Public Research & Regulation

Foundation with the objective to involve the public research sector in regulations and international agreements relevant to modern biotechnology

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GUIDE FOR NOTIFICATIONS AND RISK ASSESSMENTS FOR RELEASES INTO THE ENVIRONMENT OF GENETICALLY MODIFIED ORGANISMS

MODULE 1: GENETICALLY MODIFIED CROP PLANTS

Introduction

The objective of the Public Research and Regulation Initiative (PRRI) is to inform the public research sector about and involve it in regulations and international agreements relevant to modern biotechnology, such as the Cartagena Protocol on Biosafety (CPB). The PRRI believes that adequate biosafety regulations are essential to allow society to maximize the benefits of modern biotechnology. The PRRI is committed to utilising the scientific expertise of the organization's members in assisting with the development of workable, transparent and predictable regulations.

For most countries that have biosafety regulations in place, release into the environment of genetically modified organisms (GMOs)[1] requires a permit or approval from the relevant competent authority(ies). In countries that do not yet have biosafety regulations in place, but that are Party to the CPB, the transboundary movement of GMOs for release into the environment of those countries requires notification to the designated authority prior to such movement. Risk assessment is key in the decision making process on such notifications and requests for permits.

Since the 1986 OECD[2] Recombinant DNA safety considerations, many documents and case studies have been produced that explain the general approaches for notifications and risk assessment. While most of these documents are encouragingly consistent and useful, communications from public researchers from all over the world show that there is a need to work these general approaches out in practical guidance that goes step by step through the entire process. The PRRI has set out to collate the vast, collective experience from its members to produce such practical guidance for public researchers.

The focus of this Guide is the technical and scientific information required for notification, and in particular for risk assessment. Over recent years, there has been a tendency for not only regulators to request, but also for applicants to include, unrequested, as much technical information in the notifications as is available, regardless of whether such information is relevant to the risk assessment. This is neither in the interest of safety - as it distracts the risk assessment from focusing on relevant information - nor in the interest of public research. One of the main aims of this Guide is to assist in keeping a focus on information that is relevant to risk assessment.

This Guide will be built up in modules, whereby the first module focuses on genetically modified crop plants.

[1] In this guide the term 'genetically modified organism' (GMO) is meant to be the same as the 'living modified organism' (LMO) of the Cartagena Protocol on Biosafety
[2] Organisation for Economic Co-operation and Development, www.oecd.org

This module on genetically modified crop plants contains, among others:

- Guidance on the process of notification
- Guidance on the general, administrative information requirements
- Guidance on the technical information requirements
- Guidance on risk assessment
- An Annex with examples of a summaries of relevant characteristics of crop plants that are frequently used for genetic modification
- An Annex with examples of summaries of relevant characteristics of genes that are frequently used with genetic modification
- An Annex with examples of risk assessment.

For this Guide, 'releases into the environment' refers to activities with GMOs outside of contained facilities such as laboratories, and ranges from small-scale confined field trials to commercial production of GMOs. As in the CPB, the term 'release into the environment' in this Guide does not refer to the use of GMOs as food, feed or processing. For brevity, this Guide uses the term 'notification' to refer to both notifications and requests for permits and approvals, and the term 'release' is shorthand for 'releases of GMOs into the environment'. When referring to 'the environment', this Guide refers to the total of biotic and abiotic components and their interrelationships, including biodiversity, and recognises that humans are part of the environment and are, as such, also included in the environmental risk assessment. This Guide, however, does not address food safety specifically.

This Guide uses footnotes for references and further clarifications, and endnotes to provide background documentation or opinion articles that are sent to us by members of the PRRI. Those opinion articles are included for the purpose of stimulating further thoughts and debate on certain issues, but do not necessarily reflect the opinion of the Steering Committee of the PRRI.

The guidance in this document can be used:

– for notifications of releases that a public research institute wishes to carry out in the country where it is based as well as for releases in other countries;
– for notifications required under domestic regulations as well as for notifications required under the CPB,
– by public researchers who are preparing notifications as well as by people involved in reviewing notifications.

This Guide is a 'work in progress' and is developed in a modular, step-wise fashion. Drafts for the modules, which are produced under the guidance of the Steering Committee of the PRRI, are sent for peer review to the public scientists of the so-called 'Forum' of the PRRI[3]. A first draft was made available in August 2005, and we have enjoyed the massive and constructive feedback.

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[3] For more information about the Forum, see www.pubresreg.org, under 'Forum'.

The many comments and useful suggestions are incorporated in this second draft, which still has many sections that require further detail.

The Steering Committee invites public researchers active in this field to send any comments, suggestions proposals for additions to: pietvandermeer@cs.com with copy to: kim.meulenbroeks@efbpublic.org. Scientists are particularly called upon to send to the PRRI additional summaries of the biology of crop plants (see Annex I), additional summaries of frequently used genes (see Annex II), and peer reviewed literature references summaries of the genes already addressed in Annex II.

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Chairman of the Steering Committee
of the Public Research and Regulation Initiative

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1. Planning - checking internal institute procedures and legal requirements

A public researcher who wishes to carry out a release of a GMO into the environment, needs to plan his or her activities carefully and well in advance. Internal institute procedures together with legal requirements may take many months, and the success of field trials often depends on the time of planting.

Many public research institutes have an Institutional Biosafety Committee (IBC), which is often charged with preparing and/or screening notifications on behalf of the institute. Public researchers intending to conduct a field trial are advised to check with their IBC first.

The next step is to check the legal requirements in the country of release. If the GMO is developed in the country where the release is intended to take place, the country's domestic biosafety regulations, if any, apply. When the GMO comes from another country or will be released in another country, either the procedures of the CPB or domestic regulations of the recipient country apply.

Under the CPB, the transboundary movement of GMOs intended for release into the environment in the Party of Import[4] is subject to an Advanced Informed Agreement procedure[5] (AIA), unless:

a. that Party of Import has posted a declaration on the BCH[6] in accordance with article 14 paragraph 4 of the CPB that its domestic regulatory framework applies[7],
b. that Party has posted a declaration on the BCH in accordance with article 13 that exemptions or simplified procedures apply[8],
c. that Party has entered bilateral agreements that deal with such import, in accordance with article 14 para 1-3 of the CPB, or
d. that GMO is exempted from AIA in accordance with article 7.4 of the CPB[9].

For further explanation on these questions, see for example the IUCN[10] Guide on the Biosafety Protocol[11] and the PRRI background paper on the Cartagena Protocol and the functioning of the Meetings of the Parties (MOPs)[12].

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[4] With 'Party of Import' the CPB means the Party to the CPB to which a GMO will be sent.
[5] The AIA procedure ensures that countries are provided with the information necessary to make informed decisions before agreeing to the import of such organisms into their territory.
[6] The Biosafety Clearing-House (BCH), which can be considered the 'aorta' of the CPB, is an information exchange mechanism established by the CPB to assist Parties to implement its provisions and to facilitate sharing of information on, and experience with, living modified organisms (LMOs).
See http://www.biodiv.org/biosafety/default.aspx
[7] For example, see the declarations of Norway and the EU on the BCH. Those domestic regulatory frameworks, which need to be consistent with the protocol, can contain special procedures for confined field trials, which can include exemptions and simplified procedures.
[8] For example, see the declarations of Colombia and South Africa on the BCH
[9] To date, no such exemptions have been established
[10] ICUN – World Conservation organisation
[11] http://www.iucn.org/themes/law/pdfdocuments/Biosafety-guide.pdf, pages 99 and following.

In summary:

- for a release not involving a transboundary movement, check which domestic regulations apply,
- for a release involving a transboundary movement: check whether AIA or other domestic procedures applies (see points a. to d. above).

It is in all cases advisable to check with the BCH what the current situation in a country is and which procedures apply.

Regardless of which procedure applies, it is advisable to contact the competent authority of the country where the release will occur, prior to formally submitting a notification. Contact points for the competent authorities can also be found on BCH[13].

Many competent authorities have excellent web-sites with information on notification and permit or approval procedures[14]. However, in cases where a notification is made for the first time, it is always advisable to contact the competent authority, to explain the intended activities and to seek guidance about the procedure of notifying (including requirements such as fees, attestations of legal personality etc), the information requirements and the expected time frame. Meeting with a competent authority has the best results if the applicant arrives prepared, i.e. has examined the web site, application formats and guidance notes.

For releases, there are usually specific information requirements for notifications, which are outlined in the regulations and further detailed and differentiated in application formats.

It is important to remain aware that every case is different, and that this also applies to the information that needs to be submitted, as EC Directive 2001/18/EC states in the chapeau of Annex III:

> "*Not all the points included will apply to every case. It is to be expected that individual notifications will address only the particular subset of considerations which is appropriate to individual situations*".

Similarly, Annex III of the CPB explains that

> "*The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment"*.

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[12] See the back ground paper posted on www.pubresreg.org under 'events'.
[13] http://bch.biodiv.org/contacts/authorities.aspx
[14] In this guide, the term 'permit' is used for authorisation to carry out certain activities such as field trials, and the term 'approval' is used for authorisations for 'placing on the market' (i.e. product approvals').

Although information requirements and application formats will differ from country to country, they usually have the same overall outline:

- General administrative information
- Technical information
- Risk assessment

These points are addressed in the sections below.

With regards to the inclusion of a risk assessment in a notification; in some legal systems only the technical information is required by the applicant, and the risk assessment is done by the competent authority, based on the technical information. In most cases, however, the inclusion of a risk assessment is a requirement.

2. General and administrative information in notifications

The general information requirements are necessary for the administrative processing of the notification, as well as information that provides the context of the notification.

In short, the information requirements address the questions:

a. who? – *i.e. who submits the notification?*
b. what, where and for how long?
c. why? - *Purpose of the proposed activity*

a. Who? – i.e. who submits the notification?

In different systems, different terms are used for who submits a notification, such as 'notifier' or 'applicant'. This Guide uses the term 'applicant'.

In most legal systems, permits can be given to legal persons and sometimes also to natural persons (i.e. individuals). Usually the legal system will require that the 'applicant' will be the legal person that carries responsibility and liability for the release, not the researcher[15]. Notifying on behalf of an ad hoc collaborative group of researchers without a legal status is in most cases not possible. It is therefore advisable that the notification be submitted on behalf of a legal entity, such as a department of a university.

In cases whereby several departments work together for a field trial, it may be advisable that the university itself submits the notification, rather than a single department of that university. Notifications should make clear which legal entity requests the permit (e.g. a university department), and who the contact person for the notification is. This may be the responsible researcher or another designated responsible person. In particular in cases of release in countries other than the applying institution, is it important to have a local contact and to ensure that all contact persons speak languages that are understood in that country. In addition, it is common practice to include the responsible researchers (if other than the contact person) of the institution in the notification.

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[15] A legal person is a construct through which the law allows a group of individuals to act as if it were one person for certain purposes. The most common purposes are ownership, and contracts. This allows for easy conduct of business by having ownership, lawsuits, and agreements under the name of the legal entity instead of the several names of the people making up the entity. A legal entity is not necessarily distinct from the natural persons of which it is composed.

b. What, where and for how long?

It is important to make clear in the title what the intended activity is, e.g. a confined field trial with virus resistant papaya. It may be useful to be even more specific i.e. naming the event or identifying the virus.

With regard to 'where' and 'for how long', notifications should take into account that for a variety of reasons a field trial may not be carried out as scheduled, for example due to continued bad weather. This is why notifications often seek permission to do trials for several years. Similarly, permits for field trials are often requested for a number of sites on different locations, to allow flexibility in choice of field sites. In those cases it is usually required that for each year the exact locations are notified to the competent authority.

c. Why? – purpose of the activity

Notification requirements often include questions referring to 'purpose of the activity', which can refer to the purpose of the genetic modification (e.g. insect resistance) as well as to the purpose of the activity (e.g. performance testing) which is notified. It is advisable to include in the notification an explanation on both aspects.

It is useful for decision makers to know what the purpose of the genetic modification is (e.g. insect resistance with expected increases in yield and reduction of pesticide use), to understand the broader context of the request at hand when they are preparing their final decision.

It is also useful for decision makers to know the purpose and scale of a requested activity (e.g. a field trial testing performance of insect resistance) to decide whether certain conditions are workable and enforceable, given the nature of the activity.

3. Technical information in notifications[16].

3.1 Technical information in relation to the risk assessment.

The technical information is primarily the information that is necessary for the risk assessment and for decisions regarding risk management.

Only that information that is directly relevant to the risk assessment and management decision should be provided or cited. Providing ancillary information in the form of reports in appendices should be avoided since this imposes a needless resource burden on both applicants and reviewers.

To underline the importance that the submitted information needs to be relevant to risk assessment, this section gives a general introduction to risk assessment, followed by section 3.2 on technical information requirements. Section 4 discusses how the actual risk assessment can be carried out in a scientifically sound, systematic and transparent manner.

Risk Assessment – Annex III of the CPB
Although risk assessment is a science-based process, there may be differences between countries in the practical approach chosen. However, examination of the many existing documentations on risk assessment[17] that have been produced over the years, shows that the underlying general principles and methodology share many similarities. In this Guide we take the outline of Annex III of the CBP as a point of reference, because the PRRI believes that Annex III is a good reflection of the effective and consistent practice of risk assessment that started with the 1986 OECD Recombinant DNA Guidelines, and which was reconfirmed in the UNEP 1995 Technical Guidelines[18].

Annex III starts by explaining that the ***objective of risk assessment*** is

> *"to **identify** and **evaluate** the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health".*

It is important to recognise the two terms 'identify' and 'evaluate', i.e. this process does not stop at listing potential adverse effects, but evaluates them in terms of whether they are significant and, as Annex III states, 'acceptable or manageable'.

[16] This and next section of the Guide builds among others on the training manual that was developed in the context of the capacity building project "Implementation of National Biosafety Frameworks in pre-accession countries in Central and Eastern Europe*"*
[17] See http://bch.biodiv.org/resources/resources.aspx
[18] See: http://www.biosafetyprotocol.be/UNEPGuid/Contents.html

Annex III outlines general principles that govern risk assessment, including:

- Scientifically sound and transparent,
- Case by case,
- Comparative - risks associated with GMOs are considered in the context of the risks posed by the non-modified host organism.

Annex III also describes the ***methodology of risk assessment****,* explaining that risk assessment typically follows a number of steps[19]:

1. *Hazard identification* - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
2. *Likelihood estimation* - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;
3. *Consequence evaluation* - An evaluation of the consequences should these adverse effects be realized;
4. *Risk estimation* - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;
5. *Risk management* – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

Finally, Annex III explains that, in the process of conducting the steps outlined above, ***risk assessment takes into account*** the relevant characteristics of:

- The recipient organism, host organism or parental organisms.
- Inserted genes, sequences and related information about the donor(s) and the transformation system.
- The resulting GMO,
- Detection and identification of the GMO
- The intended use (e.g. the scale of the activity - field trial or commercial use)
- The receiving environment.

Section 4 will explain how this all can be done in a scientifically sound, systematic and transparent manner.

[19] In some systems, such as the EU Directives on GMOs, the following order of these steps may vary a bit and sometimes certain steps are split in 'sub steps'. However, the overall approach is still largely the same.

3.2 Technical information in notification.

Although the level of detail will vary from case to case, risk assessment for releases of GMOs typically takes into account the points listed in Annex III, which are discussed in the paragraphs below. The level of detail required should be appropriate to the nature of the activity being considered. Early in development of a GMO, when less information is available, information on risks is limited and so confinement is higher to reduce exposure. Thus, small-scale field trials that determine the efficacy of a gene or the suitability of several transgenic lines will require less safety information than commercial releases, but will have a higher level of risk management. Furthermore, because field trials are generally small scale, represent a temporary short-term exposure to the environment, and are carried out under confinement conditions, the likelihood and consequences of risk components can be considered "unlikely" or "highly unlikely", and "minor" or "marginal", respectively. Therefore while risk assessment for confined field trials can be simpler, risk management takes on a more important role.

a. Characteristics of the recipient organism or parental organisms.
In this document, the terms ‘recipient organism’ and ‘host organism’ refer to the organism into which the genes are introduced through genetic modification methods. The term, ‘parental organism’ refers to cases whereby there is no clear ‘recipient’, e.g. when two cells are merged through processes such as cell fusion. This section of the Guide focuses on the use of crop plants as recipients. Other cases, such as cell fusion of crop plants will be discussed in a later module.

Whether or not novel genotypic and phenotypic characteristics may have adverse effects, depends, among other things, on the characteristics of the recipient.

A good way of presenting the relevant data of a recipient crop is to include a brief summary (one or two paragraphs) in the notification itself with more detailed relevant information in an annex (few pages), with references to existing documentation, databases etc. Again, the information in the notification and in the annexes should be pertinent to the notification.

The annexes would address topics such as:

- Origin and taxonomy of the recipient plant;
- The use as a crop;
- Genetics;
- Weedy characteristics, including survival, dispersal, volunteers and dormancy;
- Potential for outcrossing – gene transfer;
- Further references, literature and databases cited.

Annex I to this Guide gives examples of summaries of the relevant characteristics of the biology of crop plants.

b. Characteristics of the inserted genes and sequences and related information about the donor(s) and the transformation system.

As Annex III of the CPB explains, the risk assessment starts with an identification of novel genotypic and phenotypic characteristics, resulting from the genetic modification, that may have adverse effects. The reason that both ‘genotypic’ and ‘phenotypic’ are mentioned is because a certain inserted gene may not be expressed enough to result in phenotypic changes, and the level of expression of individual gene insertions may result in different phenotypic changes.

The first step in this process is to specify what has been inserted and incorporated in the genetic material of the recipient plant.

Inserting genes into the genetic material of a recipient crop plant can happen in several ways, either by incorporating those genes into molecular vectors, such as a Ti-plasmid, which can then inserts the genes of interest into the nucleus of a plant cell, or as DNA coated on microscopic particles, which are ‘shot’ into the plant cells[20].

The results of these processes will be that only a small number of the plant cells will actually have taken up pieces of the new DNA. Sometimes only one ‘copy’ of the insert is taken up, and sometimes the inserted DNA is taken up in pieces of different lengths. These may include pieces of the vector and partial (‘truncated’) genes that are not expressed. After these transformation steps, selection methods and in vitro regeneration methods then make it possible for the recovery of a whole GM plant from individual transformed cells.

With regard to the characteristics of the inserted sequences, there are two approaches:

- Either a full molecular characterisation is done of the transformed plants, identifying which part or parts have actually been inserted and integrated in the plant’s genome[21],
- or if a full molecular characterization has not yet been done, it is assumed that the entire construct may have been integrated into the recipient plant, and the risk assessment is conducted on that basis.

The second option is particularly important for public research in cases where there are many transformants to test and the release is a small-scale, confined field trial. This approach also allows researchers to submit notifications well in advance and even before the actual transformants are produced.

[20] This section describes the two favoured methods i.e. Agrobacterium-mediated transformation and Biolistics respectively. However, it should also be noted that other methods do exist for the production of GMOs. Add a text block or annex with an overview of techniques.

[21] Standard molecular techniques and checklists are available for molecular characterisation. See for example: http://www.aphis.usda.gov/brs/international_coord.html

When moving to larger scale or less confined field trials, more detailed characterisation is usually requested, leading to a full characterization for unconfined (commercial) release. This is all part of the 'step by step' approach as defined in the 1986 OECD 'Blue Book'.

After establishing which of the sequences are actually incorporated in the plant (this is called 'the insert'), the next step is to identify which of those sequences in the insert need to be considered in the risk assessment.

Inserts may contain the following sequences:

- Genes that either produce proteins or RNA molecules that have metabolic functions, such as anti-sense RNA applications;
- Non-coding DNA from the insert or the vector, which may include, among others, origins of replication.

All inserted *functional genes* are, in principle, relevant to the risk assessment, regardless of whether they are the 'genes of interest' or genes that have 'travelled along' in the process, such as selectable markers. A gene with a prokaryotic promoter (i.e. which will not be expressed in a plant cell), will also be considered in the risk assessment.

While regulatory sequences such as promoters are usually considered in the context of the functional genes of which they are part, in certain cases regulatory sequences are considered individually. For example, this may be the case when tissue specific promoters are used. Examples will be discussed in later modules of this Guide.

Origins of replication (*ori*) contain DNA sequences that in bacteria are required for the start of replication and/or mobilisation. Any autonomously replicating DNA molecule will possesses one or more *ori*. The *ori* inserted will usually be of prokaryotic origin: and allows the vector to replicate in its bacterial background. These *oris* will not be functional in the eukaryotic (plant) background, but they may facilitate replication of genes in the – unlikely – event that they are taken up and recovered in a replicable form by a bacterium. *Oris* are abundant in the bacteria found in the digestive tract of humans and animals, for example, and in bacteria present on plants and animals. Examples will be discussed in later modules.

Once the relevant inserted sequences are identified, the process continues by listing, for each inserted gene:

- the name and abbreviation
- origin
- resulting new or changed traits (phenotype) and related traits of the donor organism
- the resulting gene product and its mode of action

Annex II gives examples of this type of information for a number of frequently used genes. Some of the information in those examples is extracted from the web based pilot database "The Gene Files" (www.genefiles.org), which was developed in collaboration with the Dutch institute Plant Research International Wageningen and the Slovak Academy of Sciences. The database contains information on the identity and function of the genes as well as information on environmental risk assessments that have been carried out earlier in relation to those genes. The PRRI intends to – subject to the availability of funds – continue the Gene Files initiative.

c. The resulting GMO

In some cases, data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Those data may contain useful information for the notification at hand. In particular, data that shows whether and to what extent the resulting GMO behaves differently than the non-modified host plant. However, one should always remain aware that plants can behave differently in contained situations such as greenhouses compared to growing those same plants in the open air.

d. Suggested detection and identification methods and their specificity, sensitivity and reliability.

Detection and identification are important for reasons of monitoring and enforcement.

GMOs contain one or more additional traits encoded by an introduced gene(s), which generally produce additional proteins that confers the trait of interest. This means that detection and identification could focus on the inserted DNA, the resulting proteins or both[22].

Examples of protein based testing methods include:
- Western blot
- ELISA
- Lateral flow strip
- Magnetic particles
- Protein chips

DNA based testing methods include:
- Southern blot
- Qualitative PCR
- Quantitative end-point PCR
- Quantitative real-time PCR

[22] In addition to the "classical" methods for DNA and protein analysis, e.g. polymerase chain reaction and enzyme linked immunosorbent analysis, certain types of GMO-containing matrices can be profiled by complementary chemical analysis methods such as chromatography and near infrared spectroscopy.

Before selecting a method, it is important to establish what kind of information the different tools provide.

By way of brief summary:

- Plasmid maps: will provide details of the DNA that in principle could be inserted into the plant
- Southern blots and PCR: on presence of the gene
- Northern blots: on gene expression
- Western blots: on presence of the protein
- ELISA: quantification of the protein

Each of these methods has its own advantages and disadvantages in terms of targets, ease of use, specificity, sensitivity, costs, etc. Over the years, many good articles have been produced that provide an overview of the available methods and their advantages and disadvantages[23].

e. The intended use (e.g. field trial or commercial use)
As explained in the introduction, the term 'release into the environment', in this Guide refers to non-contained use activities with GMOs, ranging from Growing GMOs in small scale confined field trials to commercial use of GMOs (e.g. seed production)[24].

The major distinction between placing on the market and field trials is that with field trials, the GMOs involved are still under various degrees of control, whereas after placing on the market for commercial production of a GMO, its use is in principle unrestricted except of course for specific product-use conditions, such as labelling or monitoring.

Some regulatory systems distinguish between confined and unconfined field trials whereas other systems take into account both scale and confinement, such as:

- small scale confined field trials,
- small scale unconfined field trials,
- large scale unconfined field trials.

[23] See for example: Elke Anklam, Ferruccio Gadani, Petra Heinze, Hans Pijnenburg, Guy Van Den Eede: *Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products* in: Eur Food Res Technol (2002) 214:3–26
[24] It should be recognised that field trials and commercial use are not the only two types of activities that can be called release into the environment. For example, certain forms of waste treatment can also be considered a release into the environment.

e. The receiving environment.

The characteristics of the receiving environment are crucial for the risk assessment.

Relevant characteristics for field trials include:

- comparison between the normal growing environment with proposed field trial environment.
- specific environmental factors influencing survival and distribution (e.g. climate, soil conditions)
- presence of sexually compatible crops
- presence of sexually compatible wild relatives[25].

3.3 Confidential information. [26]

[25] including its feral populations, see Ammann, K., Jacot, Y., & Rufener Al Mazyad, P. (2005). The ecology and detection of plant ferality in the historic records. In Crop Ferality and Volunteerism (ed J. Gressel), pp. 31-43. Taylor & Francis, Boca Raton, http://www.botanischergarten.ch/Feral-def/Feral-MS-5-20040703.pdf extended version

[26] To be completed.

4. The environmental risk assessment

As outlined in section 3.1 the risk assessment methodology typically follows a number of steps

- Hazard identification
- Likelihood estimation
- Consequence evaluation, including a baseline assessment
- Risk estimation
- Risk management
- Consideration of overall risk

Practice shows that these steps are part of a phased approach:

a. Phase 1: Consideration of each of the inserted genes and sequences individually
b. Phase 2: Consideration of the whole plant, including potential synergistic and of possible insertion effects and including available empirical information on the resulting GMO
c. Phase 3: Consideration of risk management and overall risk.

Phase 1: Consideration of the inserted genes and sequences individually

Step 1. Hazard identification
After the basic information about inserted sequences is collected (see previous section), the actual risk assessment starts with identifying any potential hazards or potential adverse effects for each of the genes selected for consideration in the risk assessment as described in 3.1. This is the process of problem-formulation and is a critical first step in any risk assessment.

It is important to recognise that identifying potential adverse effects in this first step of the risk assessment does not mean that such effects are expected to occur, but that it only means that they will be considered in the steps of the risk assessment. A risk assessment may very well start with considering many potential adverse effects, while yet coming to the conclusion that there are no significant risks involved.

Unlike risk assessments for chemicals, there is no fixed process for the identification of potential adverse effects related to the introduction of a gene. Whether or not a particular gene or sequence may have the potential to cause adverse effects on the environment or human health, depends on the characteristics of the gene and its gene product, the recipient organisms and any changes in the phenotype, the receiving environment and of the type of application (e.g small scale field trial).

In this stage of the risk assessment process, it is important to consider the **type** of potential adverse effect(s) for which it is scientifically conceivable that they may occur, given the characteristics of the gene involved, regardless of whether it is likely that such an effect would actually occur in the proposed release. That question of likelihood will be addressed in the next stage of the risk assessment.

Examples of the type of potential adverse effects that are, depending on the case, typically considered in risk assessments for GMOs are:

- *Toxicity:* This focuses on the question of whether the expressed product of inserted gene/sequence can result in toxic effects in the recipient plant in case of incidental consumption by humans or animals.
  Note: The evaluation of toxicity in the environmental safety assessment is different from the food safety assessment (which is not addressed in this Guide), in the sense that the environmental safety assessment looks at possible toxic effects in case of incidental consumption, for example in the case when someone has taken by accident a maize cob from a test field. Food safety looks at, among others, toxic effects in case of normal consumption as food, for which there are existing approaches for evaluating food safety. Relevant in the context of considering toxicity are the following considerations: 1) DNA is not toxic, and 2) proteins are very rarely toxic, and even in the case of certain toxic effects, 3) the exposure in the case of incidental consumption will be very low.

- *Allergenicity:* Similarly to the consideration of toxicity, this focuses on the question of whether the inserted gene/sequence can result in allergenic effects arising from cases of incidental consumption of the GMO by humans or animals, or in case of exposure to parts of the plants, such as pollen27. (see also back ground and opinion articles in endnote i).

- Weediness: Can the inserted gene/sequence cause changes in the weedy characteristics of the recipient plant, i.e. can the recipient – due to the genetic modification - become more persistent in agricultural habitats or more invasive in natural habitat? This could be the case when the inserted gene or sequences confer a selective advantage or changes in fitness or dispersal28. Weediness of a plant depends on many different characteristics, such as persistence, outcrossing, dispersal, etc. etc, and other factors such as the receiving environment and its climate. In general, it is therefore not very likely unlikely that a change in one particular trait would suddenly make a plant

[27] See also the section "environmental safety vs food safety".
[28] See the publications of Fredshild in the series of Methods of Risk Assessment in the Birkhäuser Verlag, now Springer. e.g. Ammann, K. & Jacot, Y. (2003) Vertical Gene Flow. In Methods for Risk Assessment of Transgenic Plants (eds K. Ammann, Jacot, Y. & R. Braun). Birkhäuser, Basel

become 'more weedy'. However, it is theoretically conceivable that a certain new trait may just 'tip a balance' of the weediness of a crop that already had a number of weedy characteristics. [29]

- Susceptibility to pathogens: Can the inserted gene/sequence cause changes in susceptibility to pathogens, which in turn can cause the dissemination of infectious diseases and/or create new reservoirs of pathogens or vectors. For example, the change in starch composition of potatoes could conceivably result in a change in frost tolerance. Changes in frost tolerance can cause potatoes to survive winters more easily, which could result in the surviving tubers becoming sources of diseases.

- *Effects on non-target organisms.* Can the inserted gene/sequence cause adverse effects on populations of non-target organisms, for example by indirect effects on population level of other insects than the target insect or, where applicable, predators, competitors, herbivores, pollinators, symbionts, parasites and pathogens [30]. See also endnote

- *Unintended effects on the target organisms*: Can the inserted gene/sequence cause unintended adverse effects on the target organisms, such as resistance development. Resistance development is not an adverse effect in itself, unless it impairs other types of treatments such as spraying with microbial pesticides (see also the background and opinion article in endnote[ii].

- Can the inserted gene/sequence result in a *change in management* of the genetically modified crop plant that has a negative impact on the environment.

- Can the inserted gene/sequence cause adverse *changes in biogeochemical processes*, such as changes in the nitrogen cycle.

- Can the inserted gene/sequence cause *other unintended adverse effects*, such as:
  - reduce effectiveness of an antibiotic used in medicine as result of horizontal transfer of antibiotic-resistance genes,
  - the development of new virus strains due to the introduction of viral sequences in a plant genome and possible recombination of genetic material.

Some potential adverse effects will be considered in the risk assessment of almost all cases, while other potential adverse effects will only be considered in specific cases,

[29] Several useful checklists are available. See for example: http://www.aphis.usda.gov/brs/international_coord.html
[30] See, for example, for ongoing research in this field the GMO Guidelines project - http://www.gmo-guidelines.info/public/science/nontarget.html.

depending on the inserted gene[31]. Nevertheless, in all cases there should be a clear 'trigger', derived from the function and the effects of the introduced gene or gene product in the host organism, in order to make further scrutiny of the role of the gene product necessary.

In the process of identifying potential adverse effects it should also be remembered that effects can be direct, indirect, immediate and delayed[32].

Adverse effects may occur directly or indirectly through mechanisms such as:

- the spread of the GMO(s) themselves,
- outcrossing or geneflow
- instability of relevant traits such as male sterility
- interactions with other organisms,
- changes in agricultural practices.

It is important to remember that mechanisms such as outcrossing are not adverse effects by themselves. Outcrossing is a natural process that happens between plants growing in nature, between crops used in agriculture, and from crops in agriculture to plants in nature and from plants in nature to crops in agriculture, and whether or not it can result in adverse effects will depend on the characteristics of the gene that is out crossed. In particular for many commercially grown crops, the question is not whether genes will outcross, because in many cases they will. The question is whether that could cause problems, which is a key point in the risk assessment. (see also endnote [iii]).

It is also important to distinguish between potential adverse environmental effects and potential adverse agronomic effects. For example, the development of resistance of the target insect is as such not an adverse environmental effect, but an agronomic and therefore commercial effect, because in the case of resistance development farmers would not buy that variety any longer. The term ' as such' is used in the previous sentence, because resistance development of the target insects could also have

[31] A parallel can be drawn with preparing international travel, in all cases one checks for travel documents, but one only checks for warm clothing if the trip goes to cold climates and one only checks for malaria medicine if the trip goes to countries where this disease is prevalent.

[32] For example, Directive 2001/18/EC describes these terms as follows:
– "direct effects" refers to primary effects on human health or the environment which are a result of the GMO itself and which do not occur through a causal chain of events;
– "indirect effects" refers to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management. Observations of indirect effects are likely to be delayed;
– "immediate effects" refers to effects on human health or the environment which are observed during the period of the release of the GMO. Immediate effects may be direct or indirect;
– "delayed effects" refers to effects on human health or the environment which may not be observed during the period of the release of the GMO but become apparent as a direct or indirect effect either at a later stage or after termination of the release.

environmental consequences, for example in cases whereby other treatments could no longer be used and need to be replaced by other less desired treatments.

Summarising: the first step in the environmental risk assessment, the hazard identification, lays the foundation for the risk assessment, and it is therefore crucial that this first step is done thoroughly and on the basis of sound science.

It is essential that the hazard identification step addresses explicitly three closely related topics:

- the 'triggers', i.e. which new genotypic or phenotypic characteristics of the GM plant may cause adverse effects on the environment,
- the scientifically conceivable scenarios that – in theory - could lead to those adverse effects,
- a clear description of those adverse effects.

Triggers
The triggers in hazard identification are any new genotypic or phenotypic characteristics of the GM plant. For example, the presence of constitutively expressed viral coat protein DNA in a crop plant has in several cases resulted in the consideration of the possibility of the development – through natural recombinations – of new virus strains. Such a consideration is valid, as it is 'triggered' by the characteristics of the inserted viral coat protein. When considering a BT gene, there would obviously be no consideration of the possibility of the development of new virus strains, as there are no 'triggers' for such consideration in the case of a BT gene.

Scientifically conceivable scenarios
This point is closely related to the 'trigger' and only starts when there is a trigger. When a certain potential adverse effect is considered in the risk assessment, transparency requires that the 'scenario' is described as how this potential adverse effects might occur, i.e. the causal steps that could end in the adverse effect. The scenario should show the scientifically supportable chain of causal events that may lead to its occurrence and why the result would be adverse. As with all scenario writing, this is a creative process, which requires both science and imagination, with the latter tempered by considerations of plausibility. Taking the example of the viral coat protein and the BT gene: there are scientifically conceivable scenarios with regard to coat protein DNA that – in theory – could lead to recombinations. There are no scientifically conceivable scenarios to assume recombinations between a BT gene and viral infections. With the example of the coat protein DNA, the situation is relatively straightforward. However, in particular in the case of possible delayed or indirect effects, the situation will be more complicated. Again, all this is still only part of the hazard identification, the likelihood consideration of whether or not such effect will occur in the concrete case will come later.

Description of the potential adverse effect
Next, it is important to formulate clearly which potential adverse effect is being considered. For example, just mentioning 'horizontal gene transfer of an antibiotic

resistance gene' does not clarify a potential adverse effect. Transparency requires that, in the example of antibiotic resistance, reference is made to the potential adverse effect of reduced effectiveness of an antibiotic used in medicine as a result of horizontal transfer of antibiotic-resistance genes to pathogenic micro-organisms.

In formulating the potential adverse effects, it is important to distinguish between the potential change in characteristics of the GM plant (for example a potential increase in weediness, which in itself is not an adverse effect) and the potential adverse effects the may arise from an increase in weediness. A potential adverse effect could be one that through an increased fitness, the GM plant (or any relatives to which it could transfer the genes that result in increased fitness) could compete with other plants and eventually have a lasting effect on the population level of other plants. Such consequences may differ in nature depending on the receiving environment, which may be agricultural ecosystems, natural ecosystems, centres of origin or diversity etc.

The usual approach is that if it has been established that a GM plant has, due to the genetic modification, an increased fitness, then the risk assessment will explore further what the potentially resulting consequences may be, i.e. what the potential receiving environment is, and in particular whether wild relatives are present with which the GM plant can outcross.

This part of the debate is best helped by being as specific as possible about the type of potential adverse effect that is being considered. It is not helpful to just refer to 'potential impacts on biodiversity', because that as such doesn't clarify the issue at hand. Most of the potential adverse effects we discuss in risk assessment are effects that would finally be relevant for biodiversity, be it impacts on non-target organisms, weediness or anything else.

Step 2. Estimation of likelihood.

The next step in the risk assessment is an estimation of the likelihood that the events will actually occur in the particular case that is being examined.

This stage follows a similar systematic approach. An estimation of likelihood is made for each potential adverse effect identified for each of the inserted genes or sequences.

Here the term 'estimation' is chosen, because exact numbers of the frequency with which something will happen in nature cannot always be given. While this may be possible in certain risk calculations such as non-target risks[33], more frequently the risk finding is qualitative on the basis of a weight-of-evidence analysis. In the risk assessment, therefore, it terms such as 'highly likely', 'likely', 'unlikely', 'highly

[33] See for example Dively et al., Environ. Ent., 33, 2004; Sears et al., PNAS, 98, 2001; Wolt et al., Environ Ent, 32, 2003

unlikely', 'negligible' or 'effectively zero' are frequently used to convey the weight-of-evidence finding.[34].

The likelihood of a certain inserted gene or sequence actually having a potential adverse effect is influenced by many different factors, such as:

- The characteristics of the inserted gene: For example, a gene that is not involved in toxicity of the donor organism, is very unlikely to cause the recipient organism to be toxic. On the other hand, it is likely that a gene product that is known to be toxic for one insect, such as the endotoxins produced by *Bacillus thurigiensis,* is also toxic for other closely related insects. In general, if the gene product is a toxin, or is involved in the production of toxic metabolites, in the donor, data describing its toxicity or toxic effects in the GMO will be required.

- The characteristics of the recipient organism: For example, the potential for outcrossing with wild relatives is negligible for sterile plants or in regions where no cross-compatible relatives exist, but is likely with fertile plants in an environment where cross-compatible wild relatives are present.

- The characteristics or the scale of the activity: For example, the likelihood of a genetically modified plant with a certain 'built-in' pesticide resulting in significant impact on insects or other organisms other than the target pest, is negligible in a small-scale confined field trial, but may be likely in wide spread commercial use[35].

As was said above, there may be elements of uncertainty in the risk assessment. This is often the case for this step, which deals with data that can only be estimates at best, and that cannot always take into account the complexity of environmental processes.

In cases where the estimation of likelihood does not result in a clear conclusion, it is sometimes advisable to proceed to the next step of the assessment, by assuming as a 'worst case scenario' that a certain event will occur. For example, rather than spending much time and effort to determine the exact frequency of outcrossing of a certain variety, it can be assumed that if the plant <u>can</u> outcross, then it <u>will</u> outcross. The attention is then focused on the next step in the risk assessment, i.e. what are the potential consequences of such outcrossing[36].

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[34] Or 'zero' for that matter, but many scientists are uncomfortable using the term 'zero' in the context of risk assessment

[35] According to Arber, this the main difference between mutation dynamics occurring in nature and transgenes inserted into a new organism through genetic engineering: the (in evolutionary terms) almost immediate release of millions of transgenic organisms into nature Arber, W. (2002) Roots, strategies and prospects of functional genomics. Current Science, 83, 7, pp 826-828 <Go to ISI>://000178662800019 and http://www.botanischergarten.ch/Mutations/Arber-Comparison-2002.pdf

[36] There are methods to assess the outcrossing potential of crops to their wild relatives by assessing plant collections with morphometric methods and by systematic crossing

Step 3. Evaluation of the consequences.

The next step in the process is an evaluation of the consequences should these adverse effects be realized. This step is different from the first step, because it evaluates the severity of a certain effect in a particular situation and environment. Something that may be of no significant consequence in one environment may be of significant consequence in another. Terms often used in this step of the risk assessment are 'major', 'intermediate', 'minor', and 'marginal'.

Evaluating the consequences that the introduction of a genetically modified plant may have on the environment is less straightforward for a number of reasons: First, the types of effects that may have to be considered differ strongly from each other, such as weediness, effects on non-target organisms, etc. Secondly, ecosystems in general are very dynamic systems in which many changes occur constantly. Thirdly, the severity of a certain effect has to be compared with the effects of using the non-modified host organism. In the case of introducing a GM variety, it should also be considered that every agricultural activity has an impact on the environment in which it takes place. For example, a simple agricultural practice such as ploughing has a severe impact on the soil organisms such as worms, insects, bacteria and fungi, because of the exposure to air and UV light.

In order to evaluate the possible consequences of the introduction of a GMO in the context of these dynamic processes, the concept of "base line" plays an important role. The assessment of the transfer of antibiotic resistance genes from plant material to microbial organisms can serve to illustrate this. Apart from the discussion of whether or not it is likely that such genes in decaying plant material can be taken up by bacteria in such a way that the gene will still function in the bacterium, one could assess what the consequence would be if such uptake would happen. For this purpose, it is important to know what the baseline is, i.e. what is the existing situation with antibiotic resistance genes in the soil population? It is known that certain antibiotic resistance genes, such as kanamycin resistance, are so abundantly present in the environment that any – theoretical - addition through horizontal gene transfer would make no measurable difference, i.e. would be of no significant consequence. However, the – theoretical - addition through horizontal gene transfer of for example vancomycin resistance would be of significant consequence, because resistance against vancomycin is not yet widespread.

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experiments. Jacot, Y., Ammann, K., Rufener Al Mazyad, P., Chueca, C., Davin, J., Gressel, J., Loureiro, I., Wang, H., & Benavente, E. (2004) Hybridization between wheat and wild relatives, a European Union research programme. *In Introgression from Genetically Modified Plants into Wild Relatives* (ed D.B. H. den Nijs, and J. Sweet), pp. 63-74. CABI Publishing, http://www.botanischergarten.ch/Geneflow/Jacot-et-al-Amsterdam-2003.pdf

Step 4. Estimation of risk

The next step in the risk assessment is the evaluation of risk, for each of the identified potential adverse effects.

Note that at this point the term used is 'risk' instead of 'potential adverse effect'. Risk follows from the combination of the severity of a potential adverse effect (i.e. consequence) and the likelihood of it occurring.
A certain event may be of a very low likelihood, but the consequences could be so severe that the risk is still high. In this stage, the first question is whether a certain effect would be measurable and significant, and – if so – how severe a certain effect would be.

In the absence of quantitative descriptions of likelihood, terms often used in this step of the risk assessment are: high, moderate, low, negligible.

This stage qualifies any identified risk; it does not yet address the question whether certain risks are acceptable. That is a political decision that is usually taken by the responsible authorities, and follows after the next steps have been addressed, i.e. the consideration of risk management and the consideration of the overall risk.

Need for systematic approach in steps 1 to 4: worksheets with matrices.
It is strongly recommended that steps 1 – 4 above be carried out in a systematic way for each inserted gene or sequence.

To facilitate a systematic approach, matrices such as the one included in Annex III to this Guide can be used. The use of such matrices helps to focus the assessment, and once a matrix is filled in properly, it can be used to formulate the text into the section 'risk assessment' of the notification.

Phase 2: Consideration of the GM plant 'as a whole.

After the systematic 'gene by gene approach', the risk assessment moves to a more 'holistic' phase by looking at the plant 'as a whole'. In this phase, the risk assessment looks at: 1) potential synergistic effects of the inserted genes and 2) available data of the GMO itself, including data on insertion effects

Potential synergistic effects
A key question is whether the introduced traits confer characteristics that may enhance or reduce the effect of the GM plant in the environment. For example, a plant with one newly introduced abiotic stress resistance, such as drought resistance, may behave differently from a plant in which genes conferring temperature and saline tolerances are also inserted. Whether this is a point for consideration depends on the traits introduced and on the biochemical pathways involved. Certain combinations of

traits may enhance the potential for adverse effects, whereas other combinations may reduce the likelihood of adverse effects. The use of two different Bt genes, for example, is sometimes applied to reduce the likelihood of resistance development in the target organism. In some instances data for the individual genes and expressed products will be sufficient to understand the potential for synergistic effects, whereas in other cases additional research may be warranted. Examples of risk assessment of 'stacked genes' will be discussed in subsequent modules.

Data on the resulting GMO

In some cases, data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Those data may contain useful information for the notification at hand. In particular data that shows whether – and to what extent - the resulting GMO behaves differently than the non-modified host plant.

Possible insertion effects.
In looking at possible effects as a result of genetic modification, some systems – in particular food safety systems – also look at possible effects as a result of insertion of a sequence within a gene, which could interfere with the pathways in the plant. This is often referred to as 'insertion effects'. Although insertion effects are to a large extent similar to the effects of genomic rearrangements that happen during plant breeding, it is practice that any such effects, which are applicable to the specific event only and bear no relation to the characteristics of the inserted gene, be checked before the crop is placed on the market. There are genes that are only normally expressed during stress, and changes to these genes - due to disruption of plant genes during the insertion process - may not be noted until that particular stress is experienced. Such effects cannot be predicted and can only be verified with a certain degree of uncertainty by looking at the GMO as a whole. This is why application formats usually contain questions about any diversions of the standard (e.g. UPOV[37]) plant characteristics observed during the field trials.

Phase 3: Consideration of risk management and a determination of overall risk.

Finally, in cases whereby, on the basis of the previous steps, the risks involved are not deemed to be 'negligible' or 'marginal', the risk assessment continues with the next phase, which is a consideration of whether the identified risk is manageable or acceptable[38].

[37] The International Union for the Protection of New Varieties of Plants (UPOV)
[38] See for example Annex III of the Biosafety Protocol

Risk management

The first part in this last step of the risk assessment is a consideration of whether the identified risk is manageable i.e. a consideration of appropriate risk management strategies[39].

In this phase of risk assessment, the question to address is whether the identified risks require specific risk management measures. If the answer is 'yes', then a risk management strategy is defined. The risk management strategy needs to recognize that oftentimes a key rationale for small scale environmental release is to clarify unknowns in prior iterations of the risk assessment through field studies. Therefore, one rationale for approval of limited release in an instance where risks are not negligible is to develop the data needed for refined risk assessments.

For cases where a risk management strategy has been defined, the risk assessment 'loops back' to the earlier steps in the risk assessment to determine whether the proposed risk management strategies sufficiently reduce the likelihood or the consequence. This is one reason why risk assessment is often called an "iterative process". Availability of new data, derived for instance from a field confined, 'risk managed', field experiment may also be a reason to revisit and possibly revise a risk assessment[40].

There are many different strategies for risk management[41] of genetically modified plants, including:

- reproductive isolation; by removing of flowers, use of isolation distances or border rows, temporal isolation etc.,
- reduction of the size or duration of an application
- special design features such as male sterility[42].

Determination of overall risk

In cases where the level of risk is intrinsically not negligible or where the application of risk management would be very costly or difficult, the question arises whether any identified risks are acceptable.

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[39] See for example Annex II of Directive 2001/18.
[40] This practice is also applied since a long time in traditional agriculture.
[41] Add Annex with risk management startegies for crop plants
[42] see also Al-Ahmad, H., Galili, S., & Gressel, J. (2004) Tandem constructs to mitigate transgene persistence: tobacco as a model. Molecular Ecology, 13, 3, pp 697-710 <Go to ISI>://000188825700016
Gressel, J. & Al-Ahmad, H. (2005) Assessing and managing biological risks of plants used for bioremediation, including risks of ransgene flow. Zeitschrift Fur Naturforschung C-a Journal of Biosciences, 60, 3-4, pp 154-165
<Go to ISI>://000230152300002

Risks can be considered acceptable in two different contexts: in the context of the overall risk to human health and the environment, and in the context of other socio-economic considerations.
Considering the broader socio-economic context of GM applications are part of the final political decision making process. As the CPB states in article 27, Parties may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity. Consideration of the socio-economic impacts resulting from the reduction of pesticide use would be consistent with that article.

A consideration of overall risk to the environment (including human health) is typically part of the risk assessment process.

In the last step of the evaluation, therefore, the overall environmental impact is evaluated. Note that at this point there is again a change in terminology. While in the previous steps the focus was on potential 'adverse effects' and 'risks', in this last step the focus is on the 'overall environmental impact', i.e. consideration and comparison of potential adverse effects as well as potential beneficial effects on the environment.

This is why any identified risk for the environment or human health related to the genetic modification is compared with the risks associated with use of the non-modified recipient. For example, the aforementioned introduced insect resistance may have a potential impact on some non-target insects, but comparison with the practice of spraying synthetic pesticides on the non-modified crop may indicate that the impact on non-target organisms of the spraying practice is far more severe.

Conclusion – examples

The level of detail and topics addressed in risk assessment for the release of GMOs is highly case-dependent, and unlike assessments of chemicals, where there are defined procedures with complete, quantitative methods. However, risk assessment for release of GMOs can be done in a scientifically sound, systematic and transparent manner, following the steps and the points to consider outlined above. Annex IV contains examples of risk assessment considerations worked out in the worksheet for a number of frequently used types of genes[43]. These examples will not discuss specific transformants or events, but will more generally discuss types of genes, such as insect resistance through BT genes, or herbicide tolerance. They examples are given as illustrations of the type of considerations that may be relevant in different cases, and do not intend to be complete.

[43] These examples will be worked out in detail in the period late 2005 – early 2006.

5. General and cross cutting issues

In addition to the systematic steps of preparing notifications and risk assessments discussed in the previous sections, there are a number of general, cross cutting issues that are very important in gaining an understanding of the whole process.

These issues include:

- The Precautionary Approach
- Dealing with scientific uncertainty
- Food Safety vs Environmental safety

The Precautionary approach

Principle 15 of the Rio Declaration on Environment and Development (UNCED, Rio de Janeiro, June 1992) states:

> *"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."*

The PRRI warmly embraces the precautionary approach described in Principle 15 of the Rio Declaration.

In many ways, most biosafety regulations worldwide illustrate the precautionary approach, because they were invoked in the 1970s and 1980s on the basis of theoretical assumptions that certain novel genetic combinations may have adverse effects on the environment[44].

Not only is the mere existence of biosafety regulations an expression of the precautionary approach, the process of risk assessment as described in this Guide takes a precautionary approach, for example where in cases of limited familiarity risk management is applied in the form of confined field trials, and also the practice of applying 'worst case scenarios' (see above) is an expression of a prudent approach.

[44] Now, 30 years later, thousands of field trials with GMOs have been carried out world wide and around 400 million hectares of GM crops have been planted by millions of farmers in developing and developed countries, consumed in billions of meals, and there have been no verifiable reports of adverse effects to human health or biodiversity. This does not mean that GMOs by themselves are inherently safe, but it does indicate that the current risk assessment methodology and practice are effective.

This Guide attempts to provide illustrations and practical ways to incorporate the precautionary approach in the process of risk assessment. This becomes an especially prominent part of a risk assessment when there is a consideration of the existing situation in which the proposed action (e.g., introduction of an LMO) is being considered. As described below, the aspect of certainty and uncertainty comes into play in these deliberations. In practice, many regulatory approaches recognize the imperfect nature of any data set when making decisions, and thus build components into their system to allow certain activities with limitations when appropriate. The use of confined field trials of GM plants is a good example of how a smaller data set may provide sufficient certainty if measures are used to limit the introduction and persistence of the plant into the environment. Once there is sufficient information, these measures are modified or eliminated as appropriate for that individual case.

However, over the last years there seems to be a tendency to interpret the precautionary approach in a way that would suggest that any question that one could raise about GMOs, regardless whether any risks (let alone threats of serious or irreversible damage) have been identified, would suffice to stop research in this field.

This is unwise from the point of view of good governance, because the assumption that any new technology would have to completely be without risks or questions marks, would result in the fact that existing technologies with known adverse impacts, such as spraying of pesticides, could never be replaced by newer, less risky technologies.

As the CPB recognises, the trigger to evoke a precautionary approach is 1) there is a scientifically sound identified threat of damage and 2) that there is scientific uncertainty about the extent of the potential adverse effects. The practical implementation of the precautionary approach will vary from case to case, with a consideration of which measures may be practical and cost-effective to achieve the goal of avoiding the identified threat.

Addressing uncertainty

Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the GMO in the receiving environment. Where there is uncertainty we are often asked to do more experiments in order to answer the question, but further experimentation may not provide the necessary information. In those cases we often apply an approach where the focus is less on determining the likelihood of an occurrence, but rather evaluating what the consequences of the occurrence would be. Some risk assessors have referred to this approach as one of assuming a ‘worst case’ scenario, and this approach is described in section 4.

Food safety aspects vs environmental aspects

It is important to differentiate between risks related to food safety and risks related to the environment. For some, this has become complicated by the language in the Protocol in which there is mention of ‘taking also into account human health. To draw these distinctions more clearly, there is a brief discussion below.

Key issues in risks to food safety are toxicity and allergenicity. For toxicity, protocols are available for its evaluation, such as the Codex Alimentarius[45] provides guidance for evaluating food safety of plants derived by genetic engineering, including approaches to assessing toxicity. For allergenicity a different approach is required, because allergenicity can usually only be scored by patients who show the allergenic reaction[46]. For an evaluation of the potential consequence of possible toxicity or allergenicity, the type of application is taken into account. For applications, such as small scale field trials, in which the material resulting from the field trial is not consumed by humans or animals, toxicity and allergenicity would generally be of no consequence. For large-scale and market releases, toxicity and allergenicity would be of consequence and therefore needs to be addressed and usually the results of toxicity and allergenicity assessments are included in risk assessment. As mentioned before, assessors should bear in mind that there is a difference between looking at toxicity in terms of food safety, where it is assumed that large quantities may be consumed frequently (i.e. scenarios in which even low levels of toxicity may have a consequence) and toxicity in the context of environmental safety, where the focus is on effects of incidental consumption. In looking at toxicity as a result of genetic modification, two aspects need to be distinguished: possible toxicity of the gene product, and, in the specific case of food safety, possible insertion effects that may cause changes in pathways in the plant, including pathways that are related to toxicity. Although the latter case can be compared with the normal effects of genomic rearrangements that happen during plant breeding, it is practice that any such insertion effects, that are applicable to the specific event only, and bear no relation to the transgene, be checked in a food safety assessment, before the crop is placed on the market. The focus of this Guide is on the possible toxicity of the gene product in the context of environmental safety assessment. (see also endnote[iv])

[45] http://www.codexalimentarius.net/web/index_en.jsp

[46] See also http://www.who.int/fsf/GMfood/Consultation_Jan2001/report20.pdf made after the 2nd Joint FAO/WHO Consultation on Foods Derived from Biotechnology, Allergenicity of genetically modified foods, 22-25 January 2001, Rome, Italy

ANNEXES

Annex I - summaries of the biology of crop plants

CURRENT CONTENTS

- Brassica napus ((Rapeseed, oilseed rape, rape, canola)
- Cucumis melo (Cantaloupe)
- Cucurbita pepo (squash)
- Glycine Max (Soybean)
- Gossypium (Cotton)
- Lycopersicon Esculentum (Tomato)
- Oryza sativa (Rice)
- Solanum Tuberosum (Potato)
- Zea Mais Linneaus (Maize, Corn)

Brassica napus (rapeseed, oilseed rape, rape, canola)[47]

Rapeseed as a Crop
Brassica napus is a mustard crop grown primarily for its seed which yields about forty percent oil and a high-protein animal feed. Recent interest in the crop has centred on cultivars that have low erucic acid and are thus desirable edible oils. Traditional and other uses have been for lamp oils, soap making, high-temperature and tenacious high-erucic acid lubricating oils, and plastics manufacturing (R™bbelen et al., 1989; Weiss, 1983). Other species of Brassica are also grown as rapeseed oil. World production of rapeseed oil in 1987-1988 was 7.5 million metric tons, ranking it number three behind soybean (15.4) and palm (11.7), and before sunflower (7.0), cottonseed (3.4), and peanut (2.8) (Jewell, 1989). China, India, Europe, and Canada are the top world producers (Niewiadomski 1990). Current production in the United States is limited.

Taxonomy of Rapeseed
The Brassica genus belongs to the Brassicaceae (Cruciferae) family, and consists of about 375 genera and 3200 species, includes crops, condiments, ornamentals, and many weeds. Brassica contains about 100 species, including cabbage, cauliflower, broccoli, brussels sprouts, turnip, various mustards and weeds (Willis, 1973). *Brassica napus* belongs to a group of six genetically inter-related species (U, 1935) (R™bbelen et al., 1989):

- *B. nigra* (Linnaeus) Koch, black mustard, a diploid species n=8, originally spread by trade over much of the Old World, and now spread as a weed throughout much of the New World, including virtually all of the United States.
- *B. oleracea* Linnaeus, cabbage, broccoli, brussels sprouts, cauliflower, kale, a diploid species n=9, originally confined to the Mediterranean, but now widely grown in temperate gardens.
- *B. rapa* Linnaeus (or *B. campestris* Linnaeus), field mustard, turnip, turnip rape, bird rape, a diploid species n=10, originally spread throughout much of Europe, Asia, northern India, and northern Africa, and now either grown as a vegetable or oil crop, or spread as an occasional weed in much of the United States.
- *B. carinata* A. Braun, Abyssinian mustard, Ethiopian mustard, an allotetraploid species n=17, derived from *B. nigra* and *B. oleracea*, presumed to come from an ancient cross or crosses in northeast Africa, and occasionally grown in the United States as a novelty.
- *B. juncea* (Linnaeus) Czerniakowska et Cosson, Indian mustard, brown mustard, mustard greens, an allotetraploid species n=18, derived from Old World crosses of *B. nigra* and *B. rapa*, and now grown for the leaves, or spread as an occasional weed in crops or waste places. *B. napus* Linnaeus, the subject of this EA, an allotetraploid species n=19, derived from ancient crosses between *B. oleracea* and *B.rapa*, and now grown widely for its oil, and an occasional weed or volunteer in cultivated fields.

[47] For further information, feel free to contact PRRI member: Dr Penny A-C Sparrow, Department of Crop Genetics, John Innes Centre, UK, Email: penelope.sparrow@bbsrc.ac.uk

Sexual Reproduction and Interspecific Crosses in Rapeseed
Brassica napus produces an inflorescence of yellow, nectar-bearing flowers. The plants are capable of both self-fertilization and intraspecific cross- fertilization. Honeybees are the primary pollinators. Partial sexual compatibility exists with some related Brassica spp. and other closely related species outside the genus.
Rapeseed has unexceptional entomophilous flowers capable of both self- and cross-pollination. In cultivated fields, cross-pollination has been reported at about 35%, but varies depending on the availability of insect pollinators, cultivar, and weather. Downey and Bing (1990) reported outcrossing rates of 2.1, 1.1, and 0.6 percent for isolation plots located 46, 137, and 366 meters from a pollen source. Seed certification requires 660 feet isolation for Foundation Seed for B. napus, and even greater distance (1320 feet) for self- incompatible species such as B. rapa. At these distances there is a tolerance of 0.05 percent offtypes, presumably derived from pollen contamination by sources beyond the specified distance (7 CFR Part 201.76).
Honey bees are the primary pollinators of rapeseed. Although a honeybee colony may collect nectar and pollen from many species and potential foraging flights can be quite distant (to 10 km), several factors limit the potential for spread (Seeley 1985). First, each individual honeybee forager almost always collects nectar and pollen from a single plant species during a single visit. Second, given abundant flowers, such as in a cultivated field, individual honeybee foragers tend to collect nectar and pollen from flowers in the same or immediately adjacent plants. Third, honeybees are very sensitive to barometric pressure, and decrease foraging distances in response to impending adverse weather. Fourth, honeybees are subject to the pressures of energy economics, and do not forage at great distances from the nest when abundant nectar and pollen sources are close by, as in many agricultural settings.
Crosses within the species B. napus occur readily. Crosses between B. napus and other species occur with varying degrees of difficulty, and depend greatly on the direction of the cross. It should be kept in mind that the three allotetraploid species mentioned above undoubtedly arose from old natural crosses of diploid species, probably several times for each species, and thus the potential for gene movement among all these species cannot be dismissed readily. Bing (1991) reported the following crosses and attempted crosses of plants that may be outside cultivation or escapes from cultivation. Data reported are, in order, (1) cross performed (pistillate plant listed first, pollen plant listed second), (2) the number of hybrid seed per 100 pollinated buds, and (3) the results of co-cultivation.

- Sinapsis arvensis x B. napus, no hybrid seeds, and no hybrids from field co-cultivation.
- B. nigra x B. napus, 0.1 hybrid seeds, and no hybrids from field co- cultivation.
- B. rapa x B. napus, 933.8 hybrid seeds, and 1.3% hybrids from field co-cultivation.
- B. juncea x B. napus, 401.9 hybrid seeds, 4.7% hybrids from field co- cultivation.
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The potential of a gene movement, at very low level, from B. napus to other Brassica spp. such as B. juncea or B. rapa, will be subject to the availability of the target organism and the reduced fertility of the hybrids.

Weediness of Rapeseed
Many species of Brassica and related mustards are weeds or have weedy tendencies. Brassica napus is mentioned as an occasional weed, escape, or volunteer in cultivated fields (Munz 1968, Bailey 1949, Muenscher 1980). B. juncea, B. nigra, B. rapa and Sinapis arvensis (=B. kaber) to some degree are agricultural weeds, sometimes serious (Gleason, 1952; Slife et al. 1960; Reed 1970; Muenscher 1980).

Modes of Gene Escape in Rapeseed
Genes of B. napus may be transferred out of the test area by seed or by pollen.
Seed is capable of germinating in subsequent seasons.

Although the survival and maintenance of hybrids is relatively unlikely, plants receptive to B. napus pollen should not be in the area. Specifically, B. napus plants should not be within bee pollination range, and B. rapa or B. oleracea plants in flower should not be within the area during the period of flowering of the transgenic crop.

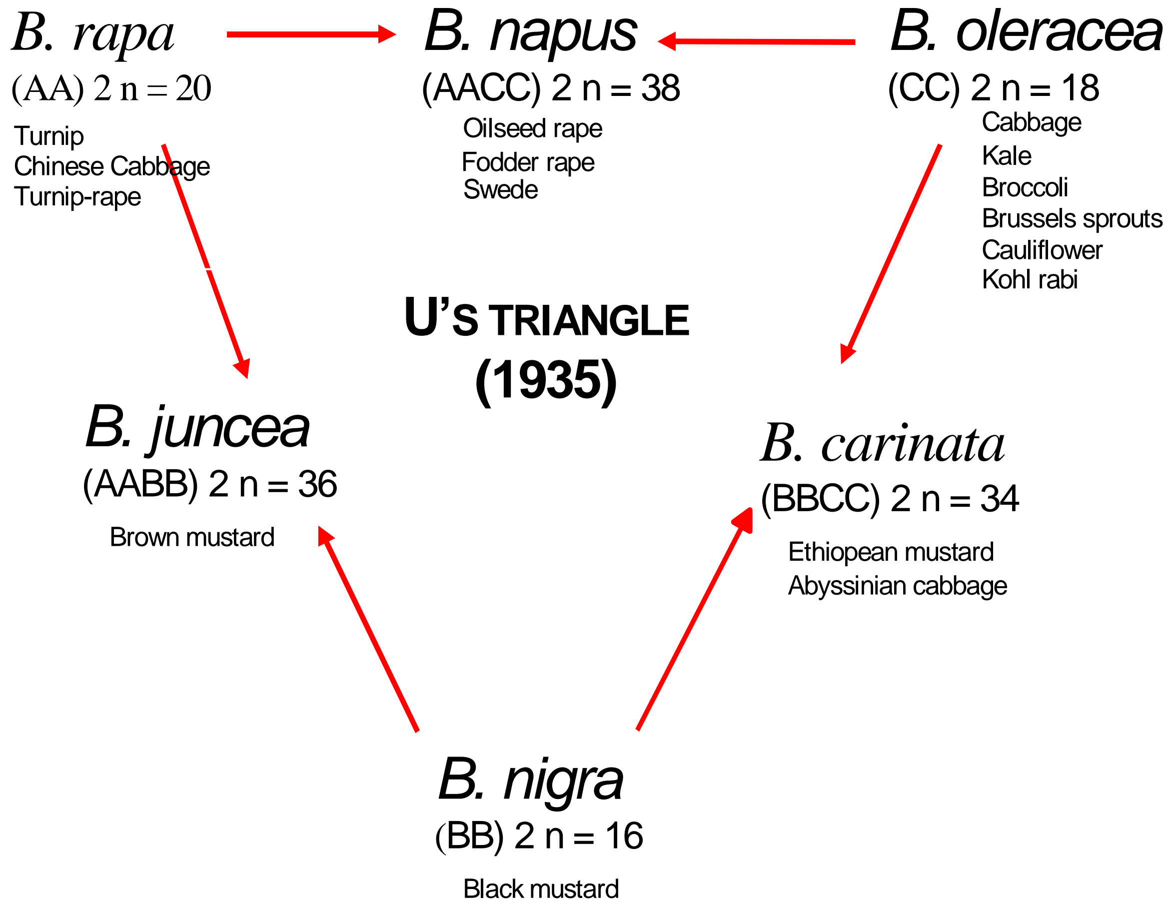

Figure 1 U's Triangle: The genetic relationship of the cultivated *Brassica* species. Redrawn from U (1935).

Genomic analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. (1935) Jpn. J. Bot. **7**:389-452.

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Cucumis melo (Cantaloupe)

Cantaloupe as a Crop
Cantaloupe is only one of the numerous cultivated melons in the highly polymorphic species, Cucumis melo Linnaeus, that is grown for the sweet edible fruit walls (Purseglove, 1968). The seeds are also eaten, and contain a high proportion of oil. The term cantaloupe refers to the common American usage of the term, to designate those melon cultivars with net-veined fruits, that are commonly referred to as cantaloupes or muskmelons in United States commerce (Everett, 1981).

Cucumis melo probably originated in Africa, from which it dispersed in cultivation to Egypt, then to the rest of Europe, China, India, the remainder of Asia, and finally throughout much of the world (Milne and Milne, 1975).

Taxonomy of Cantaloupe
Cucumis melo is a member of the Cucurbitaceae, a family of about 90 genera and 700 to 760 species, mostly of the tropics (Porter, 1967). The family includes pumpkins, squashes, gourds, watermelon, loofah, and several weeds. The genus Cucumis, to which the cantaloupe, cucumbers, and several melons belong, includes about 70 species (Hutchinson, 1967; Terrell et al., 1986). Five species of Cucumis, including C. melo and C. sativus, the cucumber, are native to or naturalized in the United States (Kartesz and Kartesz, 1980). Cucumis melo includes a wide range of cultivated plants. Although crosses outside the species are sterile, intraspecific crosses are generally fertile, resulting in a confusing range of variation (Purseglove, 1968). The more common cultivated plants fall into four main groups. First are the true cantaloupes of Europe. These have thick, scaly, rough, often deeply grooved, but not netted rinds. Second are the musk-melons, mostly grown in the United States, where they are incorrectly called cantaloupes. These have finely netted rinds with shallow ribs. Third are the casaba or winter melons with large fruits. These have smooth, often yellow rinds. The honeydew melons are in this third group. Fourth are a group of elongated melons of India, China, and Japan. These are grown as vegetables (Purseglove, 1968). Other classification schemes and peculiar cultivars could be presented (Everett, 1981).

Morphology and Reproduction of Cantaloupe
Purseglove (1968) described Cucumis melo as follows:

"A variable, trailing, softly hairy annual. Vines are monoecious or andro-monoecious. Root system large and superficial. Stems ridged or striate. Lvs orbicular or ovate to reniform, angled or shallowly 5-7 lobed, 8-15 cm in diameter, dentate, base cordate; petiole 4-10 cm long; tendrils simple. Fls staminate and clustered, pistillate and solitary, or hermaphrodite, 1.2-3.0 cm in diameter, yellow, on short stout pedicels;

calyx 5-lobed, 6-8 mm long; corolla deeply 5-partite, petals round, 2 cm long; stamens 3, free, connectives of anthers prolonged; pistil with 3-5 placentas and stigmas. Fr. very variable in size, shape and rind, globular or oblong, smooth or furrowed, rind glabrous and smooth to rough and reticulate, pale to deep yellow, yellow-brown, or green, flesh yellow, pink or green, many seeded. Seeds whitish or buff, flat, smooth, 5-15 mm long. About 30 seeds per g."
Cantaloupes fit this general description for the purposes of biology of this EA. The plants are trailing annuals. If unchecked, they spread to about 10 feed. The staminate flowers are numerous. There are fewer flowers that are hermaphroditic. One to six melon fruits develop per plant. The fruits are oblong to round and four to eight inches in diameter. There are at least 400 seeds per fruit (McGregor, 1976).

Pollination of Cantaloupe
Cantaloupe flowers open after sunrise, the exact time depends on sunlight, temperature, and humidity. The flower closes permanently in the afternoon of the same day. Almost all pollen is collected and transferred before noon. Although hermaphroditic flowers are self- fertile, they are incapable of performing self-pollination. Insects are required for the transfer of pollen. The primary pollinators are bees, particularly honey bees (McGregor, 1976).
Handel (1982) studied gene movement and bee movement in an 18 by 18 meter experimental garden of cantaloupe. He found that gene, and thus pollen, movement from the center of this garden, which had been planted with cantaloupes that bore a dominant genetic marker for green cotyledons, decreased with the distance from the central plants. Plants at 0.5 meters from the center had an average of 83 percent of the marker gene. Plants at 8.5 meters had an average of 21 percent of the gene. He also found that bee movements from plant to plant were generally short; over 99 percent of bee movements from one plant to another were of three meters or less. Most of the bees he observed were bumblebees. Bumblebees tend to have greater foraging distances than honeybees (Levin and Kerster, 1974).

Cultivation of Cantaloupe
Cantaloupes are not tolerant to frost. They perform well in hot weather. They are generally planted about one foot apart in rows about five feet apart (Everett, 1981; Lorenz and Maynard, 1988; McGregor, 1976). The plants respond well to fertilizer. They are subject to several pathogens (Purseglove, 1968).

Weediness of Cantaloupe
Cucumis melo has been reported as a weed from Colombia, Ghana, West Polynesia, and Sudan. Several other species in the genus have been reported as weeds, some as common, serious or principal weeds of some countries (Holm et al, 1979). Many other species in the family Cucurbitaceae have been reported as weeds.
Cucumis melo has been reported as spontaneous in the Northeastern United States, but it has not been reported as naturalized or weedy (Gleason, 1952).

Modes of Gene Escape in Cantaloupe
Genes of cantaloupe may escape from the test plot in three ways: as pollen, as seeds, and as vegetative parts. Physical precautions can be taken to prevent escape of vegetative parts and seeds. Escape of pollen is more difficult and requires more analysis.
For pollen to serve as a conduit of escape, it must find a receptive stigma of Cucumis melo near the test area. Cucumis melo does not grow wild in Wisconsin (Gleason, 1952). The application states that there is no commercial production of cantaloupe within two miles of the test site. Because there is a limited time each morning when pollination must be completed, two miles should serve as a reasonably effective precaution against pollen escape. Border plants of cantaloupe will further reduce the probability of bee-mediated pollen movement outside of the test area.

Cucurbita pepo (Squash)

Squash as a Crop
Cucurbita pepo is one of four related species of the genus Cucurbita that share culinary and other human uses. Various cultivars of Cucurbita pepo are called summer squash, winter squash, pumpkin, vegetable marrow, zucchini, and spaghetti squash (Purseglove, 1968; Terrell et al., 1986), which are eaten as a vegetable, fed to livestock, or used for ornament (Cobley, 1976).

Cucurbita pepo is known from archaeological sites in North America, including Mexican sites from 7000-5500 B.C. Cucurbita texana Gray grows wild in Texas and may be a wild ancestor or a modern weedy offspring (Purseglove, 1968).

Taxonomy of Squash
The genus Cucurbita consists of about 30 species of annual, tendril- bearing plants of the family Cucurbitaceae (Hutchinson, (1967). Four species are commonly cultivated: Cucurbita maxima, Cucurbita mixta, Cucurbita moschata, and Cucurbita pepo (Kernick, 1961). The fruits of these four species, and consequently the plants, are called squash, pumpkin, summer squash, winter squash, and a host of other names, based solely on the culinary or other human uses for which the plants are used, and with no regard to proper species taxonomy (Everett, 1981).

Morphology and Reproduction of Squash
Cucurbita pepo is a polymorphic species. The plants are annual herbs, monoecious, spreading or occasionally bushy, with harsh bristles. The flowers bear short sepals, united petals bright yellow to orange-yellow. Staminate flowers with three stamens. Pistillate flowers with single pistil of three inferior united carpels (Porter, 1967; Purseglove, 1968).
Because the pollen is in one flower and the stigma in another, mechanical transfer of pollen is essential for fertilization. Under normal agricultural practice, this is accomplished by honeybees. The flowers open in the morning and close by noon, the actual time depends on the weather and season. Usually pollination is most effective before 9 a.m. Other insects may also play a minor role (McGregor, 1976).

Kernick (1961) suggests an isolation distance of 400 meters between squash varieties used for seed production. Handel's (1982) studies of gene flow and bee foraging behavior in cantaloupe suggests a rapid decline in the likelihood of external gene flow in a species with a similar pollination biology.

Hybrids can be obtained between the four cultivated species of Cucurbita. However, according to Purseglove (1968): ". . . no naturally-occurring interspecific hybrid has ever been found."

Cultivation of Squash
The plants are usually planted in small hills, the spacing depending on whether the plants are spreading or bushy cultivars. The plants are usually started from seed, but can be started from cuttings. The plants respond well to fertilizer (Purseglove, 1968).

Weediness of Squash
Cucurbita pepo has been reported as a weed from Jamaica and West Polynesia (Holm et al., 1979). Other species of the genus, and related genera in the Cucurbitaceae, have also been reported as weeds. However, despite the extensive cultivation of Cucurbita pepo in the United States since prehistoric times, there is no body of scientific accounts of significant weediness in the United States.

Modes of Gene Escape in Squash
Genes of squash may escape from the test plot in three ways: as pollen, as seeds, and as vegetative parts. Physical precautions can be taken to prevent escape of vegetative parts and seeds. Escape of pollen is more difficult and requires more analysis.
For pollen to serve as a conduit of escape, it must find a receptive stigma of Cucurbita pepo near the test area. The application states that there is a commercial pumpkin farm about two miles from the test area. Because there is a limited time each morning when pollination must be completed, two miles should serve as a reasonably effective precaution against pollen escape. Border plants of squash will further reduce the probability of bee-mediated pollen movement outside of the test area.

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Glycine max (Soybean)

Soybean, Glycine max (L.) Merr., combines in one crop both the dominant world supply of edible vegetable oil, and the dominant supply of high-protein feed supplements for livestock. Other fractions and derivatives of the seed have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987).

Taxonomy of Soybean Relatives
The soybean is a papilionoid legume (family Fabaceae, subfamily Faboideae), and a member of the tribe Phaseoleae, subtribe Glycininae. The subtribe to which soybean belongs consists of 16 genera, none of which, save for soybean (Glycine) and kudzu (Pueraria), are commonly known outside of botanical science. The genus Glycine is unique within the subtribe on several morphological and chromosomal characters, and does not seem to bear an especially intimate relationship with any other genus in the subtribe (Lackey, 1977). A single exception may be the genus Sinodolichos, a rarely-collected and poorly-known genus from Asia. Sinodolichos is unknown in the living state outside of Asia (Lackey, 1981a).

The genus Glycine is divided into two questionably distinct subgenera: Glycine and Soia. The first consists of six or seven perennial species primarily from Australia. The second consists of three annual species from Asia: Glycine max, Glycine soia Sieb. & Zucc., and Glycine gracilis Skvortz. The first species is the cultivated soybean, the second species is the wild form of the soybean, and the third species is the weedy form of the soybean (Lackey, 1981a).

Morphology and Sexual Reproduction of Soybean
The soybean plant is a branched, non frost tolerant (Johnson, 1987), annual about one meter above ground level and two meters below ground level. The stem tissues are mostly primary, although the basal and more mature portions of the stems develop secondary vascular tissues during later development (Lersten and Carlson, 1987). This woody development is in accord with the derivation of soybean from tree ancestors in the rosewood tribe, Dalbergieae (Lackey, 1981a). The nodulated root system is intermediate between a taproot type and a diffuse type. The foliage leaves are alternate, pinnately trifoliolate, with pulvini, stipels, and stipules (Lersten and Carlson, 1987). The soybean flower is a standard papilionaceous flower with calyx of five united sepals; zygomorphic corolla of carina, alae, and vexillum; androecium of ten diadelphous 9+1 stamens; and gynoecium of a single carpel. Two to four seeds develop in the pods (Carlson and Lersten, 1987). The seeds have two large cotyledons and scant endosperm (Lackey, 1981b).
The anthers mature in the bud and shed their pollen directly onto the stigma of the same flower, thus ensuring a high degree of self-pollination. Cross pollination is less than one percent, often substantially so, (Carlson and Lersten, 1987; Dzikowski, 1936; McGregor, 1976). Soybean plants are thus virtually pure breeding homozygous

lines, although manual cross-pollination is practiced routinely in breeding programs (Fehr, 1987).

Culture of Soybean
Soybeans are grown in United States agriculture as monocultures of rowcrops for sale to off-farm processors. Seed is generally pure lines for each field, although blends of two or more lines are sometimes planted (Johnson, 1987). The plants are usually inoculated with Bradyrhizobium cultures (Harper, 1987; Jordan, 1982). Clean tillage has been the traditional method of field preparation, but recently no tillage and reduced tillage systems have become more common. Irrigation is not usually practiced (Van Doren and Reicosky, 1987). A complex and sophisticated system of cultivars, agricultural implements, agricultural chemicals, and processing means has been developed for the crop.

Distribution of Soybean
The United States, Brazil, China, and Argentina account for over 90 percent of world soybean production (Jewell, 1988). Soybeans are grown throughout much of the United States. The principal producing States are Illinois, Iowa, Missouri, Minnesota, Indiana, and Ohio (Jewell, 1988).

The wild and weedy forms of soybean (G. gracilis, and G. soia), and all other non-soybean species of Glycine grow naturally only in Asia, Australia, and closely associated areas (Hermann, 1962). In the United States, the wild and weedy forms of soybean are only known at university and other specialized research stations.

Modes of Gene Escape from Research Plots
Pollen is unlikely to escape from research plots. Soybeans are almost completely self pollinated (Carlson and Lersten, 1987; McGregor, 1976). Caviness (1970) showed that honey bees are responsible for the occasional cross-pollination, and that thrips are ineffective pollination vectors. Soybean seed has a short time potential for high germination and vigor, and in commercial operation, fresh soybean seed is produced annually for each new season (TeKrony et al., 1987). However, some remaining seed from one crop is capable of germinating the following season, and is therefore able to cause a temporal, if not geographic, dispersal of the soybean plant. Vegetative reproduction of soybean plants does not occur under field conditions.

Survival of Soybean Plants
Soybean plants are annuals, and do in most areas of cultivation not survive from one growing season to the next (Hymowitz and Singh, 1987). Survival from one season to the next is by seed, and for commercial varieties, this requires fresh, properly grown and handled seed for each growing season (TeKrony et al., 1987).

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Gossypium (cotton)

Origin and Taxonomy of Cotton
The genus *Gossypium*, a member of the *Malvaceae* family, consists of some 50 species, four of which are generally cultivated (Fryxell, 1992). The most commonly cultivated species, *G. hirsutum* L., is the subject of this Environmental Assessement. Other cultivated species are *G. arboreum* L., *G. barbadense* L., and *G. herbaceum* L. The centre of origin of cotton is ……..[48]

Cotton as a Crop
Four species of the genus *Gossypium* are known as cotton, which is grown primarily for the seed hairs that are made into textiles. Cotton is predominant as a textile fiber because the mature dry hairs twist in such a way that fine, strong threads can be spun from them. Other products, such as cottonseed oil, cake, and cotton linters are byproducts of fiber production. Cotton, a perennial plant cultivated as an annual, is grown in ……….

Genetics of Cotton
At least eight genome designations, A, B, C, D, E, F, G and K, are found in the genus (Endrizzi et al., 1985). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum*, and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*. By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. How and when the original crosses occurred has been subject to much speculation. Euploids of these plants have 52 somatic chromosomes, and are frequently designated as AADD. Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. *Gossypium tomentosum* has been crossed with *G. hirsutum* in breeding programs. The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean, as invaders in the constantly disturbed habitats of strand and associated environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1979).

Weedy characteristics of Cotton
Although the New World allotetraploids show some tendencies to "weediness" (Fryxell, 1979), the genus shows no particular weedy tendencies. Cotton *Gossypium hirsutum* is generally self-pollinating, but in the presence of suitable insect pollinators can exhibit cross pollination. Bumble bees (*Bombus* spp.), Melissodes bees, and honey bees (*Apis mellifera*) are the primary pollinators (McGregor, 1976). The concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use. If suitable bee pollinators are present, distribution of pollen decreases considerably with increasing distance.

[48] Include reference

McGregor (1976) reported results from an experiment in which a cotton field was surrounded by a large number of honey bee colonies, and movement of pollen was traced by means of fluorescent particles. At 150 to 200 feet, 1.6 percent of the flowers showed the presence of the particles.

Modes of Gene Transfer from Cotton

Genetic material of *G. hirsutum* may be transferred from an area of cultivation by vegetative material, by seed, or by pollen. Propagation by vegetative material is not a common method of reproduction in cotton. Movement of seed can occur on farm implements such as planters and harvesters, and can be minimized by cleaning of equipment between plots when separation of crop varieties is desired. Movement of genetic material by pollen is possible only to those plants with the proper chromosomal type, in this case, only to those allotetraploids with AADD genomes. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments would reduce the potential for pollen movement. Movement of genetic material to *G. tomentosum* is less understood. The plants are chromosomally compatible with *G. hirsutum*, but there is some doubt as to the possibility for pollination. The stigma in *G. tomentosum* is elongated, and the plant seems incapable of self-pollination until acted upon by an insect pollinator, but flowers of *G. tomentosum* seem to be pollinated by moths, not bees, and they are receptive at night, not in the day. Most *Gossypium* flowers are ephemeral; they open in the morning and wither at the end of the same day. Both these factors would seem to minimize the possibility of cross-pollination. However, Fryxell (1979) reports that *G. tomentosum* may be losing its genetic identity from introgression hybridization of cultivated cottons by unknown means.

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Lycopersicon esculentum (Tomato)

Biology of Tomato
The commercial tomato belongs to a species referred to as L. esculentum. Lycopersicon is a relatively small genus within the large and diverse family Solanaceae, consisting of approximately 90 genera. The genus is currently thought to consist of cultivated tomato, L. esculentum, and eight closely related wild Lycopersicon species (Rick, 1976). Lycopersicon species are native to Ecuador, Peru, and the Galapagos Islands; however, most evidence suggests that the site of domestication was Mexico (Taylor, 1986).

Propagation and Cultivation of Tomato
Tomato is a highly specialized crop and bred to be grown under intensive monoculture. Tomato is grown commercially wherever agronomic conditions will permit an economic yield to be obtained. All commercial tomato cultivars are exclusively self-compatible and exclusively inbreeding. As is true for most self-pollinating plants, the viability of exposed tomato pollen is limited. The distance required between foundation seed fields is 200 feet which in practical terms is the effective distance tomato pollen can travel under field conditions and remain viable (Anonymous, 1971; Rick, 1976).

Cross-pollination.
Tomato does not cross-pollinate other plant species. The factors that prevent cross-pollination are well documented and are applicable to genetically engineered tomato. Tomato can be crossed by hand-pollination to all wild Lycopersicon species with varying degrees of success. The genus has been divided into two subgenera, the one easily crossed with commercial tomato (esculentum complex), and those that cannot be easily crossed (peruvianum complex). Hybridization between these two subgenera usually leads to early embryo breakdown, which results in seed that is not viable. This problem can be circumvented by embryo culture and other laboratory techniques, albeit at great effort.
The closest genetic relatives of tomato are in the genus Solanum. Hybrids have been obtained between L. esculentum and S. lycopersicoides, but these hybrids are usually sterile (Stevens and Rick, 1986). No other member of the genus, including S. nigrum, a common weed in tomato fields, has yielded viable hybrids (Taylor, 1986).

There is no evidence that tomato plants can cross-pollinate with other plants in the area of the field test. Similarly, there is no evidence that the engineered tomato plants will cross-pollinate with any other tomato plants in the vicinity.

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Oryza sativa (Rice)[49]

Species composition and distribution
Northern India, Southeast Asia, and southern China are believed to be the centre of origin of Asian rice (*O. sativa*). The rice genus *Oryza* has a pantropical distribution and comprises approximately 23 species that include both diploids ($2n=2x=24$) and tetraploids ($2n=4x=48$), and ten different genome types: AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH, and JJKK (Vaughan 1994; Ge et al 1999). The genus *Oryza* is distributed in Asia (eg *O. rufipogon* and *O. nivara*, both AA genomes), Africa (eg *O. barthii,* and *O. longistaminata*, both AA genomes), Australia (eg *O. meridionalis*, AA genome), tropical America (*O. glumaepatula,*AA genome, and *O. grandiglumis*, *O. alta,* and *O. latifolia,* all CCDD genomes) (Akimoto 1998; Sano and Sano 1990; Vaughan 1994). Asian cultivated rice (*O. sativa*) has the diploid AA genome. Wild progenitors of African cultivated rice (*O. glaberrima*, AA genome) are grasses endemic to West Africa.

Environmental Safety Considerations
Outcrossing and Weediness Potential
Cultivated rice (*Oryza sativa* L.) is primarily an autogamous, self-pollinating plant, although gene introgression into other cultivated rice is possible. Cultivated rice is an annual, it does not shatter or disperse its seed, and has not acquired extended dormancy. Reported outcrossing rates are less than one percent and are limited by the biological characteristics of rice (Messeguer et al 2001). Factors including flower morphology, inability of pollen to remain viable longer than a few minutes, and a lack of insect vectors for pollen spread contribute to the low propensity of rice to cross-pollinate. Modern rice cultivars are often grown near older, traditional landraces in Asia, whereby only very low hybridization rates between these two groups have been measured (Rong et al 2004). This is consistent with recommended distances of six meters and less in certified seed production (Gealy et al 2003). *Oryza* species with different genome types have significant reproductive isolation, making them unlikely to hybridize with each other. Hybridization between species in different genera within the tribe Oryzeae is extremely difficult, even using artificial conditions such as embryo rescue.

In the United States, the only wild species known to be compatible to cultivated rice is *O. rufipogon*, which has been found in a single location in the Everglades of Florida, and red rice, a wild variant of cultivated *O. sativa*, thus it is considered very unlikely that cultivated rice would hybridize with *O. rufipogon* under such conditions.

Red rice, also known as *O. sativa* f. *spontanea* is considered a weedy species in the cultivation of rice, as the reproduction of red rice favours specific environmental

[49] For further information, please feel free to approach PRRI member Jorge E Mayer, PhD, MIP (Law), Golden Rice Project Manager, Campus Technologies, Freiburg, Germany, email jorge.mayer@goldenrice.org

conditions (such as flooded fields) that are typical in the cultivation of commercial rice. Outside of rice production areas, red rice is not a weed species. Gene flow from cultivated rice into red rice can occur, although the rate is likely to be very low with levels being dependent on the degree of overlapping of flowering periods. Weedy rice is readily found in tropical America. Weedy rice appears to be mainly composed of annual *Oryza* spp with feral traits including seed shattering. In contrast to Asia where manual transplanting is still predominant, direct seeding of weedy rice-contaminated seed is common for a high proportion of rice farmers in tropical America, ensuring field reinfestations and making it one of the most serious weed problems in this region (Fischer and Ramirez 1993).

Weedy rice is often referred to as red rice because of the red color of its pericarp, and it has been botanically classified as *O. sativa f. spontanea,* the same species as cultivated rice (Chu et al 1969; Diarra et al 1985; Ellstrand et al 1999; Langevin et al 1990; Oka and Chang 1961). Reports suggest that weedy rice may include other *Oryza* species including *O. barthii*, *O. glaberrima*, *O. longistaminata*, *O. nivara*, *O. punctata*, *O. sativa*, and *O. latifolia* (an American tetraploid) (Holm et al 1997). Hybrid swarms between the American form of *O. perennis* and *O. sativa* have been found in Cuba (Chu and Oka 1969). Weedy rice may also have evolved through the dedomestication of cultivated rice to weedy types (Vaughan et al 2003). In addition to seed shattering, weedy rice seeds may possess secondary dormancy, and some types are morphologically indistinguishable from rice varieties yet still shatter seed (Lentini and Espinoza 2005). Natural gene flow estimates in the field from herbicide-resistant rice into weedy rice under temperate conditions indicate hybridization rates of under one percent (Chen et al 2004; Estorninos et al 2002; Messeguer et 2004; Zhang et al 2003), as confirmed by genetic analysis. However, a cumulative hybridization rate (over a 3-year period) under temperate conditions may be from 1 to 52% (Guadaggnuolo et al 2001), indicating that genes from rice varieties may transfer and be quickly fixed into weedy rice if they have a selective value. The cumulative rate of introgression may be even higher under tropical conditions because of the lack of crop rotation and several crop cycles per year. Several biological, genetic, and environmental factors affect the level of outcross compatibility, including temperature, humidity, genotype, flower morphology, stigma receptivity, pollen viability, pollen germination, and tube development.

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Zea Mais Linneaus (Maize, Corn)

Corn as a Crop
Zea mays Linnaeus, known as maize throughout most of the world, and as corn in the United States, is a large, annual, monoecious grass, that is grown for animal feed, silage, human grain, vegetable oil, sugar syrups, and other miscellaneous uses. It is a premier cash crop and its cultivation, genetics, processing, financing, and distribution on a national and international scale is pervasive and complex.

World production in 1987/1988 was 439 million metric tons, of which the United States produced 179, China 76, Brazil 23, and France 12. Corn is grown commercially in almost all States of the United States (Jewell, 1989). United States production in 1987 was 7064 million bushels, of which the top State producers were Iowa (1306), Illinois (1201), Nebraska (812), Minnesota (635), and Indiana (632). Corn has the highest value of production of any United States crop; 1987 value was 12.1 billion dollars, compared to soybeans at 10.4, hay at 9.1, wheat at 5.4, and cotton at 5.0.

Corn has been cultivated since the earliest historic times from Peru to central North America. The region of origin is now presumed to be Mexico (Gould, 1968). Dispersal to the Old World is generally deemed to have occurred in the sixteenth and seventeenth centuries (Cobley and Steele, 1976); however, recent evidence indicates that dispersal to India may have occurred prior to the twelfth and thirteenth centuries by unknown means (Johannessen and Parker, 1989).

Taxonomy of Corn
Zea is a genus of the family Gramineae (Poaceae), commonly known as the grass family. The genus consists of some four species: Zea mays, cultivated corn and teosinte; Zea diploperennis Iltis et al., diploperennial teosinte; Zea luxurians (Durieu et Asch.) Bird; and Zea perennis (Hitchc.) Reeves et Mangelsd., perennial teosinte. Various of the species have been assigned to the segregate genus Euchlaena, which is not currently recognized, or have been divided into numerous small species within the genus Zea (Terrell et al., 1986).

The closest generic relative to Zea is Tripsacum, a genus of seven species, three of which occur in the United States (Gould, 1968). Tripsacum differs from corn in many respects, including chromosome number (n=9), in contrast to Zea (n=10). All species of Tripsacum can cross with Zea, but only with difficulty and only with extreme sterility (Galinat, 1988).

Cultivated corn is presumed to have been transformed from teosinte, Zea mays subspecies mexicana (Schrader) Iltis, more than 8000 years ago. During this transformation, cultivated corn gained several valuable agronomic traits, but lost the ability to survive in the wild. Teosinte, however, remains a successful wild grass in

Mexico and Guatemala. Despite some confusion over proper taxonomic groupings of the non-cultivated members of Zea, wild members maintain a successful array of annual or perennial plants with visible chromosomal peculiarities and ploidy levels, and many adaptive macroscopic phenotypes. Cultivated corn and the wild members of diploid and tetraploid Zea can be crossed to produce fertile F1 hybrids. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Galinat, 1988).

The second major transformation of cultivated corn occurred in the twentieth century, and particularly since the 1930's. This transformation occurred through inbred lines for hybrid seed production, and by other methods. Almost all corn grown in the United States now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are virtually unknown in commerce (Hallauer et al., 1988). This transformation has resulted in more uniform commercial plants with superior agronomic characteristics, and has contributed to the six-fold increase in per acre yields in the last sixty years.

Morphology and Reproduction of Corn

Corn is a tall, robust, monoecious annual, with overlapping sheaths and broad, conspicuously distichous blades; staminate spikelets in long spikelike racemes, these numerous, forming large spreading terminal panicles, (tassels); pistillate inflorescence in the axils of the leaves, the spikelets in 8-16 (30) rows, on a thickened, almost woody axis (cob), the whole enclosed in numerous large foliaceous bracts or spathes, the long styles (silk) protruding from the summit as a mass of silky threads; grains at maturity greatly exceeding the glumes (Hitchcock and Chase, 1951). Pollination, fertilization, and caryopsis development of corn follows a fairly standard pattern for chasmogamous wind-pollinated grasses, with the following points of exception and note:

Pollen is produced entirely in the staminate inflorescences. Eggs are produced entirely in the pistillate inflorescences.

Self-pollination and fertilization and cross- pollination and fertilization are usually possible and frequencies of each are usually determined by physical proximity and other physical influences on pollen transfer. A number of complicating factors, such as genetic sterility factors and differential growth rates of pollen tubes may also influence the frequencies of self- fertilization versus cross-fertilization.

Corn styles and corn pollen tubes are the longest known in the plant kingdom.

Shed pollen typically remains viable for 10 to 30 minutes, but may remain viable for much longer under refrigerated conditions (Coe et al., 1988).

The staminate and pistillate inflorescences do not develop at the same time. The pistillate inflorescence is precocious. However, there is the appearance of slight protandry because the elongating styles are delayed for about seven days in emergence from the bracts of the pistillate inflorescence, while the development of the later-developing staminate inflorescence is fully visible.

The genetics of corn is better known than that of any other crop plant.

Pollination of Corn
Studies of pollination of corn have mostly centered on the needs of hybrid seed production. This production involves the development and maintenance of inbred lines and the subsequent crosses to produce commercial seed. In the former, self-pollination is mandatory. In the latter, cross-pollination is mandatory. Mechanisms have been developed to ensure each kind of pollination.

Breeder seed is usually derived from self-pollinated seed at the F8 to F10 generation of inbreeding (Wych, 1988). A high degree of self- pollination is ensured by planting well isolated blocks that virtually guarantee natural random sib mating. Minimum isolation distances for foundation seed are one-eighth mile (660 feet) from the nearest contaminating source. Other safeguards, such as physical barriers or unharvested border rows, can further reduce the possibility of contamination. Fields are preferred that have not been recently planted in corn. This is to minimize the appearance of volunteer corn from a previous season.

Hybrid seed production fields also require isolation, similar to that for foundation seed. Isolation distance may be modified by such factors as high winds, additional border rows, size of field, natural barriers, and differential flowering dates. Flowering dates are often adjusted by differential planting dates, planting depth, or fertilizing. The two different parents are planted in a regular pattern of rows, such as four pistillate to one staminate (4:1), or 4:2, or 6:2, or a variety of other combinations. Detasseling or use of cytoplasmic male sterility prevents pistillate plants from shedding viable pollen, and thus ensures cross-pollination.

Weediness of Corn
Corn appears as a volunteer in some fields and roadsides, but it never has been able to establish itself outside of cultivation (Gould, 1968). Some of the other species of Zea are successful wild plants, but have no pronounced weedy tendencies (Galinat, 1988).

Modes of Gene Escape in Corn
Genes of corn may escape from the test plot in two ways. The first is by pollen transfer. The second is by movement of the grains.
If viable pollen of the transgenic plants can be transferred by wind to any receptive corn stigma within the 30 minute period of pollen viability, an escape of genetic material could take place. This potential transfer becomes more unlikely as distance increases from the transgenic plants, and from a practical standpoint becomes

increasingly unlikely at distances much beyond the foundation seed isolation distance of 660 feet. Temporal isolation would further reduce the likelihood of effective pollination and fertilization. In addition, any physical impediment to this movement, such as effective detasseling or bagging, would completely eliminate the possibility of gene escape by way of pollen.

To prevent grain from remaining in the field or otherwise escaping, all ears would have to be collected or otherwise destroyed. To ensure that no grain escaped harvest, the field would have to be monitored for volunteer corn plants in the following season.

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Solanum Tuberosum (potato, pieper)

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

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

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

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

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

The potato "seed" of commerce is not true botanical seed, but rather consists of sections of potato tuber with one or more "eyes", i.e. lateral buds (Everett, 1981). The potatoes of commerce are therefore all reproduced vegetatively, as clones. This necessarily means that once a cultivar is produced, it is genetically stable in perpetuity, barring mutation, clonal variation (Shepard et al., 1980) or some other unusual event. It also means that potato clones are especially susceptible to disease transmission via the tuber sections (Ross, 1986).

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

Potato plants are notorious for sterility, both male and female (Ross, 1986). This causes difficulties in potato breeding. Most commercial cultivars are sterile (Burbank, 1921).

Distribution of Cultivated Potatoes
The aboriginal home of cultivated potatoes, in the South American Andes, still possesses a wide range of wild potatoes, cultivated potatoes, and hybrid swarms of intermediate potatoes at various ploidy levels. But that is not the major world center of potato culture. Most potatoes are grown in temperate climates or the mountains of tropical areas. The major world producers, in order of production, are U.S.S.R, Poland, United States, East Germany, West Germany, and France (Talburt, 1987).
In the United States, potatoes are widely grown, but especially in the States of Idaho, Washington, Oregon, Colorado, North Dakota, Wisconsin, and Maine (Jewell, 1988).

Modes of Gene Escape
In most potato varieties, gene escape by pollen is usually precluded because of male sterility. In Lemhi Russet potatoes gene escape by pollen is unlikely unless sexually compatible relatives are in the immediate proximity of the test area. According to documentation in the submission, transfer by insect pollinators is unlikely even in this instance. Gene escape could occur by the mechanical removal of potato tubers from the site.

Volunteer potatoes[50]

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Annex II - relevant characteristics of inserted genes

CONTENT[51]:

- AAD
  BLA
- BAR
- BARNASE
- BARSTAR
- CMV
- CP4 EPSPS
- CRY1AB
- CRY1AC
- CRY1FA
- CRY2AB
- GUS
- NPT2
- NPT3
- PRV
- PVY

[51] In the course of 2005/2006, this annex will be further expanded.

Name(s)	**AAD**
Abbreviation(s)	**aad,** aadA, aadA1, aadA2, aadA2b, ant(9)
Origin	Escherichia coli Tn7 transposon
Intended trait(s)	antibiotic resistance resistance to streptomycin/spectinomycin antibiotics (Str^R and Sp^R) selectable marker gene
Gene product	AAD (3") streptomycin 3"-adenylyltransferase streptomycin adenylate synthetase
Mode of action	The transposon Tn7 DNA fragment contains the *aadA* gene encoding for an aminoglycoside-modifying enzyme (EC 2.7.7.47), the streptomycin 3"-adenylyltransferase, which mediates bacterial resistance to the streptomycin and spectinomycin. The enzyme inactivates these antibiotics by phosphorylation leading to 3"-adenylylstreptomycin and diphosphate as reaction products. The *aadA* gene is used as selectable marker gene during transformation process of chloroplasts. Because there are many hundreds of copies of chloroplast DNA per cell, the introduction of transgenes into chloroplasts is a two-step process. In the first step, the *aadA* gene integrates into a fraction of the chloroplast DNA molecules present in a cell. In the second step, modified chloroplast genomes containing *aadA* are selected with the antibiotics (spectinomycin and treptomycin) until they replace all wildtype chloroplast genomes after repeated cell and chloroplast divisions. Once a plant is homoplasmic (contains only modified chloroplast genomes) the *aadA* gene is no longer required.

Name(s)	**BLA**
Abbreviation(s)	***bla,*** *ampC, shv*
Origin	*Escherichia coli* (eubacterium)
Intended trait(s)	antibiotic resistance resistance to penicillin (ampicillin) antibiotics selectable marker beta-lactamase (*bla*) from *Escherichia coli* is used for selection when the construct is in *E. coli* cells
Gene product	Beta-lactamase penicillinase, cephalosporinase
Mode of action	The most common mechanism of bacterial resistance to b-Lactam antibiotics is the production of b-lactamases that belong to a group of enzymes of varying specificity hydrolysing and inactivating beta-lactams; some act more rapidly on penicillins, some more rapidly on cephalosporins. Four kinds of beta-lactamases have been identified. Class-B enzymes are zinc containing proteins whilst class -A, C and D enzymes are serine hydrolases. They act on carbon-nitrogen bonds, other than peptide bonds. These enzymes catalyze the opening of the f1-lactam ring of antibiotics such as penicillin and ampicillin, thus making them ineffective in binding to and inactivating the transpeptidase involved in cross-linking polysaccharide chains of the bacterial cell wall.

Name(s)	- **phosphinothricin acetyl transferase** - glufosinate-ammonium resistance gene - phosphinothricin-N-acetyl transferase - phosphinothricin n-acetyltransferase - ppt n-acetyltransferase - phosphinothricin-resistance gene
Abbreviation(s)	**BAR**
Origin	Streptomyces hygroscopicus (Eubacteria)
Intended trait(s)	Tolerance to the amino acid phosphinothricin, the L-isomer of phosphinothricin (L-PPT) is used as a broad spectrum herbicide. L-PPT is the active ingredient of the herbicide glufosinate ammonium (GA). Glufosinate ammonium is an equimolar, racemic mixture of the D- and L-isomers of PPT. The L-PPT tolerance is used: 1. for agronomic purposes, weed management; 2. as a selectable marker, during the breeding proces; 3. for selection during hybrid seed production, as part of a male-sterility system.
Gene product	phosphinothricin acetyl transferase (PAT)
Mode of action	Herbicides based on L-PPT are non-systemic, non-selective herbicides used for post-emergence control of many broadleaf and grassy weeds. L-PPT is a structural analogue of glutamate, the substrate of glutamine synthetase. Glutamine synthetase is the only enzyme in plants that can detoxify the ammonia generated by various normal metabolic processes within the plant. L-PPT exerts its herbicidal effect through the irreversible inhibition of glutamine synthetase, in the presence of ATP. When L-PPT inhibits glutamine synthetase, phytotoxic levels of ammonia accumulate in the plant. The bar gene encodes for the enzyme phosphinothricin acetyltransferase (PAT). . This enzyme belongs to the pat/bar subfamily of the acetyltransferase family and catalyses the biochemical reaction: acetyl-CoA + L-glutamate = CoA + N-acetyl-L-glutamate The bar gene as derived from S. hygroscopicus encodes for a PAT enzyme that can also use L-PPT as a substrate resulting in the N-acetyl-phosphinothricin, a compound that does not inactivate glutamine synthetase. The PAT enzyme thereby confers tolerance to herbicides based on L-PPT as the active ingredient.

Name(s)	- **barnase** - synbar (synth. brnase) - ribonuclease (RNase ba, RNase bi = binase, Rnase bci, Rnase bco, Rnase bp) - G specific endonuclease
Abbreviation(s)	**BARNASE**
Origin	Bacillus amyloliquefaciens (Eubacteria)
Intended trait(s)	Ribonuclease activity selective inactivation of cells expressing the enzyme from a tissue-specific promoter. The Ribonuclease activity is used: (1). as a part of a male-sterility system, barnase confers male sterility when expressed in pollen generating tissue (tapetum); (2). as means for obtaining disease resistance, e.g. when under control of promoters that are activated upon infection.
Gene product	Bacillus amyloliquefaciens extracellular ribonuclease (BARNASE)
Mode of action	BARNASE is an enzyme that that cleaves RNA and degrades it into fragments or completely to its ribonucleotide subunits. It is an extracellular ribonuclease, the enzyme is secreted by the donor organism Bacillus amyloliquefaciens cell. Barnase hydrolyses phosphodiester bonds in rna, poly- and oligo-ribonucleotides resulting in 3'-nucleoside mono-phosphates. The expression of BARNASE in plant cells results in break down of RNA, thereby disrupting the cellular processes ultimately leading to cell death. **Male sterility** Barnase is used for construction of male sterile plants. Male sterility is obtained by placing the gene under the control of a tissue specific promoter that leads to the production of BARNASE enzyme during anther development, frequently the modification aims at the disruption of the tapetum (the lining of the anther cavity the pollen generating tissue). In this way self pollination is prevented, thereby: (a) enabling hybrid production, or (b) leading to an improved quality during the production of hybrid. BARNASE activity can be blocked by the specific intracellular inhibitor BARSTAR (Bacillus amyloliquefaciens ribonulease inhibitor), thereby preventing the male sterilisation, preserving the male fertility. Hybrids of a male sterile plant (expressing barnase) and a male fertile plant (expressing barstar) can lead to male fertile offspring that express the barnase gene as well as the barstar gene. This combination is especially used for the production of hybrid

	seed of crops where seed setting is desired (e.g. oilseed rape). **Disease resistance** Barnase is used for obtaining reduced susceptibility towards diseases e.g. as caused by nematodes. Reduced susceptibility is obtained by placing the gene under the control of a promoter that is activated upon infection, leading to the death of the infected cells. The cell death reduces the ability of the pathogen to spread in the plant, thereby limiting the consequences of the infection and inhibiting the spread of the pathogen. This makes the crop less susceptible for the pathogen.

Name(s)	- **Bacillus amyloliquefaciens barnase inhibitor** - ribonuclease inhibitor - barstar-like protein
Abbreviation(s)	- **barstar** - cac0844, cac3364
Origin	Bacillus amyloliquefaciens (Eubacteria)
Intended trait(s)	inhibitor of the ribonuclease barnase The inhibitor activity is used as a part of a hybrid system that is based on the male sterility obtained via the expression of barnase. Simultaneous expression of barstar prevents that the barnase activity leads to male sterility (also called "restoration of fertility").
Gene product	ribonuclease inhibitor (BARSTAR)
Mode of action	BARSTAR is a ribonuclease inhibitor that specifically inhibits the Bacillus amyloliquefaciens RNAse. BARSTAR strongly inhibits by forming a one-to-one non-covalent complex, with BARNASE, thereby preventing the BARNASE activty of degrading RNA and thereby disrupting the cellular processes that ultimately lead to cell death. **Hybrid male sterility system** Barnase is used for construction of male sterile plants. Male sterility is obtained by placing the gene under the control of a tissue specific promoter that leads to the production of BARNASE enzyme during anther development, frequently the modification aims at the disruption of the tapetum (the lining of the anther cavity the pollen generating tissue). In this way self pollination is prevented, thereby: (a) enabling hybrid production, or (b) leading to an improved quality during the production of hybrid. BARNASE activity can be blocked by the specific intracellular inhibitor BARSTAR, thereby preventing the male sterilisation, preserving the male fertility. Hybrids of a male sterile plant (expressing barnase) and a male fertile plant (expressing barstar) can lead to male fertile offspring that express the barnase gene as well as the barstar gene. This combination is especially used for the production of hybrid seed of crops where seed setting is desired (e.g. oilseed rape).

Name(s)	**Cucumber mosaic virus coat protein gene**
Abbreviation(s)	***CMV cp***, *CMV coat protein*
Origin	Cucumber mosaic virus (cucumber mosaic cucumovirus) Viruses; ssRNA positive-strand viruses, no DNA stage; Bromoviridae; Cucumovirus.
Intended trait(s)	To resist infection by cucumber mosaic virus
Gene product	CMV coat protein
Mode of action	Cucumber mosaic virus (CMV) is an RNA plant virus with a tripartite genome and an extremely broad host range. Characteristics of the RNA: Length: 218 aa, molecular weight: 24319 Da

Name(s)	Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC2.5.1.19)
Abbreviation	**EPSPS-CP4**
origin:	*Agrobacterium sp.* strain CP4
Intended trait(s)	Glyphosate tolerance: expression of a glyphosate-tolerant EPSPS protein providing tolerance to the herbicide glyphosate, the active ingredient in the Roundup® family of agricultural herbicides. CP4 EPSPS is mainly used with the intention of providing glyphosate tolerance to the crop, allowing it to be treated with glyphosate and providing effective control of weeds during the growing season.
Gene product	CP4 EPSPS protein
Mode of action of the gene product:	The *cp4 epsps* gene from *Agrobacterium sp.* strain CP4, a common soil-borne bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Harrison *et al.,* 1996). The CP4 EPSPS protein is functionally similar to a diverse family of EPSPS proteins typically present in food and feed derived from plant, fungal and microbial sources (Levin and Sprinson, 1964; Harrison *et al.,* 1996). Similar to other EPSPS proteins, the CP4 EPSPS protein catalyzes the reaction of shikimate-3-phosphate (S3P) with phosphoenolpyruvate (PEP) into 5-enolpyruvylshikimate-3-phosphate (EPSP), a step in the production of aromatic amino acids via the shikimate pathway (Herrmann, 1983; Haslam, 1993). The shikimate pathway and, hence, EPSPS proteins are absent in mammals, fish, birds, reptiles and insects. Unlike EPSPS proteins found in plants, CP4 EPSPS has a greatly reduced affinity for glyphosate (the active ingredient in the Roundup® family of agricultural herbicides) and has relatively high catalytic efficiency compared to most EPSPS proteins (Barry *et al.,* 1992; Padgette *et al.*, 1991). Thus, in plants expressing the CP4 EPSPS protein, the biosynthesis of aromatic amino acids is maintained in the presence of glyphosate. The properties of the CP4 EPSPS protein have been reviewed by the OECD (OECD, 1999).

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Name (s):	insecticidal delta-endotoxin CryIAb, pesticidal crystal protein Cry1Ab, and crystalline entomocidal protoxin
Abbreviation(s)	**CRY1AB**
origin:	*Bacillus thuringiensis* subsp. kurstaki (Eubacteria)
Intended trait(s)	Insect resistance: expression of an insecticidal protein, resistance to specific lepidopteran plant pests, moths and butterfly insects that feed on plants (e.g. via sap sucking or leaf eating); cryIAb is mainly used with the intention of obtaining reduced crop susceptibility to infestation by the pest insect *Ostrinia nubilalis*, commonly called, European corn borer (ECB)
Gene product	insecticidal delta-endotoxin CryIAb
Mode of action of the gene product:	The *Bacillus thuringiensis* gene cryIAb encodes a 133 kDa non-toxic protein, which accumulates in (bipyramidal) crystalline inclusions during sporulation of *B. thuringiensis* (Arango et al., 2002). This protein is a prototoxin which solubilizes in the alkaline environment of the insect midgut and is converted into toxic core fragment via cleavage of the protein. This conversion process (proteolysis) takes place under the influence of crystal-associated or larval-midgut proteases. The toxic domain is localized in the N-terminal half of the protoxin. The activated toxin binds to specific high affinity receptors on the surface of the epithelial cells of the midgut of larvae of lepidopteran insects (Hofte and Whiteley, 1989), generating pores in the cell membrane. The pores in the membrane disturb the osmotic balance of the cells. Consequently, the cells swell and lyse. The larvae stop feeding and eventually die. The Cry1A(b) protein has been shown to be highly specific and is insecticidal to certain lepidopteran insects. This specificity is directly attributable to the presence of receptors in the mid gut of target insects. There are no receptors for the delta endotoxins of *Bacillus thuringiensis* subspecies on the surface of mammalian intestinal cells, therefore humans and other mammals are not susceptible to these insecticidal proteins. For the genetic modification of plants frequently only, the DNA sequence encoding the toxic core fragment of the protein is used.

known as:	Cry1Ac delta-endotoxin
origin:	*Bacillus thuringiensis* subsp. *kurstaki*
Intended trait(s)	Insect resistance: expression of a Cry1Ac insecticidal protein providing resistance to the lepidopteran plant pests that feed on cotton plants. Cry1Ac is mainly used with the intention of providing season-long protection against key lepidopteran insect pests, including tobacco budworm, pink bollworm and cotton bollworm (Wilson *et al.,* 1994; Betz *et al*., 2000).
Gene product	Cry1Ac insecticidal delta-endotoxin protein
Mode of action of the gene product:	The *cry1Ac* gene from *Bacillus thuringiensis* subsp. *kurstaki*, a common spore-forming, gram-positive bacterium, has been sequenced and shown to encode a 134.8 kDa Cry1Ac protein. The Cry1Ac protein is nearly identical in structure and activity to the Cry1Ac protein found in nature and in commercial *B.t.k.* microbial formulations. Microbial formulations of *Bacillus thuringiensis* that contain the Cry1Ac insecticidal protein have been registered in numerous countries worldwide, and they have been safely used for control of lepidopteran insect pests for more than 40 years (Luthy *et al.,* 1982, Baum *et al.,* 199). The insecticidal activity of the Cry1Ac protein requires that the protein be ingested. In the insect gut, the protein is solubilized due to the high pH of the insect gut and is proteolytically cleaved to the active core of the protein, which is resistant to further degradation by the insect gut proteases. The core protein binds to specific receptors on the mid-gut of lepidopteran insects, inserts into the membrane and forms ion-specific pores (English and Slatin, 1992). These events disrupt the digestive processes and cause the death of the insect. The digestive tract tissues of non-target insects, mammals, birds, and fish do not contain receptors that bind the Cry1Ac protein. Therefore the Cry1Ac protein cannot disrupt digestion and is, therefore, non-toxic to species other than lepidopteran insects (Betz *et al*., 2000; Hofmann *et al*., 1988).

- Name(s)	- **insecticidal delta-endotoxin cry1Fa** - pesticidal crystal protein cry1Ab - crystalline entomocidal protoxin
Abbreviation(s)	***cry1Fa***, *cry1F*, *cry1Fa2* (*cry1F* is a modified/less than full lenght form of the *cry1Fa2* gene)
Origin	*Bacillus thuringiensis* subs. *aizawai* (Eubacteria; gram+; soil-born)
Intended trait(s)	**Insect resistance**: expression of an insecticidal protein - resistance to specific lepidopteran insects larvae (including European corn borer, southwestern corn borer, fall armyworm, and black cutworm) that are pests of corn
Gene product	**Cry1F** protein; insecticidal delta-endotoxin
Mode of action	The *Bacillus thuringiensis* gene *cryI1Fa* encodes for a 134 kDa protein. The geneproduct belongs to the delta endotoxin family. The CryIF(a) crystal protein is produced during sporulation and is accumulated both as an inclusion and as part of the spore coat. The toxic segment of the protein is located in he N-terminus. Shorter version (modified/less than full length/synthetic) of the Cry1Fa2 delta-endotoxin protein, Cry1F, which has a slightly broader spectrum of activity against lepidopteran pests of corn than currently available corn varieties expressing *B.t.*Cry1A delta-endotoxins. The activated toxin binds to specific high affinity receptors on the surface of the epithelium cells of the midgut of larvae of lepidopteran insects (Hofte and Whiteley, 1989), generating pores in the cell membrane. The pores in the membrane disturb the osmotic balance of the cells. Consequently, the cells swell and lyse. The larvae stop feeding and eventually die.

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known as:	Cry2Ab2 delta-endotoxin
origin:	*Bacillus thuringiensis* subsp. *kurstaki*
Intended trait(s)	Insect resistance: expression of a Cry2Ab2 insecticidal protein providing resistance to the lepidopteran plant pests that feed on cotton plants. Cry2Ab2 is mainly used with the intention of providing season-long protection against key lepidopteran insect pests, including tobacco budworm, pink bollworm and cotton bollworm along with control of sporadic pests, such as beet and fall armyworm.
Gene product	Cry2Ab2 insecticidal delta-endotoxin protein
Mode of action of the gene product:	The *cry2Ab2* gene from *Bacillus thuringiensis* subsp. *kurstaki*, a common spore-forming, gram-positive bacterium, has been sequenced and shown to encode an approximately 63 kDa Cry2Ab2 protein. Microbial formulations of *Bacillus thuringiensis*, which include the Cry2A class of proteins, have been registered in numerous countries worldwide and have been safely used for control of lepidopteran insect pests for more than 40 years (Lüthy *et al.*, 1982; Baum *et al.*, 1999; IPCS, 1999; Betz *et al.*, 2000). *B.t.* microbial formulations have been shown to be specific to the target insect pests and do not have deleterious effects to non-target organisms such as beneficial insects, birds, fish, and mammals, including humans (U.S. EPA, 1988 and 1998). Therefore, there is a history of safe dietary and occupational exposure to Cry proteins derived from *B.t.*, including those of the Cry2A class. The Cry proteins exhibit a complex, multi-component mode of action. Insecticidal activity of the Cry proteins requires that the protein be ingested by the target insect pest. In the insect gut, the protein is solubilized due to the high pH of the insect gut and is proteolytically cleaved to the active core of the protein, which is resistant to further degradation by the insect gut proteases (Lilley *et al.*, 1980; English and Slatin, 1992). The core protein binds to specific receptors on the mid-gut epithelium cells of susceptible insects, inserts into the membrane, and forms ion-specific pores (English and Slatin, 1992). The cells swell due to an influx of ions and water, leading to cell lysis and ultimately the death of the insect (Höfte and Whitely, 1989). The digestive tract tissues of non-target insects, mammals, birds, and fish do not contain receptors that bind the Cry proteins (Noteborn, 1994; Sacchi *et al.*, 1986; Van Mellaert *et al.*, 1988). Therefore, the Cry proteins cannot disrupt digestion in on-target species. Cry proteins are considered non-toxic to species other than lepidopteran and dipteran insects because there is a strong correlation between toxicity and specific binding of Cry proteins (Siegel *et al.,* 2001; Betz *et al.,* 2000; Hofmann *et al.,* 1988).

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Name(s):	Coleopteran B.t. endotoxin
Abbreviation	**CRY3Bb1**
origin:	*Bacillus thuringiensis* subsp. *kumamotoensis*
Intended trait(s)	Insect resistance: expression of an insecticidal protein, resistance to specific coleopteran (beetles) plant pests that feed on corn roots. Cry3Bb1 is mainly used with the intention of obtaining reduced crop susceptibility to infestation by corn rootworms (western corn rootworm, *Diabrotica virgifera virgifera;* Mexican corn rootworm, *Diabrotica virgifera zeae*; northern corn rootworm. *Diabrotica barberi*; and the southern corn rootworm, *Diabrotica undecimpunctata howardi*.
Gene product	insecticidal endotoxin Cry3Bb1
Mode of action of the gene product:	The wild-type Cry3Bb1 was isolated from *B.t.* spp. *kumamotoensis,* a spore-forming, gram-positive bacterium that is found naturally in soil. It is a 74 kDa protein, which acts by disrupting the membranes of cells lining the midgut of insect larvae, is insecticidal only to coleopteran insects. Unlike the Cry1 toxins, Cry3Bb1 does not have a protoxin region and works at a near neutral pH (~ 6.5 – 7.0) instead of an alkaline pH.

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Name(s)	**beta-glucuronidase** beta-D-glucuronoside glucuronosohydrolase
Abbreviation(s)	**GUS,** *uidA, gusA, gurA, b1617*
Origin	*Escherichia coli* (eubacterium)
Intended trait(s)	**reporter gene** or **gene-fusion marker** • widely used in the developement of transgenic plants to facilitate the selection of transformed plant tissues in the laboratory
Gene product	
Mode of action	The *uidA* gene from *E. coli* encodes for an enzyme (EC 3.2.1.31) named beta-glucuronidase, which belongs to family 2 of glycosyl hydrolases. The enzyme catalyzes hydrolytic reactions of miscellaneous substrates (generally water-soluble) resulting in disruption of glycosyl bonds. One of the most commont substrates for this enzyme is beta-D-glucuronoside, which is converted to D-glucuronate. The *uidA* gene serves as a reporter gene, allowing the selection of transformed plant tissues that were successfully engineered by the genetic constructs with genes of interest. In the presence of a particular substrate the beta-glucuronidase enzyme enables the visualization of miscellaneous molecular events.

Name(s)	- **aminoglycoside-3’-O-phosphotransferase** - neomycin-kanamycin phosphotransferase - kanamycin kinase - neomycin phosphotransferase
Abbreviation(s)	aph(3’)II, kan, KmR, neo, nptii, **nptII, npt2,**
Origin	Escherichia coli transposon Tn5
Intended trait(s)	resistance to the antibiotic kanamycin used as a selectable maker
Gene product	aminoglycoside-3'-O-phosphotransferase
Mode of action	The nptII gene encodes for enzyme aminoglycoside-3’-O-phosphotransferase (EC 2.7.1.95) catalyzing biochemical reaction ATP + Kanamycin = ADP + Kanamycin 3’-phosphate The biochemical reaction provides resistance to the antibiotic kanamycin. It can also use neomycin, paromomycin, neamine, paromamine, vistamycin and gentamicin A as substrates. The NptII amino acid sequence is similar to other aminoglycoside phosphotranspherase sequences, although they differ in enzymatic activity and substrate specificity leading to differences in the resistance spectrum. (e.g. the NptII enzyme from Pseudomonas aeruginosa also acts on butirosin.) Kanamycin is an aminoglycosidic antibiotic that binds to (bacterial) ribosomes thus disrupting normal protein synthesis and killing the cell. The kanamycin-resistance gene codes for an enzyme that prevents kanamycin from binding to ribosomes by changing its chemical structure, thereby rendering the cells resistant.

Name(s)	- **aminoglycoside phosphotransferase type III** - neomycin phosphotransferase type III
Abbreviation(s)	***nptIII,*** *aph(3')III*
Origin	*Streptococcus faecalis (Enterococcus faecalis) and Staphylococcus aureus;* Bakteria, Enterococcus
Intended trait(s)	resistance to kanamycin and structurally-related aminoglycosides, including amikacin used as selection marker
Gene product	- aminoglycoside 3'-phosphotransferase - kanamycin kinase, type iii - neomycin-kanamycin phosphotransferase, type iii - aph(3')iii
Mode of action	Length of the gene is: 264 aa, molecular weight: 30974 Da. The *nptIII* gene encodes for enzyme aminoglycoside 3'-phosphotransferase (EC 2.7.1.95) catalyzing biochemical reaction: atp + kanamycin = adp + kanamycin 3'-phosphate (also acts on other antibiotics). The biochemical reaction provides resistance to the antibiotic kanamycin and structurally-related aminoglycosides, including amikacin. Also acts on the antibiotics neomycin, paromomycin, neamine, paromamine, vistamycin and gentamicin A. An enzyme from Pseudomonas aeruginosa also acts on butirosin. The kanamycin resistance gene from Staphylococcus aureus has been sequenced and its structure compared with similar genes isolated from Streptomyces fradiae and from two transposons, Tn5 and Tn903, originally isolated from Klebsiella pneumoniae and Salmonella typhimurium, respectively.

Name(s)	**Papaya ringspot virus coat protein gene**
Abbreviation(s)	**PRVcp,** PRV CP, PRSV CP
Origin	Papaya ringspot virus PRV, PRSV
Intended trait(s)	to resist infection by papaya ringspot virus (PRV)
Gene product	Papaya ringspot virus coat protein
Mode of action	ssRNA positive-strand viruses Length: 2241 BP THE VIRAL RNA OF POTYVIRUSES IS EXPRESSED AS A SINGLE POLYPROTEIN WHICH UNDERGOES POSTTRANSLATIONAL PROTEOLYTIC PROCESSING RESULTING IN THE PRODUCTION OF AT LEAST EIGHT INDIVIDUAL PROTEINS. Length: 675 aa, molecular weight: 77925 Da

Name(s)	***Potato Virus Y coat protein gene***
Abbreviation(s)	***PVY cp****, PVY coat protein, PVY-O CP*
Origin	Potato (poty) Virus Y strain O / Viruses; ssRNA positive-strand viruses, no DNA stage; Potyviridae; Potyvirus/
Intended trait(s)	To resist infection by Potato Virus Y
Gene product	PVY coat protein, PVY cp, PVY-O CP
Mode of action of the gene product	Expression of the PVY coat protein in the plants does not make the plants diseased, but rather, is designed to confer resistance to PVY, an economically important pathogen of potato. The *PVYcp* gene was obtained in from a PVY strain O isolate infecting potatoes in Washington State, USA (De Bokx and Huttinga, 1981; Murphy et al., 1995). The gene sequence of the native PVY coat protein gene was modified only by adding an ATG start codon to facilitate translation of the protein (PVY has a positive-sense RNA genome that is translated as a polyprotein which is subsequently cleaved to yield, among other proteins, the viral coat protein subunits). The *PVYcp* gene construct also contains the complete 3'-untranslated region of the PVY genome directly downstream of the coat protein coding region. (1.) PVY is the type member of the potyvirus group and is an aphid-transmissible RNA virus that commonly infects potato causing serious disease and economic loss. (2.)

Annex III – worksheet (risk assessment per gene)

Dossier/Applicant:
Plant:
Type of use:
Gene:

Identified potential adverse effect(s)	**Estimation of likelihood** Terms used: *Highly likely* *Likely* *Unlikely* *Highly unlikely*	**Evaluation of consequence** Terms used: *Major* *Intermediate* *Minor* *Marginal*	**Estimation of the risk** Terms used: *High* *Moderate* *Low* *Negligible*
Potential adverse effect 1			
Potential adverse effect 2			
Potential adverse effect 3			
Etc.			

Annex IV - Examples of risk assessment considerations[52].

[52] A number of examples will be worked out in detail in the period late 2005 – early 2006.

Example of risk assessment considerations for releases of GM crop plant with a Bt gene[53].

(e.g. CRY1AB, CRY1AC, CRY1FA, CRY2AB)

Identification of potential adverse effect	**Estimation of likelihood**	**Evaluation of consequence**	**Estimation of the risk**
Potential effects on non target organisms			
Potential unintended effects on the target organism			
Potential changes in weediness			
Potential changes in toxicity			
Potential changes in allergenicity			
Etc.			

The different considerations for different cases are discussed below:

Potential effects on non-target organisms

- *Trigger*: As the inserted genes code for insecticidal toxins, there is reason to consider in the risk assessment the question of potential effects on non-target organisms, including beneficial organisms.
  The *scenarios* that would be considered are 1) direct effects in the case of other insects or other animals eating the GM plants with the Bt gene, and 2) indirect effects in the case of other animals consuming the target insects. In the latter case there may be different types of effects, either because a) those other organisms could ingest indirectly the Bt toxin, or b) because those other organisms would have – if the Bt toxin is effective – fewer insects to prey on. Point of debate under scenario b) is the fact that large numbers of insects caused by crop fields are not a natural situation.

- *Estimation of likelihood*: In the cases of the GM crops with Bt genes to date, the gene products are well known to be highly specific and limited to a small group of Lepidoptera. The likelihood of those Lepidoptera insects being directly affected by the Bt toxin depends first of all on the type of activity, i.e. in cases of small scale field trials, any impact on the population level of those Lepidoptera insects is very unlikely. In cases of large scale commercial use, the estimation of likelihood considers the presence and feeding behaviour of those Lepidoptera insects, which depends on those insects and on the crops involved. When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact on the population level of those insects is very unlikely. When they are present and do use the crop involved as main source of food, then additional testing may be required.

- *Evaluation of consequence*: If the empirical testing results show a significant impact on the population level of those other Lepidoptera insects when exposed to GM crops with Bt Toxin, then an evaluation of the consequence will follow. If those other insects are threatened species, then an impact by growing these crops could be intermediate or major. If those insects are widely available in the country then an impact could be minor or

[53] This example is still 'in statu nascendi', but is included in this version of the Guide to provide potential users a flavour of the approach taken. Potential users are called upon t provide the PRRI with feedback.

marginal. If the other insects are also pest insects, then any impact on those insects may even be welcome. The results of any testing this stage needs to be compared with a proper baseline, derived from growing the unmodified recipient plant.

– *Estimation of risk.* If the evaluation of the consequences shows that the consequences are not marginal, then the estimation of risk will follow, and will depend on the outcome of the estimation of the likelihood and the evaluation of the consequence. What that estimation finally will be, will vary from case to case. If for example a certain crop is normally grown on a very large scale in a country, then the conclusion may be different then when a crop is only marginally grown. This part of the risk assessment can also make use of available data resulting from growing Bt crops on a commercial scale. To date, no verifiable reports have been produced of direct effects on non-target organisms in areas where Bt crops are grown. The conclusion of the risk assessment may be high, moderate, low or negligible.

– *Consideration of risk management.* As discussed under 'likelihood', in cases of small-scale confined releases, the risk management applied by confinement is usually sufficient to address the issue of effects on non-target insects. In cases of large scale, commercial use whereby the estimation of the risk of effects on certain non-target organisms is not negligible, the next step is normally to consider risk management strategies. However, it is obvious that in the case of commercial use of a crop risk management aimed at preventing certain insects to forage on the crop is practically not feasible. In those cases, the risk assessment moves ahead to the next stage

– *Determination of overall risk.* In small scale confined field trials, the risk of effects on population levels of non target organisms is negligible. In case of large scale, commercial use whereby the estimation of the risk of effects on certain non-target organisms is not negligible and cannot be managed, the risk assessment moves ahead to determine the overall risk. In doing so, one of the parameters taken into account is the risk of using the non-modified organism. In cases where the use of the non modified organism includes spraying against the pest insect with synthetic pesticides, this will be taken into account, and the resulting conclusion could be that the overall environmental impact of growing such a crop would be positive, despite a risk to affect certain non-target organisms.

Note: The October 2005 issue of Environmental Entomology introduces a new section "Transgenic Plants and Insects" with 13 papers on the longer-term assessment of potential non target effects of transgenic Bt cotton and corn active against lepidopteran and coleopteran pests. http://titania.esa.catchword.org/vl=3626685/cl=31/nw=1/rpsv/cw/vhosts/esa/0046225x/latest.htm. The articles are listed in endnote [v].

Potential unintended effects on the target organism – resistance development against Bt

– *Trigger*: Resistance development against Bt is in itself not an adverse environmental effect but an adverse agronomic and commercial effect. However, it can also be an environmental effect, in case it impairs other treatments such as spraying with microbial pesticides. Whether or not this may be the case depends on the pest insect and crop involved. For example in the case of the European Corn Borer in maize, microbial treatments are not widely used and therefore there would be no

trigger for further examination from a biosafety point of view[54], whereas in potato microbial pesticides are used.

- *Estimation of likelihood*: in the case of small scale field trials, the likelihood of resistance development is very, very low. In the case of commercial use, the likelihood can be high in cases whereby a certain Bt crop would be grown for long periods on large areas without effective resistance development strategies.

- *Evaluation of consequence*: The severity of the environmental consequence of resistant development will depend on the extent to which treatments such as microbial pesticides are used. The larger the area of microbial pesticide use, the more sever the consequence.

- *Estimation of risk.* The estimation of the risk of resistance development will depend on the likelihood of resistance development (which may be different for each type of Bt) and which depends on the availability of resistance management strategies.

- *Consideration of risk management.* Risk management strategies are available in the form of resistance development strategies, which include the use of refugia, rotation of varieties etc, etc.

- *Determination of overall risk.* The overall risk to the environment of development of resistance will be lower in cases where effective resistance management strategies are available and where the areas of microbial pesticide use are small, and will be higher in cases where effective resistance management strategies are not available and where the areas of microbial pesticide use are large.

Potential unintended effects on the target organism – Potential changes in weediness

- Trigger: weediness of a plant depends on many different characteristics, such as persistence, outcrossing, dispersal, etc. etc, and other factors such as the receiving environment and its climate. In general, it is therefore not very likely unlikely that a change in one particular trait would suddenly make a plant become 'more weedy'. However, it is theoretically conceivable that a certain new trait may just 'tip a balance' of the weediness of a crop that already had a number of weedy characteristics.

- *Estimation of likelihood*: the likelihood of potential changes in weediness depends on the weedy characteristics of the crop involved and on the characteristics of the newly inserted trait or traits. The weedy characteristics of specific crops are addressed in annex I. Whether or not a Bt gene could add to the weediness of a crop plant, depends on the question whether the pest insect involved plays a role in keeping wild populations of that crop at a low level.

- *Evaluation of consequence*: The severity of the environmental consequence of changes in weediness of a crop variety, will depend on whether that crop could have competition effects on the population level in specially protected areas and whether abundant growing

[54] From an agronomic and marketing point of view, the developer will definitely examine the issue of resistance development against Bt, because any such development would make his product less valuable to farmers.

of that crop would have to be treated with undesirable means.

- *Estimation of risk*[55].
- *Consideration of risk management.*
- *Determination of overall risk.*

[55] To be completed - This example is still 'in statu nascendi', but is included in this version of the Guide to provide potential users a flavour of the approach taken. Potential users are called upon t provide the PRRI with feedback.

Annex V - List of Abbreviations[56]

AIA	Advanced Informed Agreement procedure as defined in article 7 and following of the CPB
BCH	Biosafety Clearing House of the CPB (http://bch.biodiv.org/)
CPB	Cartagena Protocol on Biosafety (http://www.biodiv.org/biosafety/default.aspx)
GMO	Genetically Modified Organism
PRRI	Public Research and Regulation Initiative
IBC	Institute Biosafety Committee
	In this guide, the term 'permit' is used for authorisation to carry out certain activities such as field trials, and the term 'approval' is used for authorisations for 'placing on the market'
Notification	
Release	

[56] To be completed - Suggested additions: UV, MOP, CBD, EU, EC, IUCN, OECD, CFIA, UDA, DNA, RNA, UPOV, UNEP, FAO, WHO

Annex VI – Glossary of terms[57]

Permits, approvals, authorisations, consents,	In this guide, the term 'permit' is used for authorisation to carry out certain activities such as field trials, and the term 'approval' is used for authorisations for 'placing on the market'
Applicant	
Permits,	The term 'permit' is used for authorisation to carry out certain activities such as field trials.
Approval	The term 'approval' is used for authorisations for 'placing on the market'.
Applicant	The legal body submitting the notification
Recipient organism	The 'recipient' organism is the organism in which the genes are introduced through genetic modification methods.
Parental organism	

[57] To be completed

Endnotes – background information and opinion articles

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[i] **Allergies and genetically engineered foods**
September 2005 - Agricultural Biotechnology White Paper -
Alan McHughen, D. Phil.
Plant Biotechnology Extension Specialist
University of California, Riverside
http://agribiotech.info/McHughallergiesRSAS.doc

One of the greatest fears about biotechnology is that common foods might inadvertently harbor new allergens, becoming an unexpected food hazard to unknowing consumers. There is no evidence to support this fear, in spite of years of consumption of a range of biotech foods by hundreds of millions of people. Biotechnology is instead being used to overcome the hazards of common food allergies, in the process offering one of the greatest benefits of this precise science. Instead of creating new allergenic threats, biotechnology is eliminating allergens from common foods.
Q. How do food proteins cause allergy?
A. In humans and other mammals, the normal immunological response is to protect against the presence of unusual, potentially harmful proteins. But in the allergic person, the immune system overreacts to certain specific proteins, the allergens. During an allergic reaction, the immunoglobulin (IgE) defense responds to the presence of certain proteins (or to other metabolites associated with proteins). The IgE antibodies bind to mast cells, causing a release of histamine normally contained within the mast cells. The released histamine, in turn, causes the inflammation we observe as red wheals and rashes, and may constrict airways and dilate blood vessels.
Depending on the severity of the reaction, the victim may suffer from mild discomfort or irritation to, in extreme cases, potentially fatal anaphylaxis. The major food allergenic proteins occur in just eight food groups: wheat, soybeans, peanuts, tree nuts, milk, eggs, fish, and shellfish. Together, these few foods are responsible for more than 90 percent of food allergies.
Q. What is genetic engineering doing to solve the allergenic protein problem?
A. Genetic engineering of foods is now being used to alleviate the dangers of allergens, through at least three different approaches:
1) One approach is simply to remove the offending protein from the food. This strategy depends on identifying the specific allergenic protein, then engineering the plant or animal not to produce that protein. This is not as simple as it sounds, because foods contain as many as 10,000 different proteins. Even the common Brazil nut's allergenic protein was not identified until recently (and that was with the help of biotechnological methods).
Also, the allergenic factor may be not one protein, but several. Peanuts, another common allergenic food, contain at least three classes of allergenic proteins; removing just one allergen will not necessarily help if the other allergens remain.
Another complication: Sometimes the allergenic protein is a major component of the food, so removing it will alter the characteristic nature of the food. Or, the allergenic protein may play a crucial role in the growth or development of the plant or animal producing it; removing the critical protein may kill the plant or animal before it can be harvested. So, while using biotechnology to remove a protein seems simple, it is not always feasible.
2) A second strategy is to alter the protein so it still functions normally in the crop or animal, but is not recognized by the allergic person's body as the trigger for an allergic response. We may be able to use genetic engineering to change the structure of the protein at the IgE recognition portion without affecting the normal function of the protein.
This approach is being undertaken in peanuts, where researchers are altering the three major allergens to make them less recognizable by IgE antibodies.
3) A third method is to provide the body with a means to lessen the allergic response. A feature common to many allergenic proteins is that they are very stable and slow to digest in the stomach. Instead of being quickly destroyed by digestion as most proteins are, allergenic proteins remain intact longer, giving them time to prompt the allergenic response.
In this approach, researchers have identified a common mechanism that causes digestive stability in the allergens, and have sought to overcome that mechanism. Scientists have shown the potential for this approach by treating milk, one of the common allergenic foods, with a common, non-allergenic protein called Thioredoxin H, which breaks the chemical bonds in the allergenic proteins. Milk so treated was 300 times less allergenic when fed to sensitive dogs.
The researchers are now using genetic engineering to add additional Thioredoxin H to wheat, soy, and other allergenic foods in the hope that the additional enzyme (Thioredoxin is already present in small

amounts) will help break down the allergens.
All of these strategies are in early stages of research and are not ready for market. However, preliminary results from all are encouraging and show real potential for providing relief to millions of humans suffering allergic reactions to common foods. Clearly, here is a use of genetic engineering with real and important benefit to consumers.
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http://www.ars.usda.gov/is/AR/archive/sep02/soy0902.htm — Researchers Develop First Hypoallergenic Soybeans, originally published in Agricultural Research, September 2002.

[ii] **What is Bt and what is the risk of insects becoming resistant to Bt transgenic plants?**
September 2005
Agricultural Biotechnology White Paper
A.M. Shelton, Ph.D.
Professor of Entomology
Cornell University/NYSAES
http://agribiotech.info/SheltonBtRSTS.doc
One of modern agriculture’s best defenses against plant-eating insects is Bt, which either can be applied to the surfaces of crops to provide temporary protection or can be genetically engineered into the crops to protect against insects throughout the lifespan of the plants. Judicious use of Bt has allowed growers to avoid applying large quantities of costly and potentially toxic insecticides.
However, the widespread success of Bt has prompted concerns that insects might someday become resistant to this important treatment. This is a valid concern that has engaged agricultural researchers before Bt crops reached the marketplace.
Q. What is Bt?
A. Bt is short for Bacillus thuringiensis, a natural bacterium in the genus Bacillus. This diverse genus also includes more than 20 other Bacillus species and hundreds of different subspecies. Members of the genus Bacillus are generally considered soil bacteria, and Bt is common in terrestrial habitats including soil, living and dead insects, insect feces, granaries, and on the surfaces of plants. Bt occurs in nature predominantly as spores that can disseminate widely throughout the environment. Bt is very safe to humans and the environment.
A unique feature of Bt is that the bacterium produces crystalline structures, and these proteins have activity against some insect species. Bt was first isolated about 100 years ago in Japan from silkworm larvae. For over 40 years, Bt has been applied to crops in spray form as an insecticide, containing a mixture of spores and the associated protein crystals.
Rachel Carson promoted Bt as a natural insecticide in her book, Silent Spring, published in 1962. By 1995, 182 Bt-based products were registered by the United States Environmental Protection Agency (EPA). However, by 1999 the total sales of Bt formulations constituted less than 2 percent of the total sales of all insecticides. Bt lacked performance compared to many other available insecticides.
Q. How does Bt kill insects?
A. The main insecticidal effect of Bt comes from insecticidal crystal proteins (ICPs) produced during the bacterium’s sporulation phase. Different ICPs work against particular insect types — caterpillars, for example, or certain species of beetles. Key agricultural pests currently targeted with Bt insecticides include bollworms, stem borers, budworms, and leaf worms in field crops and grains; the gypsy moth and spruce budworm in forests; and the cabbage looper and diamondback moth in vegetable crops. Mosquitoes and black flies are also controlled with Bt sprays or treatment of water breeding sites with Bt.
Bt insecticides, whether in the form of a spray or in a Bt-engineered crop, do not function on contact as most insecticides do. Rather, the ICPs must be ingested by the target organism to be effective. The process takes hours or even days — somewhat longer than is required for synthetic insecticides to kill insects.
The active ICP binds to specific receptors on the midgut of the stomach, forming pores and leading to leakage of the midgut contents, paralysis, and death of the insect. Only some insect species have such receptors in the gut; humans and other organisms do not.
Q. What are Bt plants?

A. Bt plants have genes from the Bt bacterium engineered into them so that the plants produce an ICP toxic to the pest species of concern. As the insect feeds on the plant, it ingests the ICP and suffers the same fate as if it ingested leaf tissue sprayed with Bt. There are only two Bt crops registered in the United States — Bt corn and Bt cotton.

Q. How do Bt-engineered plants compare with foliar sprays of Bt?

A. There are some advantages to the use of Bt-engineered plants compared with foliar sprays of Bt, and some disadvantages. The chief advantages to Bt plants are that the pests hiding inside plant parts (stem borers, for example) can now be controlled effectively; multiple sprays are not needed; and the dose of Bt can be more effectively regulated. A disadvantage of Bt plants is that insect-specific ICPs cannot be changed during a growing season.

Q. What is resistance?

A. Resistance is a genetic change by an organism — in this case, the insect pest — that allows it to avoid harm from another organism or chemical product. Just as disease-causing bacteria can develop resistance to antibiotics, insect pests can develop resistance to synthetic insecticides. Developed resistance can impair the performance of insecticides in the field.

A recent survey found that more than 500 species of arthropods have developed strains that are resistant to one or more of the five principal classes of insecticides. Some insect species have even developed resistance in the field to foliar sprays of some Bt products.

Q. So, what are the chances that insects will develop resistance to Bt plants?

A. There are only two insect species that have developed resistance to Bt under commercial situations — the diamondback moth and the cabbage looper. In a few places in the world, some populations of these insects have developed resistance to foliar sprays of Bt.

This warns us that some insect species have the capacity to develop resistance to an ICP. However, after ten years of large-scale plantings of Bt crops, there have been no reported failures of Bt crops in the field due to resistance. The important question is: Why have we not seen resistance?

Although there are no definite answers, there are some interesting speculations. One is that the high and consistent levels of ICP production in the plant is much less favorable for the development of resistance, compared to the variable and constantly changing dose when Bt is sprayed on the plant. Also, there may be fewer genes for resistance in insect populations than was originally thought. And resistance in insects may be what geneticists call a recessive characteristic, meaning that resistance may take many more generations — if ever — to develop.

Perhaps most importantly, Bt plants are more strictly regulated than foliar sprays of Bt. The principal requirement for a resistance-management program for Bt plants is the use of a non-Bt “refuge” to allow Bt-susceptible genes to be maintained in the general population of insects.

When growers deliberately plant non-Bt crops nearby, it is a trade-off: growers sacrifice a fraction of their refuge crop to insects, in exchange for avoiding the remote possibility that all insects will become resistant to Bt. No other insecticides, including foliar sprays of Bt, are so strictly regulated.

Q. What’s the bottom line?

A. It has only been since the genes for production of Bt ICPs were engineered into plants that Bt really became a major insecticide. However, with its more widespread use there is increased risk of resistance development to Bt plants. So far, we have not seen any resistance after 10 years of use, and this is remarkable since some insects have developed resistance to other insecticides in fewer than five years. But resistance may come in the future. However, if it does come it will likely be to only a single type of ICP and other Bt ICPs will still provide control. It is also important to consider that in the years prior to the development of resistance to a specific ICP, substantial environmental and human health benefits would have accumulated compared to the use of more toxic insecticides.

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iii Gene flow or out crossing between plants is a natural process that happens between plants growing in nature, between crops used in agriculture, and from crops in agriculture to plants in nature and from plants in nature to crops in agriculture. Gene flow occurs naturally, through exchange of pollen between sexually compatible plants. In some plant species this process of outcrossing is even obligatory for reproduction. When crossable plants are grown within the distance that pollen can travel and still be viable, outcrossing may occur. Many of our most important agricultural crops are hybrids, resulting from gene flow (i.e., intercrossing) between plants of two or more species in nature, helped by human intervention for selection of specific traits that were deemed favourable. For example, wheat is derived from gene flow between three wild species. Soybean, cotton, and oilseed rape are all derived from gene flow between two species. Maize was derived from two ancestral species. Furthermore, gene flow between domesticated plants and sexually compatible wild species has led to plant varieties resistant to diseases, insects, drought, and other stresses to combine the best traits for survival in the wild. Many of these traits are common in weeds, which gives them a competitive advantage in various environments. In many cases the original varieties that were used for domestication also possessed these traits, but they have been lost either by accident, or on purpose in the domestication process. Therefore, gene flow is not a phenomenon that is novel to GM crops; it has been occurring forever through evolution, and it has been used for millennia with the development of conventionally produced crops. When gene flow occurs between crossable plants, half of the genetic material from each parent (25,000 to 50,000 genes) is transferred to their progeny. Commercially deployed GM crops are genetically the same as the progenitor line from which they were derived, except for the introduced gene (s). The novel gene is only likely to persist or increase in a wild population if it confers a selective advantage to the GMO for the environment in which it is grown. In short, outcrossing is a natural process, and whether or not it can result in adverse effects will depend on the characteristics of the gene that is out crossed. In particular for many commercially grown crops, the question is not whether genes will outcross, because in many cases they will. The question is whether that could cause problems, which is a key point in the risk assessment. It is required to carefully describe a scenario on gene flow: As an example, it is not correct to state that gene flow is detrimental to landraces in regions of high biodiversity per se, since experience in population genetics tells us that centers of species diversity react in a more robust way to introgression of genes. Celis, C., Scurrah, M., Cowgill, S., Chumbiauca, S., Green, J., Franco, J., Main, G., Kiezebrink, D., Visser, R.G.F., & Atkinson, H.J. (2004) Environmental biosafety and transgenic potato in a centre of diversity for this crop. Nature, 432, 7014, pp 222-225 - http://www.botanischergarten.ch/Potatoe/Celis-Potatoe-Nature-2004.pdf
See also Dr. Peter Raven: Transgenes in Mexican maize: Desirability or inevitability? PNAS September 13, 2005 vol. 102 no. 37 13003–13004

[iv] **Food safety of crops and foods produced through biotechnology**
September 2005
Agricultural Biotechnology White Paper
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http://agribiotech.info/ChassyFood_SafeTS.doc

Q: Are biotech foods safe to eat?
A: Most scientific experts agree that foods produced through biotechnology are as safe as, or safer than, any other food in the supermarket. Genetically modified (GM) crops aren't new.
For thousand of years plant breeders have worked to create genetically modified crop varieties. None of the crops that we eat today resembles its wild ancestor. Most ancestors were poisonous and low-yielding wild plants before early humans domesticated them.
Today we can choose among hundreds of varieties of some crops, all so genetically different that they differ in size, shape, and even color. And varieties of the same grain, fruit, or vegetable can have different compositions and nutrient contents as well. That is because they are all extensively genetically modified — the "traditional" way.

Q: Who regulates genetic modification of foods?
A: There is no regulatory oversight of traditional genetic modification. This kind of plant breeding allows the introduction of thousands of new varieties each year all over the world without any requirement for pre-

market safety review. We have learned from thousands of years of experience that plant breeding is almost always safe.

By contrast, plants modified with modern biotechnology techniques are subjected to careful pre-market safety evaluation and must be approved by government regulatory agencies before reaching the market.

Q: Who says GM foods are safe?

A: In the face of contradictory statements about the safety of GM foods, the consumer must decide whom to believe. There exists a broad scientific consensus that foods produced through biotechnology are not only as safe as foods produced through conventional plant-breeding technology. Probably they are safer because of the more precise technology that is used to produce them and the closer regulatory scrutiny they undergo.

That was the conclusion of European Union scientists who studied the safety assessment process used for biotech foods. A similar conclusion was reached in 2003 by United Kingdom scientists who were asked by their government to evaluate the potential risks of GM foods.

A large number of scientific societies, expert panels, national academies of sciences and international organizations have studied the safety of GM foods and crops and reached the same conclusion: There is no reason to be concerned about the safety of eating foods derived through biotechnology.

Q: Aren't genetically modified foods fundamentally different?

A: Opponents of crops produced through biotechnology like to call them "Frankenfoods." In fact, rather than being drastically altered monstrosities, most are crops into which a single new trait has been inserted. Since one or two genes are inserted into a plant that has some 25,000 to 40,000 genes, it's fair to say that not much has really been changed.

Q: Why tinker with plant genes in the first place?

A: Most GM crops on the market today fall into three classes:

Plants that are resistant to insects by the introduction of a gene that helps them defend themselves

Plants into which a gene has been introduced for an enzyme that makes them tolerant to weed-control herbicides

Plants containing a gene for a viral protein that makes them resistant to viruses.

Composition analysis shows that these biotech crops have the same amounts of protein, lipids, and carbohydrates as other varieties of the same crop. They also have the same vitamin and mineral content. In fact, aside from the one additional trait that is present in very small amounts in the plant, they have the same composition.

Q: Are GM foods tested first in animals?

A: New biotech varieties have been fed to a number of animal species to test their performance as feeds. No differences have been observed between GM crops and conventional crops when used as feeds. These feeding tests are not intended, however, to prove that long-term consumption of these crops by humans is absolutely safe. That is because there are no valid scientific protocols available for proving that whole foods are safe.

It is virtually impossible to provide absolute assurance that food will be safe to consume over a whole lifetime of 80 or more years. With foods that are reasonably safe — like biotech crops — scientists and regulators rely instead on the detailed analysis of composition, toxicity, and potential for allergenicity. If no safety issues are detected during these studies and the composition is unchanged, there is no reason to believe that there will be any long-term safety issues with a biotech food.

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