engaged on regular trade between 'single-commodity' export and import terminals. Even with these vessel types, however, the true sources and volumes of deballasted water may be hidden in confidential commercial records, although interviews with shipping agents and ship's officers will improve estimates or guesses.

When trying to use port records to collate BW discharge estimates for a period before the regular use of BWRFs, the most common problem is identifying 'true' BW source ports. For some types of bulk cargo trade, the recorded 'Last' and 'Next' Ports of Call may be obvious unloading or loading terminals. In the case of oil tankers and bulk carriers, however, their voyages often involve 'Last' and/or 'Next' ports of call which involve:

- a fuel bunkering stop, requiring a relatively minor (500-1800 tonnes) or no BW discharge;
- a crew-change, supply and/or in-water maintenance port (no discharge or uplift of BW);
- dry-docking for repainting and heavy maintenance (all BW will be discharged before and during docking, then up-lifted during and after the re-floating operation);
- a strategic route or hub port to wait for new sailing orders (usually no discharge/uptake);
- the first or last of 1-3 terminals for cargo part-loading (e.g. at some shallow water crude oil terminals in the ROPME Sea Area, Black Sea, Nigeria, Venezuela, etc), or for cargo part-unloading (e.g. product tankers and bulk carriers visiting coastal and river ports in many parts of the world).

In the case of bulk carriers, these may be spot-chartered from a strategic bunkering and regional hub such as Singapore, but may still be carrying BW from a different Asian port which was the final destination of its previous charter. For both liquid and dry bulk carriers departing an import berth in ballast and not engaged on a time charter/liner service (Appendix 5), their Next of Port of Call may be a 'route direction' port, based on known or anticipated sailing orders (many ships do not receive their ultimate destination instructions until after their departure). 'Direction ports' for ships in ballast or carrying product or crude oil include:

- Gibraltar (= the ship will enter or exit the Mediterranean for an unconfirmed destination);
- Istanbul (= the ship will enter or exit the Black Sea for the same reason);
- Port Said, Suez, Panama etc (= the final destination lies beyond one of these canals);
- Hong Kong, Singapore, Shanghai, Rotterdam etc (= moving towards a strategic hub).

For ports with a significant import trade via general cargo ships, small bulk carriers and chemical/products tankers (e.g. Mumbai, Odessa and Dalian), attempting a worthwhile 'Forward Risk Assessment' is made worse by the multi-trading behaviour of these vessel types, which tend not to visit the same ports on a regular or voyage-consecutive basis.

Even when fully completed, an IMO BWRF does not help identify BW Destination ports because this form does not request any detail or prediction concerning BW uptake or

destination (Appendix 6). To undertake a reliable 'Forward' risk assessment therefore requires supplementary questions. These would need to be provided on a small form very close to the time of sailing to maximise the number of useful answers.

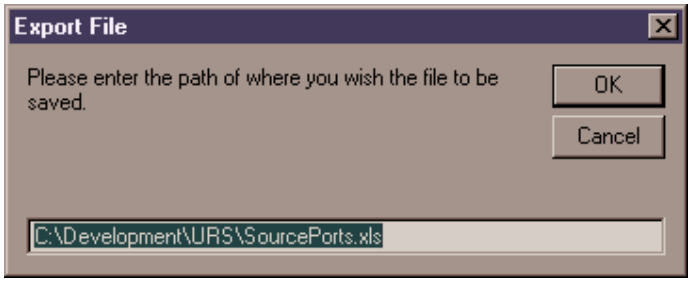
7.3 IMPORTING THE ENVIRONMENTAL MATCHING COEFFICIENT (C3)

To import the C3 values from Worksheet 5 of the Excel PED file (Section 5.4.5), it is necessary to copy them into a temporary Excel file which is generated by the Database. One file lists all BW Source Ports (even if discharged BW volumes were not added in the Database), the other lists all Next Ports of Call (C3 values for Next Ports of Call are used only for the 'forward risk assessment' of presumed BW destination ports).

Using Excel files for exporting the two lists of port names and importing the C3 coefficients avoids the problem of sending copies of the large BW Database to scientists who undertook the PRIMER analysis but who may not have MS Access 2000 on their PCs It also avoids possible accidental changes to the Database's contents. The steps for importing the C3 values are described in the following sections.

7.3.1 Importing C3 Values for the BW Source Ports

The Excel file '**SourcePorts.xls**' contains the list of BW Source Ports, and is generated by the Database. To do this, click on '**Export List of Source Ports for Environmental Matching**' (in the **BW Risk Analysis** sub-menu), then type a Directory/Folder pathway in the Request Box:



NOTE: Generating this Excel file will cause the Database to automatically review and update the list all BW Source ports, including **Deleting** any source port no longer associated with any BW tank discharge (Section 2.5) and **Deleting** the C3 values previously entered for all BW Source Ports, in preparation for receiving a new set of C3 values.

The '**SourcePorts.xls**' file lists the UN Port Code, port name, country, decimal Lat/Long position and bioregion of all source ports. It also contains two columns for the C3 values. The layout of its Worksheet is as follows:

UN Port Code	Port Name	Country	BioRegion	PortLatDecimal	PortLongDecimal	Environmental MatchingCoefficient	Coefficient Calculated
BGBOJ	Bourgas	Bulgaria	MED-IXA	42.5000	27.4800		TRUE
BGVAR	Varna	Bulgaria	MED-IXA	43.2000	27.9500		TRUE
BRMCZ	Maceio	Brazil	SA-III	-9.6800	-35.4300		TRUE
BRPNG	Paranagua	Brazil	SA-IIB	-25.5000	-48.5200		TRUE
BRSSZ	Santos	Brazil	SA-IIB	-24.0000	-46.3300		TRUE
CNSHA	Shanghai (Shihu) Sh	China	NWP-3a	31.2500	121.5000		TRUE
CUCFG	Cienfuegos	Cuba	CAR-II	22.1500	-80.4500		TRUE
CYLCA	Larnaca	Cyprus	MED-V	34.9166	33.6500		TRUE
CYLMS	Limassol	Cyprus	MED-V	34.6500	33.0200		TRUE
DEBRE	Bremen	Germany	NEA-II	53.0000	8.7800		TRUE

The C3 values can be Copy/Pasted from the green cells in Worksheet 5 of the Excel PED file into the empty "Environmental Matching Coefficient" column, after checking the ports are listed in the same order (if necessary, use Excel's *Data [Sort by…]* tool on the UN Port Codes to make both lists 100% alphabetically matched).

It is likely there will be BW Source Ports which were not included in the PRIMER multivariate analysis because of insufficient environmental data. For these ports, it is possible to take a C3 value calculated for a similar port in the same bioregion, increase it by 'rounding-up', and using this estimation. Care is required when selecting an appropriately similar port (e.g. do not use a river port to provide an estimated C3 for a nearby 'bay' or 'estuary' port, and vice versa. A generous (conservative) 'round-up' provides a precautionary estimate.

Any port given an estimated C3 can be flagged in the BWRA Results Table, by deleting the "TRUE" value (in the adjacent "Coefficient Calculated" column of the Excel file) or replacing it with a "0", "FALSE", "ESTIMATED" or other appropriate text comment.

After saving and closing the '**SourcePorts.xls**, its contents can be imported automatically into the BW Database by clicking on the '**Import List of Source Ports Environmental Coefficients…**' button (in the '**BW Risk Analysis**' sub-menu). The same file pathway 'Request Box' will then appear, asking for the Directory/Folder pathway to the saved file (the pathway previously provided for exporting this file will appear in its box).

If the BWRA results indicate that port/s with an estimated C3 have high risk, the BWRA should be repeated using a calculated C3 value (i.e. find the missing port environmental data, repeat the PRIMER analysis, then import the calculated C3 values before re-running the BWRA).

7.3.2 Importing C3 Values for the 'Next Ports of Call'

The Excel file **NextPorts.xls** is generated automatically by clicking on '**Export List of Next Ports for Environmental Matching**' then giving a Directory/Folder pathway in the Request Box (i.e. same procedure as for the SourcePorts.xls, in Section 7.3.1).

NOTE: Generating this Excel file will cause the Database to **Delete** all previous C3 values entered for Next Ports, in preparation for receiving a new set of C3 values.

This file lists all 'Next Ports of Call' stored in the BW Database, including their UN Port Code, port name, country, decimal lat/long position, bioregion and the frequency of vessel departures (= Next Port Visits). The '**Next Ports.xls**' file contains the same two columns for the C3 data and has a similar layout:

UN Port Code	Port Name	Country	Latitude	Longitude	BioRegion	Frequency Of Next Port Visit	C3 - Environmental Matching	Environmental Matching Calculated
AOCAB	Cabinda	Angola	-5.55	12.183	WA-IV	0.360000014		TRUE
AOLAD	Luanda	Angola	-8.78333	13.2667	WA-IV	0.449999988		TRUE
ARBUE	Buenos Aires	Argentina	-34.6	-58.35	SA-IIA	0.180000007		TRUE
ARCMP	Campana	Argentina	-34.25	-58.97	SA-IIA-RP	0.090000004		TRUE
ARRGA	Rio Grande	Argentina	-53.8	-67.66	SA-I	0.090000004		TRUE
AUBNE	Brisbane	Australia	-27.38	153.16	AUS-XII	0.090000004		TRUE
AUPBY	Port Bonython	Australia	-33.01	137.76	AUS-VII	0.090000004		TRUE
BEANR	Antwerpen	Belgium	51.2333	4.46667	NEA-II	1.360000014		TRUE
BRFOR	Fortaleza	Brazil	-3.68	-38.5	SA-IV	0.270000011		TRUE
BRPNG	Paranagua	Brazil	-25.5	-48.52	SA-IIB	0.090000004		TRUE
BRRIG	Rio Grande	Brazil	-32.03	-52.1	SA-IIA	0.090000004		TRUE
BRRIO	Rio de Janeiro	Brazil	-22.85	-43.13	SA-IIB	0.540000021		TRUE
BRSSZ	Santos	Brazil	-24	-46.33	SA-IIB	0.180000007		TRUE
BRVIX	Vitoria	Brazil	-20.31	-40.33	SA-III	0.090000004		TRUE

The "Frequency of Next Port Visits" column allows the most common destinations to be readily identified (e.g. use Excel's *Data [Sort by…]* tool to re-arrange the ports in descending order).

As with the 'SourcePorts.xls' file, the C3 values can be quickly copy/pasted from Worksheet 5 of the Excel PED file. For ports with estimated C3 values, these can also be flagged by deleting or changing the adjacent "TRUE" cell to any character or text comment.

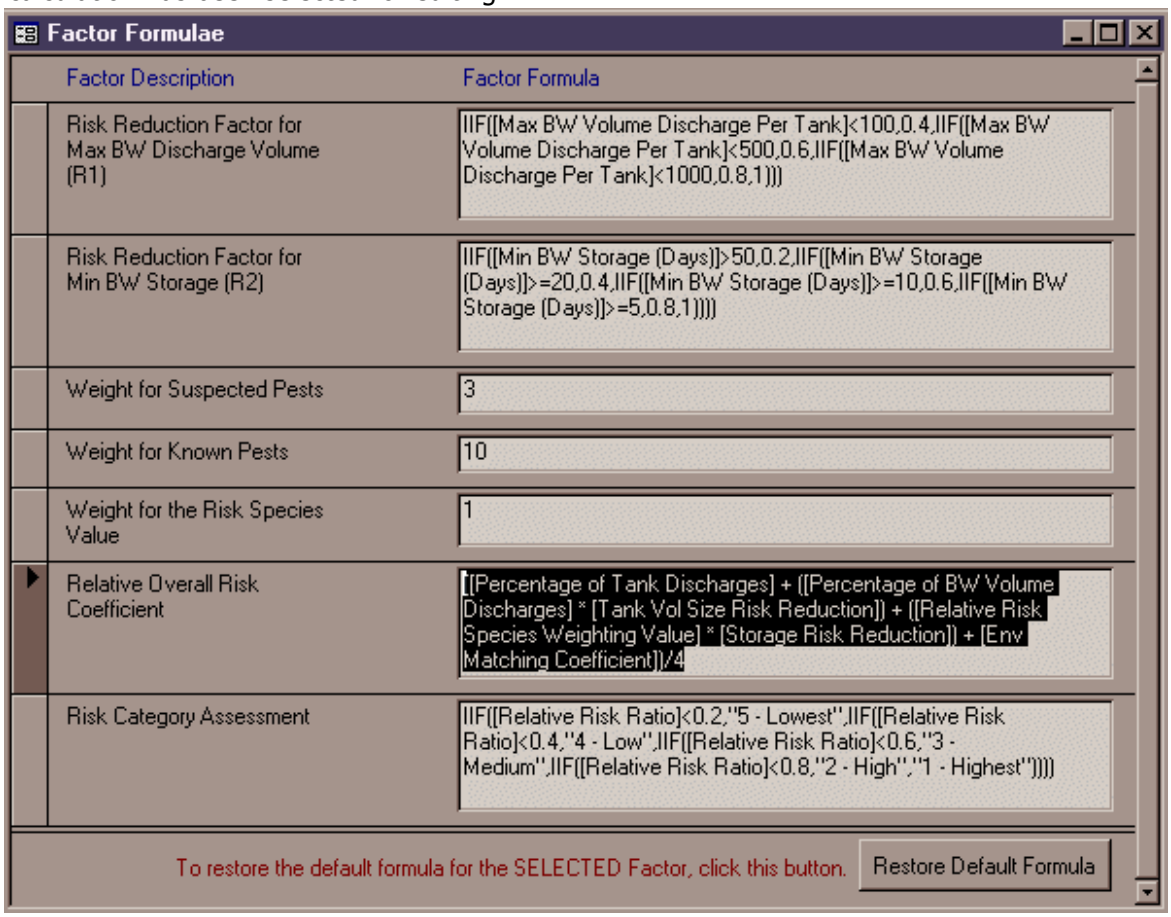
After saving and closing the file, import its contents to the BW Database by clicking on the '**Import List of Next Port Environmental Coefficients**' button (in the '**BW Risk Analysis**' sub-menu), and giving the file location pathway in the Request Box.

7.4 REVIEWING AND CHANGING THE BWRA FORMULAE

The default formulae and weightings, as used by the Database to calculate the Relative Overall Risk (ROR) for a project standard BWRA, can be checked (or changed) by clicking on '**Review Formula for Calculating the Risk**' in the '**BW Risk Analysis**' sub-menu.

This launches an interactive **Factor Formulae** display window, showing the various components of the ROR calculation. These components include the 'Risk Category Assessment', which shows the way each ROR value is 'binned' into one of five colour categories used by the GIS display (**Highest**, **High**, **Medium**, **Low**, **Lowest**).

To alter a default setting, select the text of the formula inside the white cell and make the required change. This change will not become 'active' or saved until the **Factor Formulae** window is closed (i.e. close this window before re-running the BWRA analysis). In the following example, the default text of the formula used for the project standard ROR calculation has been selected for editing:



There are many formula or weighting changes which may be made during a BWRA study. For example, if C1 (tank discharge frequency) is considered more important than C2 (tank discharge volume), the influence (weight) of C2 on the ROR result may be halved by multiplying C2 by 0.5. To achieve this, simply insert '*0.5' into the ROR default formula, as follows:

[[Percentage of Tank Discharges] + [[Percentage of BW Volume Discharges] *** 0.5** * [Tank Vol Size Risk Reduction]] + [[Relative Risk Species Weighting Value] * [Storage Risk Reduction]] + [Env Matching Coefficient]]/4

Then close the display window to save the change and re-run the BWRA (Section 7.5). Any change must follow the format, function and mathematical rules of MS Access 2000. These are similar but not 100% the same as those in Excel's calculation cells. For example, the logical 'If' is spelled IIF, and each named factor must be inside square brackets […].

The various format rules and mathematical functions are described in the MS Access 2000 user manual and also in its 'Help' function (if the application has been fully installed).

A formatting error or function-name spelling error in one of the calculation cells can cause the BW Database to abandon the calculation and report an Error Message. To remove mistakes and return the calculation to the default 'project standard', return to the Formula Display window, select the altered component, then click on the '**Restore Default Formula**' button (in the lower-right corner of the **Factor Formulae** window).

Making a change and closing the Display Window will automatically save the change. The new formula will then be used every time, even if the Database file is closed and reopened later. Any change will remain until the 'Display Formula' window is re-opened and a new change is made (or the 'Restore Default' button is clicked).

Other examples of the types of change which can be made include:

(1) For a BWRA that focuses only on toxic dinoflagellate cysts and pathogens (i.e. anaerobes that can survive in ballast tanks for long storage periods), there should be no reduction in risk due to long storage times. In this case, simply replace all the default IIF statements in the calculation cell for '**Risk Reduction Factor for Min BW Storage (R2)'** with a single 1, as follows:

1

This will make R2 = 1, so the calculation will apply a uniform 'non-reduction' factor of 1 to all storage times. The values of R2 are displayed in the results table, so these can be checked to confirm the change was made correctly.

(2) In the case of the Weight values, these influence the size of the risk species C4 coefficient, which is denoted in the ROR formula cell as the: [Relative Risk Species Weighting Value]

To uniformly change the sizes of C4 and thus their total influence on the ROR results, replace the "1" default value provided in the 'Weight for Risk Species Value' [**w3**] cell, with either a higher (=> more influence) or smaller (=> less influence) value. The same effect can be achieved by inserting the C4 multiplier directly into the formula (see the above example box).

If one or both of the Weights which are used on the suspected (**w1**) and known (**w2**) pest species are changed, this will differentially change the C4 values, according to the numbers of these species types in each bioregion (Section 6.1; see also the BWRA Country Report for Sepetiba (Brazil) for a summary of the types of outcomes that can occur by manipulating the C4 weights; http://globallast.imo.org).

7.5 DISPLAYING AND EXPORTING THE BWRA RESULTS

Every time the '**Display Port Risk Assessment**' button is clicked in the '**BW Risk Analysis**' sub-menu, the Database will re-calculate the BWRA and regenerate the results table. If any change had been made in a BWRA formula or weighting cell, make sure the **Factor Formulae** window is closed before re-running the BWRA.

The results table automatically ranks the ROR results for each BW Source Port in descending order (i.e. from Highest to Lowest risk ports). The ROR values are listed on the right side, beyond the various columns which list the port details, the C1-C4 coefficients and the risk reduction factors (R1, R2).

The results are calculated using the default 'project standard' methods unless these have been changed (Section 7.4). The 'Next Ports of Call' results are not listed in this table because there are no data for calculating the C2, R1, R2 and C4 values (see Section 7.6).

The results table can be 'copy/pasted' into a MS Word document or an Excel spreadsheet for convenient storage and review on PCs which do not have the MS Access 2000 application.

TIP:	To avoid importing non-standard characters and attributes into an Excel spreadsheet, it is best to first copy the table into a Word document. Use '*Select All*' (in Access' Edit drop-down menu), then '*Copy*' and paste into a blank Word document. Then select and copy the rows of the new Word table, and paste these into an empty Excel spreadsheet. This two-step process will provide a 'clean' worksheet that is amenable to all Excel format and calculation commands.

A copy of the results table can also be saved by the Database as a new Excel file, if the Database is opened using the '*Shift-Key/Open*' method. This method gives the Database manager access to all Access functions, including the use of the Query, Records Filter/Sort and Export features to assemble new tables.

There is no need to copy or transfer any results to the ArcView GIS. The GIS will access all BW Database tables automatically if there is an ODBC connection (Section 3; Appendix 4).

7.6 FORWARD RISK ASSESSMENT

The Forward Risk Assessment examines the relative risk of introducing unwanted species via BW uplifted at the Receiving Port and exported to other ports. The largest single quantities of ballasted water will be uplifted by large liquid and bulk carriers when offloading their cargo at a dedicated import facility. The ballasting requirements of general cargo ships, container vessels, Ro-Ro vessels, vehicle carriers are variable and often small. It is rare for these ship types to discharge all their cargo at a single port and depart in a fully ballasted condition.

Some of the problems in determining how much BW may be taken up, and where and when the ballasted or part ballasted tanks are eventually discharged, are described in Section 7.2.

A preliminary evaluation is possible using the ArcView GIS, as this displays the two coefficients which give an indication of the 'forward' risk, i.e. the percentage of vessels departing to each 'Next Port of Call' (= 'C1 Forward'), and the environmental matching of these destination ports with the Receiving port (= 'C3 Forward'). The GIS can also display tables of the species assigned by the Database to the Bioregions of the Receiving Port and its Next Ports of Call. The NextPorts.xls file also contains the list of all Next Ports of Call, plus the proportional frequency of departures to these ports (Section 7.3.2).

The preliminary 'forward' assessment can therefore be undertaken by identifying the most frequent destination ports which have a moderate to high environmental matching, then cross-checking the vessel types and probable trading patterns to these ports. The aim is to remove any apparently 'high risk' destination port which is a bunkering or 'direction' port, plus ports that are the frequent destination of laden ships (i.e. departing from cargo export terminals and carrying little or no BW). It is also possible to evaluate the types of species which are present in the bioregion of the Receiving Port and possibly absent in the bioregion of the remaining 'high risk' destination ports.

8 EVALUATING THE PROJECT-STANDARD BWRA RESULTS

The BWRA Database provides 'first-pass' risk assessment results for training, demonstration and evaluation for BW management and research purposes. The project standard (default) BWRA provides a semi-quantitative method which removes as much subjective decision taking as possible from calculating the risk rankings (Appendix 1). However the reliability of the various risk coefficient values and port rankings remains heavily dependent on the quality of the input data.

Accuracy of the Database outputs relies particularly on the number of collected BWRFs and the quality of these records, as well as on the amount and quality of the port environmental data and risk species taxonomic and presence/absence data, as sought and collated from a wide range of published, non-published and scientist sources.

Responsibility for a Pilot Country applying the BWRA Database and its results for any BW management purpose rests with the Country Focal Point (CFP) of that country. In the case of the 'first-pass' results produced by the Pilot Country BWRAs during Activity 3.1[6], these provide baseline outputs and benchmark to help explore and refine the method, including any future improvements and expansion to the input tables and risk calculations.

For example, the Database does not contain all of the NIS identified from the pilot country Port Baseline Surveys (the taxonomic identifications for many groups were still progressing during Activity 3.1). The Database also contains many risk species which are known or strongly suspected to be transferred primarily via hull fouling or aquaculture vectors rather than BW. While their inclusion was actively and unanimously sought by the pilot country scientists who participated in the activity, the project-standard BWRA is unable to discriminate against hull fouling or aquaculture-mediated species.

As noted during the *1st International BWRA Workshop* held in Melbourne in September 2003, the design and open structure of the Database allow future versions to be enhanced and include additional factors such as the principle vector for each risk species, and measure/s of the 'invader-friendliness' of the port's receiving environment, such as its eutrophication status (Appendix 1). Similarly, other tools in the PRIMER package can be used to investigate which of the present 34 port environmental parameters have provided the most value (explanatory power) for separating dissimilar port environments.

Finally, the value of treating the environmental matching coefficient (C3) as a risk-reduction factor (i.e. applied as an 'R3' in the BWRA calculation instead of a surrogate for the incomplete C4 measure of risk species threat; see Appendix 1), will progressively increase as the bioregional distributions and threat status of NIS become clearer from future baseline port surveys (e.g. see GloBallast's *Ballast Water News* ***12*** [September-December 2003] and the BWRA Pilot Country Reports[6]).

[6] GloBallast's newsletters and the six Pilot Country BWRA Reports are available at: http://globallast.imo.org

Appendix 1

Terminology and Methods of Ballast Water Risk Assessment

Table of Contents

A.1 TERMS AND DEFINITIONS

"The less a science has advanced, the more its terminology tends to rest upon uncritical assumptions of mutual understanding" (from Quine 1946, as quoted by Carlton 2002).

The understanding and modelling of marine invasions is an emerging immature science, and its terminology is continuing to change and evolve. Presently there is no widely used convenient glossary of terms providing an integrated set of consistent, logical definitions based on fully understood processes.

All aquatic species invasions are international, and successful efforts to curb and combat them require the collaboration, mutual understanding and willing cooperation of many marine scientists, advisers, bureaucrats and politicians whose first language is not English. For this reason, it is useful to avoid a large number of apparently interchangeable but potentially confusing terms.

The following terms are drawn from those defined, discussed or followed by various publications, including IMO documents as well as Carlton (1985, 1987, 1996), OTA (1993), Cohen & Carlton (1995), Hilliard *et al*. (1997a), Hilliard (1999), Boudouresque & Verlaque (2002) and Hutchings *et al*. (2002).

Non-**I**ndigenous **S**pecies (NIS) / Non-Native Species: These terms have exactly the same meaning, and both are more precise than ambiguous and potentially confusing terms such as 'adventive', 'alien', 'exotic', 'feral'[7], 'foreign', 'invasive' or 'weedy' species.

[7] A feral marine species may be used to describe a NIS which was originally imported by the Aquaculture or Aquarium trade, but then 'escaped' to establish self-maintaining populations 'in the wild'. This usage is the same as that to describe previously domesticated animals or plants which have established 'wild' populations. A feral species is therefore one which, before escaping from a mariculture operation or aquarium, had previously undergone some form of selective breeding for improved growth and husbandry (e.g. the Pacific oyster *Crassotrea gigas* and the 'aquarium strains' of *Calaupera taxifolia*).

A Cryptogenic Species is "*neither demonstratively native nor introduced*" (Cohen & Carlton 1995, Carlton 1996). Many of the widespread and so-called 'Cosmopolitan' species are cryptogenic, because their original, natural distribution has been blurred by centuries of transfers via sail ships, canoes, canal building, aquaculture etc. In some regions, several historical introductions had been assumed to be part of the native marine community until fossil bed and genetic studies have shown otherwise, or at least invoked doubt. Examples include some common foulers and wood-borers, such as the infamous bivalve 'shipworm' (*Teredo navalis*), the striped barnacle (*Balanus amphitrite*), the blue mussels (*Mytilus* spp. complex), the brown mussels (*Perna perna, Perna picta*/*Perna viridis*) and the so-called Portuguese oyster "*Ostrea angulata*" (until genetic studies in 1998 confirmed its 16th Century introduction from *Crassostrea gigas* stocks in Japan) (Carlton 1999, Zaitsev & Öztürk 2001, Leppäkoski *et al.* 2002, Dr F. Fernandes, pers comm.).

The terms Introduction and Introduced Species are still causing confusion, as definitions are often terse and poorly defined. For example, definitions in a recent APEC report glossary ("*An introduced species is a marine species that's movement has been assisted by human activities to an area outside its range*"; "*an introduction/translocation is the human-assisted movement of an animal to an area outside its natural range*") are inconsistent (plants and protists are ignored) and ambiguous. Such definitions do not separate the direct or indirect human-assisted movements of organisms or propagules, from the possible eventual establishment of a viable, self-reproducing population as a result of the introduction.

Definitions such as: *'An introduced marine species is one which, through direct or indirect human activity, has established a self-reproducing population in a natural or semi-natural habitat outside its native range*' places the introduction at the end of a chain of events. In this case, the initial pioneering population of founder organisms are not provided the same introduced status as an established, self-sustaining population of acclimated or naturalised organisms.

To avoid ambiguities and acknowledge that transitory marine introductions are known from Australia, America and Europe, definitions in Carlton (1985) and Hilliard *et al.* (1997) may be modified to define an introduced marine species as: *"A species which, by human-mediated transfer, has formed at least a transitory population in a region beyond its native range. This population may or may not develop into a viable long-term population owing to local factor/s that may affect some aspect of growth, reproduction or recruitment. In these cases only repeated inoculations may allow the continued (or sporadic) presence of a detectable population*."

Cohen & Carlton's (1995) described an established introduction as: "*an introduced species that is present and reproducing in the wild, and whose numbers, age-structure, distribution and persistence over time suggest that they will continue to be present - barring eradication efforts or a major natural catastrophic event*". This needs to be modified to include regions where major natural catastrophic events are not rare (e.g. cyclone, flood, severe winter, etc), plus the potential ability of the naturalised, self-sustaining population to spread (Hilliard *et al.* 1997, Bouderesque & Verlaque 2002). Thus an established population is "*An introduced species with self-sustaining population/s that have persisted in the wild by natural means for several years, including at least one natural climactic event or extrema, and which may be capable of spreading by natural and/or human-mediated means*."

Invasive species are a subset of established introduced species. However increasing *ad hoc* use of the term 'invasive' to convey a sense of impact and urgency for virtually any introduction, is now diluting its meaning (Carlton 2002). The IUCN (2000) definition of an invasive species ("*an alien species that becomes established in natural or semi-natural ecosystems or habitat, is an agent of change, and*

threatens native biological diversity") is unhelpful because it invokes unclear and indistinct concepts (Carlton 2002). Similarly, Bouderesque & Verlaque (2002) define an invasive species to be "*an introduced species that is ecologically and/or economically harmful species*". This definition provides (a) no differentiation between transitional or established introductions, and (b) no bearing regarding the capacity to spread into undisturbed semi-natural and natural habitats, either naturally by themselves or by local human-mediated vectors.

Following Thomson (1991), Hilliard *et al.* (1997) and Ruiz *et al*. (1997), it is more useful to define an invasive species as: "*An established introduction which:*

- *has good natural dispersal characteristics;*
- *tolerates a range of localised environmental conditions;*
- *forms a common component of the habitats and communities into which it spreads; and*
- *is demonstrably capable of colonising a wide geographical area in its new environment.*

A truly invasive aquatic species is arguably one which spreads without any form of human assistance, into natural or at least semi-natural areas that are not significantly disturbed or 'vacant' due to previous natural or human causes. As summarised by Hutchings *et al*. (2002), an invasive marine species is typically (but not always):

- Common (= relatively abundant and widespread within their native range);
- Pioneering (= among the first to colonise or utilise disturbed and 'vacant' habitats in both its native and introduced regions; includes most port and harbour foulers);
- Tolerant (= can endure a broad range of physical conditions including temperature, salinity, substrate type and pollutants, and often having a tough or quiescent life-cycle stage well-adapted for surviving extreme conditions as well as dispersal);
- Generalist (= e.g. ingests a range of food by filtering, deposit feeding or scavenging); and
- Competitive (= out-competes/overwhelms native taxa by shading, smothering, altering substrates, predating or excessively filtering the water column; achieved by developing large populations via better reproduction, recruitment, growth and/or survival rates, including relative impunity to local predators, parasites and diseases).

Whether or not an invasive species becomes labelled a 'nuisance species' (USA/ Canada), 'marine pest' (Australia/New Zealand/UK), 'harmful species' (IMO), or 'alien invader' (IUCN) simply depends on the agency and the perceived type and extent of its known or suspected impacts on ecological and socio-economic resources and values. Labels such as 'harmful', 'invader', 'nuisance' and 'pest' stem from terms used for lists and guidelines that were compiled for the emerging policies of governments and international agencies. The definition for a harmful species in the draft IMO *International Convention for the Control and Management of Ships' Ballast Water and Sediments* is: "*aquatic organisms or pathogens which, if introduced into the sea including estuaries, or into fresh water courses, may create hazards to the environment, human health, property or resources, impair biological diversity or interfere with other legitimate uses of such areas*".

An invasive marine species is typically given one of the above labels owing to its sheer density of numbers, competitive prowess, rate of spread and/or noxious trait. They are not based on any widely agreed scientific definitions. As Carlton (2002) notes, "*the concepts and thus words used to describe non-native species vary among countries and among scientists, and in the near future show no clear*

indication of achieving either intra-national or international uniformity (or understanding)". For convenience, the above terms can be considered interchangeable and may be defined in line with Carlton's (2002) call to clarify features and processes. For example, "*A harmful marine species or pest is one that has demonstrably:*

- *Reduced native biodiversity via competition, habitat alteration, infection or diverting food-chains;*
- *Infected, parasitised or otherwise directly or indirectly damaged a commercial or recreationally important fish stock;*
- *Caused gross fouling to vessel hulls, seawater intakes, jetty piles, navigation infrastructure, mariculture equipment, etc;*
- *Disrupted aquaculture activities and caused increased public health risk by infection or release of toxins (eg. toxic dinoflagellate blooms); and/or*
- *Degraded a locally important public amenity or aesthetic value.*

It has been suggested that any invasive species which is defined by a 'spread' criterion must, *ipso facto*, be exerting unwanted ecological impacts, in which case all invasive species should fall under the 'harmful/pest/nuisance' umbrella (see Carlton 2002). However, there are many examples of invasions which have not reduced native biodiversity, particularly where they have occupied 'vacant' ecological space/Eltonian niches (e.g. in the Eastern Mediterranean and Hawaii; Bouderesque & Verlaque 2002, Coles & Eldredge 2002, Leppäkoski et al 2002). Some have even become a beneficial part of a local fishery. It therefore seems prudent to allow the division between invasive and harmful, at least for the time being.

Terms such as 'suspected pest', 'suspected harmful species' or 'potential next pest' can be used when their predicted or deduced impacts have not been demonstrated through scientific research reports, refereed publications or government regulations and guidelines.

Vectors and Pathways: these are the physical means or agents by which a species is transferred. Ballast water, ships' hulls and the movements of commercial oysters and baitfish in the aquaculture industry are examples. The particular geographic routes taken by these vectors are sometimes termed pathways, although 'pathway' is used by some agencies to refer to the combined vector and route.

Evaluating the characteristics of vectors and their routes is an integral part of NIS risk management, and their routes can be divided into primary and secondary categories. Primary routes transfer aquatic NIS across significant natural barriers (i.e. trans-oceanic and intercontinental routes), while secondary routes help spread and disperse their initial incursion/s between points within a region (i.e. via routes used by domestic and local 'hub' shipping, coastal fishing vessels, pleasure craft, river barges etc).

In line with use of vector (a medical term for the agents such as mosquitos which introduce disease organisms to the human body), inoculation refers to the release, discharge, spawning or dislodgement of viable marine organisms into a new environment in sufficient numbers and/or frequencies to provide more than a negligible chance of causing an introduction (e.g. Hilliard *et al* 1997, Hayes 1997, Hewitt & Hayes 2002, Ruiz *et al*. 2000). The inoculation concept is appropriate because outcomes (port infections) depend on the viability and strength of the inoculum, the degree of exposure of the receiving 'host' environment to inoculation and its current state of 'health'. In the case of port waters, eutrophic and disturbed bays, harbours, estuaries and inland waterways typically contain damaged if not depauperate communities of native biota (= reduced host immunity), and are being increasingly recognised as 'invader-friendly' water bodies.

Although it is theoretically possible for a single release of two individuals (or a single small colony) to eventually generate a viable population in a new environment, the chances are vanishingly small for the majority of aquatic habitats and circumstances. The most likely exception to this rule-of-thumb is probably the transfer of a handful of mature adults of a highly fecund hull-fouling species into a semi-enclosed, protected area (e.g. a poorly flushed harbour basin). A few such adults may release tens of thousands or millions of gametes if sufficient spawning cues and water quality are present. The result could be a successful, if not massive, settlement on relatively vacant artificial substrates in the harbour.

A.2 QUANTITATIVE RISK ASSESSMENTS

Risk is a measure of the likelihood of an event and magnitude of its consequences. Risk can be estimated by qualitative, semi-quantitative or quantitative means, depending on the type and amount of available information and the completeness of the model of the hazard under investigation (e.g. Hayes 1997, Hay & Taylor 1999).

In a typical quantitative risk assessment (QRA) undertaken by engineers, risk is a measured social-economic consequence of an identified hazard, multiplied by the probability of the hazard occurring (a hazardous situation is one waiting for the circumstances and events to occur that produce the unwanted outcome). Careful identification of all hazards, including evaluation of the possible circumstances and consequences, is the first step in any risk assessment process, and is usually termed a detailed hazard analysis. For example, to quantify the risk of an explosion occurring inside a complex petrochemical plant, the potential consequences ('end-points') of an explosion at particular sites within the plant need to be determined by identifying and evaluating:

(a) the site of each hazardous activity or process within the plant, including its proximity to unprotected workers and other sensitive plant structures, the nearest public street and houses, and the combinations of equipment failures and human errors that would cause an explosion at each site;

(b) the possible events and circumstances that could cause these failures and errors to coincide; and

(c) the number of deaths and injuries and extent of plant and property damage from the resultant explosion at each site. The consequences are typically measured and compared in terms of the $ costs associated with compensation payments, legal fees, rebuilding, lost production, loss of reputation and marketing losses, based on long established guidelines and dollar values used by insurance companies.

A fully quantitative risk assessment will also provide measures of certainty of the calculated risks.

In the case of assessing the biological risk from introducing a marine species, attempting a conventional quantitative risk assessment is impractical as bioinvasion models remain incomplete and there are rapidly increasing uncertainties with each step of the invasion process (i.e. from ballast uptake/ballast tank infection, through voyage survival, release/inoculation and survival in a new environment, to population establishment, spread, impacts and outcomes; e.g. Hayes 1997, Hay & Taylor 1999, Hilliard & Raaymakers 1997, Hayes & Hewitt 1998, 2000, Hewitt & Hayes 2002). Quantifying the risk for unwanted marine species introductions is possible only if:

- each potentially harmful species is identified and assessed individually (i.e. a targeted approach);

- the key physical processes and features dictating the chances of uptake, journey survival, release, new environment survival and subsequent reproduction and recruitment are known for each life-cycle stage, vector and route that are involved in the introduction process for each species; and
- the possible damaging consequences following each introduction (i.e. the end-point of the risk analysis for each species) is simplified to minimise the number of steps and uncertainties,

As noted by Hayes & Hewitt (1998, 2000), if the selected end-point is *'the establishment of a NIS population at the site of interest'*, then the risk needs to express the likelihood of that establishment. If the end-point is some subsequent effect, such as displacement of local species (loss of native biodiversity), then the risk must express the probability of this type of ecological damage following the establishment of a non-native species. The latter provides useful information to coastal resource managers, but is currently impossible to measure with any useful degree of certainty because of the incompleteness of bioinvasion understanding and modelling (Hewitt & Hayes 2002).

'*Establishment of a NIS population at the site of interest*' is a relatively simple end-point that carries the implicit assumption that establishment of any non-native species is unwanted. However even this is difficult to predict with useful certainty for a particular port or harbour. A more achievable end-point is '*Inoculation of any known or suspected harmful NIS into a port where its life cycle stages are likely to tolerate local conditions and reproduce*'. This defines the release of specifically unwanted NIS into an environmentally similar port as the risk which needs to be managed (e.g. Hewitt & Hayes 2002).

Australia has experimented with quantitative risk assessment for targeted marine pests, assuming these may also act as 'surrogates' for unknown harmful NIS using the same pathways. Constraining the end-point to a small subset of total available NIS makes the task of acquiring reliable data on the environmental tolerance ranges for the principal life-cycle stages of each species more achievable, although it can still be a lengthy and expensive process.

Inoculation by one or more of a short-list of targeted species is the end-point taken by Australia's Decision Support System (DSS). The DSS is the first BWRA system in the world to experiment with a fully quantitative approach. It was developed to assess the risk of specific ballast tank discharges following individual ship voyages, and was first implemented in July 2001. It was installed by AQIS to assess the likelihood that particular ballast tank discharges by a vessel arriving from an overseas port could inoculate one or more of 12 declared pest species into a port where they could survive (Hayes & Hewitt 1998, Patterson & Colgan 1998, Colgan 1999, Hewitt & Hayes 2002)[8].

In response to the issue of using a limited targeted species approach for vessels carrying overseas BW with a wide range of 'untargeted' species, a national taskforce on marine pest incursions recommended an interim 'trigger list' of additional pest species in 2000, while CSIRO Marine Research compiled a list of predicted 'next pests' during 2001-2002 (Hayes *et al.* 2002, Williamson *et al.* 2002). Both lists contain species whose impacts reported elsewhere warranted their treatment as unwanted 'next pests', and were produced to help evaluate the priority and scale of a rapid emergency response following discovery of an incursion, and some may be added to a future version of the DSS. As described by

[8] The DSS can use several levels of assessment, with the level applied for a particular vessel and voyage determined by the amount of available data. The more quantitative 'upper' levels require detailed data about the BW source, uptake and voyage conditions. The 'lower' levels invoke an environmental-matching component (overlap of temperature & salinity ranges) when key data are absent (a typical situation for international voyages). The DSS was started in 2001 and by the second half of 2002 was using its lower levels to assess the intended BW discharges of some 10-12% of merchant ships arriving from overseas ports.

Hayes *et al* (2002) and Williamson *et al*. (2002), the deductive and inductive criteria used to justify a species inclusion on these lists comprised:

1. A demonstrable invasive history;
2. One or more relevant transport vectors are still operating;
3. A demonstrable impact in either their native or invaded ranges on economy, environment, human health and/or amenity.
4. Predicted to have potential major impacts in Australia, as inferred from overseas data and the characteristics of Australian marine environments and their communities.

In 2003, CSIRO completed Australia's web-based *National Introduced Marine Pests Information System* (NIMPIS), while the National Introduced Marine Pests Coordinating Group (NIMPCG) reviewed the outcomes of the DSS experiment. It was concluded that the DSS was not useful for overseas arrivals due to the problem of obtaining reliable species presence/absence data for many overseas ports (the DSS defaults to 'high risk' when key data are absent). However the committee recognised the DSS should be useful for managing ballast water discharges by ships trading on Australia's domestic routes (Australian port survey and environmental data are far more complete), provided Australia's seven coastal States would agree to implementing a uniform national system.

The more rigorously and explicitly can a port state identify 'high risk' ballast water, the more justification it gains for applying rigorous control and management measures on vessels intending to discharge it. The targeted DSS approach is therefore attractive to developed countries wishing to reduce the spread of declared pests, particularly via domestic 'port-hopping' and other secondary pathways in their coastal or inland waterways.

The DSS approach appears most likely to suitable for countries and states with long and/or relatively isolated coastlines such as Australia, Brazil, Canada, Chile, Iceland, Hawaii, New Zealand, South Africa and the United States. Most of these countries have busy ports located in several or more bioregions, many of which are already suffering damage from harmful invasive species. However a ballast water DSS is expensive, and its usefulness and cost-effectiveness remains to be proven since it:

- requires a large amount of data covering a range of specific vessel, port, voyage, BW exchange, target species life cycle stages and environmental parameters;
- places a high information technology and management cost burden on the port state;
- can leave the state vulnerable to the introduction or spread of non-targeted organisms unless other surveillance and response measures are implemented.

A DSS also ignores hull-fouling plus other vectors associated with fishing and pleasure craft, but this is true of any risk assessment method that is focussed on the factors which influence ballast-mediated introductions.

A.3 SEMI-QUANTITATIVE RISK ASSESSMENT

A.3.1 Pros and Cons

BWRA approaches which rank the relative risk posed by trading routes and vessel types do not attempt to quantify the risk posed by individual ballast tank discharges. The main features of a useful semi-quantitative approach for estimating the relative overall risk (ROR) of ballast-mediated introductions from particular trading routes are that it should:

- follow objective principles, allowing the data to speak for itself;
- minimise subjective input. Where this type of input cannot be avoided, it needs to logical and defensible on biological grounds, such as use of the logarithmic principle;
- treat the main coefficients of risk equally;
- produce ratio or proportional result values (e.g. 0 - 1; 0 - 100%) which permit categorisations into easily understood, simple levels of risk.

There are several reasons why a semi-quantitative approach can be more preferable than a targeted DSS approach. These include:

- Avoids the detailed data requirements and the infrastructure and manpower costs required for developing, installing, operating and responding to a 24 hour DSS;
- The end-point can cover both anticipated and unanticipated invaders;
- By identifying high risk routes, allows more effective focussing of available resources (often limited) on data gap-filling, ballast exchange compliance monitoring, verification sampling and research;
- Can be readily used or shared on a regional basis by neighboring coastal states which share a common sea, sea lanes and/or inland waterways.

Because of the wide knowledge gaps on the types and precise distributions of risk species, semi-quantitative approaches rely on 'environmental matching' as a surrogate indicator of invasion risk. The disadvantages of semi-quantitative rankings of route and vessel type risks include:

- they require regular updates to incorporate changes in shipping trade and pertinent information from the rapidly expanding invasive species databases and publications;
- they may produce overly conservative ranking results which, unless interpreted carefully, could invoke unnecessary compliance monitoring costs;
- their results provide less robust evidence for politically and economically justifying tank discharge management controls, ship inspections and associated shipping delays or penalty schemes etc.
- an over-reliance on matching temperature and salinity ranges can 'miss' invasive species which have unusually broad temperature or salinity tolerances (e.g. the Asian bag mussel, *Musculista senhousia*, has a range reported to extend from Vladivostok to Singapore, although it is unclear if there are two or more races or sub-species providing this range).

The last point highlights the value of incorporating mechanism/s into the semi-quantitative approach to reduce the chance of it missing harmful species with broad temperature and/or salinity ranges. Thus the method needs to (a) avoid overly simple environmental matching, and (b) be capable of storing,

utilising and updating data on the distributions of known and suspected harmful species in or near the world's trading ports (i.e. allowing their presence to influence the results). An ability to discriminate trading routes by the presence of known and suspected pests species influences a risk assessment's end-point and improves the value of its results (see Section A.3.3).

A3.2 Measuring Environmental Similarity

Measures of environmental matching can be based on simple overlaps of climate region (1 variable) or maximum and minimum water temperature and salinity ranges (i.e. overlaps inside simple plots), or via statistical analysis of many variables (e.g. Hilliard *et al*. 1997, Gollasch 2002, Hewitt & Hayes 2002).

The environmental matching method adopted for the GloBallast BWRA uses 34 variables in an attempt to make the method more sensitive to the tolerances and habitat preferences of marine, brackish and freshwater species. For example, if salinity range (maximum and minimum) were the only variables for identifying estuarine ports, the matching procedure would not separate highly seasonal estuaries (i.e. those which experience a major and sudden salinity decline for several weeks owing to short monsoon or spring-melt season) from those with similar temperature ranges but located in wet equatorial and temperate regions respectively (i.e. where more consistent patterns of river discharge cause tidally-induced salinity fluctuations on a 6 or 12 hour basis).

There are several multivariate methods available in statistical packages such as Statistica, Systat, SPPSX, etc for handling a large group of disparate variables. Methods which measure variable distances between samples (ports) in multi-dimensional space are considered more direct and appropriate for port environmental matching purposes than less direct approaches such as Principal Components analysis (e.g. Belbin 1991, 1995, Belbin *et al*. 1992, Hilliard *et al* 1997b; see also the PRIMER Manuals).

The most suitable distance metric in the choices provided by PRIMER is the 'normalised Euclidean distance'. 'Euclidean distance' refers to the geometric principles stemming from the Greek philosopher Euclid. It is a 'point-to-point' metric which represents the direct 'diagonal' distance in n-dimensions of space (n = the number of environmental variables used). Other distance measures for comparing environmental characteristics include the Gower-Metric and the so-called 'Manhattan' metric (e.g. Gower 1971, PRIMER Manual). Manhattan distance is equivalent to moving between points along the axes only, in the same fashion a person in Manhattan cannot take diagonal 'short-cuts' from one building to another, but must use elevators and grid of streets (i.e. X value + Y value + Z value etc).

'Normalised' refers to the need to convert a variety of scalable, integer and even categorical variables into a uniform set of variables with unitless values. For example, the port environmental variable 'Distance to nearest river mouth' is measured in kilometres, while 'Tidal range' is measured in metres, rainfall variables are measured in millimetres of precipitation and temperature variables are measured in degrees, etc. Because 'apples cannot be compared with oranges', the scale of each variable needs to be normalised to prevent it biasing the distance measure.

A categorical variable can be treated as a normalisable integer provided its numerical labelling follows a step-wise logical sequence. For example, the variable 'Port Type' is a category which progressively moves from inland to open coastal waters in six steps: River port (1), Estuary port (2), Tidal Creek port (3), Breakwater Harbour port (4), Natural Bay port (5) and Offshore jetty/Mooring terminal port (6).

The normalisation step is automatically undertaken by PRIMER when its normalised Euclidean distance metric is selected for the environmental similarity analysis. A set of unitless variables is generated for the distance calculations, with each value of a particular variable representing the number of standard deviations from the mean value of that variable. The unitless values may be calculated before input (e.g. using the formula in the PRIMER manual), in which case PRIMER's 'Euclidean Distance' should be used. However this is unnecessary except for teaching or demonstration purposes.

It is worth noting that a small set of samples (ports) may yield a number of variables with highly skewed or bimodal distributions, which will add little to (if not reduce) the outcome of the similarity analysis. These problematic distributions can be avoided by either increasing the number of ports to provide a more global range, using a suitable transformation before the normalisation step, or substituting them with an equivalent but more normally distributed parameter.

A3.3 End-Point of the GloBallast BWRA

Environmental matching allows semi-quantitative BWRAs to include both targeted and untargeted species, but the end point has to accommodate the current inability to predict the possible establishment and consequences of transferring particular NIS[9]. Thus the end point is more constrained than in the targeted DSS approach, which is aimed at identifying and so avoiding high risk inoculations for a small group of known pests.

Methods to segregate 'harmless' from 'harmful' NIS require clear-cut definitions, good data and reliable bioinvasion models - all of which are currently lacking (Carlton 2002). It is therefore not possible to use a simple dichotomous approach (harmful/harmless), other than for convenient administrative, political or public awareness purposes. A more precautionary basis for the end-point is needed. This is also appropriate for any ports which have nearby mariculture operations and/or sites declared (or informally recognised) for local fish nursery, public recreation, nature conservation or wildlife/biodiversity values.

It was therefore considered appropriate for the 'first-pass' GloBallast BWRAs to categorise NIS into three groups (those with known, suspected or apparently no harmful credentials), based on a subjective evaluation of available species lists and reports comparable to the deductive/inductive

[9] Of the large numbers of introduced marine species now documented for the coastal waters of Australasia, Europe and North America, it is generally acknowledged that between 5% and 15% have typically achieved an invasive if not harmful status in most regions, and there is consensus regarding the validity of the "10% rule of thumb" noted for terrestrial introductions. This informal measure predicts that ~10% of introduced terrestrial plants and animals will spread and ~10% of these will become pests (e.g. Ruiz *et al*. 1997, Leppäkoski *et al*. 2002, Williamson *et al*. 2002). Effects of marine pests on native biodiversity, ecosystems, fisheries and aquaculture activities range from severe basin-wide impacts (especially in biogeographically-isolated temperate regions containing a high level of endemic species such as the Ponto-Caspian and Mississippi basins) to localised and as yet unclear effect, such as in Indo-West Pacific tropical waters beyond disturbed port environments (e.g. Hutchings *et al*. 2002).

Among some of the infamous introductions of the 20th Century, several species did not spread or show other signs of their invasive prowess and harmful effects in initial years and even decades following introduction, while others have undergone 'boom-bust' cycles (e.g. the Chinese mitten crab Eriocheir sinensis in several parts of Europe). Reasons why an introduced population may take a long time to start increasing in abundance and spreading include the processes of local adaptation/gene selection, the removal of a local physical barrier (or appearance of a new local vector) allowing spread from a previously isolated location, a change to the local pollution and habitat conditions, and/or a gradual regional trend in temperature, rainfall/evaporation and salinity due to climate change. The boom-bust phenomena of the mitten crab is related to trends in climate variables that influence regional winter temperatures and rainfall intensity (in Leppäkoski *et al*. 2002).

approach described earlier (Section A.2). Allocation of NIS and weighting values to the three categories form the only component of the BWRA system requiring subjective input.

In summary, the end point in the BWRA rankings represents, for each BW source port, a portion of the total threat posed by ballast-mediated introductions of potentially harmful species, as inferred from the species that may be present in the source port, the frequency, size and quality of the ballast tank inoculations, and the capacity of the receiving environment to sustain the discharged organisms.

A.4 HYBRID APPROACHES TO BW RISK ASSESSMENT

Semi-quantitative risk assessment systems which can be shifted, as far as their data permit, into a 'hybrid' of the environmental matching and targeted species approaches avoid redundancy and facilitate development, and this flexibility is an integral feature of the BWRA system used for Activity 3.1.

Hybridising features include easy alterations to the way the environmental matching component is used for calculating overall risk, and to the weight which can be applied to individual risk species and overall risk species threat. To appreciate how components can be changed, users need to understand how the following risk coefficients and risk reduction factors can be applied in exploratory calculations of introduction threats posed by BW source ports:

Coefficient C1 - proportion of total number of BW discharges (relative frequency of inoculation),

Coefficient C2 - proportion of total BW volume discharged (relative size of total inoculation),

Coefficient C3 - environmental similarity (on a scale from 5% to 100% similar),

Coefficient C4 - proportion of total threat posed by all risk species present in all BW source ports[10,11]).

Risk factor R1 - a reduction to inoculation quality, as reflected by BW tank size (values are W4).

Risk factor R2 - a reduction to inoculation size and quality, as reflected by BW tank storage time (the values are W5).

Equation (1) is the default setting used by the project standard BWRA for calculating relative overall risk (ROR) of a BW source port:

(1) $$ROR = (C1 + [C2 \times R1.W4] + C3 + [C4 \times R2.W5]) / 4$$

This equation represents a logical formula for scientists who consider that environmental matching (C3) should be treated as an independent coefficient of risk, so that it provides a surrogate for the relatively biased information on NIS distributions and their harmful potential:

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[10] as identified in each source port's bioregion. This provides a conservative approach and will be necessary until a lot more port surveys are conducted and published.

[11] for a 'Forward' risk assessment which examines the risk posed by BW uplifted and exported from the Receiving Port, C4 is unavailable but C1 may be replaced by the frequency of intended visits to Next Ports of Call (reported on BW Reporting Forms). C2 is difficult to calculate due to lack of reported data. In some cases it is possible to provide BW volume estimates for C2, such as for empty tankers or bulkers departing from an import terminal to a next port of call which is a well-known export terminal. CD-ROMs such as the *Fairplay Ports Guide* help identify specific port trades, and the Galbraith posters which show important bulk terminals and trading routes are also useful.

In equation (1), ROR is a combined measure of the relative 'inoculation' frequency, size and quality, the ability of risk species in a source port to survive and establish at the Receiving Port, and the relative level of threat posed by species likely to be present at this port. The division by 4 keeps the result in the 0-1 range, allowing convenient expression of the ROR as a ratio (percentage) of the total risk posed by all source ports.

The BW source port of Tubarão (in Brazil) provides an example of how C3 markedly influences the ROR outcome[12]. Tubarão had a relatively low C1 (ranked 83rd of all Sepetiba's BW source ports) and a relatively low C4 value (0.117; which was 43% of that of the source port posing the highest risk species threat). However its environmental matching coefficient was relatively high (ranked 3rd at 0.79), and the overall risk (ROR) of Tubarão was 13th in the rank of all source ports exporting BW to Sepetiba.

Such outcomes are not uncommon since the default estimation of ROR (Equation [1]) uses the simple mean of the C1-C4 coefficients, and C3 is often the largest of these. This is because the C3 value represents a direct measure of port similarity and is unaffected by the number or bioregional location of other source ports. In contrast, the C1, C2 and C4 values represent proportional measures of the total inoculation frequency, volume and risk species threat posed by all BW source ports, with C4 operating at the bioregion level. Until the resolution and reliability of the C4 values can be improved, it is logical to allow the port-specific C3 values to markedly influence the ROR results.

For BWRAs involving a limited number of BW source ports for which there is reasonable risk species data, it is simple to alter the ROR formula to allow the relative level of threat posed by these species (C4) to occupy a more focal point in the risk calculation. In this case, a marine scientist may prefer to alter the ROR formula so it can:

(i) treat C3 as a 'risk reduction factor' which influences the size of C4 (rather than using it as an independent 'surrogate' measure of unidentified and unknown risk species); and/or

(ii) increase the influence of C4 by raising the value of W3 (C4's overall weighting factor) from its default level of 1 (see User Guide Section 6 for explanation of the W1-W3 weights used for C4).

The GloBallast BWRA Database allows the default ROR formula to be changed to achieve these and other options. To achieve option (i), the formula may be altered to apply C3 as follows:

(2) $ROR = (C1 + [C2 \times R1.W4] + [C3 \times C4 \times R2.W5]) / 3$

[the divisor is now 3 because of the reduced number of summed coefficients].

Equation (2) is logical if the Database contains a reasonably accurate distribution of appropriately weighted risk species for all BW source port bioregions (including native species considered potentially harmful if they establish in other areas). In the case of a source port with a very dissimilar environment (e.g. C3 = 0.2) but in a bioregion containing a relatively large number of risk species (e.g. C4 = 0.3), then Equation (2) will reduce C4 by at least 80% (i.e. to 0.06). If ballast tank storage times on ships arriving from this source port are long (e.g. for voyages between 10-20 days; W5 = 0.6), then C4 will be further reduced to 0.036 (representing an 88% reduction to its initial value).

[12] in Brazil's Pilot Country BWRA Report for the Port of Sepetiba (http://globallast.imo.org)

Equation (2) is less conservative than (1) when the C4 values do not provide reliable measures of risk species threat. The following table shows how Equation (1) produces higher ROR values for typical trading situations (i.e. when no BW source port has C1 or C2 values larger than 0.5):

		Relative Overall Risk	Proportion of discharge Frequency	Proportion of discharge Volume	Enviro-mental Matching	Relative Risk species threat
		ROR	**C1**	**C2**	**C3**	**C4**
ROR = [C1 + C2 + C3 + C4] / 4	**Equation (1)**	**0.150**	0.1	0.1	0.2	0.2
ROR = [C1 + C2 + (C3 x C4)] / 3	**Equation (2)**	**0.080**	0.1	0.1	0.2	0.2
ROR = [C1 + C2 + C3 + C4] / 4	**Equation (1)**	**0.200**	0.2	0.2	0.2	0.2
ROR = [C1 + C2 + (C3 x C4)] / 3	**Equation (2)**	**0.147**	0.2	0.2	0.2	0.2
ROR = [C1 + C2 + C3 + C4] / 4	**Equation (1)**	**0.350**	0.5	0.5	0.2	0.2
ROR = [C1 + C2 + (C3 x C4)] / 3	**Equation (2)**	**0.347**	0.5	0.5	0.2	0.2
ROR = [C1 + C2 + C3 + C4] / 4	**Equation (1)**	**0.400**	0.6	0.6	0.2	0.2
ROR = [C1 + C2 + (C3 x C4)] / 3	**Equation (2)**	**0.413**	0.6	0.6	0.2	0.2
ROR = [C1 + C2 + C3 + C4] / 4	**Equation (1)**	**0.450**	0.7	0.7	0.2	0.2
ROR = [C1 + C2 + (C3 x C4)] / 3	**Equation (2)**	**0.480**	0.7	0.7	0.2	0.2

Option (ii) above was to leave the default ROR calculation unchanged but increase the size of C4. Note that any weight change to C4 is more sensitive in Equation (1) because C3 has less influence on C4 size than in (2).

Because C4 typically exerts less influence on the ROR result than C3 in the default GloBallast BWRA, Brazilian counterparts undertaking the BWRA Activity for the Port of Sepetiba explored the effect of altering all three default weights used for calculating C4 (i.e. W1 = 3, W2 = 10, W3 = 1; see Section 6 in the User Guide), in order to evaluate their influence on C4 size and ROR outcomes.

Altering W3 to values between 0.2 and 5 showed that only source ports with moderate environmental matching values were sensitive to marked changes in C4 size (Port of Sepetiba BWRA Report; http://globallast.imo.org). Exploring changes to W2 (the weight applied to known pest species) confirmed that such alterations may cause a source port's C4 value to increase, decrease or remain virtually unchanged, depending on the relative number of NIS, suspected and known pest species that had been assigned to the bioregion of that source port. The Brazilian investigation not only showed how altering the various C4 weights can produce unexpected outcomes, but also underlined the potential trap of 'numbers games' due to the inherent behaviour of the relative approach. The trap arises whenever no clear biological rationale, data bias correction or other logical objective forms the basis for altering the default formula and weighting values. On the other hand, exploring the ROR rankings and influence of the individual risk components is an integral part of evaluating semi-quantitative and hybrid BWRA outcomes, so that the importance and contribution of each coefficient, risk reduction factor and weighting value can be recognised and understood.

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Appendix 2

Country Prefixes used in UN Port Codes

Country	Prefix for UN Port Code	Country	Prefix for UN Port Code
Albania	**AL**	Faeroe Islands	**FO**
Algeria	**DZ**	Falkland Islands (Malvinas)	**FK**
American Samoa	**AS**	Fiji	**FJ**
Angola	**AO**	Finland	**FI**
Antigua and Barbuda	**AG**	France	**FR**
Argentina	**AR**	French Guiana	**GF**
Aruba	**AW**	French Polynesia	**PF**
Australia	**AU**	Gabon	**GA**
Azerbaijan	**AZ**	Gambia	**GM**
Bahamas	**BS**	Georgia	**GE**
Bahrain	**BH**	Germany Federal Republic Of	**DE**
Bangladesh	**BD**	Ghana	**GH**
Barbados	**BB**	Gibraltar	**GI**
Belgium	**BE**	Greece	**GR**
Belize	**BZ**	Greenland	**GL**
Benin	**BJ**	Grenada	**GD**
Bermuda	**BM**	Guadeloupe	**GP**
Brazil	**BR**	Guam	**GU**
Brunei Darussalam	**BN**	Guatemala	**GT**
Bulgaria	**BG**	Guinea	**GN**
Cambodia	**KH**	Guinea-Bissau	**GW**
Cameroon	**CM**	Haiti	**HT**
Canada	**CA**	Honduras	**HN**
Cape Verde	**CV**	Hong Kong	**HK**
Chile	**CL**	Iceland	**IS**
China	**CN**	India	**IN**
Christmas Islands	**CX**	Indonesia	**ID**
Cocos (Keeling) Islands	**CC**	Iran Islamic Republic of	**IR**
Colombia	**CO**	Iraq	**IQ**
Comoros	**KM**	Ireland	**IE**
Congo	**CG**	Israel	**IL**
Congo Democratic Republic	**CD**	Italy	**IT**
Cook Islands	**CK**	Ivory Coast	**CI**
Costa Rica	**CR**	Jamaica	**JM**
Croatia	**HR**	Japan	**JP**
Cuba	**CU**	Jordan	**JO**
Cyprus	**CY**	Kazakhstan	**KZ**
Denmark	**DK**	Kenya	**KE**
Djibouti	**DJ**	Kiribati	**KI**
Dominica	**DM**	Korea Dem People's Rep	**KP**
Dominican Republic	**DO**	Korea Republic of	**KR**
Ecuador	**EC**	Kuwait	**KW**
Egypt	**EG**	Latvia	**LV**
El Salvador	**SV**	Lebanon	**LB**
Eritrea	**ER**	Liberia	**LR**

Country	Prefix for UN Port Code	Country	Prefix for UN Port Code
Estonia	**EE**	Lithuania	**LT**
Lybian Arab Jamahiriya	**LY**	Singapore	**SG**
Macau	**MO**	Slovenia	**SI**
Madagascar	**MG**	Solomon Islands	**SB**
Malaysia	**MY**	Somalia	**SO**
Malta	**MT**	South Africa	**ZA**
Marshall Islands	**MH**	Spain	**ES**
Mauritania	**MR**	Sri Lanka	**LK**
Mauritius	**MU**	St Helena	**SH**
Mexico	**MX**	St Kitts-Nevis	**KN**
Monaco	**MC**	St Pierre and Miquelon	**PM**
Morocco	**MA**	St Vincent and The Grenadines	**VC**
Mozambique	**MZ**	Sudan	**SD**
Myanmar (Former Burma)	**MM**	Suriname	**SR**
Namibia	**NA**	Sweden	**SE**
Nauru	**NR**	Syrian Arab Republic	**SY**
Netherlands	**NL**	Taiwan Province of China	**TW**
Netherlands Antilles	**AN**	Tanzania United Republic Of	**TZ**
New Caledonia	**NC**	Thailand	**TH**
New Zealand	**NZ**	Togo	**TG**
Nicaragua	**NI**	Tonga	**TO**
Nigeria	**NG**	Trinidad and Tobago	**TT**
Norfolk Island	**NF**	Tunisia	**TN**
Norway	**NO**	Turkey	**TR**
Oman	**OM**	Turks and Caicos Islands	**TC**
Pakistan	**PK**	Tuvalu	**TV**
Panama	**PA**	Ukraine	**UA**
Papua New Guinea	**PG**	United Arab Emirates	**AE**
Peru	**PE**	United Kingdom	**GB**
Philippines	**PH**	United States	**US**
Poland	**PL**	United States Virgin Islands	**VI**
Portugal	**PT**	Uruguay	**UY**
Puerto Rico	**PR**	Vanuatu	**VU**
Qatar	**QA**	Venezuela	**VE**
Reunion	**RE**	Viet Nam	**VN**
Romania	**RO**	Virgin Islands British	**VG**
Russian Federation	**RU**	Yemen	**YE**
Samoa	**WS**	Yugoslavia (Fed Rep Of)	**YU**
Sao Tome and Principe	**ST**		
Saudi Arabia	**SA**		
Senegal	**SN**		
Seychelles	**SC**		
Sierra Leone	**SL**		

APPENDIX 3

Use and Origin of the Bioregion System

1 USE OF BIOREGIONS

Bioregions serve multiple purposes and are required for several reasons. For example, many marine regions of the world remain poorly surveyed with a limited marine taxonomy literature. This results in a 'patchy' and artificial distribution of recorded marine species distributions. In addition, few marine species surveys have been undertaken in port environments, and rarely have all ports been surveyed within one bioregion.

Bioregions represent environmentally similar geographic areas. Thus if a species is present in one part of a Bioregion, there is a good chance it can spread via natural or human-mediated processes to other sites in the same bioregion.

A conservative approach can therefore be adopted for a Ballast Water Risk Assessment, whereby a species, if recorded in at least one location in a Bioregion, is considered potentially present at all ports within the boundaries of the same Bioregion.

2 ORIGINS OF BIOREGIONS

The Bioregions in the World Map largely follow those adopted by the International Union for the Conservation of Nature (IUCN) to define areas for marine biological conservation purposes (Kelleher *et al.* 1995), with specific alterations made by CSIRO-CRIMP. They also follow the recent National Introduced Marine Species Information System (NIMPIS) developed in Australia to help reduce the spread of introductions (Section 3).

The nearshore bioregions are based on Kelleher *et al.* (1995), who used groups of local marine experts within 18 separate ocean regions to identify nearshore bioregions based largely on environmental and biological characteristics.

The offshore bioregions are derived from Longhurst (1998), who primarily identified oceanic regions using their physical characteristics. In this context, it is worth noting the following 'rules of thumb' with respect to seawater temperature in commonly used regional labels:

- Cold Regions: seawater regularly freezes in winter, rarely exceeds 10°C in summer.
- Cool Temperate Regions: inshore seawater occasionally freezes in winter, rarely exceeds 20°C in summer except in the surface layer of inland seas (e.g. Black Sea, Caspian Sea).
- Warm Temperate Regions: seawater never freezes (usually always >8°C) and may reach 26°C in summer, with shallow, protected inshore areas sometimes peaking to 28-29°C.
- Sub-Tropical Regions: coastal waters typically in the 16-28°C range, but shallow inshore and estuarine waters may fall to 11-12°C during mid-winter nights. Wider ranges (10-33°C) occur in highly insolated, restricted basins and gulfs such as the ROPME Sea Area.
- Tropical/Equatorial Regions: rarely falls below 20°C and may exceed 32°C in summer or ITZ extremes.

The nearshore marine bioregions of Kelleher *et al* (1995) were modified by Hewitt *et al* (2002) for the purposes of introduced marine species distributions as follows:

- their offshore boundary is limited to 200 nautical miles, in order to represent each country's coastal zone and continental shelf; and
- additional bioregions have been added for oceanic islands and island networks (e.g., Hawaiian Islands, Galapagos Islands, Canary Islands).

Hewitt *et al* (2002) also assigned the nearshore bioregions to large-scale biological provinces derived from Sherman *et al* (1990). These provincial associations represent broad-scale patterns of transitions in biodiversity and species assemblages.

3 AUSTRALIA'S "NIMPIS" SYSTEM

The National Introduced Marine Pest Information System (NIMPIS) was developed by the Centre for Research on Introduced Marine Pests (CRIMP) at CSIRO Marine Research. It was developed to provide managers, researchers, students and public with access to up to date information on the distribution and biology of introduced marine species, and some potential control options for those species designated as 'marine pests'. Included in NIMPIS are:

(a) species known to be introduced to Australian waters, and

(b) species considered likely to become future introductions to Australia ('next pests').

The information in NIMPIS is held in a structured format that is also used by the Biological Risk Assessment module of AQIS' Decision Support System for ballast water management (AQIS BWM-DSS; see also Appendix 1). As a result, some of the text may appear technical.

NIMPIS is a dynamic information system that is regularly updated, and therefore certain functions, links, species information and applications may be unavailable at times. Information copied from the NIMPIS site, which is still undergoing development, may be cited and used for public education and research. Individual pages include a recommended citation at the bottom of the page.

4. REFERENCES

Hewitt CL, Martin RB, Sliwa C, McEnnulty FR, Murphy NE, Jones T and Cooper S (2002). Editors. *National Introduced Marine Pest Information System*: http://crimp.marine.csiro.au/nimpis (Date of access).

Kelleher, G., Bleakley, C. and Wells, S. (1995). *A Global Representative System of Marine Protected Areas*. The World Bank, Washington DC, USA

Longhurst, A.R. (1998). *Ecological Geography of the Sea*. Academic Press, San Diego, CA.

Sherman K, Alexander LM and Gold BD (1990). *Large Marine Ecosystems: Patterns, Processes and Yields*. AAAS Press, Washington DC, USA.

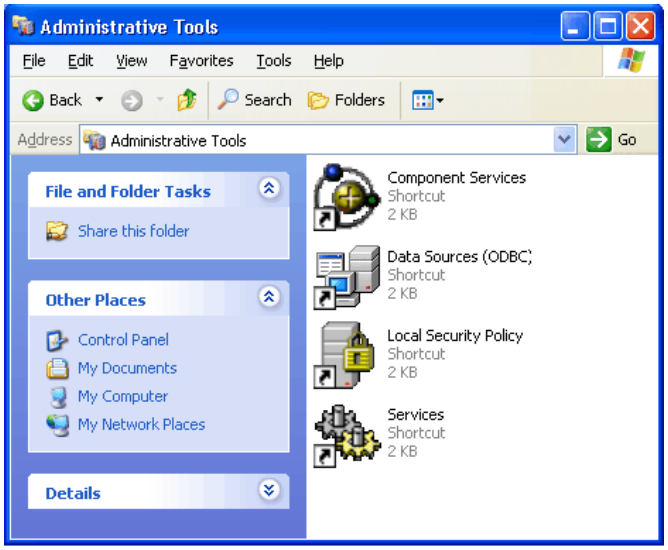
APPENDIX 4

Creating an ODBC Connection

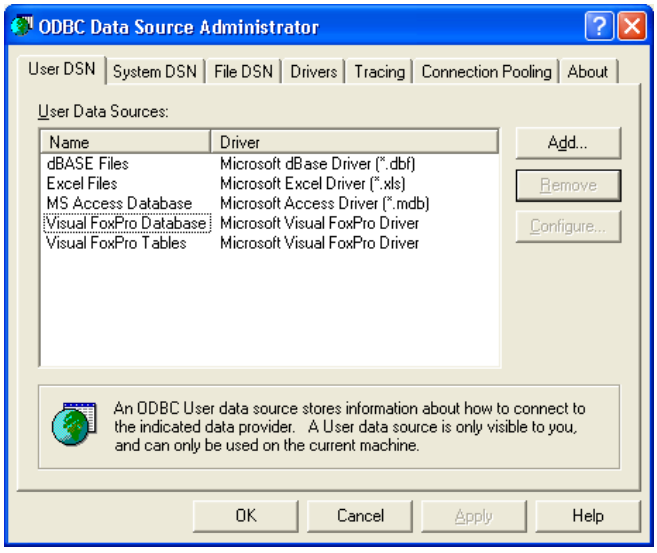
To display the BWRA results using the GIS, it is necessary to establish a connection between the BWRA Database and ArcView. An ODBC (Open Database Connectivity) link is used to do this. Information on ODBC is also at: http://webopedia.internet.com/TERM/O/ODBC.html

Follow these steps to permanently establish the ODBC connection:

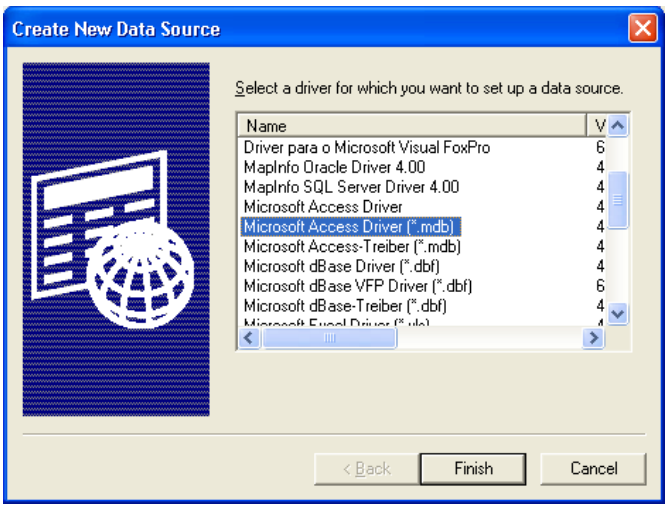
1. In **Control Panel** > **Administrative Tools** double-click **Data Sources (ODBC).**



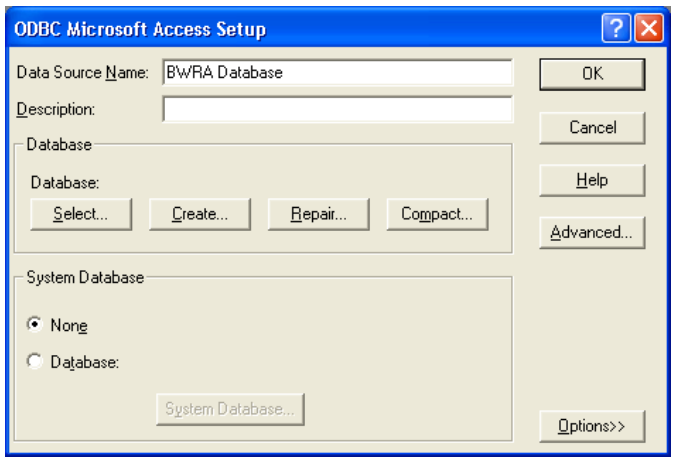
2. Choose **Add....**



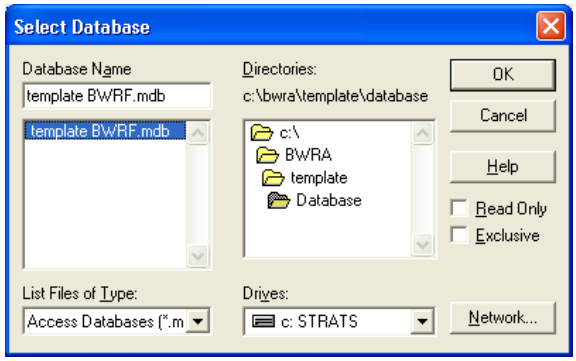
3. Select the **Microsoft Access Driver (*.mdb)** and click **Finish.**



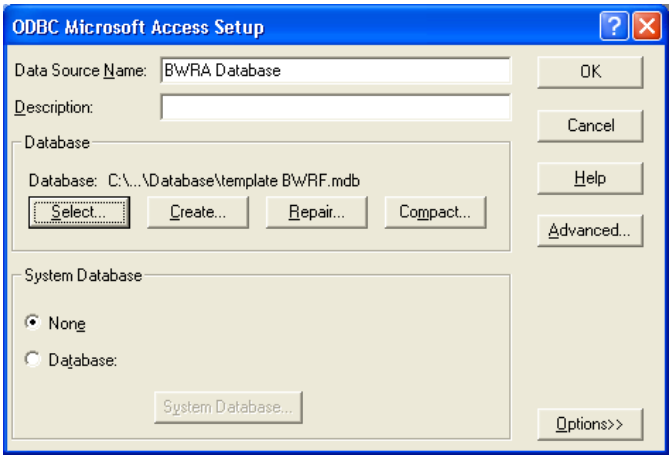
4. Add a **Data Source Name** (i.e. BWRA Database or BWRA Dalian) and then click **Database: Select**.



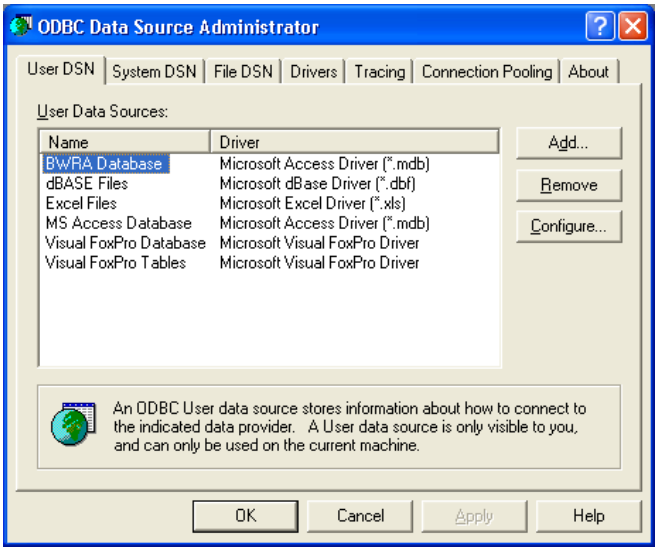
5. Navigate to the appropriate BWRA database.



6. Click **OK,** return to the **ODBC Microsoft Access Setup** dialog and click **OK** again.



7. You will now see the **BWRA Database** connection appear in the **User Data Sources** list.



The **BWRA Database** connection is now permanently available for use by ArcView.

APPENDIX 5

Glossary of Maritime Jargon and Ballasting Factors

1 MARITIME JARGON

Aframax — Tankers mainly carrying crude oil (but with separate tanks for other chemicals) designed for African routes and harbour depths. Aframax ships are typically between 97,000 and 107,000 DWT, with a maximum length of 246 meters, beam of 42 meters, and draft of 14.5 meters.

Alliances — Groupings of ocean carriers designed to increase sailing frequencies and realize economies of scale. Typically members of an alliance "pool" their ships in a particular trade, and allocate part of each ship's capacity to alliance members. In this way alliance members can offer far more frequencies than would be possible using only their own ships. Also alliances are used to achieve cost efficiencies, especially in terminal operations. Key alliances (with selected members at present) include: Grand Alliance (Hapag-Lloyd, Malaysia International Shipping Corp., NYK Line, OOCL, and P&O Nedlloyd); New World Alliance (APL-NOL, Hyundai Merchant Marine, and Mitsui O.S.K. Line); and the United Alliance (Cho Yang, DSR-Senator, Hanjin, and the United Arab Shipping Company).

Auto carriers / Vehicle Carriers — Ships similar to "Ro-Ro's" but designed to carry only automobiles. Typically auto carriers enclose a large volume with a low draft (like a big floating box). Multiple decks house thousands of cars. Mainly used on routes from Europe, Japan, Korea and United States to countries with a significant demand for automobile importations.

Ballast — Non-revenue-producing weight used to 'balance' a ship when it has little or no cargo. Bulk carriers and tankers often make the return trip "in ballast" (i.e. empty, without a cargo).

Bulk Carriers, 'Bulkers' or 'Bulkies' — Ships designed to load carry dry cargoes in bulk such as grains, oil seeds, ores, and coal, from point to point. Bulkies may be 'geared' (= has a set of cranes for self-loading/unloading) or 'ungeared' (relies on port equipment to load/unload cargo).

Bunker fuel / 'Bunkers' - typically a standard medium weight fuel oil (MFO) used by ships.

Cape-Size/Cape Class — A type of Bulk Carrier too large for the Suez Canal, and used to carry heavy cargoes (mainly iron ore) around the Cape of Good Hope to and from Asia. Cape-Class ships typically weigh between 80,000 - 199,000 DWT, with a beam between 40 to 50 meters.

CIF — Cost, Insurance and Freight. The cost of a product, plus insurance and freight charges required to deliver the shipment to a specified point.

Conferences — Groupings of carriers designed to limit competition and insure stability by setting and publishing rates for specific trade lanes. Since the U.S. enacted OSRA (Ocean Shipping Reform Act) in 1998, conferences have become largely ineffective with most shippers contracting confidentially with ocean carriers. The largest conference is the FEFE (Far Eastern Freight Conference) for carriers operating between Europe and Asia, followed by TACA (Trans Atlantic Carrier Agreement). At present there is no conference for the transpacific market; instead, there are eastbound and westbound "discussion agreements" that are much looser in structure and have no antitrust protection for joint rate making.

Combo, Combi — Ships that can carry either liquid or dry bulk cargoes (see also *OBOs*).

Container Ships - carry standards-size ocean containers, either TEUs or FEUs. Most container cargo consists of finished products, capital goods, or higher value semi-manufactured goods.

Cube — the volumetric capacity of a container. A TEU offers an internal volume of approximately 1,150 cubic feet, while an FEU offers approximately 2,300 cubic feet.

Dead Weight Tonnage — see DWT

Density — the number of kilograms per cubic meter (or, in the USA, pounds per cubic foot). Density matters because Containers often are full before they reach their weight-bearing limits. Density is measured in two ways: product density represents the density of a particular product in its packaging (weight divided by volume), whereas stowed density represents the density of a loaded ship (net payload divided by cubic capacity). Stowed density is usually lower than product density because of Container loading inefficiencies (unused space).

Distribution channels — the links through which freight shipments flow from shipper to consignee. There are three primary distribution channels for ocean freight: shipper-direct, carrier-direct and NVOCC. Most bulk trade moves in the shipper-direct channel, where shippers directly charter bulk carriers or tankers, and use the entire capacity of the ship. Most liner trade (containerized traffic) moves through the carrier-direct or NVOCC channels. In the carrier-direct channel, steamship companies employ sales forces to originate traffic from multiple shippers, and bundle other services (such as inland transport) with the core port-to-port transportation product. In the NVOCC channel, a Non-Vessel Operating Common Carrier act as a freight forwarder that combines traffic from multiple shippers into large consolidations that command volume-discounts from carriers. NVOCCs also bundle services in order to create door-to-door transportation.

Draft — depth in water of a ship. Ships typically need at least two or three feet clearance under their keel to avoid accidents. A ship's minimum depth is the draft of ship when empty and in ballast. When harbour or channel depth is less than the fully loaded depth then ships must reduce load.

Dry Bulk Carriers — Ships designed to carry dry bulk cargoes (grain, coal, mineral ores, wood chips, etc).

DWT — Dead Weight Tonnage. DWT is a measure of the carrying capacity of a ship when fully loaded, usually in ***Metric tonnes***. DWT is the weight of the cargo, stores, fuel, crew and passengers carried by a ship when fully loaded. It is measured by the difference between the ship's 'lightweight' and its displacement when loaded to its summer freeboard load-line in normal seawater (1.025 specific gravity). DWT can be used to estimate ballast water capacity for many classes of ship.

FCL — Full Container Load. A shipping container which is fully loaded.

FEU — Forty-foot Equivalent Unit. An ocean container measuring 40 feet long by 8 feet wide by 8 feet high. FEUs are used mainly for shipping manufactures, and can hold 8 and 22 metric tons of cargo, depending on the product being shipped. More of the world's trade is being shipped in 40' containers, but the standard unit of account remains the TEU (two TEUs per FEU). In weight terms, one FEU is typically equivalent to 1.4 TEUs.

FOB — Free On Board. The cost of a product excluding freight transportation and insurance charges necessary to deliver it to the buyer.

Freight forwarder — a company that offers door-to-door transportation shippers, and often sell related services (such as warehouse management) as well. Most major forwarders operate NVOCCs (Non-Vessel-Operating Common Carriers) that combine traffic from multiple shippers into large consolidations that command lower rates from ocean carriers. In most markets, NVOCCs generate the majority of containerized ocean traffic.

GDP (Gross Domestic Product) — the total value of all goods and services produced in a particular country but excluding earnings remitted from abroad.

Geared ships are equipped with their own cranes for loading and off-loading containers at smaller ports. Generally handimax ships (under 50,000 DWT) that carry cranes on board or off loading (conveyer belts and other bulk discharge systems) can be used in smaller ports, especially ports in developing regions.

GNP (Gross National Product) — the total value of all goods and services produced in a particular country including earnings remitted from abroad (by individuals or enterprises). Gross payload includes the tare weight of containers.

Gross Registered Tonnage - (GRT) is a theoretical estimate of a ship's maximum carrying capacity, as it is derived from the total volume of enclosed spaces which are available for cargo, stores, crew,

passengers etc, within the hull and superstructure. GRT does not permit reliable estimates of ballast water capacity.

Handysize — Bulk carriers between 10,000 DWT and 35,000 DWT (48% of world bulk fleet).

Handymax — Bulk carriers of between 35,000 and 50,000 DWT (24% of world bulk fleet).

Laden or Un-laden — A maritime freight term describing if the voyage included cargo or was without cargo. A vessel that is laden has cargo and one that is unladen has no cargo (i.e., ballast only).

LCL — Light Container Load. A shipping container which is not fully loaded (see ***FCL***).

Length — maximum length of the vessel.

Liner / Liner Service - Ships that travel on a regular and repetitive schedule between a set of ports (usually container ships). Net payload excludes the weight of containers.

Long ton – a British unit of weight (Avoirdupois) equivalent to 2240 pounds (see ***Short Ton*** and ***Metric Tonne***).

***LNG -* Liquid natural gas** - LNG (methane, hydrogen) is carried by purpose-built LNG carriers which have very low temperature pressurised containers to maintain a liquid form. Boil off gas is often used as fuel for the main engine/s, which are adapted to run on gas during the delivery voyage and normal bunker fuel oil on the ballasted return voyage.

LPG - Liquid petroleum gas - LPG (butane, propane etc) is generated in petroleum refineries and carried under pressure in LPG tankers to maintain its liquid form. LPG is also carried in palleted cylinders by small cargo vessels delivering fuels and supplies to remote harbours and islands etc.

Metric Tonne – a metric tonne (MT) is equivalent to 1000 kilograms (approximately 2205 pounds) and has the same weight as 1 cubic metre of freshwater (1 m^3 = 1000 litres). Note that 1 m^3 of normal seawater (35 psu) has a higher specific gravity and weight (1.035 MT).

NVO or NVOCC — Non-Vessel Operating Common Carrier. NVOCCs act as freight forwarders that consolidate multiple shipper's traffic into large consolidations that command lower rates from ocean carriers.

NWS — New Worldscale (= New World-Wide Tanker Nominal Freight Scale). A pricing scale used for measuring daily average lease rates for vessels over 10,000 DWT. The NWS is a table used by ship owners for representative routes for showing time charter rates, using a common set of cost calculations. Included in the scale are distance, port costs, port time (standardized four days), bunker oil costs and other costs, plus $12,000 per day. A daily rate of 0.8 NWS (e.g. for a representative 75,000 DWT tanker) means 80% of its daily standard rate for a standard round trip voyage.

OBO's — Ore, Bulk or Oil carrier, which is a vessel capable of carrying dry or wet bulk cargoes that can be used for 'triangular' trades.

O/O — Ore or Oil carriers (vessels similar to OBOs).

Panama Canal — A vital 82 km long international shipping lane which crosses the narrowest part of Central America. Built by US Army Engineers and opened in 1914, this canal markedly shortens the distances and voyage times between Pacific and Atlantic ports. Unlike the ***Suez Canal***, the Panama has three sets of locks and a freshwater lake sector. Two sets of locks are on the Pacific side (Miraflores and Pedro Miguel Locks) and one set is on the Atlantic side (Gatun Locks). Sea level at the Pacific end is 24 cm higher than at the Atlantic end, and the Pacific end has much greater tides. Lake Gatun is 26 meters above sea level and fed by the Chagres River, which was dammed to make the lake during canal construction. The Gaillard Cut, located between Miraflores and Pedro Miguel, is 9 meters above sea level.

Panamax — The largest types of vessel capable of transiting the Panama Canal (i.e. can just fit into the locks). Panamax vessels do not exceed 80,000 DWT, and are less than 294 m (965 feet) long and 32.3 m (106 feet) wide, and must have a transit draft no more than 12.04 metres (39.5 feet).

Reefer — A refrigerated cargo vessel. Refrigerated cargo may be carried in Reefer vessels (fast ships with insulated cold storage holds or cargo spaces within their hull) or inside Reefer Containers. Container Ships carrying reefer containers may also carry standard containers, but must provide sufficient electric power to each slot which is designated for accommodating a Reefer Container.

Ro/Ro Ships — "Roll-On/Roll-Off" ships are designed to load cargo from front or rear ramps. Designed to carry heavy cargoes of odd shapes but requiring additional rigging or protection from weather (within decks). May be used for automobiles, trucks, tractors, machine tools, generators, etc.

Short ton - a United States unit of weight equivalent to 2000 pounds (see ***Long ton***).

Spot charter — Charter of a vessel (sometimes complete with cargo) for a single voyage.

Stevedore — A longshoreman or an individual or company that loads/unloads vessels in a port.

Strings — a group of vessels all operates the same itinerary in order to provide same day-of-week ("fixed day") port calls.

Suez Canal — A 162 km long international shipping lane connecting the Mediterranean and Red Sea. The Suez Canal was built by Ferdinand de Lesseps, opened in 1869 and markedly shortens the distance between Asia and Europe (the alternative is to go around the southern tip of Africa; see ***Cape-Size/Cape Class***). There are no locks and the maximum transit draft is presently 16.1 metres (there is a plan to increase depths to allow 22 m draft vessels by 2010). Some 15,000 ships pass through the canal each year, with typical passage times of 11-16 hours.

Suezmax — The maximum size vessel able to transit the Suez Canal. With the exception of very large tankers (VLCCs and ULCCs) and Cape-size bulk carriers, most trading ships can transit the Suez Canal. In the case of tankers, the Suezmax class is typically in the 140,000 - 160,000 DWT range with lengths of 269-274 meters, beams of 44-48 meters and transit drafts up to 16.1 meter (i.e. maximum drafts may be 17.5 m, as some of the cargo can be discharged before entry into the canal). Because of the navigational constraints of the Bosphorus Straits (which links oil exports ports in the Black Sea to the Mediterranean and northwest Europe markets), the smaller dimensions of the Suezmax tankers are allowing them to serve some of the fastest growing oil producing regions in the South Caucasus and Central Asia region.

Tanker – a broad term covering a range of vessel classes which carry liquid or liquefied bulk cargo (e.g. crude oil, fuel oil, aviation spirit, LPG, LNG, naphtha, other petroleum products or liquid bulk commodities such as caustic soda, vegetable oils or molasses). Most tanker classes are either specialized or dedicated to particular types of cargo, with the majority typically carrying ballast water on their return voyage (e.g. when returning to oil export terminals or refineries in the ROPME Sea Area, Venezuela, Nigeria, etc). The oil tanker market is dominated by the multi-national oil companies. Tankers may be placed on the ***Spot Market*** or on a longterm ***Time Charter***. Tanker owners generally prefer time charters since there is rarely a 'return' cargo. Thus tankers must justify their costs on a single voyage.

Tare weight — the weight of an empty container or pallet. Subtracting tare weight from gross payload yields net payload.

TEU — Twenty foot container for shipping mainly manufactures (sometimes bulk items are containerized but only when transportation costs are low or when the number of empty containers needed to be repositioned is large). A 20 foot container can hold between 5 to 16 MT of cargo (lighter cargo such as clothing and textiles typically cube out, i.e. fill the container space, before reaching the weight limit).

Time Charter — Charter of a vessel for a specified length of time or number of voyages.

Tonne/ton — see Metric Tonne and Short ton.

ULCC/Ultra Large Crude Carrier – the largest type of oil tanker used for carrying crude oil, in the 300,000-360,000 DWT range. ULCCs cannot transit the Suez or Panama canals, having length of ~365 meters, beams of ~60 meters and laden drafts of ~22.4 meters.

VLOC/Very Large Ore Carrier — Dry bulk carriers over 180,000 DWT (0.2% of World Fleet).

VLCC /Very Large Crude Carrier – oil tankers bigger than 'Super Tankers' used mostly for carrying crude oil in the 290,000-300,000 DWT range. As with ULCCs, VLCCs are too large to transit the Suez or Panama canals, having lengths ~330 meters, beams of ~58 meters and laden drafts of 21 meters.

2 FACTORS INFLUENCING BW DISCHARGE VOLUME AND LOCATION

The amount of BW a vessel needs to discharge when visiting a port depends on its type, size and, most importantly, the amount of cargo to be loaded versus the amount of cargo already on board.

The BW is carried in various tanks which vary in number, type and distribution according to ship type and individual design. These tanks are flooded by a combination of gravity feed and pumping, usually through a single or duplicate opening located on the underside and immediately below the pump room (sea chest). In the case of typical bulk carriers and some types of tankers, the ballast tanks are:

- the topside 'wing' tanks. These are a series of tanks which are usually triangular in cross-section and located high up along both sides of the hull. They typically carry 10-20% of the fair-weather ballast capacity, and are designed to inhibit severe, undue rolling when the vessel is empty by raising the centre of gravity. Ballast water from the topside wing tanks can be released by gravity flow from a series of ports located 2-4 m below the moulded deck line, but on many vessels it can also be released via internal piping to the pump room and side ports.
- the fore and aft 'peak' tanks. These carry a further 10-20% of the fair weather ballast capacity, and are located in the bow and stern sections.
- the deep double-bottom tanks (sometimes connected to the side ('hopper') tanks). These are located deep within the hull and hold some 60-70% of the fair-weather ballast capacity. They comprise either separate or confluent compartments, depending on the type, size and age of the vessel. BW from these and the peak tanks must be pumped out through side ports located near the pump room (a compartment usually located immediately in front of the engine room).

In addition to the normal 'fair-weather' ballast capacity, one or more of the cargo holds of bulk carriers and oil tanks of empty tankers can be flooded to further deepen the ship and provide increased stability and handling characteristics during periods of storm-induced high seas and large swells. These temporary 'heavy weather' compartments comprise pre-designated holds which can be filled with 8,000-15,000 tonnes of seawater, which can increase the ballasted weight to 50-60% of DWT (Table 1). The following table compares the fair- and heavy-weather ballast water capacities of common ship types.

Comparison of typical Ballast Water capacities among different Ship Types

Vessel Type	DWT (tonnes)	Fair weather ballast (tonnes)	% of DWT	Heavy weather ballast (tonnes)	% of DWT
Bulk Carrier	250,000	75,000	30	113,000	45
" "	150,000	45,000	30	67,000	45
" "	70,000	25,000	36	40,000	57
" "	35,000	10,000	30	17,000	49
Oil Tanker	100,000	40,000	40	45,000	45
Product Tanker	40,000	12,000	30	15,000	38
Container ship	40,000	12,000	30	15,000	38
" "	15,000	5,000	30	-	-
General cargo ship	17,000	6,000	35	-	-
" "	8,000	3,000	38	-	-
RoRo Pass. ferry	3,000	<1,000	-	-	-

Ships encountering heavy weather en route usually have plenty of opportunity to discharge any extra ballast in deep water, prior to approaching the port. Discharge of heavy weather ballast is done as soon as possible to (a) reduce the amount of immersed surface and so improve fuel efficiency, and (b)

maximise the amount of cargo hold drying and airing time. Some ports have "air draft" limitations that require inbound vessels to maintain or even temporarily increase their draft prior to berthing, in order to pass safely under a low bridge or under the ship loader gantry at a particular berth. However such limitations and practises are generally rare.

In the case of empty bulk carriers and tankers, review data indicate that their fair-weather ballast capacity is typically 35-43% and 26-40% of their DWT respectively. This ballast water is carried in various tanks dedicated to the purpose (in older tankers these include some compartments that originally were cargo tanks, prior to the implementation of MARPOL regulations restricting discharge of oily BW)[13]. The BW coefficients of DWT used in an Australian study of BW discharges are shown in the next table.

BW discharge estimates used for an Australian port study

Vessel type	Bulk carriers[1]	Woodchip carriers	Crude tankers	Product tankers	Chemical tankers	LPG tankers	Container ships	General cargo ships	RoRo vessels
Loading	39%[2]	36.0%	-	35.0%	28.5%[3]	26.0%	-	17%[4]	18.0%
Unloading	2.44%	-	3.21%	3.21%	3.21%	3.21%	-	0.55%	0.09%
Both	5.00%	-	5.54%	5.54%	5.54%	5.54%	1.88%	0.68%	1.14%
Av. Capacity	41%	41%	36%	35%	33%	33%	30%	35%	38%

1 = 34% for Ore carriers; 2 = 30% for grain; 3 = 16% for tallow / veg. oil;
4 = 8.5% for refrigerated cargo ships; 15% for livestock carriers

The next table lists the various BW Coefficients used in the Excel spreadsheet for estimating BW discharges from a ship's DWT, in the absence of BW Reporting Forms (**Utilities Folder: BW estimates from port records.xls**):

BW Coefficients used in Excel file for estimating discharges from ship DWTs

Vessel Type	BW discharge as percentage of DWT		
	Cargo Loading	Cargo Unloading	Loading & Unloading
Gas (LPG/LNG) tanker	26.0%	0.0%	3.2%
Chemical tanker	28.5%	3.2%	5.5%
Crude oil tanker	35.0%	0.0%	3.2%
Oil/bulk ore carrier (OBO)	35.0%	0.0%	3.2%
Products tanker	35.0%	3.2%	5.5%
Vegetable oil tanker	16.0%	0.0%	3.2%
Dry Bulk carrier	38.0%	2.0%	5.0%
Ore carrier	34.0%	0.0%	0.0%
Grain carrier	30.0%	0.0%	0.0%
Woodchip carrier	36.0%	0.0%	0.0%
Container ship	15.0%	0.0%	1.0%
General cargo ship	17.0%	3.5%	7.0%
Refrigerated cargo ship (Reefer)	8.5%	0.0%	0.0%
Ro-Ro cargo ship	18.0%	1.0%	9.0%
Livestock carrier	15.0%	0.0%	0.0%
Vehicles carrier	18.0%	0.0%	3.0%
Passenger vessel	0.0%	0.0%	0.0%
Landing Craft/Barges	0-5%	0.0%	0.0%

Apart from ship type and size, the total volume of BW actually discharged during the ship's approach, berthed, and departure periods, is governed by the following factors:

[13] Where a tanker is chartered to load a different product after delivering an incompatible cargo (and hence must wash its cargo tanks), the refinery may operate an onshore treatment facility that may be able to receive some 5,000-10,000 tonnes of oily wash water per tanker, depending on the treatment requirements of previous ships.

- the amount and type of cargo already on board;
- the need, if empty and in ballast, to maintain manoeuvrability and minimise windage (which rapidly increases with excessive hull clearance) when negotiating the long and twisting shipping channel through Moreton Bay;
- the need to keep the propeller sufficiently immersed to provide optimum propulsion efficiency, reversing power, and minimal wake-induced propeller shaft vibration (QDoT vessel draft regulations administered by the Regional Harbourmaster require at least 90% of the propeller diameter to be immersed when the vessel is at rest); and
- the degree and success of any deep sea ballast exchange undertaken en route (due to possible Port State restrictions or penalties on discharge of un-exchanged ballast water).

Some ships entering a sheltered sea area where strong winds are not forecasted and oceanic swells and wind waves do not occur, may elect to discharge 5-15% of the normal 'fair-weather' ballast volume to reduce their wetted surface area (= slight increase in fuel efficiency, provided the propeller remains properly submerged to prevent shaft vibration and bearing wear). Such preliminary deballasting is usually completed before entering the port's final approaches), but the amount is limited to 10-15% by the need to maintain manoeuvrability, prevent vibrations and to conform to local regulations on vessel trim and navigational safety.

A 5-15% preliminary discharge is typically attained by gravity draining of some of the wing tanks, sometimes accompanied by part-deballasting of one of the peak tanks. This can be undertaken before the ship enters the port's approach channel, or when at anchor and waiting for a vacant berth. It also depends on the prevailing/forecasted weather conditions, the planned cargo loading schedule and the design of the ballast water tanks and ballast pump-out capacity of the particular ship. Gravity draining of a bulk carrier's wing tanks when alongside a berth is strongly discouraged by most terminal operators for safety and maintenance reasons. Ballast in these tanks is therefore either drained internally via piping leading to the pump room or retained, depending on vessel type and its loading/unloading requirements.

Ship masters and Port Pilots are usually very reluctant to allow significant deballasting before or during the berthing phase, with many Ports stipulating that the propeller must remain at least 90% submerged, and draft at the bow must not be less than 2% of the overall length. For a large bulk carrier in ballast with an overall length of 290 metres (>120,000 DWT), these criteria typically require a draft of approximately 8 metres at the stern and more than 5.8 metres at the bow.

If asked, most Port Pilots will agree that ships should not commence (or continue with) a BW discharge when traversing the final approaches to a port or terminal, particularly if the approach is constrained by a narrow navigation channel. Commencing or continuing a BW discharge during this critical stage can generate unwanted, complicating navigational factors in terms of a diminishing draft, unpredictable changes to manoeuvrability and an increasing windage effect ('sailing').

Thus the vast majority (85-95%) of the normal 'fair-weather' ballast carried by empty bulk carriers or tankers is typically discharged when alongside the berth, almost always in close coordination with the cargo loading program. Deballasting must be closely co-ordinated with loading to avoid placing dangerous stresses on the hull, particularly in the forward sections as a consequence of potential uneven distribution of cargo and remaining ballast.

A key exception can be an outer anchorage area if there are regular or occasional tanker-to-tanker transfers of liquid petroleum gas (LPG) which require BW discharge by the loading tanker. For container ships, general cargo ships or bulk carriers arriving at a port to 'top-off' a part-loaded cargo (taken at a previous port), there is often no need to discharge any BW.

In summary, deballasting is usually pre-programmed and carefully monitored with respect to:

- the anticipated and actual weather and sea state conditions that occur during the arrival, possible anchoring and berthing phases;
- vessel manoeuvrability, safety and port regulations on draft and trim of ballasted vessels (including possible air draft restrictions due to gantries or bridges);
- the amount and type of cargo to be loaded, and the need to record the ship's fore and aft draft at rest.

BALLAST WATER REPORTING FORM

(To be provided to the Port State Authority upon request)

1. SHIP INFORMATION **2. BALLAST WATER**

Ship's Name:	Type:	IMO Number:	Specify Units: M^3, MT, LT, ST
Owner:	Gross Tonnage:	Call Sign:	Total Ballast Water on Board:
Flag:	Arrival Date:	Agent:	
Last Port and Country:		Arrival Port:	Total Ballast Water Capacity:
Next Port and Country:			

3. BALLAST WATER TANKS Ballast Water Management Plan on board? YES NO Management Plan Implemented? YES NO

Total number of ballast tanks on board: _____ No. of tanks in ballast: _____ IF NONE IN BALLAST GO TO No. 5.

No. of tanks exchanged: _____ No. of tanks not exchanged: _____

4. BALLAST WATER HISTORY: RECORD ALL TANKS THAT WILL BE DEBALLASTED IN PORT STATE OF ARRIVAL; IF NONE GO TO No. 5.

Tanks/ Holds (List multiple sources per tank separately)	**BALLAST WATER SOURCE**				**BALLAST WATER EXCHANGE** Circle one: Empty/Refill or Flow Through					**BALLAST WATER DISCHARGE**			
	DATE DDMMYY	Port or Lat/Long	Volume (units)	Temp (units)	DATE DDMMYY	Endpoint Lat/Long.	Volume (units)	% Exch.	Sea Hgt. (m)	DATE DDMMYY	Port or Lat/Long	Volume (units)	Salinity (units)

Ballast Water Tank Codes: Forepeak = FP, Aftpeak = AP; Double Bottom = DB; Wing = WT; Topside = TS; Cargo Hold = CH; Other = O

IF EXCHANGES WERE NOT CONDUCTED, STATE OTHER CONTROL ACTION(S) TAKEN:_____

IF NONE STATE REASON WHY NOT:_____

5: IMO BALLAST WATER GUIDELINES ON BOARD (RES. A.868(20))? YES NO

RESPONSIBLE OFFICER'S NAME AND TITLE (PRINTED) AND SIGNATURE: _____

GUIDELINES FOR COMPLETING THE BALLAST WATER REPORTING FORM

SECTION 1: SHIP INFORMATION

Ship's Name: Print the name of the ship. **Owner**: The registered owners or operators of the ship. **Flag**: Country of the port of registry.
Last Port and Country: Last port and country at which the ship called before arrival in the current port - no abbreviations, please.
Next Port and Country: Next port and country at which the ship will call, upon departure from the current port - no abbreviations, please.
Type: List specific ship type, write out or use the following abbreviations:
bulk(bc); roro (rr); container (cs); tanker(ts); passenger (pa); oil/bulk ore (ob); general cargo (gc). Write out any additional ship types. **GT**: Gross tonnage.
Arrival Date: Arrival date at current port. Please use the European date format (DDMMYY)
IMO Number: Identification Number of the ship used by the International Maritime Organization. **Call Sign**: Official call sign.
Agent: Agent used for this voyage. **Arrival Port**: This is the current port. No abbreviations, please.

SECTION 2: BALLAST WATER

(Note: Segregated ballast water = clean, non-oily ballast)
Total ballast water on board: Total segregated ballast water upon arrival at current port - with units.
Total ballast water capacity: Total volume of all ballastable tanks or holds - with units.

SECTION 3: BALLAST WATER TANKS

Count all tanks and holds separately (e.g. port and starboard tanks should be counted separately)
Total No. of Tanks on board: Count all tanks and holds that can carry segregated ballast water.
Ballast Water Management Plan on board?: Do you have a ballast water management plan, specific to your ship, onboard? Circle Yes or No.
Management Plan Implemented?: Do you follow the above plan? Circle Yes or No.
No. of Tanks in Ballast: Number of segregated ballast water tanks and holds with ballast at the start of the voyage to the current port. If you have no ballast water on board, go to section 5.
No. of Tanks Exchanged: This refers only to tanks and holds with ballast at the start of the voyage to the current port.
No. of Tanks Not Exchanged: This refers only to tanks and holds with ballast at the start of the voyage to the current port.

SECTION 4: BALLAST WATER HISTORY

BW Source: Please list all tanks and holds that you have discharged or plan to discharge in this port. Carefully write out, or use codes listed below the table. Follow each tank across the page, listing all source(s), exchange events, and/or discharge events separately. If the ballast water history is identical (i.e. the same source, exchange and discharge dates and locations), sets of tanks can be combined (example: wing tank 1 with wing tank 2, both water from Belgium, exchanged 02.11.97, mid ocean). Please use an additional page if you need, being careful to include the arrival date, ship's name and IMO number at the top.

Date: Date of ballast water uptake. Use European format (DDMMYY).
Port or Latitude/Longitude: Location of ballast water uptake.
Volume: Volume of ballast water uptake, with units.
Temperature: Water temperature at time of ballast water uptake, in degrees centigrade (Celsius).
BW Exchange: Indicate Exchange Method: Circle empty/refill or flow through.
Date: Date of ballast water exchange. Use European format (DDMMYY).
Endpoint or Latitude/Longitude: Location of ballast water exchange. If it occurred over an extended distance, list the end point latitude and longitude.
Volume: Volume of ballast water exchanged, with units.
Percentage exchanged: Percentage of ballast water exchanged. Calculate this by dividing the number of units of water exchanged by the original volume of ballast water in the tank. If necessary, estimate this based on pump rate. (Note: For effective flow-through exchange this value should be at least 300%).

ANNEX 2

Guidelines for Risk Assessment under Regulation A-4 of the BWM Convention

ANNEX

GUIDELINES FOR RISK ASSESSMENT UNDER REGULATION A-4 OF THE BWM CONVENTION (G7)

1 PURPOSE

1.1 The purpose of these Guidelines is to assist Parties to ensure that provisions of regulation A-4 of the Convention are applied in a consistent manner, and based on scientifically robust risk assessment, which ensures that the general and specific obligations of a Party to the Convention are achieved.

1.2 An additional purpose is to provide assurance to affected States that exemptions granted by a Party meet the regulation A-4.3 obligations.

1.3 The Guidelines outline three risk assessment methods that will enable Parties to identify unacceptable high risk scenarios and acceptable low risk scenarios, and advise Parties on procedures for granting and withdrawing exemptions in accordance with regulation A-4.

2 INTRODUCTION

2.1 Regulation A-4 of the Convention states that a Party or Parties, in waters under their jurisdiction may grant exemptions to any requirements to apply regulation B-3 or C-1, in addition to those exemptions contained elsewhere in the Convention, but only when they are:

.1 granted to a ship or ships on a voyage or voyages between specified ports or locations; or to a ship which operates exclusively between specified ports or locations;

.2 effective for a period of no more than five years subject to intermediate review;

.3 granted to ships that do not mix ballast water or sediments other than between the ports or locations specified in paragraph 2.1.1; and

.4 granted based on the Guidelines that have been developed by the Organization.

2.2 These Guidelines provide advice and information regarding risk assessment principles and methods, data needs, advice on application of risk assessment methods, procedures for granting exemptions, consultation and communication processes, information for reviewing exemptions and advice regarding technical assistance, co-operation and regional co-operation.

2.3 These Guidelines also provide advice regarding the roles of the Organization, shipping industry, port States and other States that might be affected by granting an exemption in accordance with regulation A-4 of the Convention.

2.4 Scientifically robust risk assessment underpins the process of Parties granting exemptions under regulation A-4 of the Convention. The assessment must be sufficiently robust to distinguish between unacceptable high risk scenarios and acceptable low risk scenarios where the discharge of ballast water not meeting regulations B-3 and C-1 is unlikely to impair or damage the environment, human health, property or resources of the granting Party and of adjacent or other States.

2.5 Risk assessments should be based on best available scientific information.

2.6 The Guidelines should be kept under review in order to incorporate experiences gained during their application and any new scientific and technical knowledge.

3 APPLICATION

3.1 These Guidelines apply to Parties granting exemptions to ships under regulation A-4 of the Convention.

3.2 Shipowners or operators wanting to seek an exemption under regulation A-4 should also consult these Guidelines.

4 DEFINITIONS

4.1 For the purposes of these Guidelines, the definitions in the Convention apply.

4.2 "Anadromous": species that spawn/reproduce in freshwater environments, but spend at least part of their adult life in a marine environment.

4.3 "Biogeographic region": a large natural region defined by physiographic and biologic characteristics within which the animal and plant species show a high degree of similarity. There are no sharp and absolute boundaries but rather more or less clearly expressed transition zones.

4.4 "Catadromous": species that spawn/reproduce in marine environments, but spend at least part of their adult life in a freshwater environment.

4.5 "Cryptogenic": species that are of unknown origin, i.e. species that are not demonstrably native or introduced to a region.

4.6 "Donor Port": port or location where the ballast water is taken onboard.

4.7 "Euryhaline": species able to tolerate a wide range of salinities.

4.8 "Eurythermal": species able to tolerate a wide range of temperatures.

4.9 "Freshwater": water with salinity lower than 0.5 psu (practical salinity units).

4.10 "Marine water": Water with salinity higher than 30 psu.

4.11 "Non-indigenous species": any species outside its native range, whether transported intentionally or accidentally by humans or transported through natural processes.

4.12 "Recipient port": port or location where the ballast water is discharged.

4.13 "Target species": species identified by a Party that meet specific criteria indicating that they may impair or damage the environment, human health, property or resources and are defined for a specific port, State or biogeographic region.

5 RISK ASSESSMENT PRINCIPLES

5.1 Risk assessment is a logical process for assigning the likelihood and consequences of specific events, such as the entry, establishment, or spread of harmful aquatic organisms and pathogens. Risk assessments can be qualitative or quantitative, and can be a valuable decision aid if completed in a systematic and rigorous manner.

5.2 The following key principles define the nature and performance of risk assessment:

.1 **Effectiveness –** That risk assessments accurately measures the risks to the extent necessary to achieve an appropriate level of protection.

.2 **Transparency –** That the reasoning and evidence supporting the action recommended by risk assessments, and areas of uncertainty (and their possible consequences to those recommendations), are clearly documented and made available to decision-makers.

.3 **Consistency –** That risk assessments achieve a uniform high level of performance, using a common process and methodology.

.4 **Comprehensiveness –** That the full range of values, including economic, environmental, social and cultural, are considered when assessing risks and making recommendations.

.5 **Risk Management –** That low risk scenarios may exist, but zero risk is not obtainable, and as such risk should be managed by determining the acceptable level of risk in each instance.

.6 **Precautionary –** That risk assessments incorporate a level of precaution when making assumptions, and making recommendations, to account for uncertainty, unreliability, and inadequacy of information. The absence of, or uncertainty in, any information should therefore be considered an indicator of potential risk.

.7 **Science based –** That risk assessments are based on the best available information that has been collected and analysed using scientific methods.

.8 **Continuous improvement –** Any risk model should be periodically reviewed and updated to account for improved understanding.

5.3 In undertaking risk assessment when considering granting an exemption, the risk assessment principles should be carefully applied. The lack of full scientific certainty should be carefully considered in the decision making process. This is especially important under these Guidelines, as any decision to grant an exemption will allow for the discharge of ballast water that does not meet the standards of regulation D-1 or D-2.

6 RISK ASSESSMENT METHODS

6.1 General

6.1.1 There are three risk assessment methods outlined in these Guidelines for assessing the risks in relation to granting an exemption in accordance with regulation A-4 of the Convention:

- Environmental matching risk assessment
- Species' biogeographical risk assessment
- Species-specific risk assessment

6.1.2 Environmental matching risk assessment relies on comparing environmental conditions between locations, species' biogeographical risk assessment compares the overlap of native and non-indigenous species to evaluate environmental similarity and to identify high risk invaders, while species-specific risk assessment evaluates the distribution and characteristics of identified target species. Dependent on the scope of the assessment being performed, the three approaches could be used either individually or in any combination, recognizing that each approach has its limitations.

6.1.3 Environment matching and species' biogeographical risk assessment may be best suited to assessments between biogeographic regions. Species-specific risk assessment may be best suited to situations where the assessment can be conducted on a limited number of harmful species within a biogeographic region.

6.2 Environmental matching risk assessment

6.2.1 Environmental matching risk assessments compare environmental conditions including temperature and salinity between donor and recipient regions. The degree of similarity between the locations provides an indication of the likelihood of survival and the establishment of any species transferred between those locations.

6.2.2 Since species are widely distributed in a region, and are rarely restricted to a single port the environmental conditions of the source region should be considered.

6.2.3 These regions are typically defined as biogeographic regions. Noting that all of the existing biogeographical schemes were derived for different purposes than proposed here, it is suggested that the Large Marine Ecosystems (LME) scheme (http://www.edc.uri.edu/lme) be used based on best available information at this time, with local and regional adaptation as necessary. It is recognized that the suggested biogeographical scheme may not be appropriate in certain circumstances and in this case other recognized biogeographical schemes may need to be considered[1].

[1] Watling and Gerkin (http://marine.rutgers.edu/OBIS/index.html) based on Briggs (1953) and Springer (1982); IUCN bioregion system; Briggs (1953) and Ekman (1974; 1995); Longhurst provinces.

6.2.4 Environmental matching should therefore compare environmental conditions between the donor biogeographic region and the recipient port to determine the likelihood that any species found in the donor biogeographic region are able to survive in the recipient port in another biogeographic region. The environmental conditions that may be considered for environmental matching include salinity, temperature or other environmental conditions, such as nutrients or oxygen.

6.2.5 The difficulty in using environmental matching risk assessments is identifying the environmental conditions that are predictive of the ability of the harmful species to successfully establish and cause harm in the new location, and in determining whether the risk of ballast water discharge is sufficiently low to be acceptable. Environmental matching risk assessments have limited value where the differences between a donor biogeographic region and a recipient port are small as high similarity is likely to indicate high likelihood of successful establishment.

6.2.6 Environmental conditions should also be compared between the donor and recipient ports. Similarity in key environmental conditions between the two ports is a stronger indication that species entrained in ballast water in the donor port could survive when released into the waters of the recipient port. The environmental conditions that may be considered for environmental matching include salinity, temperature or other environmental conditions, such as nutrients or oxygen.

6.2.7 The data necessary to enable a risk assessment using environmental matching includes, but is not limited to:

.1 Origin of the ballast water to be discharged in recipient port.

.2 Biogeographic region of donor and recipient port(s).

.3 The average and range of environmental conditions, in particular salinity and temperature.

This information is used to determine the degree of environmental similarity between the donor and recipient environments. In many cases, it should be possible to use existing data for part or all of these environmental profiles.

6.2.8 The following should be considered in gathering data on the environmental conditions:

.1 The seasonal variations in surface and bottom salinities and temperatures at the recipient port and the larger water body the port is contained within (e.g., estuary or bay). Surface and bottom values are needed to determine the full range of environmental conditions available for a potential invader (e.g., low salinity surface waters allowing the invasion of a freshwater species). Salinity and temperature depth profiles are not required if available data indicates the waters are well mixed over the entire year.

.2 In recipient ports with strong tides or currents, the temporal variations in salinity should be determined over a tidal cycle.

.3 In areas with seasonal or depth variations, the salinity should be determined on a seasonal and/or depth basis.

.4 Any anthropogenic influences on freshwater flow that could temporarily or permanently alter the salinity regime of the recipient port and surrounding waters.

.5 The seasonal temperature variation of coastal waters for the biogeographic region of the recipient port. Consideration should be given to both surface waters and to how temperature varies with depth.

6.2.9 It is recommended that the analysis of environmental conditions be followed by a consideration of the species known to be in the donor region that can tolerate extreme environmental differences. If present, a species-specific approach should be used to evaluate the risks associated with these species. Such species include:

- species that utilize both fresh and marine environments to complete their life-cycle (including anadromous (e.g., Sea Lamprey) and catadromous (e.g., Chinese Mitten crab) species);

- species with a tolerance to a wide range of temperatures (eurythermal species) or salinities (euryhaline species).

6.3 Species' biogeographical risk assessment

6.3.1 Species' biogeographical risk assessment compares the biogeographical distributions of nonindigenous, cryptogenic, and harmful native species that presently exist in the donor and recipient ports and biogeographic regions. Overlapping species in the donor and recipient ports and regions are a direct indication that environmental conditions are sufficiently similar to allow a shared fauna and flora. The biogeographical analysis could also be used to identify high risk invaders. For example, native species in the donor biogeographic region that have successfully invaded other similar biogeographic regions but that are not found in the recipient biogeographic region could be considered high risk invaders for the recipient port or location. The larger the number of biogeographic regions that such species have invaded, the greater the potential that those species would be able to become established in the recipient port or biogeographic region if introduced by ballast water not meeting regulation B-3 or C-1. Another general indicator of risk would be if the donor biogeographic region is a major source of invaders to other areas.

6.3.2 The data necessary to enable a risk assessment using a species biogeographical approach includes but may not be limited to:

.1 records of invasion in the donor and recipient biogeographic regions and ports;

.2 records of native or non-indigenous species that could be transferred through ballast water in the donor biogeographic region that have invaded other biogeographic regions and the number and nature of biogeographic regions invaded;

.3 records of native species in the donor region that have the potential to affect human health or result in substantial ecological or economic impacts after introduction in the recipient region through ballast water transfer.

6.3.3 The species' biogeographical risk assessment could also be used to identify potential target species in the donor regions as indicated by native species with wide biogeographical or habitat distributions or which are known invaders in other biogeographic regions similar to that of the recipient port.

6.4 Species-specific risk assessment

6.4.1 Species-specific risk assessments use information on life history and physiological tolerances to define a species' physiological limits and thereby estimate its potential to survive or complete its life cycle in the recipient environment. That is, they compare individual species characteristics with the environmental conditions in the recipient port, to determine the likelihood of transfer and survival.

6.4.2 In order to undertake a species-specific risk assessment, species of concern that may impair or damage the environment, human health, property or resources need to be identified and selected. These are known as the target species. Target species should be selected for a specific port, State, or geographical region, and should be identified and agreed on in consultation with affected States.

6.4.3 To determine the species that are potentially harmful and invasive, parties should initially identify all species (including cryptogenic species) that are present in the donor port but not in the recipient port. Target species should then be selected based on criteria that identify the species that have the ability to invade and become harmful. The factors to consider when identifying target species include, but should not be limited to:

- evidence of prior introduction;
- demonstrated impacts on environment, economy, human health, property or resources;
- strength and type of ecological interactions, e.g. ecological engineers;
- current distribution within biogeographic region and in other biogeographic regions; and
- relationship with ballast water as a vector.

6.4.4 Species-specific risk assessments should then be conducted on a list of target species, including actual or potentially harmful non-indigenous species (including cryptogenic species). As the number of species included in the assessment increases the number of low risk scenarios decreases. This is justified if the species assessments are accurate. The difficulty arises when the assessments are conservative due to lack of data. It should be recognized however, that the fewer the number of species analyzed, the greater the uncertainty in predicting the overall risk. The uncertainty associated with limiting the analysis to a small number of species should therefore be considered in assessing the overall risk of invasion.

6.4.5 It should be noted that there are limitations involved with using a target species approach. Although some data and information can be obtained to support decision making, identifying species that may impair or damage the environment, human health, property or resources is subjective and there will be a degree of uncertainty associated with the approach. For example, it is possible that species identified as harmful in some environments may not be harmful in others and vice versa.

6.4.6 If species-specific risk assessments are undertaken when the donor and recipient ports are within different biogeographic regions, Parties should identify and consider any uncertainties

resulting from lack of data on the presence of potentially harmful species in the donor location.

6.4.7 The data necessary to enable a risk assessment using the species-specific approach includes, but is not limited to:

.1 biogeographic region of donor and recipient port(s);

.2 the presence of all non-indigenous species (including cryptogenic species) and native species in the donor port(s), port region and biogeographic region, not present in the recipient port, to allow identification of target species;

.3 the presence of all target species in the recipient port(s), port region, and biogeographic region;

.4 the difference between target species in the donor and recipient ports, port region, and biogeographic region;

.5 life history information on the target species and physiological tolerances, in particular salinity and temperature, of each life stage; and

.6 habitat type required by the target species and availability of habitat type in the recipient port.

6.4.8 If a target species is already present in the recipient port, it may be reasonable to exclude that species from the overall risk assessment for that port unless that species is under active control. It is important to recognize, however, that even when a non-indigenous species or cryptogenic species has been reported from the donor and recipient ports, its continual introduction into the recipient ports could increase the probability that it will become established and/or achieve invasive population densities.

6.4.9 A risk assessment can take different forms. A simple assessment can be undertaken as outlined in paragraph 6.4.7 of whether a target species is present in the donor port but not in a recipient port and can be transported through ballast water. However, if considered appropriate, the likelihood of target species surviving each of the following stages may be assessed, including:

.1 Uptake – probability of viable stages entering the vessel's ballast water tanks during ballast water uptake operations;

.2 Transfer – probability of survival during the voyage;

.3 Discharge – probability of viable stages entering the recipient port through ballast water discharge on arrival; and

.4 Population establishment – probability of the species establishing a self-maintaining population in the recipient port.

6.4.10 To determine the likelihood of transfer and survival of a harmful species, the probability of each species surviving each of the stages contained in paragraph 6.4.9 may be assessed. To the extent possible the different life stages of the target species may also be assessed considering seasonal variations of life stage occurrence in donor port with seasonal conditions in the recipient port. The overall risk assessment for the discharge of unmanaged ballast water is therefore determined based on the assessment of all target species surviving all these stages.

6.4.11 In assessing whether a species will survive in the recipient port, physiological tolerances of all life stages need to be considered.

.1 The ability of the adults to survive would be indicated by the physiological limits for both temperature and salinity that fall within the environmental ranges observed in the recipient port and larger water body. As a check, a comparison could be made with the native and/or introduced ranges of the species to determine if the predicted tolerances (based on lab or field studies) reflect actual distributions.

.2 For other life stages the physiological requirements of each stage in the life cycle should be compared against the environmental conditions during the season(s) of reproduction, noting that these stage(s) may live in different habitats to complete their life cycle (e.g., coastal pelagic larvae of estuarine benthic invertebrates). Data should be collected as appropriate.

.3 Comparisons of known physiological tolerances for other conditions should be conducted if the data are available and relevant.

6.4.12 To evaluate whether the species-specific risk assessment approach is sufficiently robust to predict invaders, the approach could be used to estimate the probabilities of invasion for a suite of existing invaders within the recipient port. Failure to accurately predict existing invaders may indicate that the model under predicts the risk.

6.5 Evaluation and decision-making

6.5.1 The port State granting exemptions shall, in both the evaluation and consultation processes, give special attention to regulation A-4.3 which states that any exemptions granted under this regulation shall not impair or damage the environment, human health, property or resources of adjacent or other States. Regulation A-4.3 also states that States that may be adversely affected shall be consulted, and Parties should refer to section 8 regarding consultation.

6.5.2 It is important for the transparency and consistency of the risk assessments to define a priori criteria to distinguish between unacceptable high risk scenarios and acceptable low risk scenarios where the risk of ballast water not meeting regulations B-3 and C-1 is unlikely to impair or damage the environment, human health, property or resources of the granting Party and of adjacent or other States. The specific criteria depend upon the risk assessment approach, as well as the uncertainty in the analysis.

6.5.3 For an environmental matching risk assessment:

.1 A high-risk scenario could be indicated if the environmental conditions of the donor ports overlap the environmental conditions of the recipient region.

.2 A low-risk scenario could be indicated if the environmental conditions of the donor port do not overlap the environmental conditions of the recipient region.

6.5.4 For the species' biogeographical risk assessment:

.1 A high-risk could be indicated if the recipient port presently contains non-indigenous species whose native range includes the donor biogeographic region.

.2 A high-risk could be indicated if the donor and recipient ports share non-indigenous species whose source is from other biogeographic regions.

.3 A moderate to high risk could be indicated if the recipient biogeographic region presently contains non-indigenous species whose native range includes the donor biogeographic region.

.4 A moderate to high risk could be indicated if the donor biogeographic region is a major source for invaders for other biogeographic regions.

6.5.5 For a species-specific risk assessment, an assessment could be deemed high risk if it identifies at least one target species that satisfies all of the following:

- likely to cause harm;
- present in the donor port or biogeographic region;
- likely to be transferred to the recipient port through ballast water; and
- likely to survive in the recipient port.

6.5.6 The overall probability of a successful invasion also depends in part on the number of organisms and the frequency with which they are introduced over the entire period of the exemption. Therefore, it is recommended that a risk assessment should consider estimates of at least the following four factors:

.1 the total volume of water discharged

.2 the volume of water discharged in any event (voyage)

.3 the total number of discharge events

.4 the temporal distribution of discharge events.

6.5.7 In all cases, the level of uncertainty needs to be considered in evaluating the extent of risk. High levels of uncertainty in the biogeographical distributions and/or physiological tolerances of a target species may be sufficient in themselves to classify the risk as high. Additionally, the potential ecological impact of the target species should be considered in deciding the level of acceptable risk. The absence of, or uncertainty in, any information should not be considered a reason to grant an exemption to regulation B-3 or C-1.

6.5.8 Once the level of risk and the extent of uncertainty have been assessed, the result can be compared to the levels a Party(s) is willing to accept in order to determine whether an exemption can be granted.

6.5.9 Ships on a voyage(s) or route(s) that satisfy the requirements of regulation A-4.1 and that pass(es) the terms of acceptance in the risk assessment may be granted an exemption.

6.5.10 It is recommended that an independent peer review of the risk assessment method, data and assumptions be undertaken in order to ensure that a scientifically rigorous analysis has been conducted. The peer review should be undertaken by an independent third party with biological and risk assessment expertise.

7 PROCEDURES FOR GRANTING EXEMPTIONS

7.1 The purpose of this section is to provide guidance for Parties, Administrations and ships, engaged in the process of applying for, evaluating and/or granting exemptions in accordance with the provisions of regulation A-4. The appendix also identifies minimum information required for an exemption application.

7.2 Parties may undertake the risk assessment themselves in order to grant exemptions, or require the shipowner or operator to undertake the risk assessment. In any event the Party granting an exemption is responsible for evaluating the risk assessment, verifying the data and information used, and ensuring the risk assessment is conducted in a thorough and objective manner in accordance with the Guidelines. The recipient port State(s) should reject any application for exemption found not to be in accordance with these Guidelines, and should provide reasons as to why the application was not accepted.

7.3 Shipowners or operators wanting to seek an exemption should contact the relevant Parties to ascertain the risk assessment procedures to be undertaken and the information requirements of these procedures.

7.4 Where a Party has determined that the shipowner or operator should undertake the risk assessment, the Party should provide relevant information, including any application requirements, the risk assessment model to be used, any target species to be considered, data standards and any other required information. The shipowner or operator should follow these Guidelines and submit relevant information to the Party.

7.5 The port State shall ensure that, as required by regulation A-4.1.3, exemptions are only granted to ships that do not mix ballast water or sediments other than between the locations specified in the exemption. The port State should require evidence of the specific measures undertaken to ensure compliance with this regulation at the time the exemption is granted and over the duration of the exemption. Non-compliance during the period of exemption should result in prompt suspension or revocation of the exemption.

7.6 An exemption shall not be effective for more than 5 years from the date granted. The approval may contain seasonal and time-specific or other restrictions within the time of validity.

7.7 The result of the risk assessment should be stated as:

.1 The voyage(s) or route(s) represent(s) an acceptable risk. The application for an exemption is granted.

.2 The voyage(s) or route(s) may represent an unacceptable risk. Further consideration is required.

.3 The voyage(s) or route(s) represent(s) an unacceptable risk. The exemption from the ballast water management requirements of regulation B-3 or C-1 of the Convention is not granted.

8 CONSULTATION

8.1 In accordance with regulation A-4.3, Parties shall consult any State that may be adversely affected from any exemptions that may be granted. This should include adjacent States and any other States that may be affected, including those located in the same biogeographic region as the recipient port(s). States should exchange information and endeavour to resolve any identified concerns. Sufficient time must be given for affected States to consider proposed exemptions carefully.

8.2 Affected States should be provided with information on: the risk assessment method applied; the quality of the information used in the assessment; uncertainties in the model, model inputs and/or risk assessments; the rationale for the proposed exemption; and any terms or conditions applicable to the exemption.

8.3 The risk assessment should document the following elements as appropriate:

- Criteria or reference for defining target species in the risk method.
- The inventories of native, non-indigenous, and cryptogenic species used in the species' biogeographical risk assessment.
- Acceptance criteria applied in each step of the analysis. The risk assessment has to be put in a relevant context to enable determination of whether the risk level is acceptable or not. The only transparent verifiable way of doing this is to compare the actual risk level with clear predefined acceptance criteria in paragraphs 6.5.2 to 6.5.8.

8.4 In addition, the criteria or scientific methods used in defining and delimiting the biogeographic regions shall be presented if a scheme other than that recommended in paragraph 6.2.3 is used.

8.5 The invitation for comments should contain one of the two following options for the affected State's response:

.1 Supported without comments or conditions.

.2 Supported with comments and/or conditions.

8.6 The deadline for comments from the affected State(s) should be specified in the invitation. If no response within the given time-limit is received, this may be regarded as "Accepted without comments or conditions".

8.7 If an affected State does not support the granting of the exemption(s), the appropriate reasons should be provided. Any conditions or limitations which an affected State believes to be necessary to enable them to support an exemption should be clearly identified.

9 COMMUNICATION OF INFORMATION

9.1 Each Party to the Convention that has indicated it will grant exemptions should establish a point or points of contact for receipt of applications. Relevant contact details should be submitted to the Organization. In the absence of such information from a Party, the IMO MEPC contact point should be regarded as the contact point for the purpose of these Guidelines.

9.2 The Organization should circulate the list of contacts, and keep this list updated on a regular basis.

9.3 The decision of the recipient port State(s) shall be communicated to the shipowners or operators, the affected State(s), and the Organization as soon as possible before the effective date of the exemption. The decision should explain the basis for granting the exemption and how any comments from affected States were addressed and specify the voyage or voyages in which the exemption is granted, including the specified ports or location(s), the duration of the exemption and details of any conditions or limitations on the exemption.

9.4 Exemptions granted in accordance with regulation A-4 of the Convention, shall be effective after communication to the Organization and circulation of relevant information to Parties.

9.5 Any exemption granted shall also be recorded in the ballast water record book in accordance with regulation A-4.4.

9.6 Where exemptions have been granted for a specific voyage, any changes in voyage plans must be communicated to the Party that has granted the exemption prior to undertaking the voyage or prior to discharge of ballast water.

10 REVIEW OF RISK ASSESSMENT AND WITHDRAWAL OF EXEMPTIONS

10.1 It is recommended that information used in the risk assessment be reviewed regularly as data and assumptions used in the assessment can become outdated.

10.2 It is recommended that an intermediate review be undertaken within 12 months but in any circumstances no later than 36 months after permission is granted. A recipient port State may require several reviews to be taken during the period the exemption is granted for, but more frequent than annual reviews generally should not be required.

10.3 Renewal of an exemption following the initial 60 months must not be granted without a thorough review of the risk assessment, consultation with affected States, and notice of the decision to the Organization under regulation A-4.2.

10.4 An exemption granted under regulation A-4 of the Convention may need to be withdrawn where the actual risk associated with a voyage has increased substantially since the risk assessment was conducted. This would include emergency situations such as outbreaks, incursions, infestations, or proliferations of populations of harmful aquatic organisms and pathogens (e.g., harmful algal blooms) which are likely to be taken up in ballast water (regulation C-2 of the Convention).

10.5 When a port State notifies mariners of areas under its jurisdiction where ships should not uptake ballast water due to an emergency or other high risk situation, all exemptions should be withdrawn from ships that take up ballast water in the defined area. In such circumstances the shipowners or operators should be notified of the decision to withdraw the exemption as soon as possible.

10.6 Guidelines for additional measures regarding ballast water management including emergency situations (G13) adopted by resolution MEPC.161(56) provide guidance to rapidly identify appropriate additional measures whenever emergency situations occur in relation to ballast water operations.

11 TECHNICAL ASSISTANCE, CO-OPERATION AND REGIONAL CO-OPERATION

11.1 Article 13 of the Convention provides that Parties undertake, directly or through the Organization and other international bodies, to provide support for those Parties which request technical assistance, that Parties undertake to co-operate and that Parties shall endeavour to enhance regional co-operation.

11.2 With regard to these risk assessment Guidelines, assistance should include provision of data and information required to undertake a risk assessment, technical assistance regarding the methods for undertaking risk assessment and acceptance criteria.

APPENDIX

APPLICATION TO PORT STATE

An application for exemption to the port State should as a minimum contain information on the points listed below.

1 GENERAL INFORMATION

- Period for which an application is sought; from month and year to month and year.
- Why an exemption under regulation A-4 is sought.

2 SHIP'S INFORMATION

- Ship name
- IMO number
- Port of registry
- Gross Tonnage
- Owner
- Call sign
- Ballast water management option usually undertaken by ship, including ballast water treatment technology, if installed
- A copy of the Ship's Ballast Water Management Plan should be submitted
- The Administration may also require ballast water and sediment management history for a determined period

3 ROUTE INFORMATION

- Route of application, given as donor port(s) and recipient port for ballast water discharge.
- If single voyage: Date and time of departure and arrival.
- If multiple voyages: Voyage frequency, regularity and estimated amount of ballast water discharged during the exemption period. Estimated time and dates for departures and arrivals.
- Any voyages the ship plans to take to ports other than the specified ports during the duration of the exemption.
- If multiple voyages, the estimated total number of voyages and the amount of ballast water discharged under the duration of the exemption.

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I. INTERNATIONAL PLANT PROTECTION CONVENTION

IPPC SECRETARIAT'S DISCUSSION PAPER ON INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES AND THEIR APPLICATION TO LMOs

Introduction

1. The Interim Commission on Phytosanitary Measures (ICPM), at its Third Session, in April 2001 agreed that plant pest risks associated with living modified organisms / products of modern biotechnology (LMOs) fall within the scope of the IPPC. In particular, the ICPM endorsed the results of the June 2000 Open-Ended Exploratory Working Group on the Phytosanitary Aspects of GMOs, Biosafety and Invasive Species (OEWG). The Working Group noted that, consistent with the IPPC mandate to protect plant health, plant pest concerns that may be presented by LMOs/products of modern biotechnology fall within the scope of the IPPC. The group agreed that IPPC risk analysis and management systems are appropriate for assessing and managing, if necessary, the direct or indirect risks of pests to cultivated and wild flora and plant products that may be presented by LMOs/products of modern biotechnology. It was also agreed that IPPC systems and procedures are relevant to, and adequate for, managing the risks posed by LMOs/products of modern biotechnology as they relate to the protection of plant health. Existing national mechanisms and structures for phytosanitary systems may form a basis or a model for developing other practical approaches to managing risks associated with LMOs/products of modern biotechnology. Finally, the group noted that plant pest risks associated with LMOs/products of modern biotechnology fall clearly within the scope of the IPPC.

2. The purpose of this paper is to highlight where existing ISPMs provide guidance on plant pest risk analysis of LMOs. The international standards discussed in this document can be found on the International Phytosanitary Portal (http://www.ippc.int). It should be noted that ISPMs are guidelines, and each Contracting Party interprets and applies these guidelines in the manner that best suits their infrastructure, capabilities and needs. ISPMs allow for a flexible approach to PRA and are intentionally open and flexible to allow for different approaches depending on need and application, while still ensuring members remain true to the principles of the IPPC and hence the WTO-SPS. The application of these guidelines to LMOs does not exclude consideration of the unique risks presented by LMOs.

ISPM No. 1 (2006): *Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade*

3. This standard describes the various principles reflected in the text of the IPPC.

Basic principles include:

- -sovereignty
- -necessity
- -managed risk
- -minimal impact
- -modification
- -transparency
- -harmonization
- -equivalence

Operational principles include:

- -pest free areas and areas of low pest prevalence
- -pest risk analysis
- -pest listing
- -surveillance
- -phytosanitary certification
- -dispute settlement
- -prompt action
- -emergency measures
- -notification of non-compliance
- -information exchange
- -technical assistance

4. ISPM No. 1 reflects the purpose of the IPPC-- to secure common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote measures for their control—while ensuring that measures imposed are the least trade restrictive measures available. The development and adoption of new ISPMs has strong ties to the principles identified in ISPM No. 1. Risk analysis and managed risk are the key principles that apply to plant pest risks of LMOs.

ISPM No. 2 (2007): ***Framework for pest risk analysis***

5. ISPM No. 2 applies to both quarantine and regulated non-quarantine pests. The standard elaborates on the steps for conducting PRA: initiation, risk assessment and risk management. The initiation stage (**Section 1**) involves identifying the pests or pathways for which further analysis is needed. The second step is the assessment of the likelihood of entry, establishment and spread of a pest, and the potential economic importance. Risk management is the evaluation of various options for mitigating or reducing the risk. The PRA is usually applied to a specific area, the "PRA area", which may be a country, part of a country, or parts or all of several countries.

6. Living modified organisms are discussed in Section 1.2.4.
"Types of LMOs for which a PRA may be conducted include:

- plants for use in agriculture, horticulture or silviculture, bioremediation of soil, for industrial purposes, or as therapeutic agents (e.g. LMO plants with an enhanced vitamin profile)
- biological control agents and other beneficial organisms modified to improve their performance
- pests modified to alter their pathogenic characteristics.

The modification may result in an organism with a new trait that may now present a pest risk beyond that posed by the non-modified recipient or donor organisms, or similar organisms. Risks may include:

- increased potential for establishment and spread
- those resulting from inserted gene sequences that may act independently of the organism with subsequent unintended consequences
- potential to act as a vector for the entering of a genetic sequence into domesticated or wild relatives of that organism, resulting in an increase in the pest risk of that related organism
- in case of a modified plant species, the potential to act as a vector for the entering of an injurious genetic sequence into relatives of that species.

PRA is usually concerned with phenotypic rather than genotypic characteristics. However, genotypic characteristics should also be considered when assessing the pest risks of LMOs.

Predictive indicators more specific to LMOs include intrinsic attributes such as:

- phenotypic similarities or genetic relationships to known pest species
- introduced changes in adaptive characteristics that may increase the potential for introduction or spread
- phenotypic and genotypic instability.

For LMOs, identification requires information regarding the taxonomic status of the recipient and the donor organism, and description of the vector, the nature of the genetic modification, and the genetic sequence and its insertion site in the recipient genome.

Further potential risks of LMOs are outlined in Annex 3 to ISPM No. 11 (*Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms*, 2004). A PRA may be carried out to determine whether the LMO is a pest, and subsequently assess the pest risk."
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ISPM No. 11 (2004) ***Guidelines for pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms***

7. This standard describes the PRA process for "quarantine pests". A **quarantine pest** is defined as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled."

8. Many of the same issues that arise for ISPM No. 2 with respect to LMOs also apply to ISPM No. 11 and specific considerations for LMOs have been integrated into ISPM No. 2 and marked with a "*S2*" in the left margin of the text. In particular initiation of the PRA for an LMO would most likely be considered to be "pest initiated" if the LMO was being evaluated as a potential plant pest. Likewise, the introduction potential of the LMO would be established. However, ISPM No. 11 provides considerably more guidance than ISPM No. 2 in parts of the assessment stage. In particular, **Section 2.2.1.5** discusses the probability of transfer to a suitable host, including dispersal mechanisms (including vectors), intended use of commodity and risks from by-products—each of which may be applicable to examining the pest potential of an LMO. **Section 2.2.2** also

provides extensive guidance on evaluating probability of establishment, taking into account such factors as availability of suitable hosts, alternate hosts, and vectors in the PRA area, suitability of the environment, cultural practices, reproductive strategy of the pest, and genetic adaptability. This last factor may be especially applicable to the evaluation of the pest potential of LMOs.

9. **Section 2.3** discusses the assessment of potential economic consequences. As with ISPM No. 2, a pest must have the potential to cause economic harm. However, certain indirect effects of an LMO (e.g. effects on non-target organisms) may be difficult to quantify economically. Nonetheless, **Section 2.3.1.1** and **Section 2.3.1.2** discuss direct and indirect pest effects, respectively, and include assessment of environmental effects as well as assessing the potential for crop losses.

10. The IPPC recognizes that uncertainty is a component of PRA and provides guidance on accounting for the nature and degree of uncertainty in the overall analysis (ISPM No. 2, Section 3.1; ISPM No. 11, Section 2.4). Care is taken to document areas of uncertainty and the degree of uncertainty in any analysis, and to indicate where expert judgement has been used, including in examining risk management options. It should be stressed that phytosanitary measures are intended to account for uncertainty and should be designed in proportion to the assessed risk."

11. **Section 3** provides guidance on management options. **Section 3.4.1** discusses risk management options for consignments, including pre- and post-entry quarantine, specified conditions for the consignment and restrictions on end use, all of which may be applied to LMOs.

12. Annexes 2 and 3 of ISPM No. 11 outline how the IPPC applies to risks associated with LMOs and the criteria for determining the potential for a living modified organism to be a pest. They are attached as Annex 2 and 3, respectively, to this document. Annex 1 of ISPM No. 11 outlines how environmental harm might be considered in a PRA. It is attached as Annex 1 to this document.

ISPM 5 (2008): *Glossary of phytosanitary terms*

13. The development and use of internationally agreed terminology under the auspices of the IPPC has served the phytosanitary community since the IPPC was originally adopted. This terminology is recognized by national, regional and international organizations as the appropriate terms to use with respect to matters related to plant protection and has been incorporated in national legislation and regulations throughout the world. Many terms overlap significantly with concepts currently under the CBD and the Cartagena Protocol on Biosafety. The Cartagena Protocol on Biosafety carries with it the need for the application of specific terms and definitions for consistency. In some instances, terms developed and used under the IPPC could be applied to situations relevant to the CBD, while in other cases, certain IPPC terms are characteristic to phytosanitary issues and situations (e.g. regulated non-quarantine pest; area of low pest prevalence).

14. The term "**pest**" refers to "any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products". The ICPM "Open-ended exploratory working group on the phytosanitary aspects of GMOs, biosafety and invasive species" asserted that the scope of the Convention applies to wild flora and as such "plants" includes non-cultivated plants as well as cultivated plants. It also agreed that in cases where LMOs have the potential to cause harm to plant life or health, they could be considered "pests" and treated as such.

15. The term "potential economic importance" is an important element of the definition of quarantine pest and one to which considerable emphasis is placed in the PRA process. ISPM No. 5, Supplement No. 2 provides background information and other relevant information to clarify the meaning of this term within the IPPC and ISPMs. The Supplement clarifies that "potential economic importance" can include impacts expressed in both monetary and non-monetary terms, that markets are not the sole indicator of pest consequence, and that members maintain the right to adopt phytosanitary measures with respect to pests for which the damage caused to plants, plant products or ecosystems within an area cannot easily be quantified. The Supplement further explains that economic effects should not be interpreted to mean only market effects but is intended to provide a broad framework in which a wide variety of effects including also environmental and social effects may be analysed."

ISPM 6: *Guidelines for surveillance*

16. The scope of this standard is "the components of survey and monitoring systems for the purpose of pest detection and the supply of information for use in pest risk analyses, the establishment of pest free areas, and where appropriate, the preparation of pest lists." Surveillance for LMOs may be a key component to the use and management of LMOs, including the management of risks associated with LMOs. The standard distinguishes between general and specific surveillance. **Section 1** (General surveillance) states that the information obtained through general surveillance may be used to support NPPO declarations of pest freedom, for early detection of pests, and other types of information exchange. The information may also be used to establish host and commodity pest lists and distribution records.

17. **Section 2** (Specific surveillance) describes the components of specific survey programmes designed to obtain information on pests on specific sites over defined periods of time. Like general surveillance, specific surveillance can aid in the early detection of new pests (**Section 2.1**). This section also describes how sites for specific surveys are determined, including:

-previously reported presence and distribution of the pest;
-biology of the pest;
-distribution of host plants of the pest and especially of their areas of commercial production; and
-climatic suitability of sites for the pest.

18. **Section 2.2** describes the determination of survey sites for "commodity or host surveys". Finally, **Section 2.3** discusses the uses of targeted and random sampling, stating that some random sampling should be done to "detect unexpected events". This is especially important to the detection of pests which may affect natural environments (rather than areas under cultivation). However, the relevance of this statement to detecting unpredicted spread of an LMO is important, and demonstrates that surveillance may also be an important component in the assessing and managing plant pest risks of LMOs.

19. **Section 5** outlines the type of information that should be included in pest records (see also ISPM 8). Besides such information as scientific name and collection information, "additional information" may also be appropriate. This may be "nature of host relationship, infestation status, growth stage of plant affected, or found only in greenhouses." For LMOs, additional information describing the genetic modifications should be included in the records.

20. **Monitoring**, "an official ongoing process to verify phytosanitary situations", is considered to be a part of surveillance. The use of monitoring surveys may therefore be an important component to the permitting process for the release of LMOs into the environment. For instance, monitoring may be used after the release of an LMO plant to determine if the LMO has itself become weedy, has outcrossed to wild relatives to produce new weeds, or is otherwise producing some sort of detrimental effects on plants or the environment.

ISPM No. 9 *Guidelines for pest eradication programmes*

21. ISPM No. 9 provides guidance on how countries may undertake to eradicate both quarantine and non-quarantine pests from an area. The procedures outlined in this standard may also be applicable to conducting PRAs for LMOs. One aspect of managing risks associated with LMOs is the development of emergency plans if the LMO unexpectedly causes some problem (outcrossing to wild relatives to create new weeds, becoming weedy or invasive, etc.). **Section 1** describes the formulation of "contingency plans" which is especially important in the management of plant pest risks of LMOs. In particular it specifies that such plans are advantageous in saving time during emergency outbreak situations. Likewise, **Section 2** describes reasons for undertaking an eradication programme, and provides guidance on how an NPPO would carry out the eradication programme. It includes the same types of information that may be used in a pest risk analysis, including pest biology, distribution, potential hosts, potential for spread, possible impacts, and what sort of eradication strategies might be expected to work. ISPM No. 9, therefore, may be considered to be especially important in formulating management strategies for LMOs in cases where an LMO unexpectedly becomes a pest.

Annex 1

COMMENTS ON THE SCOPE OF THE IPPC IN REGARD TO ENVIRONMENTAL RISKS (Annex 1 to ISPM No. 11)

The full range of pests covered by the IPPC extends beyond pests directly affecting cultivated plants. The coverage of the IPPC definition of plant pests includes weeds and other species that have indirect effects on plants, and the Convention applies to the protection of wild flora. The scope of the IPPC also extends to organisms which are pests because they:

- *directly affect uncultivated/unmanaged plants*
Introduction of these pests may have few commercial consequences, and therefore they have been less likely to beevaluated, regulated and/or placed under official control. An example of this type of pest is Dutch elm disease (Ophiostoma novo-ulmi).

- *indirectly affect plants*
In addition to pests that directly affect host plants, there are those, like most weeds/invasive plants, which affect plants primarily by other processes such as competition (e.g. for cultivated plants: Canada thistle (Cirsium arvense) [weed of agricultural crops], or for uncultivated/unmanaged plants: Purple loosestrife (Lythrum salicaria) [competitor in natural and semi-natural habitats]).

- *indirectly affect plants through effects on other organisms*
Some pests may primarily affect other organisms, but thereby cause deleterious effects on plant species, or plant health in habitats or ecosystems. Examples include parasites of beneficial organisms, such as biological control agents.

To protect the environment and biological diversity without creating disguised barriers to trade, environmental risks and risks to biological diversity should be analyzed in a PRA.

Annex 2

COMMENTS ON THE SCOPE OF THE IPPC IN REGARD TO PEST RISK ANALYSIS FOR LIVING MODIFIED ORGANISMS (Annex 2 of ISPM No. 11 (2004))

Phytosanitary risks that may be associated with a living modified organism (LMO) are within the scope of the International Plant Protection Convention (IPPC) and should be considered using pest risk analysis (PRA) to make decisions regarding pest risk management.

The analysis of LMOs includes consideration of the following:

- Some LMOs may present a phytosanitary risk and therefore warrant a PRA. However other LMOs will not present a phytosanitary risks beyond those posed by related non-LMOs and therefore will not warrant a complete PRA. For example, modifications to change the physiological characteristics of a plant (e.g. ripening time, storage life) may not present any phytosanitary risk. The pest risk that may be posed by an LMO is dependent on a combination of factors, including the characteristics of the donor and recipient organisms, the genetic alteration, and the specific new trait or traits. Therefore, part of the supplementary text (see Annex 3) provides guidance on how to determine if an LMO is a potential pest.
- PRA may constitute only a portion of the overall risk analysis for import and release of a LMO. For example, countries may require the assessment of risks to human or animal health, or to the environment, beyond that covered by the IPPC. This standard only relates to the assessment and management of phytosanitary risks. As with other organisms or pathways assessed by an NPPO, LMOs may present other risks not falling within the scope of the IPPC. When an NPPO discovers potential for risks that are not of phytosanitary concern it may be appropriate to notify the relevant authorities.
- Phytosanitary risks from LMOs may result from certain traits introduced into the organism, such as those that increase the potential for establishment and spread, or from inserted gene sequences that do not alter the pest characteristics of the organism but that might act independently of the organism or have unintended consequences.
- In cases of phytosanitary risks related to gene flow, the LMO is acting more as a potential vector or pathway for introduction of a genetic construct of phytosanitary concern rather than as a pest in and of itself. Therefore, the term "pest" should be understood to include the potential of an LMO to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk.
- The risk analysis procedures of the IPPC are generally concerned with phenotypic characteristics rather than genotypic characteristics. However, genotypic characteristics may need to be considered when assessing the phytosanitary risks of LMOs.
- Potential phytosanitary risks that may be associated with LMOs could also be associated with non-LMOs. It may be useful to consider risks associated with LMOs in the context of risks posed by the non-modified recipient or parental organisms, or similar organisms, in the PRA area.

Annex 3

DETERMINING THE POTENTIAL FOR A LIVING MODIFIED ORGANISM TO BE A PEST (Annex 3 of ISPM No. 11 (2004))

This annex is relevant for living modified organisms (LMOs) only where there is potential for phytosanitary risks from the LMO associated with some characteristic or property related to the genetic modification. Other phytosanitary risks associated with the organism should be assessed under other appropriate sections of ISPM No. 11 or under other appropriate ISPMs. The information requirements outlined in section 1.3 may be needed in determining the potential for an LMO to be a pest.

Potential phytosanitary risks for LMOs

Potential phytosanitary risks for LMOs may include:

a. Changes in adaptive characteristics which may increase the potential for introduction or spread, for example alterations in:
- tolerance to adverse environmental conditions (e.g. drought, freezing, salinity etc.)
- reproductive biology
- dispersal ability of pests
- growth rate or vigour
- host range
- pest resistance
- pesticide (including herbicide) resistance or tolerance.

b. Adverse effects of gene flow or gene transfer including, for example:
- transfer of pesticide or pest resistance genes to compatible species
- the potential to overcome existing reproductive and recombination barriers resulting in pest risks
- potential for hybridization with existing organisms or pathogens to result in pathogenicity or increased pathogenicity.

c. Adverse effects on non-target organisms including, for example:
- changes in host range of the LMO, including the cases where it is intended for use as a biological control agent or organism otherwise claimed to be beneficial
- effects on other organisms, such as biological control agents, beneficial organisms, or soil fauna and microflora, nitrogen-fixing bacteria, that result in a phytosanitary impact (indirect effects)
- capacity to vector other pests
- negative direct or indirect effects of plant-produced pesticides on non-target organisms beneficial to plants.

d. Genotypic and phenotypic instability including, for example:
- reversion of an organism intended as a biocontrol agent to a virulent form.

e. Other injurious effects including, for example:
- phytosanitary risks presented by new traits in organisms that do not normally pose phytosanitary risk
- novel or enhanced capacity for virus recombination, trans-encapsidation and synergy events related to the presence of virus sequences
- phytosanitary risks resulting from nucleic acid sequences (markers, promoters, terminators, etc.) present in the insert.

The potential phytosanitary risks identified above can also be associated with non-LMOs. The risk analysis procedures of the IPPC are generally concerned with phenotypic characteristics rather than genotypic characteristics. However, genotypic characteristics may need to be considered when assessing the phytosanitary risks of LMOs.

If there is no indication that new traits resulting from genetic modifications have phytosanitary risks, the LMO may require no further consideration.

It may be useful to consider potential risks in the context of risks posed by the non-modified recipients or parental organisms, or similar organisms, in the PRA area.

In cases of phytosanitary risks related to gene flow, the LMO is acting more as a potential vector or pathway for

introduction of a genetic construct of phytosanitary concern rather than as a pest in and of itself. Therefore, the term "pest" should be understood to include the potential of an LMO to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk.

Factors that may result in the need to subject a LMO to stage 2 of the PRA include:

- lack of knowledge about a particular modification event
- the credibility of information if it is an unfamiliar modification event
- insufficient data on the behaviour of the LMO in environments similar to the PRA area
- field experience, research trials or laboratory data indicating that the LMO may pose phytosanitary risks (see sub-sections a. to e. above)
- where the LMO expresses characteristics that are associated with pests under ISPM No. 11
- existing conditions in the country (or PRA area) that may result in the LMO being a pest
- where there are PRAs for similar organisms (including LMOs) or risk analyses carried out for other purposes that indicate a pest potential
- experience in other countries.

Factors that may lead to the conclusion that an LMO is not a potential pest and/or requires no further consideration under ISPM No. 11 include:

- where the genetic modification in similar or related organisms has previously been assessed by the NPPO (or other recognized experts or agencies) as having no phytosanitary risk
- where the LMO is to be confined in a reliable containment system and not be released
- evidence from research trials that the LMO is unlikely to be a pest under the use proposed
- experience in other countries.

J. PUBLIC RESEARCH AND REGULATION INITIATIVE

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To:
Dr. Ahmed Djoghlaf
Executive Secretary
Convention on Biological Diversity
Montreal, Canada
Email:
Re: Submission in Response to Notification No. 2008-140
Ref.: SCBD/BS/MPDM/ABw/65409

January, 20th 2009

Dear Dr. Djoghlaf,

Please find attached a list of documents relevant to the work of the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management.

On 19/20 February 2009, PRRI will hold in the Netherlands its annual brainstorm session on risk assessment with colleagues from various organisations involved in risk assessment and risk assessment research. The results of that brainstorm meeting will be used in updating and expanding the PRRI Guide on risk assessment. PRRI will keep you posted if any new documents or data transpire from that meeting.

Yours truly,

Dr. Lucia De Souza

Dr. Hector Quemada

Co-chairs of the PRRI Working Group of the Public Research and Regulation Initiative

Relevant existing guidance materials regarding risk assessment and risk management of living modified organisms, submitted by the Public Research and Regulation Initative:

Public Research and Regulation Initiative. 2005. Guide for notifications and risk assessments for releases into the environment of genetically modified organisms. http://pubresreg.org/index.php?option=com_docman&task=doc_download&gid=266.

Johnson, K., et al. 2006. How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends in Plant Science* 12: 1-5.

Romeis, J., et al. 2008. assessment of risk of insect resistant crops to nontarget arthropods. *Nature Biotechnology* 26: 203-208.

K. THIRD WORLD NETWORK

-----Original Message-----
Sent: Tue 1/20/2009 4:41 AM
Subject: Submission of views and/or information in preparation for the Adhoc Technical Expert Group on Risk Assessment and Risk Management

Ahmed Djoghlaf
Executive Secretary
Secretariat of the Convention on Biological Diversity
secretariat@cbd.int

20 January 2009

Dear Dr Djoghlaf

Re:Submission of views and/or information in preparation for the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management(Ref.:SCBD/BS/MPDM/ABw/65409)

In accordance with Decision BS-IV/11, Third World Network is pleased to submit information relevant to the work of the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management.

In this regard, please find attached to this email six documents as our submission.

The documents are relevant chapters from the book 'Biosafety First - Holistic Approaches to Risk and Uncertainty in Genetic Engineering and Genetically Modified Organisms', 2007, Terje Traavik and Lim Li Ching (eds.), Tapir Academic Press, Trondheim, ISBN: 9788251921139, for which permission has been obtained for their unrestricted use in connection with the AHTEG and processes and documentation arising therefrom.

1) Genetically engineered cells and organisms: Substantially equivalent or different? By Traavik, Nielsen and Quist.

2) Genetic engineering and omitted health research: Still no answers to ageing questions. By Traavik and Heinemann.

3) Biodiversity, ecosystem services and genetically modified organisms. By Lovei, Bohn and Hilbeck.

4) Vertical (trans)gene flow: Implications for crop diversity and wild relatives. By Quist.

5) Unintended horizontal transfer of recombinant DNA. By Nielsen and Daffonchio.

6) Potential health effects of foods derived from genetically modified (GM) plants - What are the issues? By Pusztai and Bardocz.

Thank you for your kind consideration.

Yours truly,
Martin Khor
Director
Third World Network

Chapter 8

Genetically Engineered Cells and Organisms: Substantially equivalent or different?

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The dynamic and interconnected regulation of the genome is now slowly being revealed. The genome does not function in a constant, stable and linear fashion, but is instructed by and fine-tunes its activities according to networks of signals received from the external ecosystem and the internal environment of the organism. The genomic signal pathways may be modified by ecosystem variation as well as by physiological changes in the organism. Thus, the chromatin structure, the genome, the epigenome, the transcriptome, the proteome, the metabolome, and the interactome are interlinked and intertwined in various ways with information transfer in multiple directions.

Integration of foreign DNA into an established genome may have unanticipated side-effects, e.g. in terms of chromatin changes, genome instability, unexpected protein products from the transgene(s), and influence on overall organismal gene expression patterns, in quantitative as well as qualitative terms, of the recipient organism. In this chapter we discuss and exemplify, from a precautionary point of view, the changes that may occur in modified genomes and the consequences they may have. We structure the discussion as follows:

1. Lack of precision in recombinant DNA techniques
2. Changes in the genome
3. Changes in the transcriptome
4. Changes in the proteome
5. Changes in the metabalome
6. Changes in the epigenome
7. Changes in the interactome
8. Concluding remarks

1. Lack of precision in recombinant DNA techniques

Genetic engineering (GE) techniques are presented by many as a tool for the safe and predictable production of GMOs. The intended change in gene expression in GMOs is, however, often not simply a matter of transcription and translation of the inserted recombinant DNA sequences, as symbolized by the Central Dogma model (see Chapters 3, 5, 9, and 13). While achieving a stable, single-copy recombinant DNA insertion is the aim of the genetic engineer, it is not the norm.
Available methods for transfer of gene constructs into cells are inefficient and imprecise (see Chapter 4). Insertional mutagenesis is a default consequence of recombinant DNA

insertions. The resulting phenotypic consequences of the insertion events are largely determined by the characteristics of the gene transfer vector and the location of and number of copies inserted per cell.

While many emphasize the precision of recombinant DNA techniques, none of the currently available methods permit predetermination of *where* in the recipient cell-DNA our gene construct will be inserted, or the number of copies that will be inserted into GMOs of commercial relevance. The specific locations of the inserts may nevertheless substantially influence the functions of the inserted DNA as well as its effects on the cell's own genes. For instance, within the same transformed/transfected mammalian cell culture we will find cells with quite different characteristics.

These, in principle indefinite number of variants arise due to varying insertion sites and number of full or partial DNA copies. In addition to full vector copies, a number of rearranged or truncated versions, some of them quite small, may be inserted into some cells. These aberrant versions can still influence the integrity and functions of the recipient genome, and they may go undetected by conventional testing.[1] Impacts arising from uncharacterized insertions cannot be predicted from characterized insertions. Furthermore, if the characterized inserts are identical between, for example, two recombinant maize lines (events), but the insertion sites are different, one *cannot* extrapolate any biosafety conclusions from one line (event) to the other. The context of the insert would obviously be different, as would be the genes that may be affected directly or indirectly and therefore also the resulting plant phenotype.

The integration of foreign DNA (transgene) in a new host genome may influence any of the gene expression control processes described in Chapters 1 and 3. New gene products may also arise and the transgene product may also vary in its properties. For instance, read-through transcription, initiated somewhere in the insert and ending outside it, or initiated in adjacent regions and ending in the insert, may be sources for novel RNAs and recombinant proteins.[2]

The consequences of insertion may, as earlier stated, vary considerably according to the exact insertional locations and/or construct organization. This is valid for the expression of the inserted transgene as well as for changes in the recipient organism's own genes and their expression levels. The insertion may have effects by introducing a change in chromatin structure, the topography as well as the proteins binding to the DNA (Recillas-Targa, 2006), or by inducing changes in DNA methylation patterns and other epigenetic characteristics (see Chapter 5). Furthermore, cis-acting regulatory DNA motifs may be present in the insert, or may arise from the 'new' sequences created by integration that

[1]It is a common phenomenon for transgene constructs to integrate in multiple places in the genome, and for very small parts of the construct to integrate independently of full-sized versions (for recent comprehensive reviews, see Filipecki & Malepszy, 2006; Latham et al., 2006).

[2]Abortive transcription from read-through might, for example, produce novel short and double-stranded (ds)RNA molecules. A risk factor emerging from the production of novel dsRNA is the potential to induce gene silencing either locally, or on other genes. The same dsRNA can have different effects at different concentrations, in some cases showing non-specific effects at concentrations lower than those needed to induce silencing (Zhao et al., 2001). It should also be appreciated that any new RNA transcript may undergo, as described in Chapter 3, a large series of modifications that result in 'a family' of different RNA molecules, all derived from the same original source. The family members do not necessarily give rise to the same proteins or even proteins with similar functions.

can alter the expression level of genes adjacent or even distant to the insert.

GE cell cultures may be used to produce recombinant products *under contained laboratory conditions*. This implies that the product that the gene is coding for (e.g. insulin) is extensively purified before it is taken out of the laboratory, while the GE cells and DNA are destroyed inside the laboratory. Such applications of GE may, in principle, be made safe. However, when recombinant cells are developed and placed in the open environment, changes in the gene expression levels and small metabolite contents will vary according to changing ecosystem conditions.

Under the influence of given sets of ecosystem variables, the recombinant organisms may over time expose phenotypic traits that have environmental or consumer health implications. 'Consumers' may include a number of wildlife species in addition to humans and domestic animals. From biosafety/risk assessment/regulatory points of view it is hence imperative to reveal whether, compared to its unmodified counterpart, a GMO has experienced changes in the interacting regulatory parts, its *'interactome'*: the genome, epigenome, transcriptome, proteome, and metabolome working as overlapping layers of information involved in cellular function (Box 8.1). Only when minimal changes are observed will it be justified to claim 'substantial equivalence'.

Box 8.1 The '-omes' and the '-omics'

Genome: 1) The entire collection of genetic material in an organism, virus or organelle. 2) The haploid set of chromosomes (DNA) of a eukaryotic organism.

Genomics: The study and development of genetic and physical maps, large-scale DNA sequencing, gene discovery, and computer-based systems for managing and analysing genomic data.

Proteome: The full complement of proteins that are found in a particular cell or tissue under a particular set of circumstances. May include information on their relative or absolute abundance.

Proteomics: The study of the structure and expression of proteins, and of the interactions between proteins.

Interactome: The complete collection of all physical protein-protein interactions that can take place within the cell.

Interactomics: The study and construction of comprehensive sets of protein-protein interactions.

Transcriptome: The full complement of expressed gene transcripts, including alternative splice variants that are found in a particular cell or tissue under a particular set of circumstances. This may include information on the relative or absolute abundance of transcripts.

Transcriptomics: The study of the full complement of expressed gene transcripts. Several techniques have been developed for parallel analysis of the expression of thousands of genes, most notably cDNA microarrays and oligonucleotide arrays.

Metabolome: The assembly of substrates, metabolites, and other small molecules that is present in a population of cells.

Metabolomics: Study of the structure and distribution of all metabolites (small molecules), particularly organic compounds.
Functional genomics: A whole spectrum of approaches, under development, to ascertain the biochemical, cellular and/or physiological properties of each and every gene product and its regulation. These include near-saturation mutagenesis (i.e. screening hundreds of thousands of mutants to identify genes that affect traits as diverse as embryogenesis, immunology and behaviour), high-through put reverse genetics (methods to systematically and specifically inactivate individual genes), and elaboration of genetic tools.
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2. Changes in the genome

The whole purpose of a transgenesis process is of course to change the genome of the recipient organism. There are a number of possible, unpredictable consequences of DNA insertions in GMOs. They may be sorted into the following categories:

1. Genome destabilization
2. Chromatin changes with consequences for transgene as well as genome gene expression
3. *De novo* methylation of the transgene or spread of the transgene methylation pattern to endogenous genes, i.e. epigenomic effects
4. Introduction of new regulatory elements, e.g. promoters, enhancers and dehancers, known or hidden splice sites, start codons, terminators, etc. These may cause:
    a. Unpredictable, environment-dependent level of transgene expression, and
    b. Unpredictable, environment-dependent influence on expression pattern of recipient genome in terms of:
        i. Signal transduction-dependent *promoter* effects
        ii. Signal transduction-dependent *enhancer/silencer* effects
        iii. Signal transduction-dependent effects of transferred DNA methylation patterns
5. Activation of endogenous mobile elements (‘jumping genes’). Once activated, they may engage in:
6. Reinsertion at new chromosomal loci
7. Horizontal gene transfer to other individuals or species
8. Unanticipated and unpredictable changes in gene products, e.g. by posttranslational modifications
9. Silencing or over-expression of genes.

Some prominent uncertainties are related to the fact that the recipient organism receives a new promoter/enhancer. These elements govern the gene expression levels of their attached transgenes, but after insertion, they may also change the gene expression and methylation patterns in the recipient chromosome(s) over long distances up- and downstream from the insertion site. Promoters/enhancers function in response to signals received from the internal or external environment of the organism. For a GMO this may result in unpredictability with regard to:

- The chromatin organization and contents of the recipient genome
- The expression level of the inserted transgene(s)
- Altered expression of a large number of the organism's own genes
- Altered influence of geographical, chemical (i.e. *xenobiotics*) and ecological variables of the environment
- Transfer of vector sequences within the chromosomes of the organism, and vertical and/or horizontal gene transfer to other organisms.

Few published studies have been devoted to the clarification of such putative changes in GMOs.

2.1 Observations from studies of GM plants[3]

Agrobacterium-mediated gene transfer to plants can result in insertion site mutations of the T-DNA, leading to truncations, interspersions, or other complex rearrangements of the recombinant DNA. Superfluous T-DNA integration frequently accompanies *Agrobacterium*-mediated transformation, where whole and partial copies of the transgenes become integrated.

For example, a molecular analysis of *Agrobacterium*-transformed *Arabidopsis thaliana* plants revealed that 80% of the transformants had a single insertion event; of these, only 22% contained a single copy of the transgene (the desired number for stable integration and expression in transgenic lines), and the remainder of these single-insertion events contained incomplete T-DNAs, tandem T-DNAs, or T-DNA fragments. These results indicate that even relatively simple T-DNA insertions undergo large- or small-scale rearrangements during the transformation process.

Plants transformed via particle bombardment methods are often more likely than *Agrobacterium*-mediated transformed plants to demonstrate complex integration patterns. The majority of integrated DNA is either arranged as multiple copies of the intact transgene, or as multiple copies with interspersed plant genomic DNA. Further, short recombinant DNA fragments may frequently integrate along with intact or rearranged multimers.

In a study of transgenes integrated into two lines of transgenic oat, 50 of the 82 transgene fragments identified (61%) were 200 bp or shorter. One study even reported the presence of bacterial DNA at a particle bombardment insertion site. As with *Agrobacterium*-mediated transformation, simple single copy insertion events tend to be the exception, and complex and errant integration the rule.

Given the complex transgene integration locus patterns accompanying transformation, developing a transgenic plant line requires careful selection of stable and high expressing transformation events for product development. However, the initial transformation process is not the only step where the transgenes might undergo significant rearrangement. Tissue culture is a common means to produce sufficient transgenic germplasm for further product development. During this process, undesirable tissue

[3] For further information and references, see the recent review by Latham et al., 2006.

culture-induced genetic rearrangements, termed *somaclonal variation*, can occur in both conventional and transformed lines.

Further along the development of the transgenic plant line is selective crossbreeding with elite crop germplasm for high agronomic performance. This process involves a number of introgressive hybridizations (introgression and subsequent backcrossing) to produce plants homozygous for the recombinant trait in the elite crop line. During this process, the complex nature of the recombinant DNA integration loci can lead to deviations in the expected Mendelian patterns of inheritance. [4] For instance, these irregular patterns have been observed during inheritance in lettuce (McCabe & Mohapatra, 1999), rice, maize, and barley. Subsequent selection procedures of the GM material may also introduce further genomic reorganizations (Hernandez et al., 2003).

2.2 Why do DNA rearrangements occur?

In plants, exogenous DNA transfer (e.g. with *A. tumefaciens* pathogenesis) elicits a wound response that activates nucleases and DNA repair enzymes. The transferred DNA is thus either degraded or used as a substrate for DNA repair, resulting in its potential rearrangement and incorporation in the genomic DNA (Takano et al., 1997). Furthermore, specific transforming plasmid structure and construct properties can enhance recombination events all along the transformation process. Indeed, some genetic elements can act as hotspots and undergo recombination at high frequency. This is, for example, the case for the 3' end of the CaMV 35S promoter, which contains an imperfect palindrome of 19 bp.

Illegitimate recombination can also occur in the borders of the Ti plasmid of *Agrobacterium tumefaciens*, especially in the right border that contains an imperfect palindromic sequence of 11 bp. The 3' end of the *nos* terminator is also theoretically highly prone to recombination (Kohli et al., 1999). Hot spots for recombination may lead to tandem transgene repeats with interspersed plant DNA sequences in a single genetic locus. Presence of several inserts may also result from multimerization in the plasmid before transformation or from multiple insertions.

A number of transgenic and genomic rearrangements have been reported for already commercialized transgenic crop plant varieties. The nature of these rearrangements and what they may mean in a risk assessment context is further discussed in Chapter 9.

3. Changes in the transcriptome

The intention of a transgenic process is to have the transgene expressed. Hence, the intended change is to add one transcript to the transcriptome of the GMO. However, as

[4]Given the likelihood of transgene reassortment during one or more of these steps in the production of a transgenic line, arriving at a stable and well-performing transgenic line requires the careful selection from many transformation events brought through development. Technical dossiers on commercial crop lines invariably suggest the stability of the inserted construct. Yet how robust are these analyses? Documentation of transgene locus structure (organization and copy number) and stability through inheritance in the scientific literature (as well as in applications for commercial approval) almost always rely on Southern blot analysis to demonstrate transgene copy number and integrity of the single-copy inserts. However, recent studies have determined that Southern blot analysis often lacks sufficient resolution to accurately determine copy number or transgene organization, and may have difficulties in detecting small rearrangments or solitary fragments (Hoebeeck et al., 2007).

discussed in Chapter 3, and earlier in this chapter, the inherent lack of insertion precision may lead to the expression of additional, unintended transcripts as well.
Although only a small number of published studies have been designed to reveal transcriptome aberrations in GMOs, there are published studies that exemplify the following:

1. Qualitative transcriptome changes, due to inefficient terminator motifs in a transgenic plant variety
2. Quantitative transcriptome changes, due to the influence of the transgene regulatory sequences on endogenous genes located close or distant to the insertion site.

3.1 Example of new transcripts originating from a plant transgene
New evidence suggests that the nos terminator sequence used in a number of transgenic plant varieties is a recombination hotspot, prone to read-through, and may contain a cryptic cis-acting splice sequence that could generate novel RNA molecules and proteins at any place it is inserted into the genome (Rang et al., 2005).

The Roundup Ready (RR) soybean varieties derive from a soybean line into which a gene coding for glyphosate-resistant enol-pyruvylshikimate-3-phosphate-synthase (EPSPS) was introduced. The insert and the flanking regions in RR soybean have recently been characterized. It was shown that a further 250-bp fragment of the *epsps* gene is localized downstream of the introduced *nos* terminator of transcription, derived from the nopaline synthase gene of *Agrobacterium tumefaciens*. At least 150 bp of this DNA region is transcribed in the RR soybean variety.

Transcription of the additional fragment depends on whether read-through events ignore the *nos* terminator signal located upstream. The data indicate that the read-through product is further processed, resulting in four different RNA variants from which the transcribed region of the *nos* terminator is completely deleted. Deletion results in the generation of open reading frames which might code for (as yet unknown) EPSPS fusion proteins. The *nos* terminator is used as a regulatory element in several other transgenic plants intended for food production. This implies that read-through products and transcription of RNA variants might be a common feature in such plants.

3.2 Examples of the activity of the 35S CaMV plant promoter in mammalian cells
In most of the transgenic crop plants commercialized, the transcription of the transgene is governed by the 35S promoter taken from the Cauliflower Mosaic Virus (CaMV). CaMV is a DNA-containing para-retrovirus that replicates by means of reverse transcription. It was earlier assumed that the 35S promoter exclusively functions in plants, and that it would therefore not represent a food/feed safety issue if the transgene under the control of such promoter would transfer horizontally. The following quote is representative of this assumption: ‘There have also been (scientifically unfounded) concerns that the strong plant virus promoter used to express transgenic DNA might be active in mammalian cells’ (Gasson & Burke, 2001).

There have now been published studies indicating that the 35S CaMV promoter has potential for transcriptional activation in mammalian systems, in addition to studies in

different yeast species. First, 35S promoter activity was demonstrated in human fibroblast cell cultures, thereafter in hamster cells, and very recently 35S promoter activity was established in human enterocyte-like cells (Myhre et al., 2006). Such cells line the surface of human intestines, and are hence highly relevant to whether uptake of transgenic DNA from the gastro-intestinal tract may have effects on the host if unintentionally taken up. However, no published studies have investigated 35S CaMV activity *in vivo*, and this is hence an obvious area of omitted research. This example illustrates how safety assumptions/claims made in the absence of experimental investigation on the issue can be misleading.

3.3 Example of upregulation of an endogenous gene under the influence of a transgene promoter

X-Scid is a disease linked to a defective gene on the X chromosome that leads to a total breakdown of the immune system due to lack of T cells. Victims are known in the media as 'bubble boys', having to live their short lives within totally contained plastic cubicles, since every kind of innocent infection will kill them.

A gene therapy protocol was developed in order to cure, or at least alleviate the symptoms of X-Scid victims. Bone marrow cells were taken from the patient and grown in culture. The cells were transfected with a vector that contains a healthy copy of the defective gene. The vector was a deletion mutant of MLV (murine leukaemia virus), with the transgene under control of a strong promoter. After having the bone marrow cells controlled for expression of the transgene, and observing a lack of any unwanted phenotypic characteristics, the cells were returned to the patient. The rationale was that the transferred healthy gene, following integration into the genomes of the bone marrow cells, should produce the proteins that make production of T cells possible, and hence provide the patient with a functional immune system.

In an initial series of 11 treated patients, the strategy seemed to work according to plan, until a tragic setback was recognized: one of the treated patients developed a highly aggressive type of cancer. It turned out that in treated cells from this patient, the gene transfer vector had integrated into a genomic location next to the *Lmo2* gene. This gene encodes a protein product that is known to be cancer causing when over-expressed. In the present case, the strong promoter of the gene therapy vector had forced the *Lmo2* gene to over-express. In a commentary article in *New Scientist* these events were dubbed 'Gene therapy's worst nightmare'. Yet what was observed was an illustration of the known insertion site unpredictability of current recombinant DNA techniques.

3.4 Does 'transvection' occur during transgenesis in mammalian cells?

A relevant question to ask is whether known, unknown or hidden DNA motifs in the gene vector, including its plasmid backbone sequences, may act as transcriptional enhancers and hence influence transcription of endogenous genes, whether integrated in the host genome or present on an un-integrated vector. Transcriptional enhancers are relatively short (30–500bp), *cis*-acting DNA sequences usually comprised of several binding sites for TF (transcription factor; see Chapter 3) activator proteins. The hallmark of enhancers is their ability to communicate with promoters, often activating genes over a large

distance. Some enhancers are able to activate promoters in *trans*, i.e. when the enhancer is on a different genomic entity than the promoter.

Recent studies (D'Aiuto et al., 2006) have demonstrated that a CMV (human cytomegalovirus) enhancer can increase the activity of its cognate promoter in *trans*, in the absence of factors that physically bring the enhancer into close proximity of the promoter. A process like this is called *transvection*. Interestingly, the authors also provided evidence that the CMV enhancer may activate other promoters in the modified host genome. Because such transactivation effects may result in unwanted or unexpected transcriptional activation of endogenous genes, these findings are important for conception of the range of transcriptional effects expected in various genetic engineering and gene therapy approaches.

4. Changes in the proteome

Inherent to a recombinant organism is one or more intended proteomic changes, namely the expression of the transgenic protein(s) that will confer the desired new trait or property.

As earlier indicated in the present chapter, integration of foreign DNA may lead to additional quantitative and qualitative differences in the expressed proteins in a modified cell. Chapter 3 outlined some of the cellular processes that may lead to unexpected protein products from any given gene sequence. All these processes also apply to transgenes as well. Unfortunately, there are few published studies that have systematically compared the proteomes of GMOs to their unmodified counterparts. There are, however, two examples that illustrate the profound and unpredictable differences in the biological functions of a recombinant protein when it is being post-translationally modified, i.e. glycosylated, in its new host organism.

4.1 An α-amylase inhibitor-1 gene transferred from common bean to pea

It was recently shown that expression of a recombinant plant protein (α-amylase inhibitor-1, αAI) from the common bean in a non-native host plant, i.e. transgenic pea, led to the synthesis of a structurally modified, probably aberrantly glycosylated form, of this inhibitor (Prescott et al., 2005). Employing models of inflammation, it was demonstrated that consumption of the modified αAI and not the native form predisposed the mice to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the modified αAI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these usually non-immunogenic proteins. This investigation demonstrated that recombinant expression of non-native proteins in plants may lead to the synthesis of structural variants with altered immunogenicity. The frequency at which alterations in structure and immunogenicity of recombinant proteins in new hosts occur is most often not known.

4.2 Production of recombinant protein in milk

The European Medicine Agency's (EMEA) decision in February 2006 to approve a recombinant product containing anthithrombin-α, had been eagerly awaited because it would be the first drug produced in a transgenic farm animal to reach the market. The

active ingredient, *human anthithrombin-α*, is produced by and purified from the milk of transgenic goats. GTC Biotherapeutics has been developing Atryn since 1993, principally for treating patients suffering from hereditary anthithrombin deficiency, a rare condition affecting one person in every 3–5000, that puts them at increased risk of deep vein thrombosis.

The decision of EMEA was, however, based on a lack of appropriate data to allay concerns about Atryn's immunogenicity. As pointed out by an anonymous editorial commentator in *Nature Biotechnology* (2006, 24: 368), the EMEA decision '*rather skirts around some of the underlying issues that transgenic protein producers have to face*'. These issues are discussed in Chapters 3 and 4, in addition to the present chapter of this book.

Of particular concern are different and unpredictable posttranslational modifications compared to native proteins. In the case of Atryn, this really seems to matter. Compared with a conventional anthithrombin-α product, Atryn's serum half-life was reduced seven- to ten-fold, necessitating infusion of the protein rather than a one-off injection.
One of EMEA's main concerns with Atryn was, however, its potential immunogenicity. The underlying problem is that it is extremely difficult to produce 'nature-identical' proteins in milk from transgenic animals. For instance, in cows, sheep and goats, glycosylated proteins typically contain N-glycolylneuraminic acid (NGNA), a modification which is virtually absent in native human proteins. Furthermore, the high concentration of protein produced in milk, around a gram per litre, overrides the glycosylation capacity of the mammary gland. Only rabbits and chickens have human-like glycosylation patterns. The *Nature Biotechnology* commentator concluded: '*Thus, if immunogenicity of milk-produced proteins turns out to be a generic problem, then a whole class of transgenic production methods may turn out to have a limited future. Chicken milk, anyone*?'

5. Changes in the metabolome

Unintended effects of transgenesis are closely related to changes in the metabolite levels. One of the major challenges is how to analyze the overall metabolite composition of GMOs in comparison to their unmodified counterparts. Metabolomics offer one possible solution.

The quality of crop plants is a direct function of the metabolite content. The metabolome determines the flavour, aroma and texture of crops, their storage properties, nutritional values and performance in the field. Genetic (metabolic) engineering has the potential to improve plant properties. However, problems may arise from such approaches because the organismal metabolism forms a large interconnected network. '*Just as the flap of a butterfly wing might cause a hurricane, changes in the flux of one branch might lead to unexpected changes in other parts of the network*' (Memelink, 2005).

A number of unexpected changes following genetic engineering have been seen in experimental studies with, for instance, *Arabidopsis* sp. and tomatoes (e.g. Romer et al., 2000; Hemm et al., 2003). Field trials with transgenic wheat lines have demonstrated how

profoundly the environment affects the metabolome of transgenic as well as unmodified varieties, but have also demonstrated important differences between a transgenic wheat line and its parental, unmodified counterpart (Baker et al., 2006).

Potatoes produce a number of toxic secondary metabolites, which are divided into two groups: the sesquiterpenes and the glycoalkaloids (PGAs). Whereas PGAs are largely produced and present in toxic quantities in both the foliage and 'green' potatoes, it is well documented that the levels of PGAs and sesquiterpenes are affected by biotic and abiotic stress. The development of GM potato varieties has made it prudent to ascertain whether there may be changes in the amounts or types of these secondary metabolites, either as a direct effect of the transgene or due to its interactions with environmental variables. One such study has been published by Matthews et al. (2005). Transgenic potato lines were exposed, along with non-transgenic lines, to a range of biotic and abiotic stresses and a range of environmental conditions in the field and store. Following stress, a comparison was made of levels of potato glycoalkaloid and sesquiterpene levels between the two groups. Significant differences were observed in the levels of both glycoalkaloid and sesquiterpene levels between transgenic and control material and between infected and noninfected material. The study did, however, also illustrate the profound impact that environmental parameters may have on the metabolome of transgenic as well as unmodified potatoes.

6. Changes in the epigenome

Epigenetic changes[5] can be induced in cells during the transgenesis process, and to become inherited in the consecutive generations (Filipecki & Malepszy, 2006). It is, however, difficult to ascertain whether epigenetic imprinting is due to the transgenesis or cell regeneration techniques. It is known from a number of organisms that an inserted DNA fragment may both transfer its own methylation pattern to the surrounding DNA and have its own pattern changed by the surrounding recipient DNA.
The transgenesis process may induce mutagenic-stress related mechanisms described as 'programmed loss of cellular control'. According to Filipecki and Malepszy (2006), this may lead to (i) genetic changes such as polyploidy, aneuploidy, chromosome rearrangements, somatic recombination, gene amplifications, point mutations, and excisions and insertions of retrotransposons, and (ii) epigenetic changes, including DNA methylation and histone modifications.

Regulation of gene expression by induced changes in DNA methylation is a very potent regulatory mechanism. DNA methylation is based on the existence of 'the 5th base' (see Chapter 5). Transgenesis may induce methylation changes in both directions:

- DNA *hypomethylation* leading to
    - Gene activation

[5]Epigenetics (see also Chapter 5) was introduced by Conrad Waddington in 1942 as the study of the processes by which genotype gives rise to phenotype. In 1987, Robin Holliday redefined epigenetics as: 'Nuclear inheritance which is not based on differences in DNA sequence'. Epigenetics encompasses heritable changes in DNA or its associated proteins except mutations in gene sequence. Many investigators in the field of epigenetics focus on histone modifications and DNA methylation, two molecular mechanisms that are often linked and interdependent.

    - Chromosome instability
- DNA *hypermethylation* leading to
    - Gene silencing
    - Chromatin remodelling
    - RNA-associated silencing.

In recombinant plants, DNA methylation changes may occur in both directions, but *hypomethylation* has been more frequently reported. Already in 1996 it was clearly demonstrated that different epigenetic expression states might arise in transgenic plants regenerated from the same material (Matzke & Matzke, 1996), and that these states are stably inherited to the following generations.

As pointed out earlier, the influence of the environment on the initiation and persistence of epigenomic programmes cannot be overestimated, but this is an area of omitted research. In spite of a considerable number of peer-reviewed articles concerning epigenetic consequences of transgenesis in model organisms such as *Arabidopsis*, the epigenomes of marketed, transgenic crop plants are virtually unknown.

7. Changes in the interactome

The concepts and technologies of classical molecular biology have dominated genetic engineering approaches during the last 50 years. This has favoured methods that have approached complex processes by separation and isolation of single pathways and molecules. Nonetheless, biologists have continually been aware that a fundamental characteristic of all biological organization is that functional units never exist in isolation. Biological complexity is based on synergistic cooperation achieved by interactions between the components of the cell (Uhrig, 2006). Proteins are essential for almost all biological processes. They operate entirely on the basis of interactions with other molecules, i.e. other proteins, nucleic acids, lipids, or low molecular metabolites and other compounds.

Only rarely is the protein monomer the functionally active form, as most often assumed when using transgenes. Comprehensive knowledge of protein interactions is therefore an important source of information to functionally annotate proteins and to understand and model processes on a genome-wide level (see also Chapter 3). That the transgenic protein product provides the intended function and trait (e.g. insecticidal effects or herbicide tolerance in plants) does not preclude that it contains additional active domains that become evident in its new genomic, biological and environmental host context. Such ‘novel’ domains may be inherent in the amino acid chain, or arise as a result of alternative folding due to host-specific post-translational modifications (see Chapter 3). The recombinant protein may therefore engage in complex formations with endogenous proteins and other cellular components when present in novel environments. This may, in turn, lead to activation or inhibition of cellular processes, or even create new intracellular processes. To what extent this occurs is unknown, since the studies needed for clarification are rarely conducted.

8. Concluding remarks

As stated by Haslberger (2006), there is a general need for a holistic and integrated basis for assessment of the properties and effects of GMOs. This conclusion was also drawn by a recent World Health Organization (WHO) report (2005). Lack of knowledge concerning the putative and unpredictable changes in the contents of GMOs discussed in this chapter have won increasing acceptance during recent years. A fact that has been reflected in a number of expert committee reports from international organizations such as WHO, the Food and Agriculture Organization (FAO), and the Organization for Economic Cooperation and Development (OECD). Many of the risk issues identified here that lack answers (see also Chapter 9) were identified before the first transgenic plants were commercially grown in 1996. The application of the modern '-omics' techniques can contribute to reveal many risk-relevant differences in composition between recombinant organisms and their isogenic, parental counterparts under relevant environmental conditions.

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Chapter 9

Genetic Engineering and Omitted Health Research: Still No Answers to Ageing Questions

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Introduction

Some of the most crucial scientific questions concerning the health effects of genetic engineering (GE) and genetically modified organisms (GMOs) were raised up to twenty years ago.[1] Most of them have still not been answered at all, or have found unsatisfactory answers. We believe, as Mayer and Stirling[2] said, 'in the end it is often the case that those who choose the questions determine the answers'. Will another twenty years pass before societies realize the urgent need for public funding of genuinely independent risk- and hazard-related research? The time for such investment is now, so that a new scientific culture with working hypotheses rooted in the Precautionary Principle (PP)[3] can discover other, possibly even more important questions of safety.

In this chapter we will mainly confine ourselves to putative health hazards related to GM plants used as food or feed, with some brief notes on GM vaccines as well as the novel RNAi- and nanobio-technologies. Our focus is not because we do not recognize the paramount, indirect threats to public health posed by social, cultural, ethical, and economic issues, as well as the complexities posed by the relevant legal and regulatory environments, but for reasons of space. In the specific context of food or feed safety assessment, 'hazard' may be defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The hypothetical hazards of whole GM foods, i.e. those hazards that have been realized so far, fall into a few broad categories.

First, there are those either related to the random and inaccurate integration of transgenes into recipient plant genomes, with uncertainty with regard to direct or indirect effects of the polypeptide product of the transgene, or uncertainty with regard to DNA types and circumstances promoting uptake and organ establishment of foreign DNA from mammalian gastro-intestinal tracts.[4] Second, there are those that might come from the purposeful production of potential hazards, such as allergens or powerful pharmaceutical products.

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[1]See for instance: Freese, W. and Schubert, D. (2004). Safety testing and regulation of genetically engineered foods. Biotechnology and Genetic Engineering Reviews 21: 299-324, or Pusztai, A. (2002). Can science give us the tools for recognizing possible public health risks for GM food? Nutrition and Health 16: 73-84.
[2]Mayer, S. and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024.
[3]Myhr, A.I. and Traavik, T. (2002). The precautionary principle: scientific uncertainty and omitted research in the context of GMO use and release. Journal of Agricultural and Environmental Ethics 15: 73-86.
[4]For a recent, authoritative review see: The Royal Society of Canada (2001). Elements of Precaution: Recommendations for the regulation of food biotechnology in Canada. An expert panel report on the future of food biotechnology prepared by the Royal Society of Canada for Health Canada, Canadian Food Inspection Agency and Environment Canada (ISBN 0-920064-71-x), www.rsc.ca/foodbiotechnology/index/EN.html

A number of scientific concerns have been raised in connection with public and animal health. In the following sections we will discuss, in some detail, a few of these. Some of them have been thoroughly discussed in excellent, recent reviews.[5]

Our contribution is based on 'gene ecology', *a new, cross-disciplinary scientific field aimed at providing holistic knowledge based on the Precautionary Principle.*[6] Some of the concerns we raise will also be relevant for environmental risk assessments of GMOs, due to the fact that the processes discussed can take place in large ecosystems as well as in the ecosystems at the scale of the human being.

Do we know whether any GM food/feed is safe for consumption?

For a composite material such as food/feed, reductionist approaches testing single components *in vitro* are highly unsatisfactory and cannot clarify important safety issues. In spite of the obvious need, very few studies designed to investigate putative effects of GM nucleic acids or food/feed on potential animal or human consumers have been published in peer-reviewed journals.[7] A consensus has emerged that the effects observed in some published studies[8] must be experimentally followed up. To date, this has not been done.

Most of the animal feeding studies conducted so far have been designed exclusively to reveal husbandry production differences between GMOs and their unmodified counterparts. Studies designed to reveal physiological or pathological effects are extremely few, and they demonstrate a quite worrisome trend[9]: Studies performed by the GM plant producers find no problems, while studies from independent research groups often reveal effects that should have merited immediate follow-up, confirmation and extension. Such follow-up studies have not been performed. There are two main factors accounting for this situation: The lack of funds for independent research, and the reluctance of producers to deliver GM materials for analysis.[10]

Can we rely on the transgenic DNA sequences given by GM food/feed producers?

If the transgenic DNA sequences given in the notifications differ from the inserted sequences found in the GM plants, the risk assessments made prior to approval of the GM plants for marketing do not necessarily cover the potential risks associated with the GM plants.
The most thoroughly studied transgenic events are:

- Bt-transgenic maize Mon810
- Bt- and glufosinate-transgenic maize Bt176
- Glyphosate-transgenic maize GA21
- Glufosinate-transgenic maize T25 (Liberty Link)
- Glyphosate-transgenic soybean GTS 40-3-2.

[5]See Footnote 1, and e.g. Pusztai, A., Bardocz S. and Ewen S.W.B. (2003). Genetically modified foods: potential human health effects. Pp. 347-371, in Food Safety: Contaminants and Toxins, edited by JPF D'Mello. CAB International.

[6] For further information see the homepages of GENOK-Norwegian Institute of Gene Ecology, www.genok.org and INBI-Centre for Integrated Research in Biosafety, www.inbi.canterbury.ac.nz

[7]Domingo, J.L. (2000). Health Risks of GM Foods: Many opinions but few data. Science 288: 1748-1749.

[8]E.g. Fares and El-Sayed (1998). Fine structural changes in the ileum of mice fed on endotoxin-treated potatoes and transgenic potatoes. Natural Toxins 6(6): 219-233; Ewen and Pusztai (1999). Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. The Lancet, Vol. 354, 16 October 1999.

[9]Pryme, I.F. and Lembcke, R. (2003). In vivo studies on possible health consequences of genetically modified food and feed – with particular regard to ingredients consisting of genetically modified plant materials. Nutr Health 17(1): 1-8.

[10]For documentation and further reading see Footnotes 1 and 2 and references therein.

Even amongst the most thoroughly studied and some of the oldest commercial GM plants, recent independent work has revealed that rearrangements occur in transgene inserts and the nature of the rearrangements varies. Deletions (Mon810, GA21, Bt176), recombination (T25, GTS 40-3-2, Bt176), tandem or inverted repeats (T25, GA21, Bt176), as well as rearranged transgenic fragments scattered through the genome (Mon810) have been reported.[11]

The transgenic modification techniques are prone to introduce such rearrangements because exogenous DNA transfer in plants elicits a 'wound' response, which activates nucleases and DNA repair enzymes. This may result in either degradation of the incoming DNA, or insertion of rearranged copies into the plant DNA.[12] In addition, the nature of the DNA constructs used to make transgenic plants may influence the rearrangement tendencies for a given transgenic event. Some genetic elements in the constructs may act as 'hotspots' and elicit recombination at high frequencies.[13]

While it was earlier assumed that integration of transgenic constructs took place at random locations in the recipient plant genome, it has now become apparent that integration sites are often concentrated in or near elements such as retrotransposons (T25, Mon810, GA21) and repeated sequences (Bt11 maize),[14] and this poses additional risks. Firstly, by introducing a new promoter or new enhancer motifs, transgenic insertions into, or close to, such elements may lead to altered spatial and temporal expression patterns of plant genes located close to and even far from, the insert. Secondly, a strong retrotransposon LTR promoter may upregulate the transgene expression level. Thirdly, defective retrotransposons may start 'jumping' under the influence of transacting factors recruited by the insert.[15] All these events may have unpredictable effects on the long-term genetic stability of the GMOs, as well as on their nutritional value, allergenicity and toxicant contents. These putative processes represent areas of omitted research with regard to health effects of GMOs.

[11]Hernandez et al. (2003). A specific real-time quantitative PCR detection system for event MON810 in maize YieldGuard based on the 3'-transgene integration sequence. Transgenic Research 12: 179-189; Holck et al. (2002). 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified MON810 MaisGard maize. Eur Food Res Technol 214: 449-453; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity?; Windels et al. (2001). Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 213: 107-112; Rönning et al. (2003). Event specific real-time quantitative PCR for genetically modified Bt11 maize (Zea Mays). Eur Food Res Technol 216: 347-354.

[12]Takano et al. (1997). The structures of integration sites in transgenic rice. The Plant Journal 11(3): 353-361; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity? In addition to cellular mechanisms controlling the transgene integration, subsequent selection procedures of the GE material may introduce further genomic reorganisations (Hernandez et al. (2003). A specific real-time quantitative PCR detection system for event MON810 in maize YieldGuard based on the 3'-transgene integration sequence. Transgenic Research 12: 179-189).

[13]This is the case for the 35S CaMV promoter that is present in most GEPs marketed so far, and also for the Ti plasmid of Agrobacterium tumefasciens and the nos terminator (Kohli et al. (1999). Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. The Plant Journal 17(6): 591-601; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity? Hot spots may lead to tandem transgene repeats with interspersed plant DNA sequences in a single genetic locus. Presence of several inserts may also result from multimerisation in the plasmid before transformation or from multiple insertions.

[14]Rönning et al. (2003). Event specific real-time quantitative PCR for genetically modified Bt11 maize (Zea Mays). Eur Food Res Technol 216: 347-354.

[15]Jank and Haslberger (2000). Recombinant DNA insertions into plant retrotransposons. Trends in Biotechnology 18: 326.

Are transgenic DNA and proteins taken up from the mammalian GIT (gastro-intestinal tract)?

If DNA and proteins from GMOs persist in, and are taken up from the mammalian GIT, this could theoretically (as will be explained further) ultimately lead to development of chronic disease conditions. The fate and consequences of DNA persistence and uptake is, however, not extensively studied, and therefore represents yet another area of uncertainty connected to GM plants.

It has generally been claimed that DNA and proteins are effectively degraded in mammalian GITs. This has been based on assumptions that have never been systematically examined.[16] A restricted number of recent publications have shown that foreign DNA and also proteins may escape degradation, persist in the GIT and even be taken up from the intestines and transported by the blood to internal organs in biologically meaningful versions.[17] These findings should not have come as such a surprise, since scientific articles from the 1990s[18] strongly indicated that this was an area of omitted research, as stated by a number of reports.[19]

Briefly summarized, there is evidence that relatively long fragments of DNA survive for extended periods after ingestion. DNA may be detected in the faeces, the intestinal wall, peripheral white blood cells, liver, spleen, and kidney, and the foreign DNA may be found integrated in the recipient genome. When pregnant animals are fed foreign DNA, fragments may be traced to small cell clusters in foetuses and newborns. The state of GIT filling, and the feed composition may influence DNA persistence and uptake. Complexing of DNA with proteins or other macromolecules may protect against degradation.

So far, only two published reports have investigated the fate of foreign/transgenic DNA in humans.[20] The consequences of DNA persistence and uptake thus represent yet another area of

[16]Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215.

[17]Schubbert et al. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Mol Gen Genet. 242(5): 495-504; Schubbert et al. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. Proc Natl Acad Sci USA 94(3): 961-6; Schubbert et al. (1998) On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet. 259(6): 569-76; Hohlweg and Doerfler (2001). On the fate of plants or other foreign genes upon the uptake in food or after intramuscular injection in mice. Mol Genet Genomics 265: 225-233; Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215; Einspanier et al. (2001). The fate of forage plant DNA in farm animals; a collaborative case-study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212: 129-134; Klotz et al. (2002). Degradation and possible carry over of feed DNA monitored in pigs and poultry. Eur Food Res Technol 214: 271-275; Forsman et al. (2003). Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract. Mol Gen Genomics 270: 362-368; Chen et al. (2004). Transfection of mEpo gene to intestinal epithelium in vivo mediated by oral delivery of chitosan-DNA nanoparticles. World Journal of Gastroenterology 10(1): 112-116; Phipps et al. (2003). Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci. 86(12): 4070-8.

[18]Wolff et al. (1990). Direct gene transfer into mouse muscle in vivo. Science 247: 1465; Jones et al. (1997). Oral delivery of poly(lactide-co-glycolide) encapsulated vaccines. Behring Inst Mitt. Feb (98): 220-8.

[19]E.g. a number of articles cited in Traavik, T. (1999). An orphan in science. Research Report for DN No. 1999-6, www.naturforvaltning.no/archive/attachments/01/05/Vacci006.pdf

[20]Forsman et al. (2003). Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract. Mol Gen Genomics 270: 362-368; Netherwood et al. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat Biotechnol 22(2): 204-209. In the former study, volunteers were fed rabbit meat. Rabbit retrotransposon sequences (RERV-H) were detected in the blood stream and in peripheral white blood cells for a considerable length of time after ingestion. In the latter study volunteers were fed epsps-transgenic (glyphosate-tolerant) soy as burgers and soy-milk. The transgenic DNA was detected in the small intestinal contents and bacteria. The volunteers were ileostomists, i.e. individuals in which the terminal ileum is resected and digesta are diverted from the body via a syoma to a colostomy bag.

omitted research. Extrapolating from a number of experiments in mammalian cell cultures and in experimental animals, it is conceivable that in some instances insertion of foreign DNA may lead to alterations in the methylation and transcription patterns of the recipient cell genome, resulting in unpredictable levels of gene expression levels and products. Furthermore, even small inserts may result in a 'destabilization' process, the end-point of which may be malignant cancer cells.[21] The BSE/new variant Creutzfeld-Jacob's Disease epidemics caused by prion proteins painfully illustrated the phenomenon of protein persistence, uptake and biological effects. Two recent publications indicate that this phenomenon may be more general that realized.[22] A hallmark of prion diseases and a number of other debilitating, degenerative diseases, e.g. Alzheimer's and Huntington's diseases, is deposition of 'amyloid fibrils'. Recent studies indicate that any protein can adopt a confirmation known as 'amyloid'[23] upon exposure to appropriate environmental conditions. Whether such conditions are more likely when proteins are expressed in different species and at very different concentrations, as is often the case for GM food/feed that are already in the marketplace, is unknown.

The consequences of protein persistence and uptake will vary with the given situation. Generally speaking, there is a possibility that toxic, immunogenic/allergenic or carcinogenic molecules may gain entry to the organism via cells in the gastrointestinal walls. The persistence of the Bt toxin Cry1Ab in faeces means a potential for spread on fields through manure. The ecological effects, e.g. on insect larvae and earthworms,[24] are presently a matter of sheer speculation.

Have the protein contents of GM food been altered in unpredictable ways?

Transgenes or upregulated plant genes may give rise to toxicants, anti-nutrients, allergens, and, putatively, also carcinogenic or co-carcinogenic substances. The concentration of a given transgenic protein may vary according to the location(s) in the recipient host cell genome of inserted GM construct DNA, and to environmental factors influencing the activity of the transgenic regulatory elements, e.g. the 35S CaMV promoter. The biological effects of a given transgenic protein, e.g. the Cry1Ab Bt toxin or the α-amylase inhibitor from beans when expressed in peas,[25] may be unpredictably influenced by post-translational modifications, alternative splicing,[26] alternative start codons for transcription, chimeric reading frames resulting

[21]E.g. Misteli, T. (2004). Spatial positioning: a new dimension in genome function. Cell 119: 153-156; Deininger, P.L. et al. (2003). Mobile elements and mammalian genome evolution. Curr Opin Genet Develop 13: 651-658; Costello, J.F. and Plass, C. (2001). Methylation matters. J Med Genet 38: 285-303; Gatza, M.L. et al. (2005). Impact of transforming viruses on cellular mutagenesis, genome stability, and cellular transformation. Environmental and Molecular Mutagenesis 45(2-3): 304-325.

[22]The first (Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215), based on feeding of gluthathione-S-transferase to mice, demonstrated undegraded protein in stomach/small intestinal contents, and trace amounts in kidney extracts, 30 minutes or more after feeding. Very significantly, incubation with stomach contents of control mice resulted in faster degradation than in feeding experiments. The second study concerned cattle fed cry1ab-transgenic maize Bt176 (Einspanier et al. (2001). The fate of forage plant DNA in farm animals; a collaborative case study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212: 129-134). Cry1Ab protein was detected in all parts of the GIT, and it was still detectable in the faeces.

[23]Demonstrated in a series of recent articles, e.g. Bucciantini et al. (2004). Prefibrillar amyloid protein aggregates share common features of cytotoxicity. J. Biol Chem 279: 31374-31382; Kayed et al. (2003). Common structure of soluble amyloid oligomers implies common mechanisms of pathogenesis. Science 300: 486-489.

[24]Zwahlen et al. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12: 1077-1086.

[25]Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T.J.V. and Hogan, S.P. (2005). Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. J Agric Food Chem 53: 9023-9030.

[26]Rang, A., Linke, B. and Jansen, B. (2005). Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. Eur Food Res Technol 220: 438-443.

from integration into the reading frame of a plant gene, and complex formation with endogenous plant proteins.

The influence of foreign DNA insertion on endogenous plant gene expression patterns may vary with local environmental factors, the actual insertion site(s), the number and stability of the inserts, transgenic promoter effects, methylation patterns of the insert(s), and post-transformational mutations in the transgenic protein coding as well as in regulatory sequences. Even a single nucleotide change may affect the properties of a protein, or it may create a new transcription factor binding motif. Detailed studies of these phenomena under authentic conditions are lacking, and hence we are confronted with yet another area of omitted research.

Could GM food/feed cause allergies?

One of the major health concerns related to GM plants is that the transgenic product itself, e.g. a Bt toxin, changed expression of endogenous plant genes, or chemical reactions that occur during the cooking of novel foods, may result in exposure to *allergenic* compounds. The risk assessment of allergens often follows an *allergenicity decision tree.*[27] These 'trees' are based on *in vitro* tests comparing a limited number of structures, usually only one, of the transgenic protein with known allergens. Hence, these comparisons are made in the hope that the protein isolated for the test matches all proteins produced from the same gene in the GM plant. In fact, this is unlikely because allergenicity tests are usually carried out with bacteria-, not *in planta*-produced versions of the transgenic protein. Glycosylation invariably takes place in plants, but not in bacteria, so this form of post-translational modification of both the transgenic protein and endogenous proteins would not be tested. Allergenic characteristics of proteins, and also their resistance to degradation in the organism, can be affected by glycosylation. Other protein modifications may also take place, adding to the unpredictability of transgenic products.[28]

Another important question related to allergenicity is whether post marketing surveillance can provide useful information about allergens in GM foods. For a number of reasons, this is not likely to happen.[29] Treatment of allergy is symptomatic, whatever the cause may be. The allergic case is often isolated, and the potential allergen is rarely identified. The number of allergy-related medical visits is not tabulated. Even repeated visits due to well-known allergens are not counted as part of any established surveillance system. Thus, during the October 2000 Starlink episode, it proved very difficult to evaluate Starlink (containing Bt toxin Cry9C) as a human allergen.[30] An additional reason for this was that the ELISA tests, used by FDA, that found no anti-Cry9C antibodies in suspected human cases, were dubious because bacterial, recombinant antigens were used instead of the Cry9C maize versions that the individuals had been exposed to.

Case: Bt toxins in Bt-transgenic GM plants

It is very important to be aware of the fact that the Bt toxins expressed in GM plants have never been carefully analysed, and accordingly, their characteristics and properties are not known. What is clear from the starting point, however, is that they are vastly different from the bacterial *Bacillus thuringiensis* protoxins, used in organic and traditional farming and forestry for

[27]Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 111: 1114-1121.
[28]Schubert, D. (2002). A different perspective on GM food. Nat Biotechnol 20: 969; Submissions on A549 High Lysine Corn LY038 http://www.inbi.canterbury.ac.nz/ly038.shtml
[29]Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 111: 1114-1121.
[30]Bucchini, L. and Goldman, L.R. (2002). Starlink corn: a risk analysis. Environ Health Perspect 110: 5-13.

decennia.[31] The difference is evident already at the gene level, since the versions found in GMOs are engineered to produce active Bt toxins. By extrapolation, these have a number of potentially unwanted biological characteristics, ranging from solubilization of the protein under natural conditions and effects on insect and mammalian cells, to persistence and non-target effects in the environment.[32] In addition, the post-translational modifications that may influence conformations, cellular targets and biological effects of GM plant-expressed Bt toxins are unknown, and hence we once more identify an area of omitted research.

During the last few years a number of observations that may be perceived as 'early warnings' of potential health and environmental risks have appeared in the literature.[33] Most of them have, however, not been followed up by extended studies.

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[31]Stotzky, G. (2002). Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis. Pp. 187-222 in: Deborah K. Letourneau and Beth E. Burrows: Genetically Engineered Organisms. Assessing Environmental and Human Health Effects. CRC Press LLC (ISBN 0-8493-0439-3).

[32]Andow, D.A. (2002). Resisting resistance to Bt-corn. Pp. 99-124 in: Deborah K. Letourneau and Beth E. Burrows: Genetically Engineered Organisms. Assessing Environmental and Human Health Effects. CRC Press LLC (ISBN 0-8493-0439-3).

[33]Human and monkey cells exposed to Bt-toxins from the extra- or intra-cellular environment are killed or functionally disabled (Taybali and Seligy (2000). Human cell exposure assays of Bacillus thuringiensis commercial insecticides: Production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environ Health Perspect online, 18 August 2000; Tsuda et al. (2003). Cytotoxic activity of Bacillus thuringiensis Cry proteins on mammalian cells transferred with cadherine-like Cry receptor gene of Bombyx mori (silkworm). Biochem J 369: 697-703; Namba et al. (2003). The cytotoxicity of Bacillus thuringiensis subsp. coreanensis A 1519 strain against the human leukemic T cell. Biochimica et Biophysica Acta 1622: 29-35). Influenza A infections in mice were changed from silent to lethal encounters by co-exposing the animals to Bt-toxin (Hernandez et al. (2000). Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus. FEMS Immunology and Med Microbiol 209: 177-181). Farm workers exposed to Bt spores developed IgG and IgE antibodies to Bt-toxin (Cry1Ab) (Taylor et al. (2001). Will genetically modified foods be allergenic? Journal of Allergy and Clinical Immunology, May 2001, 765-771). The Bt-toxin Cry1Ac was found to have very strong direct and indirect immunological effects in rodents (Vazquez et al. (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Brazilian Journal of Medical and Biological Research 33: 147-155; Moreno-Fierros et al. (2000). Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from Bacillus thuringiensis induces compartmentalized serum, intestinal, vaginal and pulmonary immune response in Balb/c mice. Microbes and Infection 2: 885-890; Moreno-Fierros et al. (2002). Slight influence of the oestrous cycle stage on the mucosal and systemic specific antibody response induced after vaginal and intraperitoneal immunization with protoxin CryA1c from Bacillus thuringiensis in mice. ELSEVIER Life Sciences 71: 2667-2680). Earthworms exposed to Bt toxin Cry1Ab experience weight loss (Zwahlen et al. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12: 1077-1086). Cattle fed the Bt176 maize variety demonstrated undegraded Cry1Ab through the whole alimentary tract, and the intact toxin was shed in faeces (Einspanier et al. (2004). Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. Eur Food Res Technol 218: 269-273). Cry1Ab is much more resistant to degradation under field soil conditions than earlier assumed (Zwahlen et al. (2003). Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Mol Ecol 12: 765-775). Potentially IgE-binding epitopes have been identified in two Bt-toxins (Kleter and Peijnenburg (2002). Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential IgE-binding linear epitopes of allergens. BMC Structural Biology 2:8), and it should be added that many IgE-binding epitopes are conformationally not linearly determined. Finally, it is a matter of concern that Bt-toxins have lectin characteristics (Akao et al. (2001). Specificity of lectin activity of Bacillus thuringiensis parasporal inclusion proteins. J Basic Microbiol. 41(1): 3-6). Lectins are notorious for finding receptors on mammalian cells. This may lead to internalization and intracellular effects of the toxins. Occupational exposure to novel proteins, and potential allergic sensitization, has had little study, but could be of public health significance. An amazing number of foods have been proven to evoke allergic reactions by inhalation (Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Genetically Modified Foods, Mini-Monograph, Volume 111, No. 8, June 2003). In this connection the findings of serum IgG/IgE antibodies to B. thuringiensis spore extracts (Bernstein et al. (1999). Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environmental Health Perspectives 107(7): 575-582), in exposed farm workers should be given further attention. Inhalant exposure to Bt-toxin containing GMP materials may take place through pollen in rural settlements and also through dust in workplaces where foods are handled or processed.

Case: Transgenic, glyphosate-tolerant (Roundup Ready) GM plants

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS) necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition. The transgene, *cp4 epsps*, used in genetically modified crops was isolated from an *Agrobacterium* strain. The whole idea is the combined use of the GM plant and the herbicide. Recent studies indicate that in some cases such GM plants are associated with greater usage of glyphosate than the conventional counterparts.[34] A very restricted number of experimental studies have been devoted to health or environmental effects of the GM plants or the herbicide itself. Some of these may be considered 'early warnings' of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings.[35] Consequently, this is yet another area of omitted research.

Is the 35S CaMV promoter inactive in mammalian cells?

Cauliflower mosaic virus (CaMV) is a DNA containing para-retrovirus replicating by means of reverse transcription. One of the viral promoters, called 35S, is a general, strong plant promoter. It has been used to secure expression of the transgenes in most of the GMOs commercialized so far. Industry proponents have claimed unconditionally that the 35S is an exclusive plant promoter, and hence cannot, even theoretically, represent a food/feed safety issue.[36]

[34]Benbrook, C. Impacts of genetically engineered crops on pesticide use in the United States: The first eight years. Biotech InfoNet Paper No. 6, November 2003. www.biotech-info.net/technicalpaper6.html

[35]Mice fed GE soybean demonstrated significant morphological changes in their liver cells (Malatesta et al. (2002). Ultrastructural morphometrical and immunocytochemical analysis of hepatocyte nuclei from mice fed on genetically modified soy bean. Cell Structure and Function 27: 173-180). The data suggested that epsps-transgenic soybean intake was influencing liver cell nuclear features in both young and adult mice, but the mechanisms responsible for the alterations could not be identified by the experimental design of these studies. Treatment with glyphosate (Roundup) is an integrated part of the epsps-transgenic GMP application. A number of recent publications indicate unwanted effects of glyphosate on aquatic (Solomon & Thompson (2003). Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J Toxicol Environ Health B Crit Rev. 6(3): 289-324) and terrestric (Ono et al. (2002). Inhibition of Paracoccidioides brasiliensis by pesticides: is this a partial explanation for the difficulty in isolating this fungus from the soil? Med Mycol 40(5): 493-9; Blackburn and Boutin (2003). Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup). Ecotoxicol 12: 271-285) organisms and ecosystems. Recent studies in animals and cell cultures point directly to health effects in humans as well as rodents and fish. Female rats fed glyphosate during pregnancy demonstrated increased foetal mortality and malformations of the skeleton (Dallegrave et al. (2003). The teracogenic potential of the herbicide glyphosate Roundup in Wistar rats. Toxicology letters 142: 45-52). Nile Tilapia (Oreochromis niloticus) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul et al. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia. Environ Toxicol 18(4): 260-7). A study of Roundup effects on the first cell divisions of sea urchins (Marc et al. (2002). Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyklin B activation. Chem Res Toxicol 15: 326-331) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrod et al. (2003). The effect of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicoloy 185: 67-78) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard, S. et al. (2005). Differential effects of glyphosate and Roundup on human placental cells, Environ. Health Perspect. 113: 716-720). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

[36]E.g. Gasson, M. and Burke, D. (2001). Scientific perspectives on regulating the safety of genetically modified foods. Nat Rev Genet 2: 217-222.

In addition to studies in yeast[37] and in *Schizosaccharomyces pombe*,[38] there are published studies indicating that the 35S CaMV promoter *might* have potential for transcriptional activation in mammalian systems.[39] The final proof has become available during the last couple of years. First, 35S promoter activity was demonstrated in human fibroblast cell cultures,[40] thereafter in hamster cells,[41] and very recently a research group led by Terje Traavik (co-author of this chapter) has demonstrated substantial 35S promoter activity in human enterocyte-like cell cultures.[42] Such cells line the surface of human intestines. However, no published studies have investigated 35S CaMV activity *in vivo*, and this is therefore yet another area of omitted research.

Could the use of antibiotic resistance marker genes (e.g. nptII) present health hazards?

The antibiotic kanamycin is used extensively in crop genetic engineering as a selectable marker, *inter alia* in GM oilseed rape event lines such as MS1Bn x RF1Bn and Topas 19/2.
A selectable marker is a gene inserted into a cell or organism to allow the modified form to be selectively amplified while unmodified organisms are eliminated. In crop genetic engineering, the selectable marker is used in the laboratory to identify cells or embryos that carry the genetic modifications that the engineer wishes to commercialize. The selection gene is used once briefly in the laboratory, but thereafter the genetically modified crop has the unused marker gene in each and every one of its cells.

[37]Hirt, H. et al. (1990). Evolutionary conservation of transcriptional machinery between yeast and plants as shown by the efficient expression from the CaMV 35S promoter and 35S terminator. Curr Genet 17: 473-9.

[38]Gmunder and Kohli (1989). Cauliflower mosaic virus promoters direct efficient expression of a bacterial G418 resistance gene in Schizosaccharomyces pombe. Mol Gen Genet 220(1): 95-101; Probjecky et al. (1990). Expression of the beta-glucuronidase gene under the control of the CaMV 35s promoter in Schizosaccharomyces pombe. Mol Gen Genet 220(2): 314-6.

[39]The promoter initiates transcription in rabbit reticulocyte lysate (Ryabova and Hohn (2000). Ribosome shunting in the cauliflower mosaic virus 35S RNA leader is a special case of reinitiation of translation functioning in plant and animal systems. Genes & Development 14: 817-829) and in Xenopus oocytes (Ballas et al. (1989). Efficient functioning of plant promoters and Poly(A) sites in Xenopus oocytes. Nucleic Acids Research 17(19): 7891-7903). In the latter studies it was found that circular, supercoiled 35S CaMV driven expression plasmids were more active than linear forms. The CaMV genome carries structural and functional resemblance to mammalian Retroviridae and to Hepadnaviridae, which contains the human hepatitis B virus (HBV). A 19 bp palindromic sequence, including the TATA box of the 35S CaMV promoter, may act as a recombination hotspot in plants (Kohli et al. (1999). Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. Plant Journal 17(6): 591-601), and it is unknown whether this is also the case in mammalian cells. In a recent review article (Ho et al. (2000). Hazardous CaMV? Nat Biotechnol 18(4): 363) it was hypothesized that the 35S CaMV promoter might represent health hazards to human and animal consumers of transgenic plant materials. Against this it was argued that humans and mammals are continuously being exposed to CaMV particles through infected plant materials. This is true enough, but it is then forgotten that there are documented examples of animal species being resistant to intact viruses, but highly susceptible to infection by DNA from the same virus (Refs: Rekvig et al. (1992). Antibodies to eukaryotic, including autologous, native DNA are produced during BK virus infection, but not after immunization with non-infectious BK DNA. Scand J Immunol 36(3): 487-95; Zhao et al. (1996). Infectivity of chimeric human T-cell leukaemia virus type I molecular clones assessed by naked DNA inoculation. Procedures of National Academy of Sciences USA 93: 6653-6658; reviews: Traavik, T. (1999). An orphan in science. Research Report for DN No. 1999-6; Ho et al. (2000). Hazardous CaMV promoter? Nat Biotechnol 18(4): 363).

[40]Vlasak, J., Smahel, M., Pavlik, A., Pavingerova, D., and Briza, J. (2003). Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells, J Biotechnol 103: 197-202.

[41]Tepfer, M., Gaubert, S., Leroux-Coyau, M., Prince, S., and Houdebine, LM. (2004). Transient expression in mammalian cells of transgenes transcribed from the Cauliflower mosaic virus 35S promoter. Environ Biosafety Res 3: 91-97.

[42]Myhre, M.R., Fenton, K.A., Eggert, J., Nielsen, K.M. and Traavik, T. (2006). The 35S CaMV plant virus promoter is active in human enterocyte-like cells. Eur Food Res Technol 222: 185–193.

There are multiple well-known mechanisms for cross-resistance to antibiotics of a particular type.[43] Kanamycin is a member of the family aminoglycoside antibiotics. There are approximately 17 different classes of aminoglycoside-modifying enzymes. Some of these inactivate up to four different aminoglycosides. Cross-resistance between kanamycin and other aminoglycosides, e.g. gentamycin and tobramycin, was found to vary markedly between isolates.[44] All of the antibiotics mentioned are used to treat human diseases.
In spite of the belief of many genetic engineers that kanamycin is no longer employed in medical applications, there is evidence that the antibiotic is used extensively for some applications.[45]

Concluding remarks: Where do we go from here?

We have discussed in some detail a handful of selected, unanswered risk questions related to the first generation of transgenic GMOs. There are many more risk issues. Among them are issues of Horizontal Gene Transfer (HGT),[46] the new generations of multitransgenic GMOs for pharmaceutical and industrial purposes,[47] safety questions related to GM vaccines,[48] the new nanobiotechnology approaches,[49] and the applications of small double-stranded (ds)RNAs (which can cause RNAi) for a number of medical purposes.[50] Furthermore, we have the 'questions not yet asked', and we have the problem of whether available methods and regulatory frameworks will be able to pick up and manage the conceived risks once they become reality.
In recent publications it has been demonstrated that the presently used sampling and detection methods may fail to detect GM materials in food and feed.[51] In another article it was demonstrated that HGT events, that potentially carry very serious public health consequences, would not be detected in time for any meaningful preventive actions.[52] In addition, it has been shown that the dsRNA techniques are not as 'surgically targeted' as initially indicated.[53]

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[43]Heinemann, J.A., Ankenbauer, R.G., and Amábile-Cuevas, C.F. (2000). Do antibiotics maintain antibiotic resistance? Drug Discov Today 5: 195-204.

[44]The aminoglycoside antibiotic neomycin was found to cross react with kanamycin B in inhibiting RNase P ribozyme 16s ribosomal RNA and tRNA maturation (Mikkelsen et al. (1999). Inhibition of RNase P RNA cleavage by aminoglycosides. Proc Natl Acad Sci USA 96: 6155-6160).

[45]Kanamycin is used prior to endoscopy of colon and rectum (Ishikawa et al. (1999). Prevention of infectious complications subsequent to endoscopic treatment of the colon and rectum. J Infect Chemother 5: 86-90) and to treat ocular infections (Hehl et al. (1999). Improved penetration of aminoglycosides and fluorozuinolones into the aqueous humour of patients by means of Acuvue contact lenses. Eur J Clin Pharmacol. 55(4): 317-23). It is used in blunt trauma emergency treatment (Yelon et al. (1996). Efficacy of an intraperitoneal antibiotic to reduce the incidence of infection in the trauma patient: a prospective, randomized study. J Am Coll Surg 182(6): 509-14), and has been found to be effective against E coli 0157 without causing release of verotoxin (Ito et al. (1997). Evaluation of antibiotics used for enterohemorrhagic Escherichia coli O157 enteritis-effect of various antibiotics on extracellular release of verotoxin. Kansenshogaku Zasshi 71(2): 130-5).

[46]Heinemann, J.A. and Billington, C. (2004). How do genomes emerge from genes? Horizontal gene transfers can lead to critical differences between species when those genes begin reproducing vertically. ASM News 70: 464-471.

[47]Twyman, R.M. et al. (2003). Molecular pharming in plants: host systems and expression technology. Trends in Biotechnology 21: 570-578.

[48]Traavik, T. (2002).Environmental risks of genetically engineered vaccines. In: DK Letourneau and BE Burrows (eds): Genetically Engineered Organisms: Assessing Environmental and Health Effects. CRC Books, La Boca, Florida (ISBN 0849304393).

[49]Mazzola, L. (2003). Commercializing nanotechnology. Nat Biotechnol 21: 1137-1143; Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. Nat Biotechnol 21: 1166-1170.

[50]Hannon, G.J. and Rossi, J.J. (2004). Unlocking the potential of the human genome with RNA interference. Nature 431: 371-378.

[51]Heinemann J.A., Sparrow A.D. and Traavik T. (2004). Is confidence in the monitoring of GM foods justified? Trend Biotechnol 22: 331-336.

[52]Heinemann J.A. and Traavik, T. (2004). Problems in monitoring horizontal gene transfer in field trials of transgenic plants. Nat Biotechnol 22: 331-336; Heinemann J.A. and Traavik T. (2004). Monitoring horizontal gene transfer. Reply. Nat Biotechnol 22: 1349-1350.

[53]E.g. Jackson, A.L. et al. (2003). Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol 21: 635-637, and a number of other recent articles.

We are therefore left with a high number of risk issues lacking answers, adding up to a vast area of omitted research, and this falls together in time with a strong tendency towards corporate take-over of publicly funded research institutions and scientists.[54]
We must, as citizens and professionals, join together to reverse the present situation. Publicly funded, independent research grants need to become a hot political issue. This would be the most efficient remedy for chronically unanswered questions and the corporate take-over of science. In conclusion, we once more quote Mayer and Stirling:[55] 'Deciding on the questions to be asked and the comparisons to be made has to be an inclusive process and not the provenance of experts alone'. Then again, whom should society rely on for answers and advice should the time come when all science resource persons work directly or indirectly for the GM producers?

[54]Mayer, S. and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024; Martin, B. (1999), in Science and Technology Policy Year Book. Washington DC, USA: American Association for the Advancement of Science, www.aaas.org/spp/yearbook/chap15.htm; Graff GD et al. (2003). The public-private structure of intellectual ownership in agricultural biotechnology. Nat Biotechnol 21: 989-995; Heinemann, J.A. and Goven, J. The social context of drug discovery and safety testing. In Multiple Drug Resistant Bacteria (C.F. Amábile-Cuevas, ed., second edition). Horizon Scientific Press, in press.
[55]Mayer, S and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024.

Chapter 10

Biodiversity, ecosystem services and genetically modified organisms

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Introduction

Genetically modified (GM) crops have been commercially grown for 10 years. During this time the debate about them and about genetic engineering in general has continued to rage. The general public eagerly follows the developments as well as the arguments; the level of attention is possibly unparalleled since the appearance of the atomic bomb. Some argue that this is the triumph of ignorance, the result of manipulation by environmental protection organizations such as Greenpeace and/or media hype. Sometimes 'risk assessment' is pictured as a strategy to block the spread of growing GM crops. Few ecologists subscribe to any of the aforementioned. The debate about the benefits, risks and overall impact of genetic engineering is complex and so it should be. After all, genetic engineering introduces new combinations of genes that may irreversibly be a part of future evolution, and affect the environment and natural resources. The scale of this issue is thus huge and beyond the short-term scientific and political agendas: it triggers ideological, ethical and religious evaluations. In this chapter, we consider one limited but significant part of this problem circle – the potential environmental impact – and link it to the concepts of biodiversity and ecosystem services.

The overall reason to test GM plants before field release is because humankind's total impact on ecosystem services from previous introductions of new technologies is substantial (Millennium Ecosystem Assessment (MEA) 2005), including habitat destruction, introduction of exotic species, chemical pollution, and global warming, all of which, in themselves and in combination, lead to loss of biodiversity, but also to substantial pressure on all kinds of ecosystems and their services. We have learned from over 100 years of industrial-technological development that all environmentally relevant technologies come with a price – many of which outweigh the benefits in the long run (Harremoës 2002). Consequently, all new potential environmental stressors need to be carefully assessed.

Ecosystem services are ecological processes that operate on vast scales, and we derive substantial benefits from them. Production of goods such as fish and timber, generation of soils and maintenance of their fertility, decomposition, detoxification of wastes, mitigation of climatic extremes, biological control of potential pests, weeds and pathogens, and crop pollination are just some examples of ecosystem services. Their continued functioning is essential for humankind's survival – they cannot be replaced by technology. Until recently, ecosystem services have been treated as inexhaustible, but the global human population size and its use of resources have reached the point where ecosystem services show evident signs of strain.

Agriculture is one of the human activities that have a large 'ecological footprint' (Wackernagel & Rees 1997), meaning that it is a crucial factor in the global ecology. Agriculture is an important driver of environmental quality. In developed countries, there are few farmers (typically < 5% of the population) and they produce food and feed in mostly large-scale, high-input agricultural

systems, including expensive machinery and combustion of fossil fuels. In the developing countries, the situation is different. For example, approximately 70% of Africa's population is engaged in agriculture. Natural processes that underpin agricultural diversity and productivity are both recognized and needed in these regions as most of them have no means to compensate with external inputs.

The concept of biodiversity

According to a recent definition, biological diversity as a concept refers to the variety and variability of living organisms (MEA 2005). Diversity is a multifaceted concept, and ranges from intra-cellular (genetic diversity) to supra-individual (community, landscape and ecosystem diversities) levels (Magurran 2003). Ecologists have long struggled with the concept of diversity and how to quantify it. After decades of intensive search for the best index or formula describing diversity, it was finally realized that there is no single, 'best' diversity description. There exists a 'diversity of diversities' (Juhasz-Nagy 1993), including genetic, physiological, species, functional group, landscape, and ecosystem diversity (Box 10.1). In the interests of preserving biodiversity, we also have to recognize the significance of the processes that create, maintain and further develop biodiversity. In a short-term perspective, this means the ecological processes (i.e. competition, predation, etc.); over the long-term, it includes the process of evolution (Bøhn & Amundsen 2004). Too often, biodiversity is viewed as a static characteristic of communities. However, biodiversity is the emergent outcome of dynamics at ecological and evolutionary timescales.

Box 10.1 Definitions

Genetic diversity: This concept refers to the variability of genes within a species. The total number of genes that can be found in one species is never present in one individual: individuals of the same species contain a lot of identical genes but also many different ones. Genetic variability is the key to the adaptation potential to changing conditions. A species that has lost its genetic diversity is either unable or severely impaired to adapt to new conditions.

Physiological diversity: As genes only provide a 'set of instructions', the realization of this programme, depending on the environmental conditions during development, always results in slightly different physiological outcomes in individuals. They will differ in their physiology: heat tolerance, ability to resist starvation, digestion efficiency, etc.

Species-individual diversity: Communities of living organisms are composed of individuals that are classified into species. Intuitively, the more species there are in a community, the more diverse it is. The minimum diversity in a community occurs when all individuals belong to the same species. A theoretical maximum level of species diversity would be reached when all individuals belong to different species. A characterization of species diversity depends on our ability to recognize individuals as belonging to different species, and to count them.

Functional diversity: Species have different characteristics and are distinguishable, but they may be grouped according to their activity in habitats and food webs. One possibility is to group them by their feeding habits. Plants use inorganic materials and energy (mostly sunlight) to grow, in the process of producing more plant material. They can be classified into the functional group of *primary producers*. Organisms feeding on plants form the *primary consumers*, while those feeding on these are called *secondary consumers*. At the top of some food-chains are the *top predators*, often large animals. Functional groups can be further refined. One aspect of functional diversity is the diversity of such groups themselves (not all of them are present everywhere), while another is to assess the diversity within each group.

Landscape diversity: At a wider spatial scale, different habitats (for example, forests, meadows, streams, marshes, cultivated fields) form landscapes. Both the types and distribution of these compositional elements are important in determining the diversity at this level. For example, if the elements occur in one block each, the landscape-level diversity is considered lower than when the same total area of the composing elements occurs in several smaller blocks. The transition between landscape and ecosystem diversity is not always straightforward.

Ecosystem diversity: Ecosystems can be larger units, composed of several landscapes (but some argue the opposite). An ecosystem is defined as a recognizable, self-sustaining unit, but it is more plausible to consider this a theoretical.

Different biodiversity concepts, as detailed in Box 10.1, range from intra-individual (genetic) to supra-individual (species, landscape, etc.) levels, and all are relevant, depending on *context*. However, it has to be added that the most frequent use of the word biodiversity (sometimes even without definition) implies the species-individual based diversity, i.e. the word 'diversity' means the number of species. In nature, most communities contain a small number of 'common' and a much larger number of 'rare' species. Some diversity indices account for such differences but all diversity representations contain different simplifications. For example, for most diversity indices, the species identity is not important – only the density of the species present is taken into account. Two communities with the same number of species and identical relative densities would have the same diversity value even if there were no common species in them.

The functions of biodiversity

Diversity, in all of its manifestations, is valued for several different reasons. Biodiversity is also important for the functioning of ecological systems (Loreau et al. 2002), but the central question is: just how important? There are different theories to explain the significance of biodiversity for ecological systems. These theories are vigorously studied, hotly debated and not always mutually exclusive (Loreau et al. 2002; Hooper et al. 2005). The main ideas are briefly presented as follows.

1. Biodiversity has a (positive) impact on productivity

Several experiments have indicated that a more diverse ecological community of plants will produce a higher biomass than a less species-rich one (Loreau et al. 2002). The existing evidence supporting this claim is equivocal and has been debated (Hooper et al. 2005). More species can utilize the available resources more efficiently, but there seem to be some key species that have disproportionate influence on this and consequently also on productivity (Wardle & van der Putten 2002). In a more species-rich assemblage, it is more probable that such species can be found. Another hypothesis claims that a more diverse system will experience less year-to-year fluctuations in plant biomass production than a species-poor one.

2. Insurance against change (resistance and resilience)

In terms of energy efficiency, most biodiversity is unnecessary (redundant) for ecological functioning *under stable conditions*. However, elements that seem redundant under one set of conditions may become necessary if conditions change, since the organisms have to adapt. Changing conditions occur naturally, for example by extreme weather conditions, but also due to human activities, such as global warming and introduction of exotic species. It may be hard to separate natural- and human-triggered changes. For example, global warming tends to increase the occurrence of extreme weather events. Whereas resistance refers to the ability to resist change under the pressure of stressful conditions, resilience refers to the ability to return to a previous

state after a disturbance. Both traits are important for continued functioning of ecological systems.

3. Providing ecosystem services
Ecosystem services are linked to points 1 and 2 above. A more detailed explanation of their nature and importance will follow.

Human domination of the Earth
We now recognize that human impact over all of the Earth is substantial, whether we consider land conversion, use of resources, or impact on other species. Today, 25% of the global terrestrial surface has been converted to cropland (Fig. 10.1). The conversion rate is accelerating: more land was converted in the 30 years since 1950 than during the 150 years from 1700 to 1850. More than two-thirds of the area of two biomes (temperate forest; tropical dry forest) and more than half of the area of four others (Mediterranean forests; flooded grassland and savannas; tropical and subtropical savannas and grasslands; tropical and sub-tropical coniferous forests) had been converted by 1990. Our impact on other parts of the globe is also large. For example, 20% of all coral reefs had been exterminated, a further 20% damaged, and 35% of the global mangrove area had been destroyed by 1990 (MEA 2005).

Increases in fertilizer application have followed suit, and biologically available nitrogen in terrestrial systems has doubled, and that of phosphorus tripled since 1960. However, this change is extremely disproportionately distributed, with overuse in industrial countries to the point of polluting water bodies and lack of it in developing countries to the point where agriculture production is severely limited (e.g. Africa). For example, the average application in 1992 of N fertilizer was 323 kg/ha in Western Europe while only 7 kg/ha in Africa (FAO 1993). Nevertheless, at a global level, more than 50% of all the synthetic nitrogen fertilizer ever used has been used since 1985, and 60% of the increase in the atmospheric concentration of CO_2 since 1750 has taken place since 1959 (MEA 2005).

Another limited vital resource is water and we claim more and more of the available freshwater resources. The amount of water in reservoirs has quadrupled since 1960, and today there is 3–6 times more water in reservoirs than in all natural rivers combined (MEA 2005). Water withdrawal from rivers and lakes has doubled since 1960. As a result of combined erosion and river regulation, the sediment load of many major rivers has been substantially altered from pre-human conditions (Syvitski et al. 2005). In some rivers, sedimentation has increased by up to 200% and even large rivers hardly reach the coast. For example, only 10% of the Nile manages to meet the ocean. Increased sedimentation rates have caused death zones in deltas where depositing sediments are often loaded with poisonous chemicals (Syvitski et al. 2005).

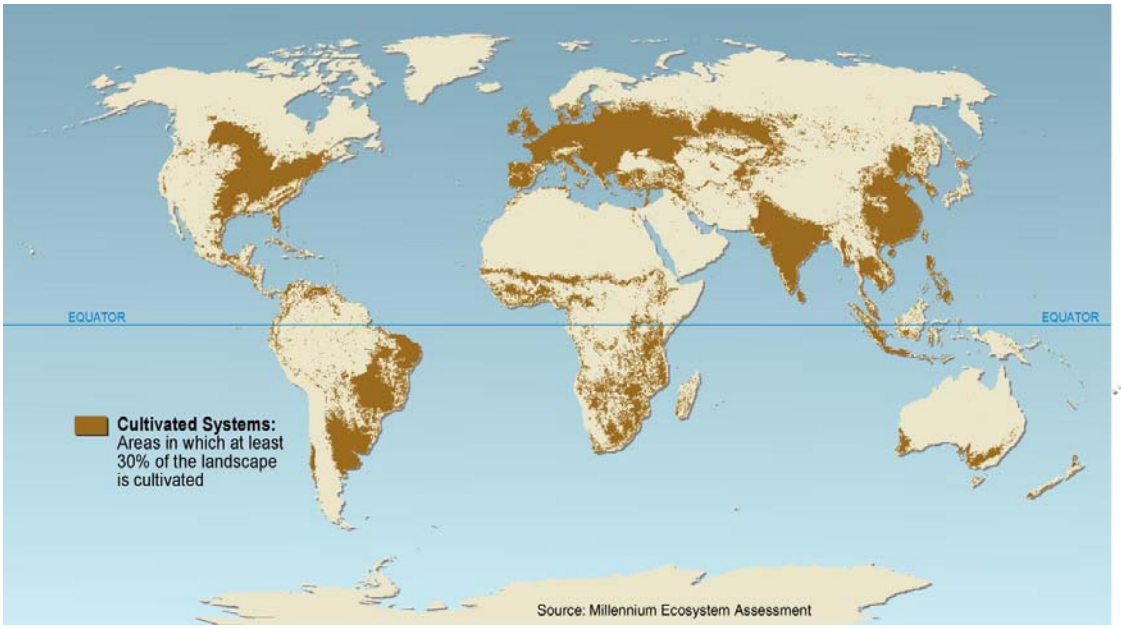

Figure 10.1. The terrestrial areas converted to cropland worldwide. Source: Millennium Ecosystem Assessment, 2005.

Concerns about biodiversity

The impacts of agriculture on resources together with other human activities have had significant impacts on global biodiversity. Introduced species have had particularly broad impact. In historic times, numerous intentional introductions of species deemed useful or merely desirable at new locations have been made. Their effects are often considered beneficial, but we have numerous examples of unwanted, significant negative effects (Baskin 2002), and the number of invasive species is steadily increasing (for an example, see Fig. 10.2). Together with unintended introductions, invasions have become a significant problem, and an element of global change (Vitousek et al. 1997). One significant consequence of this is the increasing homogenization of the distribution of species on Earth (Lövei 1997). The breakdown of biogeographical barriers leads to reduced global biodiversity (Vitousek et al. 1997).

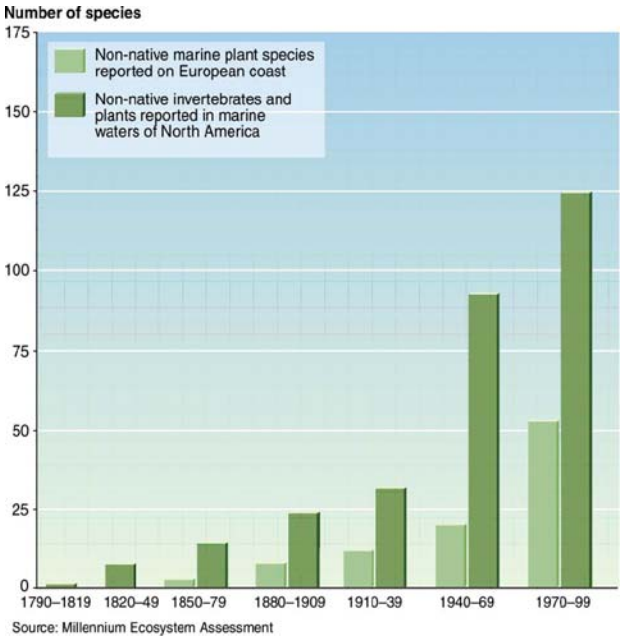

Figure 10.2. The number of non-native species reported from marine habitats in Europe and North America, 1790–1999. Source: Millennium Ecosystem Assessment, 2005.

Further signs of stress in the global biodiversity is that the population size or range (or both) of the majority of species across a range of taxonomic groups is declining (MEA 2005). Currently, estimated species extinction rates are 1000 times higher than background rates typical of the planet's history (Fig. 10.3) (MEA 2005; Lövei 2007). A total of 10–30% of mammal, bird, and amphibian species are currently threatened with extinction (Secretariat CBD 2006).

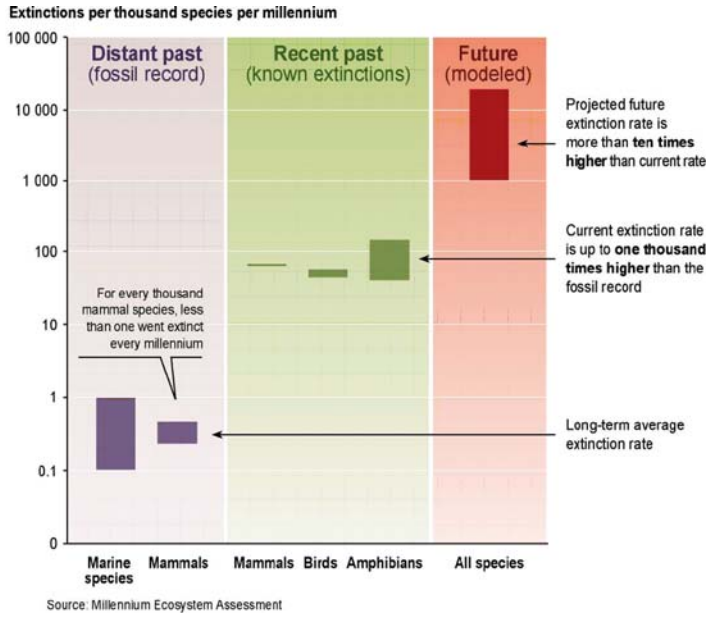

Figure 10.3. Estimated extinction rates: historical, recent and predicted. Source: Millennium Ecosystem Assessment, 2005.

Ecosystem services
Ecosystem services denote ecological processes that humankind benefits from (Daily 1997). These processes operate on vast scales, are irreplaceable, and have been formerly perceived as inexhaustible. Several types of ecosystem services ensure agricultural productivity, including soil formation, decomposition of plant residues, pollination, and natural pest control, to name a few. Several of these are already under pressure and their ability to continue at desired rates is in peril (MEA 2005).
The Millennium Ecosystem Assessment (MEA) recognizes four categories of ecosystem services (Box 10.2).

Box 10.2
Provisioning services are simply used or harvested, and in most cases humans do not do anything to manage them. Provisioning services include the provision (harvesting from the wild) of food, freshwater, medicine, fibre, and timber, energy, or industrial products (e.g. rubber). Genetic resources used for plant breeding also belong to this category.

Supporting services include services that, by their functioning, support the normal functioning of ecosystems. This includes the removal of waste products through detoxification, decomposition, air and water purification, but also soil formation and fertility maintenance, and supporting plant production through seed dispersal, and pollination.

Regulating services provide coastal and river channel stability, moderation of weather extremes, floods and drought, as well as the natural control of pests. Most organisms can occur at high densities but they do not (i.e. they do not become pests). This is due to the activity of natural enemies.

Cultural services provide numerous valuables to humans and human culture. Humankind is psychologically closely linked to nature (the 'biophilia' hypothesis, Wilson 1984). Nature is a constant source of aesthetic beauty, provides cultural and spiritual inspiration, inspires scientific discovery, and endless varieties of recreation.

Why do ecosystem services have to be considered in GM impact assessment?
As described, ecosystem services are essential for agricultural production. As the MEA concluded, humankind already is using many of the ecosystem services in a non-sustainable manner. Any further damage must be avoided. Also, the negative trends in biodiversity and natural resources must be taken very seriously. Consequently, when introducing new technologies today, such as GM crops, their potential impact on ecosystem services must be tested (Lövei 2001). Such testing is even more important in tropical countries, where agricultural producers often depend on ecosystem services more closely than farmers in the developed countries. Modern high-input agricultural practices use several external inputs that at least partially replace ecosystem services (fertilizers, pesticides, irrigation, and even pollination). Irrespective of the questionable sustainability of this practice (Tilman et al. 2002), these external inputs are often not available to farmers in developing countries, hence they have to rely more on natural ecosystem services. As GM crops will be grown outdoors, in contact with surrounding ecosystems, and they certainly have the potential to substantially modify current agricultural practices (Hawes et al. 2003), the environmental impact of genetic engineering on ecosystem services will have to be examined thoroughly (Hails 2002). Box 10.3 lists the most important potential adverse impacts currently discussed and partly investigated.

Box 10.3 Possible environmental impacts of GM crops

At intra-individual (genetic) level:

- damage to genetic resources (particular genes, gene combinations, seeds, varieties, etc.)
- uncontrolled gene flow to other species

At population level:

- species shifts due to altered traits, consciously or accidentally (via unintended gene flow)
- development of secondary pests
- development of resistant populations, curtailing the usefulness of the GM trait
- damaging of protected/endangered species (nature conservation)

At ecosystem level:

- decline in agricultural biodiversity due to the homogenization of the primary producer base (a centralized production of a relatively few, patented events, traits and varieties).

Loss of ecosystem services:

- damaging naturally-occurring biocontrol organisms
- loss of pollination services
- impact on soil organisms involved in recycling of soil nutrients and maintaining soil fertility (can be positive, due to reduced soil tillage, or negative)

For agricultural production systems:

- decrease in pesticide use, soil tillage, environmental contamination
- threatening of GM-free production reducing future choices
- loss or reduction in practices that uphold and develop varieties (i.e. diversity) with adaptations to local environmental conditions
- food or agricultural production in areas where it was not possible earlier (e.g. due to high levels of stress, lack of water, etc.)
- rearrangement of agricultural production systems, in space and time, and its resulting consequences for landscape management

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Incorporating ecosystem services into risk/impact assessment poses several challenges:
The structure and function in relevant ecosystems and food-webs have to be recognized. For example, an ecosystem may contain predator-prey relationships that keep a number of pests under control (i.e. at low densities, so we do not recognize them as a pest). Productivity may also depend on insect pollination services (e.g. cotton).

The significant functional links must be established where structure and function are reasonably well understood. Following the aforementioned example, it may turn out that pollination is much more significant than pest control for productivity in the ecosystem where a GM crop is to be introduced.

Most important species fulfilling identified relevant ecological roles that should be subjected to pre-release testing have to be identified. However, we should not forget that even the most important functions will typically be performed by numerous species. Again, following the

aforementioned example, pollination services may be provided by more than 30 bee species, but the most important could be just one, or a handful of them.

Pre-release testing should focus on these functionally important species. When such species are identified, suitable testing and monitoring methods must be developed for them. If there is no option to identify species responsible for the execution of important ecological services – as, for instance, is the case with most soil microorganisms – the relevant processes must be identified and a potential adverse impact of the GMO tested. There may or may not be suitable laboratory culture systems or field monitoring methods already available for these functionally important species or processes. If such tools are lacking, they should be developed.

Current testing regimes for GM plants

Understanding the importance of ecosystem services and the need to avoid any further adverse impacts on them through the introduction of GMOs begs the question as to what degree current regulatory testing actually addresses the issues raised so far in this chapter and how they are tested. Today, applicants applying for regulatory approval of GM plants follow largely the guidelines originally developed for testing the environmental effects of chemicals (pesticide model). The *strategy used in ecotoxicology testing of chemicals* is to expose single species (standard set) to single chemicals in a hierarchical tiered system. Tests commence with simple inexpensive range finding tests on single species and measure acute toxicological response to a chemical stressor. Further testing proceeds to more expensive higher tiered levels (including some chronic toxicity tests), only if first-tier experiments yield results of concern. In practice, this results in the testing of a standard set of species exposed individually to high concentrations of the toxin.

In the case of a GM plant producing the *Bacillus thuringiensis* toxin (Bt plant), for example microbially produced Bt-toxins are fed directly to testing organisms (bi-trophic exposition) in an experimental set-up originally developed to assess acute toxicity of synthetic chemicals. Acute toxicity measures the physiological toxicological response of an organism after being directly exposed to the isolated test substance within a short period of time (sometimes hours rather than days).

The standard set of species is representative of model ecosystem compartments, such as a generalized aquatic or terrestrial compartment. An algae species is tested as a representative for primary producers in aquatic systems (plants), water fleas (*Daphnia* spp.) as a representative of a primary consumer, and a fish species representing a secondary consumer (i.e. predator). The endpoint measured is mortality after hours or a few days (Table 10.1) (Andow & Hilbeck 2004). Further criteria for their selection as standard organisms are their documented sensitivity to certain groups of chemicals and/or their capability of accumulating high concentrations of heavy metals (e.g. springtails or earthworms). Hence, the concept of toxicity (and ecotoxicity) testing of chemicals is exceeding the notion of a case-specific testing regime related to the given receiving environment. A standard test performed in temperate Europe is (erroneously) considered applicable to tropical Africa, and vice versa.

Table 10.1. Some standardized guidelines for ecotoxicological testing of pesticides and GMOs (OECD 1998).

Test organism	*Test method*	*Duration*	*OECD Guideline No.*
Water fleas, Daphnia	*Acute immobilization/toxicity*	*24-96 h*	*202*

Fish sp. (rainbow trout)	*Acute toxicity*	*24-96 h*	*203*
Fish sp.	*Toxicity to juvenile life stages*	*4-12 wk*	*210*
Eisenia foetida (compost worm)	*Acute toxicity*	*7-14 d*	*207*
Bobwhite quail & mallard duck	*Acute toxicity*	*14-21 d*	*205*
Honey bees	*Acute toxicity (oral & contact)*	*4-24 h*	*New (1998)* *213* *214*

http: ecb.jr.it/testing-methods
www.oecd.org/dataoecd/9/11/33663321.pdf

The pesticide model as a testing guideline for insecticidal GM plants is problematic for a number of reasons. Plants are not chemicals and regulations and scientifically sound testing procedures must account for the differences:

i) In GM plants, the plant-expressed transgene product is an integral component of the plant and coupled to its metabolism. This leads to variable expression levels of the transgene product that is additionally modulated by environmental conditions, including seasonal changes in temperature, soil type, moisture, and light. On the other hand, due to the wide use of universally functioning viral promoters and terminators, the transgene products of most, if not all, currently commercially available GM plants are expressed essentially in all plant parts throughout the entire growing season. When comparing with pesticides, this is equivalent to a long persistence of the pesticidal substance and an almost complete coverage of the plant.

ii) GM plants are capable of self-reproduction. This is a fundamental difference to chemicals. Because of this capability, biological traits and organisms can increase in the environment and potentially spread and exist for unlimited time. In contrast, chemicals cannot reproduce and, thus, their absolute amount will, at best (or worst), remain stable for a long time, but over time will always decline. Most disappear within humanly conceivable time periods due to degradation.

iii) GMOs can actively spread and with them their transgene products will also spread. In addition, all passive mechanisms of spread for chemicals also apply to transgene products released into the environment from the living GM plants (e.g. exudates, leaching from living and dead material). The potential of human-aided spread of seeds, plants and animals (as already realized and exemplified in invasion biology) should not be underestimated (Baskin 2002, see Box 10.4).

Box 10.4 Spread of GM plants: Control or chaos?

Unwanted and uncontrollable spread of GM plants is a highly visible process on a global scale. By the end of 2006, over 100 cases of confirmed, unwanted contamination and 26 cases of illegal releases were registered (mostly by civil society organizations) (see GM contamination register, http://www.gmcontaminationregister.org/). A total of 39 countries on five continents have been affected, almost twice the number of countries that currently grow GM crops. In 2005, there were 7 documented cases of contamination and 8 illegal releases. In 2006, the number of

contamination cases more than doubled to 15. Most prominently, two unapproved GM events were found in rice (a herbicide-tolerant transgene from the USA and a Bt transgene from China) – these were detected at the consumer level (in shipments intended for human consumption). These were possible to detect because the necessary detection methods were available. More problematic is the detection of plants with GM traits that have not yet been commercialized. Several such lines are at the field trial stage, among them many pharmaceutical traits, for which the necessary detection methods are not yet widely available and therefore detection is more difficult. The global, illegal or unwanted spread of transgenes and their products shows a worrying tendency and it is likely that this trend will continue, perhaps even accelerate, over the coming years.

For these reasons, it is extraordinarily more difficult if not impossible to determine the exact exposure concentrations in a given environmental compartment for GM plants as compared to chemicals. In contrast, chemical pesticides (i.e. sprayed in the field) are controlled by the applicator: the timing, the point location, etc. Degradation begins immediately after application and the mode of action is typically acute (also for non-target species). A scientifically sound testing strategy and methodology for GM plants require case-specific risk assessment and must account for the whole transgenic organism. It must also treat a GM plant within an integrated biological system consisting of the plant, the novel trait and the receiving environment. Sub-lethal, chronic effects might be even more important to test for than acute effects, as the mode of action for the toxin is not immediate (it normally takes two days or longer before the 'target' dies).

Selection of test organisms

Even for chemical testing, it is problematic to use test organisms of higher trophic levels because the test substance is often not ingested directly by these organisms but is ingested via one or several intoxicated prey species. These prey species may contain the test substance, or metabolites thereof, in unknown concentrations. From our knowledge of persistent chemicals such as DDT and PCB, we know that they can accumulate and even become more toxic along the food chain. This means they can reach concentrations and toxicity levels that, at the end of the food chain, are multi-fold above the levels originally introduced into the ecosystem (Woodwell et al. 1967). We also know from research on insect-plant interactions, that insects can use toxic proteins in their host plants to turn them into defence mechanisms against their enemies. One example is the monarch butterfly (*Danais plexippus*), whose larvae accumulate an alkaloid from the host plant, milkweed, that makes them unpalatable. We do not know how herbivore species, which are not affected by novel transgene compounds, may be using them against their enemies. These complications make it currently unlikely that a few selected species could universally be used for pre-release risk assessment of GM plants.

Representativeness of test materials

As already mentioned, in toxicological and ecotoxicological testing of pesticidal GM plants, high concentrations of the microbially produced transgene product, e.g. the Bt-toxin, are applied. The significance of such tests is limited because the Bt-toxin expressed in GM plants can be quite different from the microbially derived toxin. For example, the Bt-toxin of the Cry1-class used in the regulatory tests has been derived either from the original *Bacillus* or from genetically modified *Escherichia coli*. After the microbial synthesis, the product is a protoxin of 130 kDa in size which is inactive (Höfte & Whiteley 1989; Müller-Cohn et al. 1996). Before use in the tests, the protoxin is cleaved by trypsin to create the toxic fragment of 65 kDa size. However, in transgenic Bt-plants, fragments of different sizes of the Cry1-class toxins are produced. For example, the Bt-corn event MON810 expresses a 91 kDA fragment, whereas Bt-corn event 176

expresses a 64 kDa fragment (Andow & Hilbeck 2004). From other events, it is known that the Bt-toxins degrade within the plant to fragments of even smaller size (36, 40, 55, 60 kDa) of unknown activity[1](Andow & Hilbeck 2004; AGBIOS 2006). In conclusion, this means that the Bt-toxins expressed in GM plants may vary significantly in size and activity from the test substances used to assess safety, i.e. in standard toxicological and ecotoxicological testing.
In summary, a GM plant is not a chemical. Any environmental testing must therefore account for the difference. Test strategies for case-specific risk assessment of GM plants must include the transgene product, the transformed plant and the environment of deployment as an integrated system. This is even more important in the case of GM plants that do not express a toxin, but have, for instance, an altered metabolism (e.g. herbicide tolerant plants or altered starch composition). In these cases, the adoption of test principles from chemical testing is even less relevant because environmental effects of these GM plants may become evident on other levels altogether. Following the logic for strict toxicity testing, for those GM plants that do not express a novel toxin, no testing would be required at all. This is the case for most herbicide tolerant plants to date. As the ecological impact will arise through the application of registered chemicals, no toxicity or ecotoxicity testing will need to be conducted with these plants.

A proposed new approach for environmental impact testing
Conceptual and methodological uncertainties of studying the ecological effects of GM crop plants on non-target arthropods (insects) have raised several intriguing general problems. What species or ecosystem functions should be chosen to test? By what routes might these species or functions be exposed directly or indirectly to GM crop plant products? How can meaningful scientific hypotheses be constructed to provide rapid assessments of the magnitude of the potential risks? In contrast to toxicological and ecotoxicological methods for addressing these problems, assessment of the impacts of GM crop plants must be case specific and contextualized to the environment in which they will be used. An international project in which two of the authors (Gábor Lövei and Angelika Hilbeck) have been involved, developed an 'ecosystem representative approach' for selecting species and ecosystem function as foci for further testing (Birch et al. 2004; Andow et al. 2006). This approach combines ideas and methods from a 'community approach', which emphasizes analysis of intact biodiversity, a 'functional approach', which emphasizes community reactions, a 'key species approach', which emphasizes the individuality of species, and an 'indicator species approach', which is central in ecotoxicological testing. We used classic qualitative methods of risk assessment formalized in selection matrices and directed questions, which provide transparent summaries of scientific data and expert judgement that then serve as basis for constructing testing hypotheses and designing proper experiments that address the hypotheses.

The process of ranking and species selection in the above-ground functional groups (herbivores, decomposers, natural enemies, and pollinators), allows the identification and prioritization of non-target species for some key ecological groups; it also reflects the current state of knowledge and expertise available, and identifies gaps in knowledge and uncertainties. When analysing the available information to assess the relative importance of parasitoids in maize in Kenya, for example, the information gaps could be recognized, as well as the realization that the two main maize growing regions, the lowland and the Western highlands, have to be considered separately (Table 10.2). It is also important to consider the process of exposure as part of the overall species selection. The species selection can identify missing information, for example the varying expression of Bt-toxin in different plant tissues in the Kenyan example, and is also crucial for the above-ground exposure analysis. An example of an analysis of significance and exposure is presented in Table 10.3.

[1]www.agbios.com/main.php

Table 10.2. An example of the filled-in selection matrix for parasitoids in maize agroecosystems in Kenya, following the system proposed by Birch et al. (2004).

Sub-guild	Species	Occurrence	Abundance	Presence	Linkage	Rank
Lowland, Kenyan coast						
Egg parasitoid	*Trichogramma* spp.	Certain	Medium	All season	Strong	1
Larval parasitoid	*Cotesia flavipes*	Certain	Medium	All season	Strong	1
Larval parasitoid	C.sesamiae	Certain	Low-medium	All season	Strong	2
Larval parasitoid	*Goniozus indicus*	**Not completed**				
Egg & larval parasitoid	***Chelonus curvimaculatus***	Not completed	Short rains?			
Pupal parasitoid	Pediobus furvus	Certain	Low	All season	Strong	2
Pupal parasitoid	*Dentichasmias busseolae*	Occasional	Low	All season	Strong	3
Highland, Western Kenya						
Egg parasitoid	*Trichogramma* spp., native	Likely	Medium	All season	Strong	2
Egg parasitoid	*Telenomus* spp.	**Not completed**				
Larval parasitoid	*Cotesia sesamiae*	Certain	Medium	All season	Strong	1
Larval parasitoid	*C. flavipes*	Occasional	Low	All season	Strong	3
Pupal parasitoid	*Dentichasmias busseolae*	Occasional	Low	All season	Strong	3
Pupal parasitoid	*Pediobus furvus*	Certain	Low	All season	Strong	2

Table 10.3. An example of the exposure analysis assessment as suggested by Birch et al. (2004). The example is plant-feeding arthropods in maize agroecosystems in Kenya.

Species	Feeding category	Significance	Assessment of exposure
			Spodoptera spp.
		Acarid spp.	Leaf feeder
		Locusts	Leaf feeder
		Sitophilus zeamays	Grain feeder
		Prostephanus truncatus	Grain feeder
		Plant- and leafhoppers	Phloem feeder
		Carpophilus spp.	Saprovore
		Honey bee (*Apis mellifera*)	Pollen feeder
		Wild bee spp.	Pollen feeder
		Coccinellid spp.	Pollen feeder, predator
		Forficulidae	Pollen feeder, predator
		Trichogramma spp.	Parasitoid
		Trichogrammatoidea spp	
		Cotesia flavipes	Parasitoid
		Cotesia sesamiae	Parasitoid
		Other predators: ants, anthocorids, chrysopids	Predators

This underlines the role of this approach to identify and assess the significance of knowledge gaps and uncertainty. Rather than only moving on as a 'decision has to be made', significant knowledge gaps will not be overlooked and can trigger specific action, either to stop an assessment procedure, or to initiate specific, targeted research.

The ranking and selection matrix for soil ecosystem functions has a slightly modified format, to rank and select ecosystem functions. Here, key interactions are to be identified in a systematic and transparent way; species and food-webs affected by, e.g. Bt maize, might be studied in a more relevant manner than performed until present.

Conclusions

In this chapter, we suggested that the basis of environmental risk/impact assessment should be the concepts of biodiversity and ecosystem services. Biodiversity is under threat by mainly human activities. Apart from a moral obligation to protect biodiversity, there is also a utilitarian reason, as biodiversity is important for the functioning of ecosystem services. Ecosystem services are vital for our continued existence, but recent summaries have indicated that humankind is using many of them in unsustainable ways. Consequently, it is mandatory that the impact of new kinds of activities, such as growing GM plants, be tested for their impacts on ecosystem services. Ecological systems are, however, complex and often imperfectly known. We have suggested a transparent, knowledge-based assessment procedure by which important functions and the species or groups that are most significant for this function are identified. This provides one way to develop specific pre-release testing and monitoring systems to assess the environmental impact of GM plants. This system also allows for the identification and evaluation of the significance of knowledge gaps, thus making the precautionary approach in risk assessment operational.

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Chapter 12

Vertical (trans)gene flow: Implications for crop diversity and wild relatives

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The purpose of this chapter is to present an overview of the potential evolutionary consequences of (trans)gene flow, focusing on crop plants. From a scientific standpoint, the challenge is to determine and gather all of the relevant scientific knowledge possible, identify uncertainties and known gaps of knowledge, and use this information to design a context-specific framework to guide the safe use of a particular technology. Likewise, understanding and minimizing the potential safety impacts of GMO crops requires identifying the relevant issues and information – not only genetic and biological information, but also socio-cultural and legal dimensions as well. In this case, I will introduce rudimentary concepts of gene flow, discuss the current state of knowledge, assumptions and future needs in biosafety research. The objective is to contextualize the scientific issues to help understand the issues for developing a sound scientific assessment of the potential implications of vertical transgene flow for crop biodiversity, weed and target resistance evolution, and food security. From this, a series of critical questions and needs emerge, and can be added to discussion and decision making within the realm of a particular country, crop, and/or policy regime. Other emerging issues, such as the impacts on human health and environment, are discussed in Chapters 14 and 10, respectively, and are outside the scope of this chapter. Note that this chapter is intended to give only a basic introduction to the subject, yet provides references to key literature in the field for further reading (for extensive reviews on the subject, see Ellstrand et al. 1999; Ellstrand 2001; Eastham & Sweet 2002; Gepts & Papa 2003; Messeguer 2003; Snow et al. 2004).

In Section 1, I will introduce the basic concepts in biology of gene flow. Section 2 will be dedicated to discussing the potential evolutionary significance of transgene flow from a) crop to wild relative, b) crop to landrace, and c) crop to crop, each of which have their own set of emergent socio-cultural, political and economic considerations. These will be illustrated by recent research and actual transgene flow events. In Section 3, I will discuss some of the means of tracking transgenes. Section 4 contains a discussion of some critical gaps in scientific understanding and uncertainties that should be communicated to policy makers, and the general public, for making informed decisions on the safety of transgenic crops. In the fifth and final section, I discuss some questions that may be useful in consideration of policy and risk assessment concerning GMOs with respect to crop biodiversity and food security issues.

1. Overview of vertical gene transfer (gene flow)

1.1 What is gene flow?

Gene flow is the movement of genes from one population to another, conferring new traits – the biophysical characteristics of the organism – to individuals of the recipient population. This happens by *cross-pollination* (also called *hybridization*), that is, the pollination of members of one population or genetic pool with that of another. The outcrossing of genes is said to be 'vertical' as the genetic information is passed 'down' from parents to offspring. This is contrasted with *horizontal gene transfer* (discussed in Chapter 13), where the acquisition of genes is passed over, i.e. 'horizontally', from one organism to another by means other than inheritance. Vertical gene flow often results in *introgression*, the establishment of *alleles* (gene variants), or wholly new genes (as is the case with transgenes) in the recipient population.

Therefore, vertical gene flow is restricted to organisms that can mate with one another and make offspring. In the case of crop plants, which are domesticated forms of wild plants, a high degree of compatibility can therefore exist between the crop and wild and weedy relatives. Gene flow can be from crop to crop (or landrace), from crop to wild relative, and even from wild relative to crop plant. Gene flow has been a natural, and in some cases desirable, part of evolution and speciation in flowering plants (Anderson 1961; Reiseberg & Wendel 1993; Ellstrand et al. 1999). Thus, gene flow per se is not the main concern, but rather the *types of genes*, and the level of genetic heterogeneity or homogeneity (genetic diversity) that is spread through gene flow and its effect on recipient populations, are the relevant issues. Whether flow of new genes or gene variants results in a change in *fitness*, i.e. the ability of the organism to survive *and* produce viable offspring (either positively or negatively), has been a central focus in population biology. It should be clearly pointed out that considerations of gene flow discussed here are not unique to transgenic crop varieties, but are relevant for all commercial crops. The introduction of wholly new genomic identities into recipient populations from commercial traits should be equally scrutinized, but is outside the scope of this chapter.

If commercial crops have been exchanging genes with related species for some time, why are transgenic varieties of particular concern? With transgenics, completely novel traits are passed on that could dramatically affect the fitness of individuals receiving the given gene in a population. Thus, the commercialization of transgenic plants has sparked widespread interest in the potential evolutionary significance of transgene flow. The central question is how transgene introgression may impact fitness in the new transgenic hybrids, and consequently, the significance for maintaining important crop genetic diversity for future crop breeding.

1.2 Under what conditions does gene flow occur?

Hybridization and subsequent gene flow depend on a number of biological and ecological conditions. First, the sexually compatible plants need to be growing within sufficient pollen or seed dispersal range of the transgenic crop. In many cases, there is no overlap between crops and wild/landrace relatives, and they do not pose a concern, yet crop to crop gene flow often is a concern. The possible dispersal range of reproductive propagules (i.e. pollen) is dependent on many different climactic (including wind, humidity, temperature, etc.) and biological factors (height of plant, size of propagule, natural outcrossing rates, etc.), but human dispersal can also broaden this range. Second, in order for gene flow to occur, there must be an overlap in *phenology* (flowering and fertility times) between the transgenic crop and recipient population. Flowering times may be affected by ecological and or biological factors in some circumstances, leading to partial or total reproductive isolation among neighbouring populations. Third, any mating between a transgenic crop and a landrace or wild relative must produce fertile and viable offspring. Reproductive barriers to introgression are strong, especially where *ploidy number* (genomic copy number) differs between domesticated and wild crop relatives (Jenczewski et al. 2003).This may only occur in limited scenarios. Plants that normally are only self-compatible, i.e. have the capacity to only mate with itself, also represent a type of reproductive barrier. Fourth, the offspring of the new transgenic-hybrid plant must also be viable and fertile to some extent, and a lack of survivors means that any potential gene flow would cease at this point. Yet even a low level of fertility can lead to fully viable populations in subsequent generations, as would be the case with *backcrossing* (mating 'back' or again, with the parent population) into the wild progenitor populations.

When these four conditions are met, transgene flow is likely. In some of these cases, offspring will have reduced fitness, or produce sterile (unviable) seeds. In other cases they will have improved vigour (Singh et al. 1995; Hauser et al. 1998) and fitness, yet the advantages may reduce or reverse over time. Thus, there must be a minimum level of fertility in order for the

recipient population to maintain the transgene(s) and survive to the next generation. Lastly, it should be mentioned that the dispersal of seeds themselves can also be an agent of gene flow. The movement of seeds can occur in a range of ways, mostly by human activities, such as transportation (Figure 12.1), or by wind or wild animals.

Figure 12.1. A maize plant growing on the side of the highway outside Guadalajara, Mexico. This plant presumably arrived as a seed fallen from a transport truck. (Photo: D. Quist, 2002)

1.3 In what species or kinds of crops could transgene flow occur?

Almost all of the world's most important crop plants are known to hybridize with wild relatives. At least 44 cultivated crops have demonstrated the capacity for hybridization with wild and weedy relatives, including 12 of the 13 most widely cultivated crops (Ellstrand et al. 1999), and 11 of the 20 most important US crops, including sunflower, radish, sorghum, canola, squash, rice, wheat, sugar beet, lettuce, poplar, strawberry, and bentgrass (Ellstrand 2003). As discussed, gene flow to wild relatives and landraces will depend on the availability of such species near the area of cultivation (Messeguer 2003). Crop to crop gene transfer often occurs where transgenic and non-transgenic crops are planted in close proximity. Many of these crop plants are primarily

outcrossing species, including maize, canola (rapeseed), tomato, sorghum, wheat, sugar beet, alfalfa, cucumber, radish, and strawberries (NRC 2000).

2. (Trans)gene flow and its potential evolutionary consequences

So why might changes in plant fitness (its ability to survive and reproduce) resulting from transgene flow be significant? Effects on fitness are largely dependent on the nature of the genetically engineered traits, and the external and internal factors that influence their expression (Gepts & Papa 2003; Jenczewski et al. 2003). As approximately 97% of all transgenic crops involve insect resistance or herbicide resistance, these are the main traits under consideration (yet the history of unintended transgene flow events, and the coming generation of plant-made pharmaceuticals are perhaps signs of things to come). In the case of insect resistance transgenes, levels of pest pressure in wild or landrace populations may be lower compared to crop populations, reducing the selective value of the trait. Herbicide resistance genes might exhibit an energetic cost on the hybridized plant that would have no value if the herbicide is not applied (but alternatively, great value if it is). In the case of stress-tolerant transgenic crops (drought tolerance, salt tolerance, etc.), with traits that allow survival in a broader range of ecological conditions, hybridization is likely to increase fitness and invasiveness.

Hence, whether transgenes from a source population will establish in wild or landrace sink populations will depend on a number of independent and interrelated factors – genetic, ecological and even human management variables. Identifying the most important components to survival is not straightforward, and must be considered within the ecology of transgenic hybrids. Variation in fitness is also likely across hybrid generations. With such little knowledge on the behaviour of transgenes in unintended and new genomic and ecological backgrounds, prediction of real-world effects is particularly challenging.

One principal concern of transgene flow is the loss of potentially useful crop genetic diversity in the recipient population (whether other crops, landraces or wild relatives). *Outbreeding depression* (the reduction of fitness from hybridization) can lead to a decrease in allelic diversity by extinction of members of a diverse gene pool that are less adapted to survive because of the particular introgressed transgenic trait. This is loss of diversity through negative selection. On the other hand, when transgene hybrids have an increased fitness, and can survive into the next generation, *genetic assimilation* (loss of unique genetic identity through continual hybridization and backcrossing) will have a homogenizing affect on the recipient population, also leading to a less diverse gene pool. Thus, both instances can have negative effects on genetic diversity. The magnitude of these selective forces within the new genomic and ecological background of the recipient population will largely determine the rate of evolutionary change in the recipient population (Gepts & Papa 2003).

So how can we predict the outcomes of transgene flows on a recipient population? Population matrix models have been suggested as useful ways to estimate this risk (Parker & Kareiva 1996; Bullock 1999). However, the magnitude and evidence of effects is idiosyncratic, and may take years to develop (Ellstrand & Hoffman 1990). Few direct studies have been conducted to measure the fitness effects of transgenes in wild populations (Linder 1998; Linder et al. 1998; Snow et al. 2001; Spencer & Snow 2001; Gueritaine et al. 2002; Snow et al. 2003). Of these, many were conducted under ideal agricultural conditions, where water and nutrients were not limiting, and interspecific competition was low, rather than stress conditions often faced by low- or unmanaged populations. Further, many studies seeking to understand persistence of transgenes in natural populations have only studied the first hybrid generation. Some investigators have questioned the value of such estimates in early hybrid generations (Linder et al. 1998), as variation in fitness

may occur across generations due to recombination and selection (Hauser et al. 1998). Models to quantify such changes over subsequent hybrid generations have been useful to help predict potential outcomes of such events through time (Lavigne et al. 1998).

A useful model to study survivorship after gene flow is *migration-selection balance*. This model demonstrates (Lenormand 2002) that in crop to crop, or crop to wild gene flow, even *negatively selected traits* (traits that decrease the plants' ability to survive) are still likely to be maintained (in balance) in the recipient population. Whether this allele is maintained or not depends on the level of gene flow to the population. If there are sufficient rates of gene flow of the negative selected allele, a threshold value will be reached, leading to *fixation* (permanent maintenance of the allele in the population). In this case, the sub-optimal allele would predominate purely by magnitude of gene flow coming into the population.

Given the importance of introgression for the evolution of land plants, and the ubiquity of gene flow between crops and wild relatives, the impacts on native genetic diversity is a broad concern (NRC 2000; Pilson & Prendeville 2004; Snow et al. 2004). Some investigators downplay these risks, assuming that if transgene flow produced offspring of low fitness, the transgene would not survive in the population at all. Yet, research contradicts this assertion. Theoretical studies suggest that introgression rates of genes from one population to another can be quite rapid even when the fitness advantage is small (Barton & Dracup 2000), or when there is a high frequency of transgressive hybrids (Reiseberg & Wendel 1993). A modelling study conducted by Haygood et al. (2003) demonstrated that crop alleles can be rapidly fixed in a recipient population when the migration frequency exceeds the selection threshold, even when they have a negative impact on fitness. Their study expands on how *demographic swamping* (reduced fitness in the hybrid's offspring populations) can facilitate genetic assimilation just where high rates of gene flow occur from agricultural populations. In this situation, gene flow that reduces fitness will become stable in the population when the migration rate of the alleles exceeds the level of selection, leading to reduced population size and perhaps local extinction. Further, extinction through hybridization is a valid concern not only when it involves transgenic plants, but in any situation of non-native biological or genetic invasions (see Chapter 11 on invasives) where hybridization may increase a plant's invasiveness (Ellstrand & Schierenbeck 2000).

2.1 Types of transgene flows and their implications

With the now decade-long history of GMO commercialization, the world has already witnessed a number of cases of transgene flow, from crop to wild relatives, crop to landrace and crop to crop. Within each type of transgene flow, a host of environmental, agronomic, cultural, and intellectual property concerns emerge in conjunction with the biological and evolutionary considerations of gene flow. While research has made some progress, there is still much to be learned.

2.1.1 Gene flow from crops to wild and weedy relatives

Transgene flow, generally regarded as undesirable and hence often regarded as 'transgenic contamination', presents a number of management challenges with the formation of transgenic hybrids in sexually compatible weed species (Darmency 1994; Snow & Palma 1997). Hybridization may give distinct selective advantage over non-hybrids in a population, particularly where certain herbicides are used to control these weeds – and can allow the hybrids to become more invasive in natural and agricultural habitats (Ellstrand 2003). Increased weediness of some wild relatives also augments their invasive potential into new environments whereas resistance to insect damage is inherited from insect-resistant crops. Gene flow from crops to wild relatives has been linked to the evolution of weediness in seven out of the thirteen most important crop plants (Ellstrand et al. 1999).

A good example of transgene flow between a crop and its wild relative is that of transgenic oilseed rape (also called canola) *Brassica napus*, and its wild relative *B. rapa*. Early research suggested that hybrids between oilseed rape and the weedy *B. rapa*, would be minimal, due to gene flow barriers and low survival (Crawley et al. 1993). However, later research by Mikkellsen et al. (1996) and Hall et al. (2000) have shown wide dispersal of herbicide tolerance genes in weedy *B. napa*. Gene flow has subsequently been shown to persist for many years (Pessel et al. 2001; Simard et al. 2002). This has led to a number of distinct challenges for weed management near agricultural lands.

Another example involves the escape of transgenes from glyphosate-resistant (a herbicide) bentgrass (*Agrostis stolonifera*) in the United States. Reichman et al. (2006) detected transgenic hybrids with weedy *Agrostis species* some 3.8 km downwind of transgenic field trials, in federally-protected grassland. The ecological consequences of such outcrossings are uncertain, yet any decrease in genetic diversity would lead to a change in community structure with the introgressed regions. As a result, in 2007 a federal judge ordered a temporary halt in new approvals of GM field trials, citing an inadequate environmental review of the potential environmental impacts.[1] The ruling requires that future GM trials in the US must undergo more rigorous environmental reviews.

Whether or not any resulting gene flow has an evolutionarily significant effect on wild and weedy relatives must be tested carefully. Few studies have directly addressed crop to wild transgene flow in the field (Linder & Schmitt 1995; Linder et al. 1998; Bartsch et al. 1999; Spencer & Snow 2001; Gueritaine et al. 2002; Snow et al. 2003). Researching the impacts is difficult, as the selective value of a transgene in a wild population may be different within its ecological and biological context, where a host of factors (including epistasis, genetic drift, etc.) may influence the magnitude of evolutionary impact. Nonetheless, cases such as with the aforementioned creeping bentgrass signal the need for more intensive research in this area.

2.1.2 Crop to landrace gene flow

Gene flow between modern crops and *landraces* – the genetically diverse domesticated, local, farmer-selected cultivars – has been an area of concern since the early inception of modern plant breeding. Many landraces are still being cultivated within their areas of origin, and hence, local farmers play an important role in the maintenance of in situ diversity and conservation (Gepts & Papa 2003). Landraces act as important sources of genetic diversity – the genetic stock that plant breeders must rely on for future crop improvement. For this reason protection of this diversity has been a concern of international crop research centres, international agencies, and national governments alike. The loss of this diversity involves not only food security considerations, but also cultural notions of patrimony and locally-derived genetic resources.

Centres of crop origin and diversification therefore both play crucial roles for future crop breeding. Figure 12.2 details some centres of origin for some of the world's most important food crops.

[1]http://www.centerforfoodsafety.org/GTBC_DecisionPR_2_7_07.cfm accessed 10 February 2007

Figure 12.2. Centres of origin and diversification for major crops. Other geographic areas may as well contain important sources of genetic diversity for these crops. (Modified from Crop Genetic Resources: An Economic Appraisal/EIB-2, Economic Research Service/USDA, 2002).

A number of important transgene flow cases have been reported in centres of crop origin and diversity. Perhaps most widely known is the case of transgene introgression of maize in Oaxaca, Mexico (Quist & Chapela 2001; 2002). The substantial attention paid to reports on the status of transgenes in Mexican maize (Quist & Chapela 2001; NAFTA-CEC 2002; Alvarez Morales 2002; Quist & Chapela 2002; Cleveland et al. 2005; Ortiz-Garcia et al. 2005) has not translated into follow-up empirical studies on the evolutionary significance of transgenes in maize landrace populations. Given the occurrence of transgenic introgression events in Mexico, concerns have emerged over similar events taking place in other important crop plants, including rice and soya in China (Huang et al. 2003). The impending commercialization of GM rice has been met with considerable concern over gene flow to wild and weedy rice relatives (Lu & Snow 2005), and to non-transgenic commercial varieties. Given these events, and the uncertainties over the significance of transgenic hybridization, the introduction of transgenic crops in their centres of origin and diversification represents a broad concern with socio-economic and agricultural implications. Some of these impacts, particularly evolutionary implications, may be irreversible. For these reasons, transgenic introductions in centres of origin and diversification merit special consideration.

The issue of intellectual property rights (IPR) on crop cultivars adds another dimension to the issue of transgene flow. While IPRs are in conflict with the age-old practice of seed exchange amongst local farmers who use landraces, the introduction of identifiable transgenic technologies opens up the possibility that legal action could be taken against local farmers by the patent holders.[2]

While there has been greater attention paid to gene flow to wild relatives, there has been very little scientific study, descriptive or experimental, over the potential impacts of transgene

[2]See the case of Percy Schmeiser, a canola farmer from Canada (http://www.percyschmeiser.com/)

introgression in landraces. Clearly, establishment of transgenic hybrids in landrace populations is undesirable, given the high level of uncertainty as to their effects and incidence of gene movement. Policies that limit the planting of transgenic varieties in centres of origin have been widely recommended (NRC 2000; Eastham & Sweet 2002; Gepts & Papa 2003). Yet well-intentioned policies have been largely ineffective to date.

2.1.3 Crop to crop gene flow

Crop to crop gene flow, as previously mentioned, is a broad concern in areas of GM and non-GM cultivation or use of offspring's seeds. A number of 'gene spill' events of transgenics 'contaminating' non-transgenic crops, resulting from cross pollination (Friesen et al. 2003; Mellon & Rissler 2004), and sometimes seed mixing (Mellon & Rissler 2004) have been recorded. Transgenic introgression of conventional crops has its own share of biological, socio-economic, policy, and intellectual property concerns.

Of the biological considerations, the most significant is loss of non-transgenic genetic varieties, many of which are 'heirloom varieties' (landraces) of important crop diversity. It is important to note that this is also an issue with non-transgenic commercial hybrids, where the process of domestication of crops has led to genetic bottlenecks in virtually all crops analysed to date (Doebley 1992; Gepts 1993). This has the effect of limiting the genetic stocks available to farmers and breeders.

Socio-economically, many of the same concerns mentioned for landraces also exist with crop to crop transgene flow. A number of cases of inadvertent contamination of the food supply – particularly in the USA – with varieties not approved for human consumption have made recent headlines. Cases such as the Starlink corn contamination in 2000 (Kaufman 2000) and rice in the US with multiple transgenic varieties,[3] are just a few examples of inevitable gene flow. Nations that do not accept (certain) GMO products have been forced to ban the import of grains or foods from these countries, causing a loss of markets for farmers and food distributors. Contamination events of organic crops can affect the premium value and genetic stocks of the crops for the affected farmers. Quite clearly, the unintended spread of transgenes has been a result of cultivation and seed distribution systems that were never designed for segregation of particular crop varieties. Human error and negligence of laws are also often to blame. Lastly, patent infringement lawsuits might be brought against farmers affected by transgene flow, as previously mentioned.

As a result of the many documented cases of transgene flow, robust monitoring programmes have been an important initiative for many countries, especially those with policies limiting GMOs in their food supply. Hence, tracking transgenes has not only biological but political implications.

3. Tracking transgenes

An essential initial component of understanding the ecological and environmental impact of transgene flow is first documenting the movement or presence of transgenes in a population, food shipment, or processed food item. This involves employing molecular methods to detect the synthetic transgenic DNA constructs, or target marker proteins introduced into the gene-modified commodity (Holst-Jensen et al. 2003; Nesvold et al. 2006).

[3]http://www.guardian.co.uk/gmdebate/Story/0,,1884523,00.html and http://www.aphis.usda.gov/publications/biotechnology/content/printable_version/ia_ge_rice.pdf

Successful monitoring and surveillance of transgenes in the environment or food shipment is reliant on a number of factors. First, one must be able to detect the transgenic sequences or proteins (see Chapter 33). Therefore *a priori* knowledge of the genes one is looking for is essential. Further, the gene sequence or protein one is targeting in the monitoring efforts must be intact and/or expressing. In addition, the sampling regime, limit of detection and reproducibility of results can further effect the outcome of any monitoring efforts, usually leading to false-negative results (Holst-Jensen et al. 2003). Hence, any sampling for GMOs is likely to underestimate the presence and/or frequency of GM DNA in a sampled population. Thus, not detecting a transgene in a sample population is no guarantee that the population is transgene free (Heinemann & Traavik 2004). Only with a careful multifaceted monitoring strategy can the accuracy and precision of our monitoring efforts be reasonably assured. Agencies dedicated to the detection of transgenes, such as the European Network of GMO Laboratories (ENGL) in the EU, have devised validated methods for the detection of transgenic DNA from approved GMOs in the European community. Thus, tracking transgenes is difficult, but not impossible.

4. Research needs, gaps in knowledge and uncertainties in gene flow assessments

In the first years following the commercialization of genetically modified organisms, the primary research focus has gone into developing detection systems and monitoring to account for unwanted GM DNA is foodstuffs and crops (as discussed). This has been motivated largely by policies of low or no GMO components in grain and foodstuffs in some countries, and has been a driving force in the science of GMO-related research. The salient question is the significance of gene flow when it occurs. Ecological studies of transgene flow have shed significant light on many of the unanticipated or unintended effects of transgenic biology, and have highlighted the need for robust science as the driving force behind risk assessments. Where ‘early warnings’ are identified (Harremoes et al. 2002), there is a need for careful consideration where lasting effects might otherwise be mitigated. The importance of context should not be lost on transgenic biology, where the behaviour of transgenes and their proteins might be very different within different biological (organismal) or ecological backgrounds.

While a much greater degree of risk science on transgene flow to date has focused on the direct ecological implications of specific transgenes, investigations into the ecological and evolutionary significance of transgene flow for genetic diversity in centres of origin are lacking. The case of transgenic maize in Mexico is one clear example of where such studies are urgently needed (Garcia et al. 1998; Quist & Chapela 2001; NAFTA-CEC 2002; Cleveland et al. 2005). As a result, many critical gaps in understanding remain on gene flow potential and barriers, including sexual compatibility, hybrid viability and fitness for many crop species.

Part of the difficulty in such studies is the lack of *a priori* predictive power given the likely variable behaviour of the transgene in new ecological and genetic backgrounds (Gepts & Papa 2003). Transgenic plants, like most commercial crop varieties, are designed for use within very specific environmental and cultural conditions of the agricultural field over one generation. They were never intended for new genomic or ecological backgrounds, or for use over subsequent generations that occur with gene flow. Much research has focused on the notion of fitness of a transgenic hybrid population to be substantially equivalent to transgenic crops within the intended agricultural setting. Conceptually, one must consider that the setting of the transgenic organism may grossly affect the effect or impact it may have within a particular milieu. For example, pest and competition pressures may be different depending on ecological setting, affecting fitness of the population much differently outside its intended agricultural context, such that equivalence of outcomes cannot be assumed. Further, hybridization into new genetic backgrounds may have a range of effects on the fitness of the recipient population. These responses may include a

metabolic cost decreasing its fitness, to a hybrid vigour increasing its costs. Outcomes may not be consistent across generations, growing ranges, climatic fluctuations, or stress pressures. A further consideration is the lack of understanding of the fate and stability of transgenes across generations (McCabe et al. 1999; Quist & Chapela 2001; Svitashev et al. 2002; Wilson et al. 2006) and post-translational silencing (Matzke et al. 2000), non-Mendelian inheritance, *pleiotropic* or *epistatic* effects (i.e. unintended changes in phenotype by the transgene introduction or interaction with other genes) that are important considerations for assessing gene establishment, expression, and hence fitness effects. Further, other levels of biological organization within the plant (transcriptome, proteome, metabolome; see Chapter 8) may also have direct impacts on fitness of gene flow. Another consideration is that the dominant currency of gene flow research as genes conferring traits assumes that all genes transferred will be protein-coding genes. This fails to consider the vast array of non-protein encoding DNA and RNA derivatives that are also implicated in the transfer of genetic information and the outcomes from one population to another (Mattick 2003).

Thus, the evolutionary implications of hybridization and introgression from crop to crop or crop to landrace/wild populations where it actually occurs are dependent on a number of factors, where the fitness effects cannot be predicted *a priori* to GM crop release, and may change over hybrid generations. Therefore, studies must be conducted on a case by case basis within any given context (country, environment, GMO, etc.) where relevant scientific questions can be addressed.

5. Practical considerations for policy and risk assessment on gene flow

5.1 Strategies for mitigating transgene flow

The knowledge gained from transgene flow studies has been useful in developing appropriate measures to limit gene flow from transgenic plants. A number of strategies have been outlined to document and minimize gene flow from transgenic sources.

Given the uncertainties over the ecological and evolutionary impacts of gene flow, the means to minimize potential gene flow are active areas of investigation. Most of these will involve temporal and spatial isolation of the transgenic crops from potential gene flow scenarios. Containment and confinement strategies span the range from the physical (Morris et al. 1994; Staniland et al. 2000) to the chemical (Schemthaner et al. 2003) to the molecular (Daniell 2002). No single strategy is failsafe, and overlapping approaches will be necessary to adequately ensure minimal transgene escape, yet must also be investigated for their own biosafety.

5.2 Context-specific considerations

The country, crop, and/or transgenic trait under consideration may be relevant to policy decisions on transgenic crops. For example, gene flow to landraces and wild relatives of maize may be an issue for a country such as Mexico, but not for Canada. Certain types of transgenic products may also trigger policy implications if they may impact sensitive non-target biodiversity. Foremost is a robust detection and monitoring system, whereby specific information on the marker DNA sequences, molecular characterizations and background knowledge on gene flow potential will all be important in any biosafety policy on transgenic crops. Lastly, beyond the possible ecological and economic implications of gene flow, the possible socio-economic costs of unintended gene flow must also be taken into account in any policy decision or risk assessment (Gepts & Papa 2003).

6. Conclusions

Emerging knowledge over the importance of the ecological, genetic and political backgrounds of GMO introductions is bringing new insights into the complexities surrounding the use of GMOs in agriculture. There is still much to be learned. Quite clearly, GMOs represent a new challenge in the management of agriculture where external costs and potential consequences must be duly measured along with and contrasted with any potential benefits. This is even more critical with the emerging use of crop plants to manufacture bioactive compounds, such as pharmaceuticals, that have an even greater risk magnitude. Given the scope, irreversibility and uncertainty surrounding the impacts of transgene flow, a critical analysis of the biological, ecological and social ramifications needs be thoroughly examined to arrive at sound policy decisions. This requires asking the right questions – the relevant types of 'what if' risk questions—regarding the GMO under consideration within the right social, political and agroecological dimensions.

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Chapter 13

Unintended Horizontal Transfer of Recombinant DNA

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DNA is usually transferred over generations following the normal reproduction pathway of the organism involved (e.g. sexual reproduction/inheritance by descent). This process is called *vertical gene transfer* and an example is pollen flow between the same or related plant species.[1] Thus, vertical gene transfer is the normal mode in which DNA is shared among individuals and passed on to the following generations. DNA can, however, also more infrequently spread to unrelated species through a process called *horizontal gene transfer* (HGT). HGT, sometimes also called lateral gene transfer, occurs independently of normal sexual reproduction and is more common among single-celled organisms such as bacteria. HGT is a one-way transfer of a limited amount of DNA from a donor cell/organism into single recipient cells (Figure 13.1). Examples of HGT are the spread of antibiotic resistance among bacterial species, gene therapy in humans, and *Agrobacterium*-infection in plants. HGT of recombinant DNA from GMOs to bacteria is a potential biosafety concern (Nielsen et al. 2005). In this chapter we introduce the main biosafety aspects of unintended[2] HGT processes as they relate to the use of recombinant DNA, as follows:

1. Introduction to some biosafety aspects of recombinant DNA

Genetically modified organisms (GMOs) often contain recombined genes (transgenes) collected from different species to enable the expression of new traits. Most commercialized GMOs harbour < 5 protein-encoding transgenes assembled into unique genetic combinations and regulatory contexts that provide new functions to the host organism. The intended horizontal transfer and recombination of genetic material across species barriers is thought to be of little concern by many scientists active in genetic engineering, as genes are considered to be mechanistic entities or modules that can function equally well in many organisms, regardless of

[1]Pollen transfer between related plant species is less frequent than within species, and is also called outcrossing or hybridization. Note that hybridization processes still follow the normal ways of plant reproduction and are therefore vertical gene transfer events. The participating plants contribute c.50% each to the DNA composition of the seeds, in contrast to HGT events where most often much less than 1% of the genome of one organism is transferred to another.

[2]This chapter focuses on the likelihood of unintentional HGT. Intentional HGT, i.e. the insertion of defined DNA fragments into the target organism, is the basis for all genetic engineering and production of GMOs.

their historical and evolutionary context. This reductionistic understanding of genes as functional modules acting more or less independent of their organismal background and genetic networks underlies also the way risks of potential subsequent horizontal transfer of recombinant DNA to unintended recipients are presented and addressed in the biosafety assessment of GMOs.

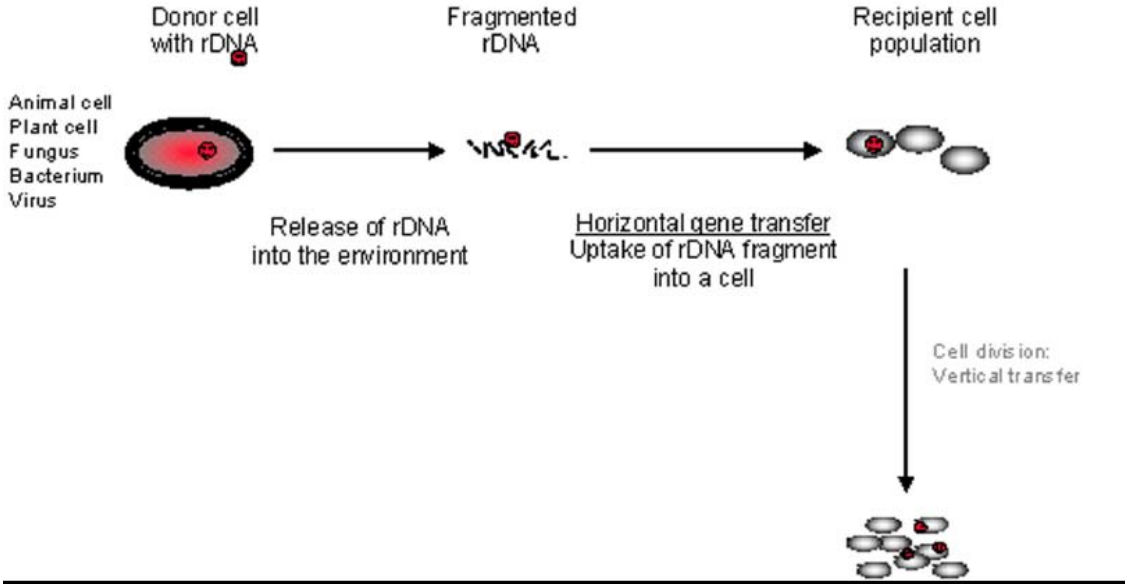

Figure 13.1. A schematic representation of horizontal gene transfer. A donor cell (of any origin) can release DNA (the presence of a particular gene is indicated with a red dot in the figure) that can persist in the environment. The subsequent uptake of DNA fragments by exposed recipient cells is called HGT. Such HGT can occur deliberately, e.g. by gene therapy in humans, and genetic engineering of plants. Bacteria have several processes that can facilitate HGT, including transformation, conjugation and transduction.

The prevailing gene-centric perspective on GMO production is also shaping the approaches to, and understanding of, biological-mechanistic consequences of unintended HGT events.[3] The health and environmental impact of potential unintended HGT from GMOs is a debated concern and risk scenario (Nielsen et al. 1998; 2001; 2005; van den Eede 2004). For instance, whereas *vertical* spread of recombinant DNA from GMOs (e.g. GM plants) to conventional crops, landraces and to some wild relatives has been documented in several studies (see Chapter 12), no studies have conclusively proven *horizontal* spread of recombinant DNA from GMOs into naturally occurring host tissues or bacteria. The reason for the absence of observations of horizontal transfer of DNA from GMOs is currently debated and can be due to:

- Lack of receptive host cells or bacteria, conducive environments, or available recombinant DNA in a given environment (e.g. the gastrointestinal tract, agricultural fields).
- Lack of a selective advantage of the horizontally transferred recombinant DNA so that rare host cells or bacterial transformants never surface in investigations working with limited sample sizes.

[3]We recognize that an implicit utilitarian value set frames the presentation of the biological aspects of unintended HGT of transgenes in this chapter. Nevertheless, we acknowledge a non-consequentialist view on HGT processes: that any unintended HGT of a man-made, recombined gene construct with traits derived from many unrelated organisms represents an unacceptable violation of nature. This latter argument may be seen an ethical objection. However, most gene constructs used in GMOs today could not have arisen by natural genetic processes or traditional breeding within the timescale of modern civilization. Ethical concerns related to the novel origin, genome and biochemical composition of GMOs are, however, also founded in a comparative perspective taking into account the long-term complex processes underlying the evolution and composition of extant organisms.

- Lack of funding, and hence, conducted and published studies that have examined the process with a reasonable effort and detection limit.
- Lack of motivation among scientists to investigate such HGT processes due to the many levels of conflicts of interest and highly vocal opinion leaders in the field.[4]
- Lack of methods preventing an investigation of HGT processes with a sensitivity that is relevant to somatic cell dynamics or bacterial evolutionary processes.

As outlined in Nielsen (2003a), some commonly occurring characteristics of recombinant DNA in GMOs can make their transgenes more likely to be taken up and expressed in unintended host or bacterial cell recipients than the majority of the genes present in naturally occurring higher organisms (Table 13.1). Given the many specific characteristics of transgenes exemplified in Table 13.1, it is clear that the argument that 'native plant genes are not observed in bacterial genomes, therefore plant transgenes will have the same constraints and, hence hypothesized occurrence of HGT processes from GM plants should be dismissed' is not relevant.

Here, we briefly present the state of knowledge concerning horizontal transfer of recombinant DNA from GM plants into human cells or into bacteria present in the gastrointestinal tract or in agricultural fields. We discuss knowledge gaps and describe various types of uncertainty embedded in the prevailing biological paradigms underlying the evaluation of HGT processes in biological risk assessments.

2. Recombinant DNA introduction and potential impact in various environments

The large-scale approval, cultivation and consumption of GM commodity crops will necessarily lead to the release and, to some extent, persistence of recombinant DNA in the environment. DNA is continually released from living organisms (e.g. crop plants) shedding tissues or cells or from their decaying debris. The release of DNA is therefore not specific to GMOs and the effect thereof should be seen in the context of DNA released from other organisms present in the same natural system (e.g. by conventional agriculture).

All living cells harbour long DNA molecules. In higher organisms, some of the DNA is broken down (fragmented) within the host during controlled cell death (apoptosis). In contrast, in single-celled organisms such as bacteria, DNA breakdown is mainly facilitated by nearby organisms with specific enzymes (called nucleases or DNases) that facilitate the degradation process. Thus, released DNA is routinely and continually degraded and recycled into nutrients in all ecosystems. Yet, evidence obtained both from DNA sequencing of whole organismal genomes and laboratory studies of DNA exchange between organisms demonstrate that some, often minor fragments of DNA, can be integrated into the genome of the exposed recipient organism (Ochman et al. 2000; Rosewich & Kistler 2000; Nakamura et al. 2004; Thomas & Nielsen 2005).

[4]A rapid transition from a scientific debate to personal attacks and attempts to discredit the researcher may soon follow if 'unwelcome' paradigm-challenging results are published. Hence, potential threats to a further scientific career development are to be considered prior to initiating risk-focused studies.

Table 13.1. Characteristics of recombinant DNA that may alter the likelihood of horizontal transfer, expression and stabilization in unintended hosts.

Modification	Recombinant DNA has an altered likelihood of mediating:
Use of bacterial gene constructs and vector sequences	- Recombination with prokaryotic genomes because the bacterial genes and mobile elements (vector sequences) have high sequence similarity to commonly occurring bacteria.[a]
Functional assembly into a single genetic unit	- Transfer of entire novel multi-gene encoded traits because only a single transfer event is necessary for a recipient to acquire a functionally optimized genetic trait complex. The trait may have previously been distributed across the donor genome (with a lower likelihood for simultaneous multi-gene transfer), or the trait was absent from the evolving species/lineages.
Introduced changes in gene expression and protein composition	- Expression of the modified traits in novel hosts, if horizontally acquired, because broad host range promoters (derived from microbial pathogens) are used to drive the expression of the engineered trait. Codon and promoter modifications may also change the expression levels and protein characteristics (e.g. mRNA processing and editing, post-translational modifications) affecting protein composition, function, stability, and location in some unintended recipients.
Insertion of a transgene construct into an unrelated genome	- Host-specific differences in the gene expression and regulation systems between the transgene's original host and the modified recipient host, can lead to unpredictable changes in the global gene regulation in the new host and in the transgene's transcription level and mRNA modifications, the translation process and composition of the translation product, altered post-translational modifications, and hence protein stability, activity and degradation.
Removal of introns from cDNA cloned genes	- Expression of the modified traits in a broader set of species and domains because intron processing (specific to eukaryotes) is regarded as a main barrier for functional assembly and expression of eukaryotic genes in bacteria.
Insertion of transgenes into organelles	- Increased exposure rates (relative to nuclear-inserted genes) to unintended recipients due to high transgene copy number in organelles, recombination (homology-based) and functional expression of the modified traits in unintended bacterial recipients because organellar genomes resemble bacteria in overall genome organization and regulation.
Large-scale release of modified gene constructs	- The large-scale and continual cultivation, processing and consumption of GMOs may result in a very low frequency horizontal gene transfer event becoming statistically likely. Empirically derived HGT frequencies obtained in laboratory-scale models are therefore of little use to understand the occurrence and impact of HGT in field scales.[b]

[a] De Vries et al. 2001; Bensasson et al. 2004
[b] Heinemann & Traavik 2004; Nielsen & Townsend 2004; Pettersen et al. 2005

The uptake process of DNA molecules into the cytoplasm of a cell is considered to be random and independent of the DNA's subsequent biological utility. Most foreign DNA taken up and integrated into the genome of an organism will have a deleterious effect due to its interference with the host cell biology and genome structure (Elena et al. 1998; Doerfler 2000). HGT processes thus resemble mutational processes, that is, they may occur by chance and repeatedly over time, but a very low proportion of the HGT events will confer a benefit, and be retained in the host over time (Heinemann & Bungard 2005). For multi-cellular organisms, HGT events occurring in somatic (i.e. not germ-line cells) will be lost when the organism dies. In contrast, HGT events occurring into germ-line cells or single-celled organisms such as bacteria will be passed on to the following generations. Predicting the long-term survival and competitive ability (*fitness*) of the transformed host organism is therefore essential to understanding whether the transformant cells will expand in numbers or eventually die out.

The potential impact of unintended HGT of recombinant DNA from GMOs to exposed organisms must be seen within the broader picture of naturally occurring processes, including i) the continual large-scale release of genetically diverse DNA molecules from a broad range of naturally occurring or introduced species in a given environment, ii) the infrequent and random HGT events occurring naturally in the same environment that the GMO will be released into, and iii) the extremely low likelihood that any DNA taken up will improve the fitness of the exposed host organism. Within the aforementioned naturally occurring HGT context one can ask biosafety relevant questions such as:

Will recombined DNA released from GMOs have an altered and increased capacity to be transferred to, and change the fitness of, exposed host cells and bacteria?
Can the likelihood of this HGT process and the subsequent population genetic trajectories of the transformed cell be accurately predicted?

Do the currently available scientific literature and empirically-founded knowledge base on HGT processes allow a scientifically-robust impact assessment to be made?

Some scientists would argue that a hypothesized low frequency HGT event is irrelevant from a GMO risk perspective, others may argue that the HGT issues are case- and transgene specific, requiring a more detailed understanding of the natural selection context of each GMO case. Common to all biosafety viewpoints is that they are founded on expert *opinion*, familiarity with the gene donor and inference, rather than conclusive empirical *evidence*. The latter is unachievable given the limited understanding of the complexity of host cells and microbial communities exposed to GMOs.

Familiarity with the gene donor as a starting point for safety assessment is important. For instance, a GMO-specific and credible risk hypothesis can be difficult to design and test if the protein-coding regions of the recombined DNA ('the transgene') are already present naturally in the same environment as the GMO is being introduced to. If the recombined DNA sequences (present in the transgene) are also present naturally, then the HGT risk aspect would be narrowed to the potential biological effects caused by the recombinant DNA's altered genome location, context and regulation. Identifying and understanding the effects of the novel genetic compositions in GMOs are thus key elements in HGT risk assessment. Risk assessments based on absence of effects due to a predicted low frequency of HGT events are invalid, given the minor (non-linear) relationship between gene transfer frequencies and environmental impact (Pettersen et al. 2005).

We encourage a shift in the focus of the further development of GMOs to the use of intragenic and genomic modifications; that is, to limit the genetic modification to within the genome of an organism without the introduction of recombined DNA from several unrelated species. Doing so may alleviate many of the current HGT concerns (Nielsen 2003b). The interest in developing an intragenic approach is currently limited by a prevailing gene-centric approach to GE (that assumes a gene's biological performance is independent of genome context) and a lack of in-depth understanding of the regulation and traits in the genomes of organisms that are of commercial interest.

2.1 Human exposure to foreign DNA
Humans are continually exposed to DNA in inhaled organisms (e.g. bacteria, viruses, pollen etc.), from a broad variety of food sources including the microorganisms present in food, via microorganisms normally present in and on humans, and infectious agents entering the body.

Thus, the human body has mechanisms to protect host cells, and utilize and degrade or remove foreign DNA molecules.

For instance, free bacterial DNA in the blood triggers immune system reactions (Stacey et al. 1996; Cohen 2002). It is estimated that humans ingest 0.1 g to 1 g of DNA per day (Doerfler 2000). Moreover, DNA is also released continually in the gastrointestinal tract from dead microorganisms and shed intestinal cells. The quantity of any recombinant DNA ingested will be a minor fraction of the total DNA consumed per human per day. Transgenes are considered chemically equivalent to any other gene present in food (Jonas et al. 2001) (with the possible exception of transgene-induced epigenetic modifications and protein interactions). Therefore, risk hypotheses of an unintended impact of recombinant DNA are mainly focused on the novel genetic composition of the recombinant DNA and not the overall chemical structure.
In the following sections, the presence of DNA in food, and its subsequent degradation in the intestine are briefly discussed. We then consider potential uptake of food-derived DNA into host intestinal cells or tissues, or into exposed bacterial cells present in the gut or in agricultural settings.

2.1.1. DNA in food
DNA molecules of broad size ranges are present in large numbers in all raw and unprocessed food sources. Depending on the extent of processing, various fractions of DNA molecules of a reduced size may be present in the consumed product. The proven persistence of DNA molecules in raw or many types of processed food is crucial for the identification of GMO ingredients (see Chapter 33). The broad application of sensitive PCR technology has thus exemplified the widespread occurrence and persistence of DNA molecules in various food sources, including processed food such as corn chips and chocolate (Rizzi et al. 2001; 2003; 2004). However, the PCR protocols applied for GMO detection routinely target small DNA fragments, typically 100–400 nucleotides long. This size range is less than the length of a single transgene with a complete protein coding sequence. Thus, the overall concentration and distribution of DNA of a size that enables entire protein coding genes to be horizontally acquired from various food sources by host cells or bacteria remains largely undetermined. Many studies have demonstrated the persistence of DNA in food, for instance in canned food, whole seeds, cracked seeds and meal of canola, wet sugar beet pulp, cereal grains, and silage (Bauer et al. 1999; Chiter et al. 2000; Einspanier et al. 2001; Duggan et al. 2003). Processing often decreases the size of DNA, and such molecules can be undetectable in extensively processed food (Pauli et al. 2000; Kharazmi et al. 2003). See Nielsen et al. (2007) for a more extensive review of DNA in various environments. Table 13.2 lists several major knowledge gaps related to the general state of knowledge of the fate of DNA in food and during digestion.

2.1.2. DNA stability in the digestive tract
Most free DNA molecules entering the digestive system undergo substantial degradation by enzymes attacking DNA (nucleases, DNases), released from the pancreas and by bacteria present in the intestine (Wilcks et al. 2004). In addition, the low pH of the stomach may chemically modify the DNA molecules. Remaining DNA fragments are excreted in the faeces with variation in the degradation efficiency between mammals. For instance, Chowdhury et al. (2003a; 2003b) reported that maize DNA could be detected in pig faeces. Few studies have been conducted on the digestion of food-derived DNA within the 6–8 m long digestive tract of adult humans. One study by Netherwood et al. (2004) reported that whereas some DNA fragments survived passage through the small bowel, transgenes could not be detected in the faeces of human volunteers feed GM soy products.

In general, studies of the degradation of DNA in the gastrointestinal tract face many methodological challenges. Ingested food contains DNA present within tissues and cells or as complex biochemical mixtures in heat- or mechanically-damaged cells. Therefore, each food source, preparation conditions, and host physiology will determine the DNA degradation efficiencies in the digestive tract. Most studies on DNA stability in the digestive systems of mammals have used purified DNA and may therefore not capture the impact of various food components, treatments and locations on DNA degradation and stability (Martín-Orúe et al. 2002). Whereas it is generally acknowledged that DNA molecules in food are substantially degraded upon digestion in animals, there are many knowledge gaps related to the specific circumstances leading to survival of smaller DNA fragments during digestion (Table 13.2).

Table 13.2. Knowledge gaps in the understanding of the fate of (recombinant) DNA in food and the GIT.

Location / process	Lack of detailed biological understanding of:
DNA in food	- The amount, size distribution, stability and degradation dynamics in various types of raw food sources. - The effects of various types of processing and subsequent storage. - The protective or degradative role of cellular/nuclear proteins, the cytoplasmic content and cell membranes/walls. - The combined effects of the above in complex food sources.
Food-derived DNA in the GIT	- The amount, size distribution, stability, and degradation dynamics in various compartments of the GIT as a function of food source, food mixtures and prior processing. - The specific degradation mechanisms active and their relative role. - The relationship between degradation mechanisms, degradation rate and DNA availability to epithelial or bacterial cells. - Quantitative DNA exposure rates to epithelial or bacterial cells. - Intra- and interspecies host variation in the above parameters.
HGT of DNA in the GIT to host cells	- The DNA uptake mechanisms, transport pathways and degradation mechanisms in host tissues and cells. - The quantitative aspects of DNA uptake from the GIT into the bloodstream of mammals. - The cellular locations of DNA after uptake, the potential transcription, and the elimination mechanisms active. - The overall uptake process such that sensitive methods and models can be developed to adequately address the fate and possible biological effects of DNA taken up into host cells from the GIT.
HGT of DNA in the GIT to intestinal bacteria	- The proportion, size distribution, location and nature of DNA complexes exposed to bacteria in various parts of the GIT. - The diversity, function, variability, and population dynamics of the microbiota in the GIT of mammals. - The species distribution of, and tempo-spatial variability in natural transformation of bacteria present in the GIT. - The host, microbial and food factors influencing uptake of feed-DNA into bacteria. - The overall uptake process such that sensitive methods and models can be developed to adequately address the occurrence of, the relevant recipient bacterial species, and the possible biological effects of bacterial DNA uptake in the GIT.

Revised from Nielsen et al. 2005. HGT: horizontal gene transfer, GIT: gastrointestinal tract.

3. HGT of recombinant DNA to eukaryotic cells (e.g. human cells)

The uptake of food-derived DNA into host intestinal cells or tissues has been raised as a potential concern related to the introduction of GMO-based food sources. As discussed, such exposure must be seen in relation to the broad variety of DNA naturally present in food, and hence, whether specific qualitative or quantitative genetic changes are present in the GMO that would create a higher risk/impact of DNA exposure from this source.

Experimental data are readily available that support the notion that intestinal cells of the host will be exposed to DNA molecules present in food (see the following). The potential transfer of transgenes from GM food into epithelial cells of the gastrointestinal tract can thus be hypothesized to take place but experimental studies have not yet shown such transfer to occur. The lack of such observation is likely due to the fact that the total surface area of the small intestine (microvillus) alone is more than 40 m^2, with approximately 100,000,000,000 mucosal cells. Rare gene transfer events into a few of these cells are practically impossible to detect with currently available methods. In risk assessment, such hypothesized HGT events are considered to have little effect on the host because intestinal cells are shed from the lumen wall continually. The life span of mucosal cells of the small intestine is 1–2 days, and less than 10 days for most epithelial cells in the human gastrointestinal tract.

Humans eat natural food products that when combined contain > 1 million genes, some that would likely cause adverse effects if inadvertently inserted and expressed in human cells. The high general genetic diversity of DNA that enters and undergoes degradation in the intestinal system is astonishing. For instance, a simple meal consisting of chicken and two vegetables will contain a genetic diversity of more than 1 million different unique (non-overlapping) DNA fragments of 1000 bp and more than 10 million unique (non-overlapping) DNA fragments of 100 bp. Assuming a normal diet will consist of at least 50 different food sources over a limited time period, the routine exposure to DNA fragments with different compositions is between 50 to 500 million. This rough calculation does not take into account the highly diverse DNA leaking from microorganisms (eaten or present in the intestine). Thus, it can be concluded that humans are continually and naturally exposed to a genetic diversity ranging from between 50 million to 5 billion different and unique DNA compositions in the size range of 100–1000 bp. Given the high variety of DNA compositions already present in conventional food sources, few, if any, specific and testable hypotheses have been put forward that suggest commercially-used transgenes would elicit more adverse effects if horizontally acquired by intestinal cells than their conventional counterparts.[5]

Whereas potential events of uptake and integration of food-derived DNA into exposed lumen (epithelial) cells remain unidentified, many studies have shown that food-ingested DNA can pass luminal cells in the gastrointestinal tract, and be detected in the bloodstream and tissues of mammals. Specific examples are feed-derived DNA taken up from the gastrointestinal tract and detection in leucocytes, spleen, liver, and kidneys in mice (M13 DNA), in the brain, eyes, liver, and heart of the offspring of mice (plasmid DNA), detection in the liver and spleen of mice following feeding with soybean leaves (Schubbert et al. 1994; 1997; 1998; Hohlweg & Doerfler 2001), and detection of fragments of plant DNA in muscle, liver, spleen, and kidneys in chicken and cattle (Einspanier et al. 2001) It has been estimated that approximately 0.1% to 1% of dietary DNA is absorbed from the gastrointestinal tract (Nielsen et al. 2005a; 2006). A precise measurement of this process is complicated because absorption from the gastrointestinal tract takes place over several hours and absorbed DNA undergoes continuous transport, degradation

[5]This argument assumes that there are no genome positional effects, epigenetic modifications or protein associations specific to the transgene that will affect its stability and likelihood of HGT.

and elimination. Nevertheless it is clear that DNA in food may reach the bloodstream and be exposed to and localized to various host cells and tissues. Some infrequent horizontal transfer events can thus be hypothesized to take place. Thus, the genetic composition of transgenes must be assessed in the 'worst-case-scenario' of being inadvertently taken up into the body from the gastrointestinal system.

This gene-centric assessment may still be ignorant of yet to be identified effects of higher order genome structures and chromosome modifications of importance for the HGT potential and subsequent inheritance. It can be concluded from Table 13.2 that the many gaps in the general biological understanding of food DNA limits the scientific basis and quality of the current risk assessment of HGT processes in this environment. The final risk assessment may therefore often be founded on expert opinion, experience and inference, rather than an in-depth understanding of the biological fate of food DNA in the gastrointestinal tract.

4. HGT of recombinant DNA to prokaryotic cells (e.g. bacterial cells)

HGT of transgenes into pathogenic, beneficial or environmental microorganisms, resulting in potential unanticipated (absolute and relative) fitness effects, has been voiced as a potential biosafety issue. As discussed so far in this chapter, a broad range of DNA compositions is continually released from decaying organic matter. Microorganisms are responsible for the majority of organic matter decomposition and therefore also DNA degradation. Thus, microorganisms present in the human gastrointestinal tract and in agricultural environments experience continual exposure to DNA released from themselves and the organisms in their immediate surroundings.

DNA fragments exposed to bacteria will most often be utilized as a nutrient source (Nielsen et al. 2007). However, in rare circumstances, foreign DNA may also be integrated into the bacterial genome (Dröge et al. 1998; Davison 1999). Many experimental observations show that bacteria can integrate DNA molecules from their environment at measurable frequencies in the laboratory. The mosaic genetic composition of bacterial genomes also strongly suggests that horizontal transfer of chromosomal DNA has shaped their composition over evolutionary timescales (Ochman et al. 2000; Feil & Spratt 2001). However, the comparative analysis of bacterial genomes identifies HGT events that are evolutionary stable and have occurred over a time span of million of years. Comparative DNA analysis does not provide information on the gene transfer frequency itself or provide a historical account of the diversity of prior DNA exposure into the bacterium in question (Pettersen et al. 2005). Thus, it remains unclear to what extent chromosomal DNA from unrelated higher organisms is taken up into bacterial cells under natural conditions over the time course of modern agriculture.[6]

Experimental studies do not suggest bacteria integrate foreign unrelated chromosomal DNA at measurable frequencies over the limited time span (hours to days) and population size examined in laboratories (De Vries et al. 2001; Nielsen et al. 1998; 2005). A high uptake frequency is also unlikely because bacteria are continually exposed to a high diversity of DNA compositions in their environments, and unchecked uptake of DNA would quickly reduce the fitness of the bacterium and soon become lethal (Elena et al. 1998). Thus, an advantage of carrying the horizontally transferred DNA is assumed necessary to cause a biologically significant

[6]The spread of antibiotic resistance genes in clinical bacterial communities demonstrates that strongly selected genes can spread between bacterial species and communities within a short time. Although most of these resistance genes are localized on mobile genetic elements, these events demonstrate that genes can spread rapidly between microbial species when they confer a strong selective advantage to the new host.

amplification and impact of the transfer event (see Figure 13.2). It is therefore suggested that biosafety risk assessments question, determine, and identify qualitative changes in the transgenes of GMOs that would make them likely to:

Transfer horizontally, establish, and be expressed in exposed bacterial recipients.
Increase the fitness of transformed bacteria more extensively than any other transforming DNA source present in the same environment, so that altered bacterial population size or habitat utilization can be expected.

For example, many of the commercially introduced first-generation of plant transgenes are derived from soil microorganisms. Thus, microbial communities are in some cases already exposed to naturally occurring counterparts to these protein encoding genes (Nielsen 2003a; EFSA 2004; Nielsen et al. 2005b) although the combinations of associated regulatory elements are unique. The introduction of similar protein coding genes from recombinant sources to soil is therefore often inferred in biological risk assessments to cause little additional environmental impact, if a HGT event occurred (Nielsen 2003a; EFSA 2004). The HGT risk of some of the commercialized GM commodity crops currently cultivated may thus be confined to the altered genetic locations, context and regulation, and overall gene copy number concentrations. See Nielsen et al. (2005) for a further discussion on some risk considerations related to the use of antibiotic marker genes in GM plants.[7]

The novelty of the transgenes inserted into GMOs is likely to increase in the future due to development of novel gene constructs (synthetic and artificial bifunctional and multifunctional proteins) obtained through gene fusions, reshuffling and *de novo* construction of novel protein encoding domains (Nielsen, 2003b). For instance, GM plants producing novel pharmaceuticals or chemicals are in development and have already been tested in field trials. Specific, reasonable and testable hypotheses can be put forward that some of these novel plant varieties may release recombinant genes that will cause a selective advantage if taken up by exposed bacteria. Thus, HGT of recombinant DNA into bacteria will become a bigger biosafety issue in the future if the current directions in GMO production are continued. The current genetic modification approaches have little focus on the gene sources and the cellular context of the recombinations made.

[7]A precautionary-based decision to phase out antibiotic resistance plant marker genes has been made in the EU (EFSA 2004; Nielsen et al. 2005). Such a decision also exemplifies the gaps in the knowledge of resistance development in bacteria. Some of the antibiotics to which the plant marker genes encode resistance are among the most widely used in the world. Thus, whereas resistance genes to these antibiotics are known to be distributed also in non-clinical environments, they are still not a part of the majority of the antibiotic treated population of clinically troublesome bacteria. We have currently no predictive understanding to identify the specific environments, locations and conditions that will lead to the acquisition of resistance in previously sensitive bacterial populations. In the absence of such knowledge, it is impossible to accurately predict the contribution of, and long-term impact of, plant marker genes to overall resistance development in bacteria. It is also noteworthy that most emerging bacterial pathogens arise from positive selection of single HGT events. Thus, most HGT events that have had an ecological impact are not a proportional result of a high DNA exposure or HGT rate. The lack of a direct relationship between exposure/bacterial uptake, and a subsequent biological population scale impact suggest that qualitative aspects and the selection present for a given HGT event are the most important contributor and predictors of risk, and that DNA exposure or HGT rates is of little informative value (Pettersen et al. 2005).

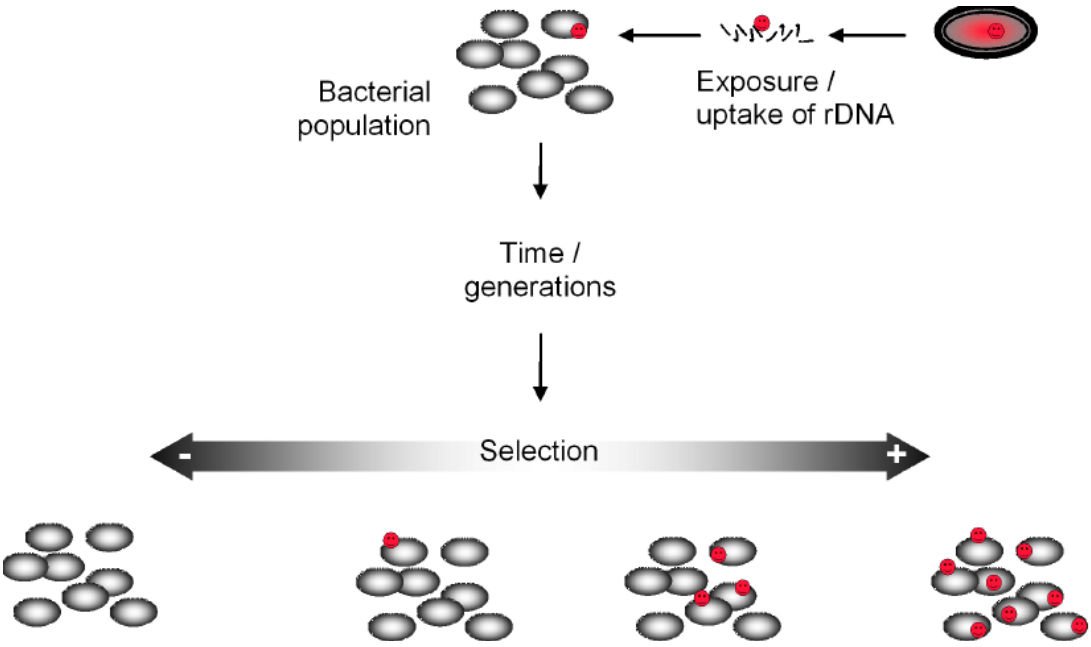

Figure 13.2. Schematic illustration of the fate of a horizontally acquired gene (red dot) over time. As shown, depending on directional selection, loss maintenance or amplification of the transformant population will occur. If the acquired gene has little effect on fitness of the transformed bacterium, random processes will determine the survival and distribution of the transformant population (this process is called genetic drift). Because most bacterial populations consist of high numbers of individuals, rare transformants will in most cases disappear from the bacterial population by chance, unless they confer a clear fitness gain to their host. Such disappearance is explained by the fact that only some members of a bacterial population will contribute to the next generation with daughter cells.

5. Concluding remarks

There are a number of knowledge gaps relating to the fate of DNA in the environment and if, when, and how exposed cells and bacteria will take up and incorporate such DNA. Knowledge gaps are themselves not indicative of harm, but are the driving motivation for new hypothesis formation and data collection. Discrepancy between the regulatory agencies' need for exact information on HGT processes and the iterative, dynamic process of knowledge formation create a situation with no clear scientific answers or regulatory or consumer consensus.
Assumption-based reasoning and a variety of information sources of variable quality have been used to aid in the assessment of potential HGT of recombinant DNA. The basis for the current risk assumptions consists of:

Laboratory test results submitted by the GMO developers.
Experimentally collected laboratory data available in the peer-reviewed literature.
Published and/or communicated historical and comparative experiences and observations of HGT processes in similar biological systems.

Submitted or conducted expert evaluations of the outcomes of conceived worst-case scenarios.
Public trust in, and scientific consensus, confidence and support of HGT risk assessment conducted by regulatory bodies depends on the quality of the data used and how uncertainty has been addressed, acknowledged and communicated (see Chapter 6). Public trust also depends on the value sets underlying scientific expert opinion formation and to what extent the consumer

adheres to the same values. The current lack of standards in HGT research that can guide hypothesis construction, choice of models and methods, and data interpretation and presentation result in sometimes heavily contextualized and motivationally biased research communications. Thus, the regulatory agencies have a challenging job separating facts from opinions, keeping in mind that even the experimental study design may bias the study to lead to a certain outcome. HGT processes occurring in nature are still not well understood and many years of further study and biological knowledge accumulation are required before precise predictions can be made on the effect or absence of effects of introduced, novel recombinant DNA. The acknowledgement of broad empirical knowledge gaps contrasts with some of the risk conclusions (the absence or presence of a HGT risk outcome) made by perhaps overly confident researchers drawing on poor data sets on HGT processes. A transparent communication of the current scientific understanding of HGT processes, the data basis applied for risk assessment, and the knowledge gaps addressed, are necessary to build public confidence in the regulatory process and to direct further HGT research on transgene ecology.

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Chapter 14

Potential Health Effects of Foods Derived from Genetically Modified (GM) plants – What are the issues?

ARPAD PUSZTAI AND SUSAN BARDOCZ

Abstract

In the European Union, the acceptance and regulation of GM crops/foods is based on the safety data which the biotech companies provide for EFSA (European Food Safety Authority) and not on the results of the EFSA's own investigations. The situation is worse in the USA where there is effectively no regulation and the commercialization of GM crops/foods is based on the flawed concept of 'substantial equivalence'. This, without stringent quantitative criteria can only serve at best, as an indication of comparability, but at worst, it can be misleading. It is therefore imperative that each GM crop is subjected to, as a minimum, the following:

- comparison of the composition of the GM- and isogenic lines with up-to-date analytical techniques, such as proteomic analysis (2D electrophoresis and mass spectrometric analysis of components)
- full biochemical, nutritional and toxicological comparison of the in planta expressed transgene product with that of the original gene used for the transformation
- microarray analysis of all novel RNA species in the genetically modified plant
- molecular examination of possible secondary DNA inserts into the plant genome
- full obligatory metabolomic NMR, etc. analysis of the transformed plant
- assessment of the variation of known toxins of GM plants grown under different agronomic conditions
- determination of the stability to degradation by acid or pepsin or other proteases/hydrolases of GM products, foreign DNA, including the gene construct, promoter, antibiotic resistance marker gene, etc. in the gut of animals in vivo
- with GM lectins, including the Bt-toxins, estimation by immunohistology of the presence/absence of epithelial binding in the gut
- investigation of the nutritional, immunological, hormonal properties, and allergenicity of GM products using the transgene product isolated from the GM crop and not with recombinant material from *E. coli*
- short- and long-term independent biological risk-assessment tests, first with laboratory animals, followed by human clinical studies of all GM crops/foods themselves and not just the transgene products. This chapter describes a suggested protocol for the testing of GM crops and foods derived from them.

Introduction

The basic tenet of the biotechnology industry engaged in the production of genetically modified (GM) crop plants and foods is that no 'credible' evidence exists that GM crops damage the environment or that GM foods harm human/animal health. Accordingly, they are as safe as their 'substantially equivalent conventional counterparts' and need no safety testing. The general acceptance of such a view could, of course, save a great deal of money for the biotechnology industry that otherwise would have to be spent on very expensive environmental- and health risk assessments of their GM products.

However, practically all recent reviews that have critically assessed the results of GM crop/food safety research data published in peer-reviewed science journals have come to the conclusion that, at best, their safety has not yet been adequately established, or at worst, that the results of risk assessment studies, particularly (but not exclusively) those carried out independent of the biotechnology industry, have raised important safety concerns which have not been properly settled. Thus, one review concluded that the most pertinent questions on environmental safety of GM crops have not yet been asked (Wolfanberger & Phifer 2000). A more recent update (Snow et al. 2005) came up with a long list of important questions that regulatory authorities should ask before any GM crops are released into the environment. Unfortunately, few of these questions have been addressed in the biotechnology companies' submissions to the regulatory authorities. The situation is not much better with the results of studies in which the potential health effects of GM foods have been investigated. Thus, an early review (Domingo 2000) found only eight peer-reviewed papers published on the potential health aspects of GM food. Pryme & Lembcke (2003) reported a rather curious aspect of the results of health risk assessment studies using laboratory animals. It appeared that most independently funded research scientists who performed animal testing of GM crops reported some potential health problems, while the results of the studies sponsored by the industry indicated none. Further reviews confirmed the scarcity of GM risk assessment research, particularly research carried out independent of the biotechnology industry. Thus, there were just over a dozen academic research papers on the health aspects of GM crops published by 2003 (Pusztai et al. 2003) and this number had increased to approximately 20 by 2005 (Pusztai & Bardocz 2006).

A report by the Canadian Royal Society stated that without in-depth biological testing of GM crops, 'substantial equivalence' is a fatally flawed concept and regulation based on it exposes Canadians to potential health risks of toxic and allergic reactions. Neither did the British Medical Association accept that all that GM crops/foods are safe, and therefore no testing is needed. In their report (The Medical Research Council 2000, recently updated) it was stated that 'any conclusion upon the safety of introducing GM material into the UK is premature as there is insufficient evidence to inform the decision making process at present'. It is, therefore, not surprising that the majority of British consumers think that GM foods are unsafe. As there is no demand for them most supermarkets in the UK have phased them out. Most consumers in Europe demand, as a minimum, the labelling and rigorous, transparent and independent safety testing of all GM foods.

Most GM crops are grown in America, the bulk in the USA. It is therefore regrettable that effectively there is no regulation in the USA that would guarantee their safety. The food regulatory agency in the USA, the Food and Drug Administration (FDA), almost totally relies on voluntary notification by the biotechnology companies that they carried out their own safety assessment of the GM crops they want to release commercially and found them to be safe. The FDA has no laboratory of its own and never, in fact, underwrites the safety of GM crops/foods. It only accepts the assurances of the biotechnology companies that their product is safe. This, in most instances, relies on a safety assessment that is based on the poorly defined and not legally binding concept of substantial equivalence.

However, similarity in composition is no guarantee that GM food is as safe as conventional food. Thus, the content of proteins, lipids and carbohydrate components of a BSE cow (a cow suffering from a condition known as bovine spongiform encephalopathy) will be similar to that of a healthy cow but, obviously, these two cows cannot be regarded as substantially equivalent for consumer health. True, compositional analysis is an obligatory starting point in risk assessment but it cannot be its endpoint. Whether GM food is toxic or allergenic cannot be decided on the basis of chemical analyses but only by biological testing with animals.

Furthermore, the biotechnology companies try to claim as much 'confidential business information' concerning their risk assessments as possible, and therefore most of the time these are unavailable in full for public or independent scrutiny or even for some national regulatory bodies.

Present state of GM food science

One of the most important reasons for the present scarcity of GM safety data is the lack of funding for basic physiological and nutritional studies of the possible health effects of GM foods on consumers. The attitude of the industry is that GM foods are safe and therefore there is no need for independent risk assessment studies. Thus, it is not surprising that ten years after the commercialization of the first GM crop, the FLAVR-SAVR tomato, there is still no generally agreed protocol for the risk assessment of GM products.

Although the EU has recently made an attempt to present a safety testing protocol for GM foods (Kuiper et al. 2004), the only previous independently funded research to set up a blueprint for GM risk assessment was the GM potato study carried out in Scotland between 1995 and 1998. Even though a blueprint for GM risk assessment based on this study was presented at an OECD meeting in Edinburgh in 2000 and subsequently published (Pusztai 2002), neither this nor the EU protocol has been generally accepted and put into practice. Accordingly, if there is any risk assessment carried out at all by the biotechnology companies this is usually an ad hoc study to suit their requirements. In the case of the more rare independent investigations into the possible biological effects of GM foods, the results obtained are non-binding on the regulatory authorities. Our database on the likely biological effects of GM foods is woefully inadequate. This is not surprising, because from the published results of one human clinical trial and a few animal studies published to date it is impossible to establish reliable and reproducible factual conclusions that are fully supported by the experimental evidence. Neither is it much help that data obtained by the biotechnology companies are seldom published and therefore these results are unavailable for most scientists. In the few cases when the industry's own risk assessment results have become public knowledge and they revealed statistically significant differences between the GM- and non-GM crop/food, the GM biotech industry denied that these differences had any biological significance. When independent scientists find such differences they are vilified.

The complexity of GM foods makes their biological testing difficult even when funding for such studies can be obtained. Thus, any protocol that may be devised must take into account that, in addition to the generally recognized importance of testing for the direct effects of the expression of the transgene, its insertion into the plant genome via a gene construct may also cause significant, indirect and unintended physiological effects by disturbing the functionality of the plant's own genes (Ewen & Pusztai 1999a; Schubert 2002, Freese & Schubert 2003; Wilson et al. 2004) and special testing methods are needed to recognize these. The number of copies of the construct inserted and their location in the plant genome (positioning effect) is also of importance.

Although the presence and consequences of such unintended effects in GM foods has long been ignored by the GM biotechnology industry, their importance is now beginning to be recognized by the regulatory agencies. Indeed, testing for these is now recommended in the Codex Alimentarius guidelines (Haslberger 2003).

Unfortunately, most currently used methods to detect unintended changes in GM products are largely inadequate. Positioning effects in plants often occur with both conventional crossbreeding and genetic engineering and empirically selecting for the desired trait and discarding the potentially harmful ones, usually to eliminate their unwanted consequences (Haslberger 2003, Pusztai & Bardocz 2006). However, it may be difficult to have appropriate selection criteria for

establishing which trait is harmful or beneficial. As it is only possible to compare the known properties and constituents of GM and conventional plants but not to look for, and even less to analyse, unknown newly created components, the limitations on our selection criteria are severe. Reliance based solely on chemical analysis of macro/micronutrients and known toxins is at best inadequate and, at worst dangerous, even when new and more sophisticated analytical methods are used, such as mRNA fingerprinting, proteomics, secondary metabolite profiling, and other profiling techniques (Kuiper et al. 2003). However, and most importantly, there is an urgent need to develop a protocol for experimental investigations using comprehensive toxicological/nutritional methods which will equally be applicable to scientifically examine the veracity of the claimed benefits of genetic manipulation and screen for its unintended and potentially harmful consequences for human/animal health. As the first contact point of exposure to any foods/feeds, including that which has been genetically modified, is the gastrointestinal tract (GIT), the first task in any proper risk assessment protocol should be to establish the consequences for the gut of short- or long-term exposure to diets that contain such foods/feeds (Ewen & Pusztai 1999a; Pusztai 2002). It is also important to point out here that any risk assessment protocol must take into account that it is not only the biological effects of the transgene product(s) that need to be unravelled but also the direct and indirect effects of the DNA vector constructs.

Alimentary tract as the first target of GM food risk assessment

To show by chemical methods the presence of new toxins/allergens in GM food products is, at best, difficult. In contrast, the presence of even minute amounts of unexpected but harmful potent bioagents in GM foods could be more easily established from their possibly disproportionally large effect on health. Thus, exposure of individuals to biologically active transgenic proteins can have major effects on their gastrointestinal tract. As most proteins are immunogenic their consumption may trigger immune/allergic effects both in the mucosal immune system of the gut and the body. It is also likely that, in addition to the effects on the gastrointestinal tract, the size, structure, and function of other internal organs will be affected, particularly in young and rapidly growing humans or animals. According to some recent unconfirmed reports, the dietary exposure to GM foods may also have harmful effects on reproduction. In addition, the risks will also have to be investigated as to whether measurable amounts of the transgenic DNA constructs in GM crops/foods survive in a functionally active state/size in the gastrointestinal tract of the human/animal ingesting them, and whether they can incorporate into the genome of the cells of their gut and body organs and what will be the consequences, if any, for the individual. The GM risk assessment protocol presented in the following outlines a gradual, step-by-step course of investigation by reliable and up-to-date methodology that addresses all these possible effects. These steps must be regarded as a minimum before any foods/feeds based on GM crops should be allowed into the human/animal food chain.

Suggested protocol for GM crop/food health risk assessment

Before any new GM crop could be made potentially safe transgenes must be identified and selected in preliminary model studies. The main criterion of the selection should be that the selected transgene and its protein product must have no toxic effects on humans or animals when given orally. However, the process of selection must be taken a step further by verifying that the selected transgene does function in the GM plant as intended. The transgene product must therefore be isolated from the GM plant and show unequivocally that its chemical and biological properties are the same as those of the gene product expressed in the original source from which the transgene was taken. It is absolutely essential that all safety studies be carried out on this isolated transgene product and not on *E. coli* recombinant surrogates.

In the GM safety studies performed by the biotechnology industry great emphasis is laid on the assertion that, according to their in vitro tests, all transgene products rapidly break down in simulated intestinal proteolytic digestion tests. Obviously, should a transgenic protein quickly break down to amino acids and small peptides in the alimentary tract its toxic effects or allergenicity could not be more than minimal and thus the safety of the GM crop should apparently be assured. However, in contrast to the protocols used in the biotechnology industry's safety assessment, true proteolytic digestibility must be established in the gut in vivo and not in a test tube in vitro. Clearly, one of the most important differences between the digestion of a protein in the alimentary canal and in a test tube using only pancreatic proteases is that in vivo, the binding of the transgene product to the intestinal wall and/or to the food matrix reduces the availability of the transgene protein (particularly in the case of the widely used transgenic lectins, such as the various *Bacillus thuringiensis*-, Bt-toxins) to the action of the proteases. Thus, an in vitro assay may give a false assurance of safety. In addition, as the structure, conformation and stability of a transgenic protein expressed in and isolated from *E. coli* is very different from that expressed in GM plants, no scientifically valid conclusions may be drawn from the results of experiments in which the assessment of the digestibility of a plant transgenic protein is attempted with an *E. coli* recombinant. Plants and eukaryotic bacteria are eons apart on an evolutionary scale and therefore no bacterial recombinants may be used in tests aimed to establish the true properties of transgenic proteins expressed in GM plants even though they are coded for by the same DNA.

Chemical composition

One of the first steps in any proper risk assessment protocol should be the characterization of the GM plant using well-authenticated and up-to-date methods of chemical analysis to estimate the contents of its major and minor components and to compare their amounts to those of the corresponding parent line. Although the results of such analysis and comparison can also be used to establish whether the GM and non-GM plants are 'substantially equivalent', first and foremost, this is an obligatory step that will allow us to carry out further biological risk assessment tests. However, for such a comparison to be scientifically valid large numbers of the GM- and the isogenic lines grown side-by-side and harvested at the same time are needed to be tested for the measurement of their major and minor constituents in parallel by classical and new analytical methods (proteomics, finger-printing, DNA/metabolic profiling, microarray analysis of all novel RNA species, full molecular biological examination with particular attention to the possibility of secondary DNA insertions into the plant genome, obligatory metabolomic NMR analysis of the transformed plant, stability of expression of foreign DNA, including the gene construct, promoter, antibiotic resistance marker gene, etc.).

Nutritional/toxicological testing with animals

As outlined, GM crops/foods will need to be examined in obligatory short- and long-term nutritional/toxicological tests with laboratory animals under controlled conditions. The intention is to find out whether there are any toxic effects in the animals fed on diets containing GM foods that would make the progression to human clinical trials unsafe. The animal tests are therefore designed to establish the effects of the GM crop/food on growth, metabolism, organ-development, immune and endocrine functions (Pusztai & Bardocz 2006), with particular emphasis on how diets based on GM food will affect the structure, function and bacterial flora of the animal gut. As the normality of these functions determine the development of young animals into healthy adults, the absence of significant differences between the health statuses of animals fed on GM- and non-GM diets may possibly indicate that the GM crop is not unsafe, at least in animal nutrition.

Diet

It is of paramount importance that the conditions of nutritional testing are rigorously standardized. Thus, all diets must be *iso*-proteinic and *iso*-energetic (i.e. contain the same amounts of protein and energy) and are fully supplemented with vitamins and essential minerals. The composition of the control diet containing the parent line should be as close to the GM diet as possible. Diet formulation is therefore – particularly when there are significant compositional differences between the GM- and its corresponding non-GM parent-line crops (e.g. see data for GM potatoes in Table 14.1) – not an easy task and supplementation with pure ingredients may be necessary to make good the compositional differences. In a second control diet, the parent line should be supplemented with the gene product isolated from the GM crop whose concentration should be the same as in the GM crop. All crops/foods should be fed both raw and after heat-treatment.

Table 14.1. Compositional values for 'Desiree' potato tubers and two GM lines expressing the snowdrop (*Galanthus nivalis*) bulb lectin, GNA (Pusztai 2002).

Constituent	Parent line	GM lines	
		Line 71	Line 74
Protein (% w/w)	7.2[a]	7.2[a]	5.6[b]
Lectin (μg/g)	6.7 (0.4)[b]	7.9 (<0.1)[a]	5.8 (0.8)[c]
Trypsin inhibitor (mg/g)	3.4 (<0.1)[a]	3.1 (0.1)[b]	2.7 (0.1)[c]
Chymotrypsin inhibitor (mg/g)	2.7 (0.1)[a]	2.6 (0.1)[a]	2.2 (0.1)[b]

The plants were grown side-by-side in field tunnels. The values are means (sd) of analyses of at least four determinations of each constituent independently carried out by two workers. Values with different superscripts are significantly different ($p<0.05$).

Experimental protocol

Groups of young rapidly growing animals (5–6 in each group) closely matched in weight (less than ± 2% w/w), housed separately, should be strictly pair-fed these diets in short- and long-term experiments. Both males and females should be tested. The progress of the animals should be closely monitored, urine and faecal samples collected throughout the experiment and the nutritional performance of the animals and the nutritional value of the diets assessed by Net Protein Utilization (NPU), and with measurements of nitrogen- and dry weight balances and feed utilization ratios. The animals should be weighed daily and any possible abnormalities observed. Blood samples should be taken before, during and at the end of the feeding experiments for immune studies (immune responsiveness assays (Table 14.2), Elispot, etc.), hormone assays (insulin, CCK, etc.) and determination of blood constituents. At the end of the experiments the animals should be killed, dissected, and their guts rinsed and the contents saved for further studies (enzyme contents, GM products, DNA, etc.), gut sections taken for histology, the wet- and dry weights (after freeze-drying of the tissues) of organs recorded (Table 14.3), and the organs subjected to compositional analyses. All these data could be used to comprehensively characterize the health and metabolic status of the animals and the behaviour of the GM fed animals could be directly compared with that of the controls. The results could then be evaluated by appropriate methods of statistics.

If any of the effects of the diet containing the GM crop on the rats is significantly different from that of the non-GM parental line control diet, the inclusion of the GM crop in food is unsafe and therefore not recommended. If the effects of feeding rats with the parent line control diet are significantly changed when this is spiked with the isolated transgene product, the *transgene is*

unsafe. Most importantly, if the effects of the diets containing the GM plant and the parent line control spiked with the gene product differ, the harm is likely to be due to the use of the particular construct vector or caused by an unintended and unforeseen effect of the *transgene insertion or position* in the plant genome. Accordingly, this method of gene transfer and the resulting GM crop is unacceptable. Thus, further research is needed to find other, more precise and safer methods of genetic modification.

Table 14.2. Results of lymphocyte proliferation assays in rats fed for 10 days on diets containing raw GM-, control/non-GM potatoes, or control/non-GM potatoes supplemented with the gene product, GNA, *Galanthus nivalis* agglutinin (Pusztai 2002).

	μg Con A/well				
Diet	0.3	1.0	3.0	6.0	9.0
Parent	10.3 (13.4)	16.0 (18.5)	4.4 (4.9)	1.9 (1.0)	1.6 (1.6)
Parent + GNA	2.5 (4.3)	2.6 (3.5)	2.0 (3.6)	1.1 (0.5)	0.9 (0.6)
GM	1.5 (0.9)		1.7 (1.1)	1.0 (0.4)	1.6 (1.1)
1.6 (1.5)					
Significance (p<)					
Parent vs					
Parent+GNA	ns	p<0.05	ns	p<0.05	ns
Parent vs GM	p<0.05	p<0.05	p<0.05	ns	ns

Rats were fed on different diets for 10 days. At the end of the experiment blood samples were taken and subjected to standard lymphocyte stimulation assay with Concanavalin A (Con A) as the mitogenic signal. The results are expressed as stimulation indexes vs control. Values are means (sd) and significance was assessed by Student t test.

Table 14.3. Relative dry organ weights of rats significantly affected by feeding with diets containing raw or boiled GM potatoes and/or parent potatoes spiked with the gene product (GNA, *Galanthus nivalis* agglutinin) (Pusztai 2002).

	Raw potatoes			Boiled potatoes
Diet	Pancreas	Jejunum	Prostate	Liver
Parent	0.68 (0.08)	0.62 (0.06)	0.24 (0.08)	3.78 (0.14)
GM	0.81 (0.05)	0.72 (0.07)	0.16 (0.02)	3.28 (0.21)
Parent + GNA	0.70 (0.08)	0.67 (0.04)	0.18 (0.02)	3.40 (0.28)
Significance (p<)				
Parent vs GM	0.01	0.03	0.05	0.001
Parent+GNA vs GM	0.03	ns	ns	ns

Rats were fed with the diets for 10 days. The values of relative dry organ weights (g organ weight/100 g dry body weight) are means (sd), n=6, by multivariate statistical analysis.

Differences in nutritional performance useful for diagnosis of harm

Organ weight changes are useful indicators of metabolic events after feeding laboratory animals with diets containing GM foodstuffs, particularly if followed up by histological examinations as part of the safety assessment of GM crops. Assessment of potential deviations in the normal development of key organs is of great diagnostic value, as shown in one of our GM-potato rat feeding studies. Sections of the various compartments of the gut taken for histology (Ewen & Pusztai 1999b) (Figure 14.1) indicated a strong trophic effect of the GM potatoes on the rats' small intestine and, to a lesser extent, on their stomach. This hyperplastic gut growth was of particular significance because the jejunum was not enlarged when the parent line diet was

supplemented with the gene product, GNA (*Galanthus nivalis* lectin), confirming previous observations which showed that the gene product had negligible growth factor effect on the jejunum, even when included in the diet at a several hundredfold concentration in comparison with that expressed in the GM potato lines (Pusztai et al. 1990). This was, in fact, one of the main reasons for selecting the gene of the natural insecticidal GNA for the genetic transformation of potatoes (Gatehouse et al. 1996) to make them pest-resistant but nutritionally safe.

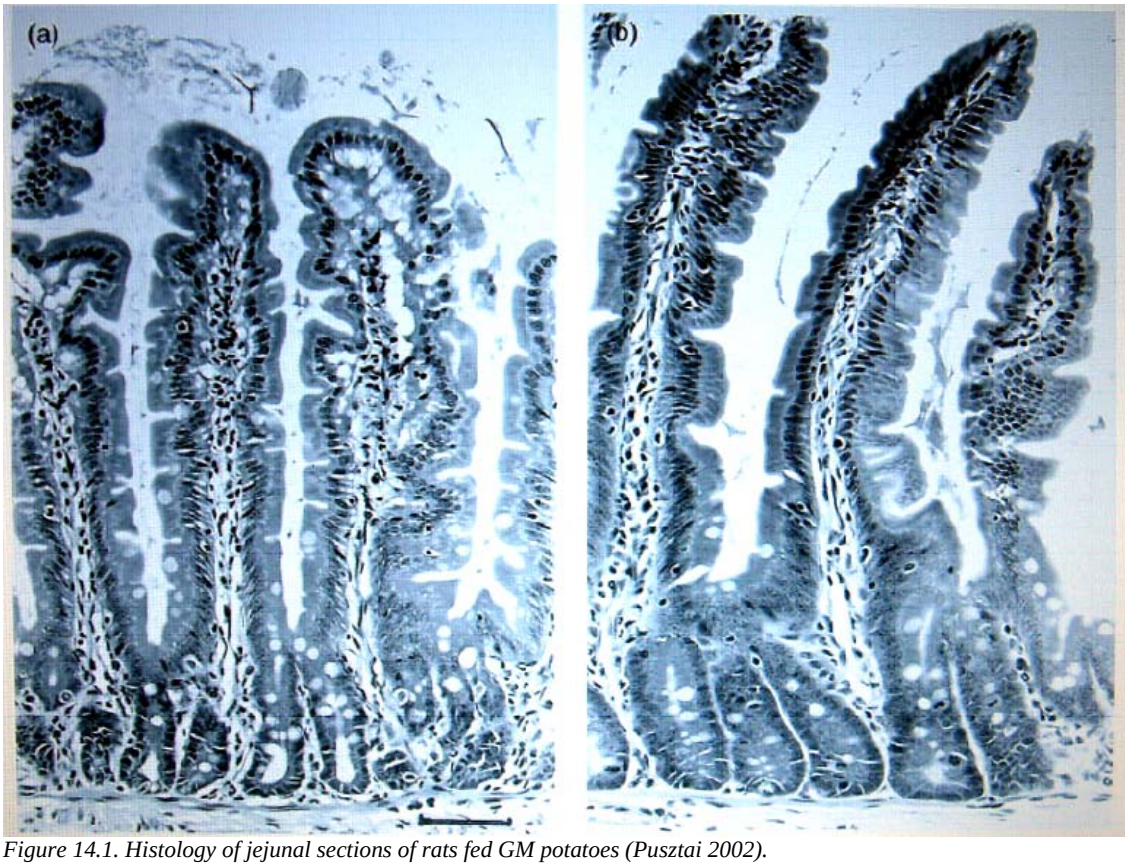

Figure 14.1. Histology of jejunal sections of rats fed GM potatoes (Pusztai 2002).
Jejunal crypt length and cells exhibit marked enlargement after feeding rats a diet of raw GM potato for 10 days, (b) in comparison with that of rats given a parental line potato diet (a). The villus length is similar in both but intraepithelial lymphocyte cell counts appear to be increased on GM potato diet. (14 mm bar = 100 μm).

As similar hypertrophic and other similar changes in gut ultrastructure in the ileum of mice fed GM potatoes expressing *Bacillus thuringiensis* var. *kurstaki* Cry 1 toxin gene or the toxin itself were shown in a different study (for reference see Pusztai et al. 2003), GM potatoes of different origins may have common trophic effects on the gut. Changes in the ultrastructure of other organs, such as the liver, pancreas, etc., on feeding with GM crop containing diets, as shown by the work of the Malatesta group (for references see Pusztai & Bardocz 2006), may also be taken as a first indication of possible harmful effects that should make follow-up studies mandatory. Changes in blood cells and blood protein levels in GM-fed animals may also suggest serious health problems, including disturbances in erithropoiesis, blood protein synthesis and the immune system. Thus, measurement of immune responsiveness could be a useful follow-up study when blood cell counts show significant differences in lymphocyte numbers that may point to one of the potentially serious hazards of the ingestion of GM foodstuffs (e.g. see our GM-potato studies, Table 14.2). This is a particularly useful method because it is in general clinical use and could therefore be easily carried out with humans. Although no hormone assays were performed on rats

fed GM or non-GM diets in our GM potato study, the consistently strong pancreatic growth stimulated by GM potato diets in the feeding studies suggests that this possibly was the result of the release of CCK (cholecystokinin) or some other humoral growth factor from the duodenum by an unknown growth/proliferative signal only found in the GM potatoes. Again, GNA (*Galanthus nivalis* lectin) could not be responsible for this because it does not stimulate the enlargement of the pancreas when fed to rats in its original source (Pusztai et al. 1990).

The measurement of circulating insulin levels after ingestion of GM diets would also be a good indicator for possible disturbances in the general metabolic state of the animals, particularly as insulin assays can be easily done on humans. Changes in blood basophile counts may also suggest possible problems of allergenicity that need to be followed up by more dynamic studies. Although the recommended decision-tree approach is a useful start to look at the allergenic potential of the GM crop, the criteria used in this, such as the lack of structural similarities of the GM protein to known allergens, the lack of glycosylation, small molecular size, or the in vitro digestibility of the GM protein, etc., are not sufficiently decisive to exclude the possibility that the GM protein is an allergen. The development of delayed hyper-sensitivity reaction found recently in GM peas expressing the kidney bean α-amylase inhibitor gene has demonstrated that proteins that are not known to be allergens in the original plant source can develop allergenic reactivity when their genes are transferred to other plant species by genetic engineering, even in the case of closely related species (Prescott et al. 2005). Finding immune-reactive antibodies to GM proteins in blood circulation, particularly of IgE-type, in humans or animals should, of course, be strong evidence for the occurrence of immune/allergenic reactions. Although there is at present no satisfactory animal model for allergenicity testing of GM proteins, immunization studies in brown Norway rats (*Rattus norvegicus*) show some promise.

Problems and perspectives

Compositional studies and animal tests are but the first steps in GM risk assessment. Next, long-term, preferably lifetime-long metabolic, immune and reproduction studies with both male and female laboratory and other animal species should also be conducted under controlled conditions. However, setting up proper protocols for these is a task that has not been accomplished yet. If none of the short- or long-term risk assessment tests on animals show harm, only then could the safety of the GM food be further tested in double-blind placebo-controlled clinical studies with human volunteers. However, it should be pointed out that most clinical studies rely on volunteers in a reasonably good state of health even though any possibly harmful effects of GM foods are expected to be more serious with the old, young and the diseased. Thus, even the results of human clinical investigations may not be representative for the whole population, particularly when it is considered that, according to some estimates, up to 40% of the population may suffer from some sort of disease of the gastrointestinal tract. It also has to be taken into consideration that because it is an irreversible technology once a GM crop is generally grown on the land and foods based on these are released into the human food chain and included in animal rations, its removal or recall will become nearly impossible.

Effects of transgenic plant DNA

In addition to the changes in protein/metabolite profiles and the possible formation of new toxins and allergens in the plant resulting from the unanticipated effects of transgene insertion and the destabilization of the recipient genome and the interference with the expression of the plant's own genes, the effects of transgenic plant DNA should also be considered. Thus, it is essential in any risk assessment protocol to determine in humans/animals ingesting GM foods whether appreciable amounts of the DNA vector construct used for developing the GM plant survive in

the gut in functional form, whether they are taken up and integrated into the genome of the individual, and what, if any, effects the foreign transgenic DNA will have on them.

GM DNA safety studies in the gastrointestinal tract

The tasks in these safety studies should follow closely the principles outlined for GM proteins and will be described in detail separately in a separate chapter in this book. Here, only the main principles of a possible GM DNA risk assessment are outlined.

The first task is to trace the GM DNA used for the development of the GM crop, such as the Bt toxin-expressing maize lines, through the intestinal tract, measure the proportion of the construct DNA surviving in functional form, establish by appropriate methods whether it is absorbed by the gut epithelial cells or by gut bacteria and integrated into the genome of these cells and whether they will express the transgene. Next, it has to be shown whether the GM DNA is absorbed into the systemic circulation and taken up by cells of body organs. In addition, it has to be investigated whether the GM DNA can pass into the placenta in pregnant females, foetus and brain, and, if so, what the biological consequences are.

In these investigations, special emphasis should be laid on whether parts of the DNA constructs, particularly the promoter, such as the cauliflower mosaic virus 35 s (CaMV 35s) are taken up by the gut and have biological effects. Obviously, as discussed in previous sections, it is of particular relevance whether the Bt toxin expressed in the GM plant has any harmful effect on the gut, body organs and the immune system. When an antibiotic resistance gene is used in the DNA construct as a selection marker gene, one of the most important questions that the risk assessment protocol will have to answer is whether this antibiotic resistance gene can transform gut bacteria in vivo. This has become highly pertinent since it was shown that functional DNA constructs used in the development of GM soybean survived in sufficient quantities in human volunteers and were found to be taken up by the bacteria in the gut (Netherwood et al. 2004 and also see Pusztai & Bardocz 2006).

Final general considerations and conclusions

In the absence of safety studies, the lack of evidence that GM food is unsafe cannot be interpreted as proof that it is safe, particularly as all well-designed GM safety studies published to date and carried out independently of the biotechnology industry have demonstrated potentially worrisome biological effects of GM food as referred to in this paper and recently documented by Smith (2007). Unfortunately, the regulators have largely ignored these.

In the light of these problems one can ask whether the future of the present generation of GM crops/foods rests on solid scientific foundations. If not, as it appears, the question is whether it is it needed at all, particularly as according to the FAO apparently there is sufficient food for feeding the world population, providing that it is evenly and properly distributed. It is possible that GM foods may be needed in future but should such a need arise we ought to first find more reliable and safer genetic transformation techniques for the development of GM crops. However, even then, their safety must be rigorously tested with biological methods, as without proper, transparent, inclusive, and independent testing the sceptical public is unlikely to be convinced of their safety and accept any present-day or future GM foods.

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