Overview of Environmental Risk Assessment

An Environmental Risk Assessment (ERA) for a transgenic crop provides scientific information on the potential for harm to the receiving environment arising from the crop’s environmental release that could arise as a result of the alteration made to the crop. Figure 1 provides an overview of the standard ERA approach for transgenic crops. The definition of what would constitute harm is determined not by the risk assessment itself, but by protection goals that arise from public policy and societal values. From those protection goals, risk assessment methodology is followed to generate rational hypotheses of harm that could arise directly or indirectly from the use of the transgenic crop. The generation and filtering of hypotheses of harm to arrive at the subset of reasonable hypotheses warranting additional investigation is known as problem formulation. The potential harms to be investigated must always be in the context of the protection goals and be derived from properties of the transgenic crop that differ from its conventional counterparts. Most hypotheses of harm and use the scientific method to test those hypotheses can be tested using existing knowledge about the crop, the trait, and/or the agricultural environment in which it is proposed to be released. Some hypotheses of harm may require the generation of additional data using the scientific method. Using a tiered approach, risk characterization integrates the likelihood of harm and the potential level of harm that could occur upon exposure to the crop. Risk characterization should compare the effects of the transgenic crop with its conventional counterpart(s) and can include comparison with the agricultural practices that the transgenic crop would replace. Hypothesis testing generally begins with unrealistic “worst case” assumptions and if the worst case scenarios suggest unacceptable harm could arise, the assessment is refined using more realistic scenarios. The final step in the process is risk communication, whereby the risk assessors inform the decision makers on the outcome of the analysis. In this way, the risk assessment methodology enables regulators to distinguish between “need-to-know” and “nice-to-know” information and enables objective science-based decisions on the environmental release of the transgenic crop.

Protection Goals

Independently of risk assessment and risk management, countries or other geopolitical units establish environmental protection goals based on societal values and priorities. In the agricultural context, protection goals are those aspects of the agricultural environment that are important to the country’s
and society’s ability to achieve their priorities for their agricultural system. To the extent that the primary function of agriculture is to efficiently produce and provide food, fiber and biomass fuel for the long term, protection goals can be set that maintain or promote productivity, cost effectiveness and environmental and economic sustainability. Other protection goals could include biodiversity, aesthetics, cultural preservation, wilderness preservation, and other valued aspects of rural life.

For example, a country may seek that a transgenic crop does not cause new unreasonable adverse effects on non-target organisms that provide important benefits to the agricultural ecosystem, such as predators and parasitoids of pests, and decomposers that are important in maintaining and recycling soil nutrients. In some cases, “non-target organisms” may be defined collectively as an assemblage of species that provide the ecosystem function, or they may be defined as individual species that perform specific ecological roles. This may also be extended to non-target organisms that are deemed to have other societal benefits, such as charismatic species (like monarch butterflies) or endangered and threatened species. Another goal may be to avoid increasing the likelihood that, as a result of the introduced transgene, the crop or a sexually compatible wild relative becomes a weed within the agricultural system or becomes invasive outside of the agricultural environment.

Protection goals need to be accompanied by standards by which to judge what adverse effect would be unreasonable in the agricultural context, or to ascertain what level of certainty is required in the risk assessment. These are generally defined in legislation and regulations covering agriculture or environmental protection.

**Problem Formulation**
Problem formulation is the process of developing rational testable hypotheses of potentially unacceptable adverse effects to the environment that would conflict with the stated protection goals.

**Conceptual Model**
A conceptual model describes key relationships between the transgenic crop and possible environmental consequences of its release. The conceptual model establishes the proposed relationships between exposure and effect and is developed from knowledge of the receiving environment, the conventional crop, and the introduced trait. The conceptual model thereby enables comparison the potential effects of the transgenic crop with effects of its conventional counterpart.

**Receiving Environment**
The agricultural environment into which a transgenic crop is to be released is a non-natural habitat that has been created by humans for food, fiber, and/or fuel production. Agricultural fields are not generally managed to support high biodiversity, but instead are dominated by one or a few cultivated plant species in order to maximize yield. Human intervention in an annual cropping system may include preparation of soil for planting, often involving removal of any existing vegetation through cultivation or herbicide applications. This is followed by planting the crop seeds, post-emergent weed management, and management of insect pests and plant pathogens until the crop is ready for harvest. Harvesting will
often involve destruction of the crop plants. Fields are therefore ecologically disturbed, highly unstable and dynamic.

Field margins, hedgerows, and other disturbed areas associated with the agricultural environment could be important to the stated protection goals, for example if a protection goal is biodiversity and these areas contain significant biodiversity. In some cases such areas can be managed in a way that is intended to harbor populations of organisms, such as insect predators, that are valued for their function within the agricultural ecosystem. These may also harbor plant species that are sexually compatible with the crop species and thus may cross-pollinate with the crop, in the process potentially gaining genes and traits from the crop.

Individual fields exist in a patchwork of other fields, and the fields exist in a landscape also containing field margins, fallow land, natural and urban areas. The landscape therefore consists of a wide range of flora and fauna. This landscape should also be considered as the receiving environment and can be especially relevant when considering risks to mobile organisms.

**Crop**

A key component of problem formulation is an understanding of important elements of the conventional crop’s biology and ecology as they pertain to the protection goals. A primary question is whether the crop possesses any characteristics of a weed. Can the conventional crop persist without human help? Can the conventional crop establish in non-agricultural areas? Does the conventional crop outcompete other flora? Does the conventional crop cause injury to public health, agriculture, or wildlife? Many, if not most, agricultural crops have been artificially selected over hundreds or thousands of years for characteristics that make them efficient at meeting human needs under cultivation. Many of these same characteristics make them poor competitors with natural vegetation in the absence of human intervention. For example, to maximize grain harvest, crop plants have been bred to not release their seeds without assistance from the farmer: soybean pods don’t shatter; maize seeds don’t fall from the ear. Similarly crops are bred to remove characteristics that make them injurious to people and wildlife.

A second primary question is whether there any sexually-compatible wild relatives in the region where the crop is cultivated in close enough proximity that cross-pollination could occur. Can the crop hybridize with wild plants? If so, can the offspring of crop x wild relative establish in natural habitats? Are the wild relatives or crop x relative hybrids important within the context of the protection goals?

**Transgenic Trait**

As the ERA focuses on important differences between the transgenic crop and its conventional counterparts, the final element of the conceptual model the transgenic trait itself. In general, the agronomics and biochemical composition of a transgenic crop are substantially equivalent to conventional varieties with the exception of the introduced trait. There is no reason to expect, and no evidence to support, transgenic crops having unexpected harmful properties that differ from conventionally-bred varieties. With this in mind, the problem formulation can generate testable hypotheses of harm that are caused by the transgenic trait. The context for the hypotheses must
consider the normal diversity and variability of the agricultural system and the conventional crop. The conceptual model therefore takes a comparative assessment approach to determine if the transgenic crop is as safe as its conventional counterparts.

Risk Scenarios
The conceptual model leads to the development of rational scenarios whereby unacceptable adverse effects to the environment could arise as a result of the environmental release of a transgenic crop that would conflict with the stated protection goals. A causal chain of events is developed whereby environmental harm, as defined in the context of protection goals, may arise as a direct or indirect result of release of the transgenic crop. Risk scenarios that are plausible are those that could be reasonably envisaged to occur based on the conceptual model. For plausible risk scenarios, the chain of events that make up the risk scenario allows the generation of one or more scientific hypotheses that can be tested.

For example, if a protection goal is functioning populations of a beneficial insect species, the associated risk scenario could be that release of the transgenic crop would reduce the population of the beneficial species by some proportion (e.g., 50%), within a specific timeframe, (e.g. a cropping season). Figure 2 provides an illustration of a scenario and the chain of events within it that could lead from environmental release of a transgenic crop to reduced abundance of a valued species owing to increased weediness of the crop.

Importantly, demonstration that just one of the events in the risk scenario is highly unlikely to occur can be sufficient to conclude that the specific risk is negligible and additional events in the chain do not need to be evaluated. For example, if existing information indicates that cross-pollination does not occur between a crop and wild relative, evaluation of the impact of the transgenic trait on the fitness of crop x wild relative hybrids is unnecessary.

It is also important that risk scenarios focus on the differences between the transgenic crop and its conventional counterpart – risks that are the same for both the transgenic crop and its conventional counterpart are not relevant to the regulatory decision-making process for release of the transgenic crop. So in this example, cross pollination itself does not represent a new risk; however, increased risk could arise for example if the transgenic trait increases the frequency of cross-pollination and crop x wild relative hybrid plants are regarded as harmful to a valued entity under one of the stated protection goals. In a comparative assessment, therefore, the conclusion of the risk assessment is whether or not the transgenic crop is as safe for the environment as its non-transgenic counterpart or other agricultural practices that may affect the same valued aspects of the environment.

Testable risk hypotheses
Understanding how the receiving environment, the crop, the transgenic trait and potential effects are relevant to the protection goals enable the objective filtering of the risk scenarios. Problem formulation is completed with the statement of testable hypotheses and development of an analysis plan that enables evaluation of the subset of scenarios by which there is a plausible potential of harm to an entity
that, in the context of the protection goals, is valued. For such scenarios of harm that cannot reasonably be ruled out based on existing information on the environment, crop, and trait, examination of the chain of events enables the statement of risk hypotheses. For example, in a risk scenario that release of a transgenic crop will cause harm to a population of beneficial non-target organisms feeding on the crop’s pollen, a step in the chain of events could be that the non-target organism is exposed to transgenic protein. In this case, a testable hypothesis could be that the pollen contains the transgenic protein. Hypotheses are testable if they can be experimentally evaluated and shown to be false during the risk characterization stage.

As indicated in figure 2, risk scenarios can be investigated by testing hypotheses at several steps in the chain of events. In the same example of a non-target organism harmed by a protein in pollen, hypotheses could also be tested that the crop produces pollen, that pollen is dispersed to the non-target organisms’ habitat, that populations of the non-target organism are present at the same time as the pollen, that the non-target organism consumes pollen, that the introduced protein is toxic to the non-target organism, that the level of protein in the pollen is sufficient to adversely affect the non-target organism, or that the level of the effect is sufficient to have ecologically relevant effects on the non-target organism population.

**Risk Characterization**

Where analysis of a risk scenario using existing data and information does not indicate that the scenario is implausible and there is a potential risk to a valued entity within the context of the protection goals, risk characterization is conducted to evaluate both the probability that harm may occur and the level of harm that may occur. Demonstration that just one of the events in a chain does not occur can be sufficient to conclude lack of risk. Alternatively, evidence that several of the steps occur rarely or to a very low extent can be sufficient to conclude that risk is negligible. A risk scenario can therefore be evaluated by testing one or a few hypotheses to conclude lack of unacceptable risk.

For example, in a non-target organism risk assessment of an insect-protected GM crop, the level of harm could be a function of the toxicity of an insecticidal protein to a non-target arthropod, and the probability of harm could be a function of the exposure of the non-target species to the insecticidal protein. In other cases, risk characterization can be accomplished by first evaluating a worst case situation and, only if that situation indicates potential for harm, progressing to more realistic situations. Importantly, if evaluation of the specific risk hypotheses for a given scenario using the worst case approach indicates the probability of harm is extremely low there is no need to evaluate the potential level of harm, and vice versa. As before, generation of information that supports the hypothesis that a step in the chain of events within a given risk scenario will not occur or that the likelihood that one or a few steps occurring is very low can be sufficient to rule out the scenario as leading to harm.

**Tiered testing for non-target organisms**

Risk characterization for harm to non-target organisms resulting from exposure to a new transgene product is often accomplished following the tiered-testing paradigm (Figure 3). Under this approach, the non-target organism of concern (or a surrogate that is functionally or phylogenetically similar to the
organism of concern), is tested in a bioassay with the purified transgene product at a concentration many fold higher than the highest estimated exposure in the field (Tier 1). If the test population is not affected at this high concentration, or if the effects are moderate (for example, less than 50% mortality) then there is a high likelihood that exposure to the transgene product in the field will not have significant effects under field conditions. If however, effects are seen at this high concentration, further bioassays are conducted using more realistic exposure levels, perhaps using the tissues from the transgenic plant rather than purified transgene product (Tier 2). Again, if the test population is not affected at realistic exposure levels, if effects observed would be acceptable within the protection goal, additional testing is not warranted. If significant effects are seen in Tier 2, additional testing can be conducted with whole plants in a green house or field cages (Tier 3). Such tests allow more realistic spatial processes to function that may more accurately reflect actual exposure under field conditions. Finally, if these lower tier studies indicate potential for unacceptable harm, a field study may be warranted whereby natural populations are monitored under the same conditions as the proposed environmental release (Tier 4). Progressing through the tiers increases the ecological relevance of the study to the actual proposed release but decrease the ability to detect effects due to greater variability in the test system. Methods or guidance for testing many non-target organisms at several of the tiers are available in published literature (e.g. Romeis et al 2011) or from regulatory agencies (e.g. US Environmental Protection Agency 2007).

Use of Existing Data

Often, existing data and information are available to address the hypotheses and new data do not need to be generated. For example, the Cry1 insecticidal proteins from Bacillus thuringiensis are known to be extremely narrow in their spectrum of activity. Based on data from a large number of laboratory and field studies it is apparent that any given Cry1 protein will affect only a small subset of insects within the order Lepidoptera, and often the affected insects belong to a single or small number of related families. Insects of the order Coleoptera are known not to be sensitive to Cry1 proteins, and therefore a risk scenario whereby release of a Cry1-producing transgenic crop leads to reduction of populations of ladybird beetles (Coleoptera: Coccinelidae) through direct toxicity is highly unlikely to occur.

Other considerations for risk characterization

Selection of Non-Target Test Organisms and Use of Surrogate Species

Where non-target organism sensitivity data are needed to perform the risk characterization, it is not possible to test all the species that may potentially be exposed to the transgenic crop or the active ingredient in the field. Instead, risk assessors use data from a small number of species that are representative of the larger guild of species present, and in many cases will use surrogate species that are related to the actual species of concern. The justification and selection criteria for non-target organism testing have been well covered in the literature (Romeis et al 2013, CERA 2013, US Environmental Protection Agency 2007).

Data Transportability - The Use of Data from other Environments

It is important that risk assessors have access to and utilize data on the crop and trait that are generated in other geographies. For example, it is not necessary to test agronomic properties and transgene
product expression levels in the environment of the proposed release if sufficient data are available from a range of other agricultural systems and crops that establishes the range of values, or if data are available from an environment that is functionally similar to the proposed receiving environment.

ERA for import approvals: low exposure scenarios

In some regulatory systems, it is necessary to perform environmental risk assessments for transgenic crops when the requested approval is not for commercial environmental release, but instead for importation of grain that is for food and feed use. In these situations, the problem formulation must specifically consider the potential for inadvertent environmental release of the transgenic crop and the scenario analyses must account for the very low exposure potential. Usually for a properly conducted problem formulation, a conclusion about acceptability of risk in the context of the protection goals can be reached with very limited risk characterization necessary.

Confirmatory field data

It is important to recognize that environmental risk assessment as described here may largely or completely be accomplished without generating environmental biosafety field data in the region of the proposed environmental release. The process of problem formulation to arrive at plausible risk hypotheses and the availability of data from laboratories or field studies in other geographies can enable the risk assessment to be completed. Only if there is remaining uncertainty in the risk characterization that would affect the regulatory decision may it be warranted to conduct field studies to evaluate the actual effects in the environment. Often field studies are completed after commercialization of a transgenic crop, but these are used more to confirm the findings of the risk assessment and to provide additional assurance to non-regulatory stakeholders of the environmental safety profile of the crop, but not as part of the risk assessment itself.

Conclusions

Transgenic crops have been commercially grown on over a billion hectares by over 15 million farmers in 30 countries over 17 years. As anticipated in environmental risk assessments, no unexpected unreasonable adverse environment effects have been documented. In many cases, transgenic cropping systems have been shown to have less effects on the agricultural environment than the conventional cropping systems they replace (for example, insect-protected maize and cotton that produce Bt proteins have repeatedly been shown to have lower impact on non-target arthropods than insecticide-treated conventional crops). The risk assessment paradigm described here has been appropriate for anticipating environmental effects, and for assessing the acceptability of identified risks in the context of agreed protection goals. It is reasonably straightforward to assess the risks arising from the introduction of one or a few well characterized genes and traits is associated within the context of natural variability of the agricultural ecosystem, conventional breeding, crop husbandry, and environmental management. Within clearly defined protection goals, the process of problem formulation and risk characterization described here provides information appropriate for decision making.
References and further reading

The International Life Sciences Institute’s Center for Environmental Risk Assessment (ILSI CERA) serves as a scientific resource for governments, academic and private sector organizations developing and applying sound science to the environmental risk assessment of agricultural biotechnologies. [www.cera-gmc.org](http://www.cera-gmc.org)


Raybould A (2011). The bucket and the searchlight: formulating and testing risk hypotheses about the weediness and invasiveness potential of transgenic crops. Environ Biosafety Res 9: 123-133. [http://dx.doi.org/10.1051/ebr/2011101](http://dx.doi.org/10.1051/ebr/2011101)


Figure 1. Generalized overview of Environmental Risk Assessment methodology for transgenic crops

Protection Goals
- Key aspects of environment that are important to society

Problem Formulation
- Identify potential pathways to harm to valued entities (functions, populations etc. indicated by the Protection Goals)

Risk characterization
- Evaluate probability of unreasonable harm to valued entities through the identified pathways. For example, Risk = Hazard x Exposure

Risk Communication
- Provide the outcome of the risk characterization to decision makers to inform regulatory decisions
Figure 2. Problem formulation: risk scenario for transgene causing the crop to form a weed. At each step in the chain, the risk assessor evaluates whether the transgenic crop has increased potential compared with the range of conventional equivalents. Adapted from Raybould (2011).
Figure 3. Overview of tiered testing system for non-target organisms that may be affected by a transgenic trait. Progression from one tier to the next occurs only if the lower tier test indicates potential for unacceptable harm under the conditions of the test. Progression through the tiers increases realism of the exposure but decreases the power to detect meaningful effects.