Planning Environmental Risk Assessment for Genetically Modified Crops: Problem Formulation for Stress-Tolerant Crops

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A scientifically sound environmental risk assessment is required for crops derived from modern biotechnology (also referred to as genetically modified [GM]) prior to unrestricted release into the environment. The scientific principles underlying the environmental risk assessments completed for herbicide-tolerant and insect-protected GM crops commercialized to date are now being applied to crops currently under development that are modified for improved tolerance to abiotic stresses. These principles, and the processes built upon them, have been shown to be sufficiently robust to provide the appropriate information for regulatory decision making and to ensure an adequate level of environmental protection. This article describes the initial steps in the environmental risk assessment process and illustrates an approach that could be taken for GM crops tolerant to an abiotic stress (e.g. water, salt, cold, and heat). The discussion below begins with an overview of the initial steps in an environmental risk assessment, known as problem formulation (US EPA, 1998). A general overview describing how problem formulation has been applied for the first GM crops is presented next. Finally, the approach is applied to a hypothetical drought-tolerant maize (*Zea mays*) product as an example of how problem formulation can guide the environmental risk assessment for a specific abiotic stress tolerant crop.

Recent advances in functional genomics have led to the discovery of genes associated with tolerance to abiotic stresses such as cold, heat, water, and salt (Vij and Tyagi, 2007). Some of these genes show promise in major crops like maize (Nelson et al., 2007) and rice (*Oryza sativa*; Hu et al., 2006). As such, a discussion on planning an environmental risk assessment of GM abiotic stress-tolerant crops is timely. Regulators are now confronting the challenges involved in evaluating data from these new and potentially beneficial products. Products expressing stress tolerance phenotypes are now being widely tested in field trials around the world. Very soon, technology providers will submit data and information on GM crops with stress-tolerant phenotypes to regulatory authorities for review that will include an environmental risk assessment as part of a request for commercial release.

PROBLEM FORMULATION IN ENVIRONMENTAL RISK ASSESSMENT

Formal risk assessment is relatively young as a scientific discipline. Its practice arose from the need to make informed decisions in a more objective way. Regulators today rely on risk assessment methodologies to collect, analyze, and communicate information to decision makers and the public (National Research Council, 1996). The basis for formal human health risk assessment in the United States took shape in 1983 with the publication of the ''Red Book'' (National Research Council, 1983). Ecological risk assessment for chemical substances was also outlined within this timeframe (Suter, 1993; US EPA, 1998) based largely upon principles outlined in the Red Book. These early efforts fixed the current paradigm that is well accepted today: risk is a function of a defined harm (hazard) and its likelihood of occurrence (exposure). Publications on environmental risk assessment (Suter, 1993; US EPA, 1998) have outlined coherent and logical steps to progressively and iteratively proceed to a point where a risk is characterized and the evidence supporting the conclusion is clearly communicated. This process has been used for chemical stressors successfully and has been described in detail by the U.S. Environmental Protection Agency (US EPA, 1998). The process (Fig. 1) follows steps of: problem formulation as the beginning; assessment of the exposure, including levels and likelihood of exposure; hazard identification and assessment that examine the potential hazard(s) using effects testing and the magnitude of the potential outcome; and risk characterization that integrates the hazard, the magnitude of the consequences, and the likelihood of occurrence. Regulatory decisions regarding the acceptability of introducing a potentially harmful agent into the environment are based on the risk characterized.

Define Assessment Endpoints

In accordance with the model (US EPA, 1998), problem formulation is the first step in the risk assessment

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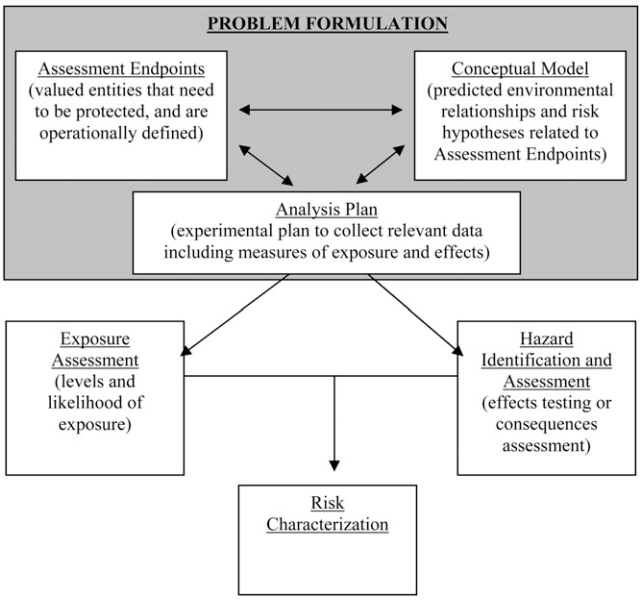

Figure 1. Schematic of problem formulation in the context of the risk assessment process as proposed by the U.S. Environmental Protection Agency (US EPA, 1998).

process (Fig. 1). It involves defining assessment endpoints, and developing a conceptual model and an analysis plan (US EPA, 1998). Assessment endpoints are valued ecological entities that can be defined in a way that risk to them can be identified and their attributes can be protected. Clearly defined policy goals and assessment endpoints are essential to the environmental risk assessment because they direct the efforts to protect that which the public wants protected. Assessment endpoints are ''ecological values defined by specific entities and their measurable attributes, providing a framework for measuring stress-response relationships'' (US EPA, 1998, p. 36). For example, honey bees are important pollinators both ecologically and economically. Depending on the nature of a stressor (a chemical or a GM trait that is regulated and is intended for release into the environment) and the problem that is ultimately formulated, an appropriate assessment endpoint could be honey bee abundance. In this example, honey bee abundance is an ecological entity that is valued, and measuring effects of the stressors on honey bees can be done using appropriate scientific methods. Typically abundance can be evaluated by measuring toxicity of a stressor to honey bees using suitable experimental methods that ideally would be validated for this purpose. A reduction in honey bee abundance (e.g. >20% used as a threshold of concern) would be considered an adverse effect on an assessment endpoint.

The U.S. Environmental Protection Agency (US EPA, 1998) points out that assessment endpoints are different from environmental goals. Protecting honey bees from a toxic or any other adverse effect of a stressor is an example of an environmental or risk management goal and, as such, would not be an assessment endpoint. Because assessment endpoints must be measurable they should not be confused with statements of policy; though their basis is found in environmental policy and they denote public input into the risk assessment process (National Research Council, 1996).

Develop a Conceptual Model

A conceptual model describes relationships between the valued entity, the stressor, and pathways of exposure and potential effects in the environment (Fig. 1). The conceptual model for an environmental risk assessment would include the available information on the nature of the stressor, its proposed use, reasonable environmental pathways whereby exposure could occur, and potential responses of the assessment endpoint as a result of exposure. For example, the conceptual model for a soil applied, systemic chemical insecticide (chemical A) used in maize would account for movement in soil as well as in the plant to various tissues where exposure to pests and other organisms (birds, insects, and mammals) might result.

The conceptual model is useful to generate risk hypotheses that are necessary to make assumptions and predictions about how a stressor could affect an assessment endpoint. Risk hypotheses are not null hypotheses, but rather they are proposed answers to reasonable questions about how the assessment endpoint(s) will respond to the stressor(s). An example of a risk hypothesis is: chemical A is translocated in a maize plant to the floral tissues; bees and other insects foraging on maize pollen will be exposed to chemical A in the pollen; populations of bees and other beneficial insects will not be adversely affected from the use of chemical A. As depicted in this example, risk hypotheses are developed using existing information for exposure and the potential for the stressor to cause harm to the entity of value. One referee correctly pointed out that proper construction of a hypothesis can either predict harm or the absence of it. In a case like chemical A where harm to honey bees could be evaluated directly, the preferred hypothesis might be ''chemical A is not harmful to bees.'' If the hypothesis is not falsified, the risk assessment should be able to conclude minimal risk.

Develop an Analysis Plan

The last step of the problem formulation, the analysis plan, delineates the data needed and the approach to be taken for data acquisition and synthesis (Fig. 1). Two important aspects included in the analysis plan are the selection of measurement endpoints and prioritization of the data needed. For example, if chemical A were active against corn rootworms (*Diabrotica* sp.) that are members of the order Coleoptera, it might be reasonable to run an effects/toxicity test on specific, beneficial, ground-dwelling members of this

order, e.g. carabids. Early in the risk assessment process some hypotheses can be adequately answered with the available information. For example, based on the known properties of a stressor it may be reasonable to conclude that toxicity or exposure will be minimal and impacts to assessment endpoints could be characterized as negligible. Using the earlier example of honey bee abundance, if there is information indicating that a stressor has no known toxicity or reasonable mechanism to be toxic to honey bees, there would be no reason to expect the assessment endpoint to be impacted. Consequently, there would be no need for additional experiments to be conducted when the knowledge of lack of harm or exposure is deemed to be adequate.

The analysis plan is also used to prioritize testing, which is important so that resources are focused to collect only the data that are essential to characterizing the risk. In the example of chemical A, testing carabids would be a higher priority than testing a species from another order that is not typically found in a maize field or that is not susceptible to the chemical. Collecting data on potential adverse effects to nontarget insects within a family that is known to be uniquely susceptible to an insecticide would clearly be higher priority than examining effects on insects in distant taxonomic groups.

Once specific measurements are chosen and given a priority, appropriate methods of measurement are selected and noted in the analysis plan. In addition, the analysis plan may describe potential higher tier experiments that could be conducted depending on the results of the first tier experiments (US EPA, 1998). Tiering describes the case where conservative assumptions are made in the initial first tier experiments and more refinements and realism are introduced in higher tier studies. For example, in first tier laboratory tests with single species that represent a group of nontarget organisms, doses should significantly exceed the expected environmental exposures. Detecting effects in first tier experiments may trigger a higher tiered evaluation that could either refine the exposure or involve a controlled examination mimicking field conditions. Using a tiered approach reduces the uncertainty in the risk assessment because each progressive tier is guided by the results of the previous one, and specific, testable, and relevant hypotheses are formulated based on data. Stated another way, higher tiers in a risk assessment are refinements of the hypotheses generated earlier and relevant to the overall goals of the risk assessment. Higher tiered experiments are often unnecessary when sufficient conservatism (e.g. toxicity testing at high doses in the laboratory) is built into the first tiers and the data provide no evidence of risk.

The information from the problem formulation and the processes described above are the starting point for an environmental risk assessment. Having a properly constructed analysis plan based on a conceptual model that is clearly linked to assessment endpoints helps guide the collection of relevant data useful for a risk assessor to evaluate hazard and exposure and ultimately estimate and characterize risk. In summary, constructing a plan in an organized manner with the available information at the beginning of the environmental risk assessment is essential for success. The following sections describe how this process has been applied to GM crops and how it could be applied to GM abiotic stress-tolerant crops.

PROBLEM FORMULATION APPLIED TO GM CROPS

Information concerning the history and evolution of environmental risk assessment applied to GM crops is useful background for this discussion. Deployment of GM crops in agriculture began in the mid-1990s with the commercialization of FlavrSavr tomatoes (*Solanum lycopersicum*), virus-resistant squash (*Cucurbita pepo*), NewLeaf potatoes (*Solanum tuberosum*), and Roundup Ready soybeans (*Glycine max*; see Biotech Crop Database, http://www.agbios.com/main.php). Over approximately 20 years, numerous frameworks for environmental risk assessment (UNEP, 1995; Rissler and Mellon, 1996; Kjellsson, 1997; Edmonds Institute, 1998; Kjaer et al., 1999; Nickson and McKee, 2002; Hancock, 2003; Wilkinson et al., 2003; Evaristo de Jesus et al., 2006) and guidance documents (National Research Council, 1987; Tiedje et al., 1989; OECD, 1993) have been published. Many of the first substantive reports on the subject of evaluating the safety of GM crops did not clearly explain the relationship between the approach they were describing and established risk assessment principles. Most of these seminal publications were largely based in biological sciences, ecological theories, and practical experience with crop plants, what Raybould (2007) has recently termed an ecological approach. In this publication, Raybould (2007) described the problems associated with approaching risk assessment without first structuring the problem using a proper problem formulation according to an ecotoxicological approach. The intent here is to reinforce the appropriateness of using the chemical model or ecotoxicological approach starting with problem formulation for GM crops.

Emerging from the development of frameworks and guidance for risk assessment of GM crops are five consensus principles and an important idea known as the concept of familiarity. The five consensus principles that should be considered in the problem formulation state that risk assessments for GM crops should: (1) be science based where quantitative information is used as available and uncertainty is considered; (2) use qualitative information in the form of expert judgment; (3) use a comparative approach; (4) be case-specific; (5) be iterative/recursive and examine conclusions already made based on new information. All of these consensus principles are consistent with the conceptual basis of risk assessment developed for chemicals (Hill and Sendashonga, 2003), and they are harmonized with international treaties developed to

protect and conserve biodiversity from the potential risks posed by GM crops (BSP, 2000).

An important concept useful in problem formulation for GM crops is familiarity (OECD, 1993). As described by the OECD (1993), familiarity is knowledge gained through experience over time (OECD, 1993; Nickson and Horak, 2006). Familiarity considers the nature of the crop that was modified, the characteristics of the trait that was introduced, the likely receiving environment for the GM crop, and the likely interactions between these (OECD, 1993; Nickson and McKee, 2002). Importantly, familiarity is not an endpoint in the assessment. Furthermore, to be familiar does not necessarily mean to be safe. Rather, it is to have sufficient information and appropriate context from which to assess the risk, which is consistent with the ideas behind the conceptual model as described above.

Other important points have been brought forward in a thoughtful retrospective analysis of the state of environmental risk assessment of GM crops (Hill and Sendashonga, 2003), where they described the lessons learned from adopting the chemical risk assessment approach to living modified organisms (LMOs). The term LMOs has been adopted by the Convention on Biological Diversity and the Cartagena Protocol on Biosafety where it is defined. Among the lessons Hill and Sendashonga (2003) note are the need for flexibility and the need to consider multiple lines of evidence all within a comparative context. Subsequently, Hill (2005) pointed out that standardization of some of the unique structural and procedural elements published is unnecessary. However, Hill (2005) clearly noted that a common understanding of the conceptual elements is essential. One thesis of this article is that the conceptual elements must be appropriate to avoid problems like uncertain relevance to the risk assessment. Therefore, as will be illustrated below, constructing suitable problem formulation will utilize a standardized conceptual basis derived from chemical risk assessment, and integrate consensus principles along with the concept of familiarity that have been developed for GM crops.

Because a risk assessment is directed by the assessment endpoints, or the environmental entities of concern and the attributes measured that need to be protected, these must be clearly defined and identified by the developer at the start. Regulatory authorities define assessment endpoints based on their legal basis for regulation. For example, the U.S. Department of Agriculture Animal and Plant Health Inspection Service requires that an assessment be made of ''pest potential,'' including the potential to become a noxious weed (Belson, 2000). For a GM plant that will be used in conventional agriculture, one possible broad description of an assessment endpoint of the risk assessment could be ''the abundance of pests and beneficial organisms in agricultural fields compared to the conventional crop.'' This construction of the assessment endpoint reflects the value that agricultural fields provides and includes a measurable element (abundance). It also allows the measurement to be quantitative, qualitative, or a combination of both as appropriate. A frequent mistake has been to propose broader environmental goals as assessment endpoints for GM crops such as protecting biodiversity or specific groups of organisms, e.g. birds. The Cartagena Protocol on Biosafety (BSP, 2000) calls for the ''conservation and sustainable use of biodiversity….'' Because conservation and sustainable use are not operational and because biodiversity in this context is a concept rather than a measurable entity, this statement is an environmental goal and not an assessment endpoint. In addition to being measurable and meaningful, this example of an assessment endpoint is consistent with the principle that the risk assessment for GM crops is comparative. Figure 2 depicts a specific example of how specific, testable hypotheses can be posited after formulating an assessment endpoint.

A conceptual model for a GM crop is constructed by collecting all available information on the crop and the trait, the likely receiving environment, and interactions among these. Familiarity with the crop, the trait, the receiving environment, and known interactions along with the product concept provides context for the conceptual model that is then used to build the analysis plan. Fundamental to any analysis plan for a GM crop is an extensive characterization of the product that includes appropriate expression and molecular analyses as well as a detailed assessment of the plant in the field and compositional components (Fig. 3). Plant characterization is another term for this detailed assessment of a crop in the field. The purpose of plant characterization is to confirm or falsify the risk hypothesis that the GM crop is not different compared to the non-GM crop other than the presence of the introduced gene(s), the expression of the gene(s), and the intended phenotype. As such, plant characterization is designed to define meaningful differences be-

Environmental Entity of Value:	• Agricultural field that supports production of food, feed and fiber
Potential Attribute of the GM Crop:	• Weediness of the GM crop – volunteer potential, persistence
↓	
Assessment Endpoint:	• Abundance of GM volunteers in subsequent rotations compared to the non-GM volunteers
Adverse Effect on the Assessment Endpoint:	• A significant increase in the number and persistence of volunteer plants
↓	
Risk Hypothesis:	• Commercial deployment of the GM crop will not result in increased weediness in the form of volunteers.
Scientific Hypotheses:	• The GM crop produces no difference in the number of volunteers compared to the conventional crop. • Dispersal of the GM crop to adjacent fields is not different compared to the conventional crop.

Figure 2. Example of formulating and integrating an assessment endpoint into the problem formulation process for a GM crop.

Basis of the Conceptual Model for a GM Crop	Consider: • The nature of the crop • The nature of the trait • The characteristics of the likely receiving environment • The likely interactions between these elements
Basic Elements of an Analysis Plan for a GM Crop	**Product Characterization** (define intended differences) • Molecular Analyses • Expression Analyses (as appropriate) **Plant Characterization** (detect meaningful differences) • Compositional Analyses • Agronomic/Phenotypic Analyses Typical risk hypothesis for plant characterization – *There are no meaningful differences between the GM crop and the non-GM crop other than the presence of the introduced gene(s), the intended expression of the introduced gene(s) and the intended phenotype.*

Figure 3. Basic elements of the conceptual model and analysis plan for a GM crop.

tween the GM crop and its conventional counterpart. Detected meaningful differences are then subjected to more detailed risk assessment (Nickson and Horak, 2006; Fig. 3). Typically, plant characterization uses endpoints that are familiar to plant breeders and experts familiar with the crop so as to leverage expert judgment in regards to understanding differences between the GM crop and its conventional counterpart (comparative assessment). In this way data analysis can leverage both quantitative information as well as a wealth of experience from traditional breeding and the use of the crop in agriculture. Based on the information gathered, a refined experimental plan may need to be drafted that outlines the experimental approach to be taken to provide additional information on the hazard and exposure potential. The implications of meaningful differences identified in the plant characterization subsequently are considered in more detail in the risk assessment.

A risk assessor examining a GM crop will use the information gathered to select and prioritize data collection within the analysis plan. Decisions must be made as to if and how much information needs to be collected on a GM plant. For example, existing knowledge of gene flow including frequency, distance, and the presence of wild relatives (exposure information) is considered within the conceptual model along with information on the introduced trait. Based on this information a decision may be made and reflected in the analysis plan as to what, if any, additional information on gene flow should be collected. In cases where a reasonable hypothesis can be developed that the introduced gene could adversely affect nontarget organisms, the analysis plan would reflect appropriate gene flow and other experiments to collect necessary information. Raybould (2007) provides an excellent discussion on the appropriateness of data selection to avoid collecting superfluous information.

In some cases with GM crops, this aspect of the problem formulation has been skipped by scientists who simply believe that measuring the frequency of gene flow at a given distance is a requirement for the risk assessment. For example, the original risk assessments for Liberty Link and Roundup Ready canola (*Brassica napus* var. *oleifera*) conducted in Canada assumed a probability of 1.0 that gene flow will occur to other canola plants and possibly wild relatives like *Brassica rapa*. The Canadian decision for Roundup Ready canola GT73 states: ''The above considerations led CFIA to conclude that gene flow from GT73 to relatives is indeed possible, but would not result in increased weediness or invasiveness of these relatives'' (available at http://www.inspection.gc.ca/english/plaveg/bio/dd/dd9502e.shtml#A9). Nevertheless, numerous studies of gene flow have been conducted that in the end provided no better estimation of the environmental risk associated with commercial release of this GM crop. The preceding statement does not minimize the fact that resistance to a herbicide like glyphosate in volunteer GM plants and weedy relatives is a significant stewardship concern (Beckie et al., 2004; Brimner et al., 2005). However, the environmental risk assessment for Roundup Ready crops has consistently shown that these GM crops pose no greater risks to the environment than their conventional counterparts.

In an earlier article, Raybould (2006) provided valuable insights into the importance of proper problem formulation in an environmental risk assessment for GM crops. Specifically, he has examined the relationship between hypothesis testing and assessment endpoints in the context of problem formulation (Fig. 2). A good analysis plan is based on risk hypotheses, which when tested will support the risk characterization because they are relevant to the risk assessment. Risk hypotheses are theories that predict the likelihood of harmful outcomes to assessment endpoints, and are distinct from other scientific hypotheses that are not logically derived from the problem formulation (Raybould, 2006). Using one assessment endpoint described above (abundance of pest organisms in an agricultural field compared to the conventional crop), an appropriate testable hypothesis would be ''the GM crop is not different from conventional crop A in its potential to volunteer,'' i.e. persist as a pest/weed in a farmer's field (Fig. 2).

However, a common mistake in risk assessment is to formulate the hypothesis as a basic question in ecology ''GM crop A is more fit than conventional crop A'' (Raybould, 2007). Testing the appropriate risk hypothesis above involves collecting experimental evidence that could falsify the hypothesis and thus inform the risk assessment. Testing the latter hypothesis would indeed broaden basic knowledge, but would not answer the safety question because its link to the assessment endpoint is indirect at best. In fact, testing the latter hypothesis could (and often does) lead to more basic scientific questions that require additional experiments to resolve the resulting uncertainty. Raybould (2006) correctly points out that proposing inappropriate hypotheses and formulating a problem poorly can result in greater uncertainty in the risk

assessment, e.g. a focus on measuring fitness averts attention from addressing whether there is an adverse impact on the plant community. In this example, a significant difference between a GM plant and its non-GM counterpart for components of fitness like seed yield is not necessarily indicative of increased risk. If elements of exposure like seed dispersal are unchanged, the risk would still be characterized as minimal. Careful selection of appropriate measurement endpoints will clearly indicate effects that are meaningful in regards to the assessment endpoints. Time spent answering irrelevant questions creates costs to regulatory agencies and delays in approving and adopting potentially improved agricultural practices.

One lesson from experience with GM crops is that developers should share an outline of their problem formulation with regulatory authorities before doing a lot of work on the risk assessment. Early conversations with experts including regulators can help define both the scientific soundness of the approach as well as the regulatory acceptability. The following section applies these principles to stress-tolerant crops such as drought-tolerant maize.

Problem Formulation for a Hypothetical Drought-Tolerant Maize

The first steps in developing an environmental risk assessment for a drought-tolerant GM maize as discussed above are to: (1) identify the assessment endpoints; (2) develop a conceptual model that is used to develop risk hypotheses; and (3) draft an analysis plan based on the conceptual model and assessment endpoints (Fig. 3). If the drought-tolerant maize plants will be deployed in conventional agriculture systems in the same way as the first GM crops, the assessment endpoints would be the same, i.e. abundance of plants and animals (pests and beneficials) and valued soil processes. As with other GM crops, the conceptual model for the problem formulation would include available information on the following: the nature of the trait (drought tolerance); the nature of the crop (maize); the likely receiving environment (maize production fields); and the interactions among these factors (Fig. 3). Based on the product concept, the problem formulation might consider whether the trait could expand the range in which the plant will be cultivated or could grow. The conceptual model for a product like drought-tolerant maize would consider the plant phenotype and how it could alter the plant's interactions in biotic communities outside the maize field. Finally, an analysis plan would include a product and plant characterization aimed at defining and detecting meaningful differences between the GM crop and its conventional counterpart (Fig. 3).

Problem formulation for a hypothetical drought-tolerant maize product must consider the defining characteristics of this particular plant for the risk assessor to determine what information is needed to assess the risk. One could begin by questioning whether detailed knowledge of the mechanism of action is needed to assess the safety of the product. For insect-protected products based on proteins from *Bacillus thuringiensis* (Bt), knowledge of the mechanism is relevant to building the conceptual model and analysis plan. In this case, Bt proteins are specifically toxic to certain pests and pose minimal risk to other organisms based on their mode of action (OECD, 2007) and levels of expression in plants. Conversely, if the gene conferring drought tolerance has no reasonable mechanism for conferring toxicity to organisms, it is unlikely that detailed knowledge of the mechanism by which a gene confers drought tolerance will be necessary for the risk assessment. Knowledge that a maize plant is tolerant to water stress is sufficient to guide the development of the conceptual model and analysis plan.

Another important defining characteristic for a drought-tolerant maize is the definition of the product in terms of its intended effect in the environmental, also known as the product concept. Terms like drought tolerance are descriptive, subject to wide interpretation (Passioura, 2007), and may be difficult to quantify due to complexities of water balance between the plant and its environment and their effect on the crop yield. Unlike a herbicide-tolerant maize where the plant response and phenotype are predictable, tolerance to drought is likely to be sensitive to the presence and level of water stress thus making stress a challenge to quantify across a wide geographic region. In addition, possible low levels of cross talk with other stress response pathways (Cassells and Doyle, 2003), might have a small, detectable change in response to another stress, e.g. heat. This response would likely not be commercially useful because it is low magnitude and inconsistent across regions. Importantly, the phenotypic characterization of the plant in the field outlined in the analysis plan should be sufficiently robust to inform the risk assessment as to whether the response is relevant to the environmental safety of the product.

Based on this information, a conceptual model should be constructed in a way that guides the risk assessor to develop an analysis plan whereby relevant information is incorporated (Fig. 4). As noted earlier, the purpose of comparative product and plant characterization is to define and identify meaningful differences between the GM crop and the conventional crop. Thus, having a good understanding of the response of the conventional plant to drought and optimal water conditions is essential as is the ability to assess interactions of the plant with other stresses. In this case, the plant characterization studies should be conducted under conditions where water applications are carefully controlled. The overarching hypothesis concerning a drought-tolerant maize designed for use in commercial agriculture would likely be that the plant poses no greater risk to the environment compared to conventional maize. However, to accurately define differences, plant characterization of the

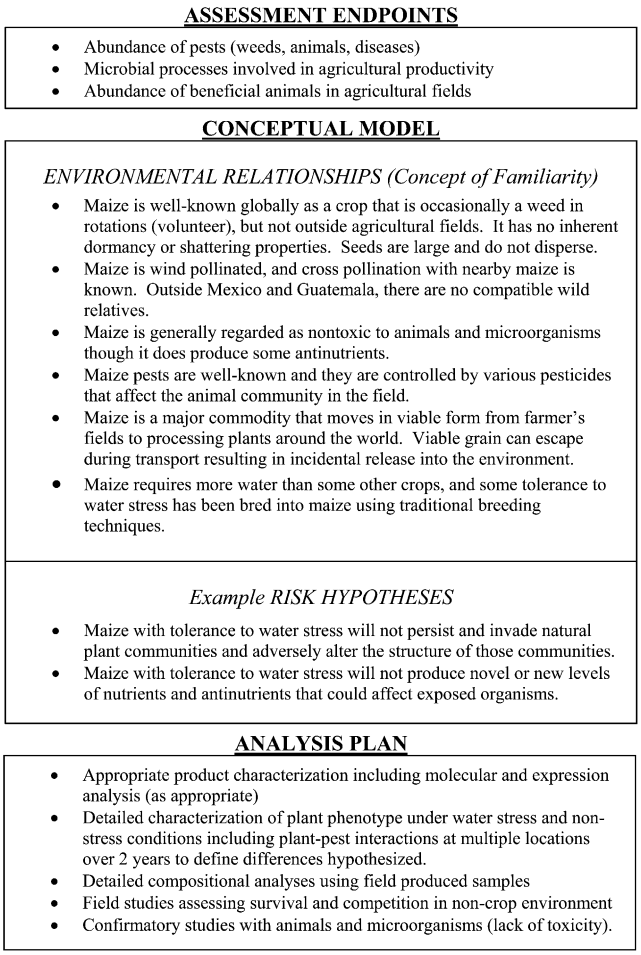

Figure 4. An overview of problem formulation for a hypothetical drought-tolerant maize.

drought-tolerant maize could require testing two unique hypotheses that are not the risk hypotheses shown in Figure 4. The first hypothesis is that there are no phenotypic differences between the GM and conventional maize when optimal water is applied. The second hypothesis would be that a phenotypic difference between the test and control when water stress is present is an increase in yield compared to the control. This hypothesis confirms an intended difference between the test and control, which will ultimately have to be addressed in the risk assessment. Water stress will have to be carefully controlled across the experiment, which will also have to be properly designed to detect a defined difference between a test and control hybrid. Utilization of a variety of commercial hybrids as reference maize lines in these experiments to validate a response to water stress expected for maize is another important factor for the comparative assessment.

For a stress-tolerant crop, it is expected that the plant will respond to the stress in a consistent manner in the agricultural setting under its intended conditions of use (hypothesis 2). A likely outcome would be that the drought-tolerant maize plant produces more seed under water stress conditions compared to the conventional control. But, as pointed out earlier, more seed does not necessarily indicate a greater level of environmental risk. As such, additional higher tiered testing could be required to define the hazard potential of the increased yield under water stress. Higher tier experiments designed to look at potential effects of drought-tolerant maize on plant communities, e.g. competition or replacement capacity studies may be necessary to refine the risk characterization (Fig. 4). Tiered testing is a proven and accepted approach to refine the environmental risk assessment (Touart and Maciorowski, 1997; Garcia-Alonzo et al., 2006). Importantly, hypothesis 2 helps define the anticipated difference between the test and control, and higher tiered testing addresses the risk hypotheses that the drought-tolerant maize has no greater persistence or invasiveness in the environment compared to conventional maize.

Comparative compositional analysis of small molecules typically found in maize would likely be recommended within the conceptual model. Compositional analyses are a risk assessment requirement for all GM crops. This point highlights the value of compositional data in environmental risk assessment and assessment of potential effects primarily on nontarget animals. Meaningful changes in plant composition could alter the ecological interactions of the maize plant with the biotic community, particularly interactions with pests. If this were the case, the analysis plan would include confirmatory testing to collect data on appropriate analytes. These confirmatory tests would be reflected in the analysis plan for the environmental risk assessment for the drought-tolerant maize (Fig. 4).

The analysis plan outlined in Figure 4 should be sufficiently robust to address the concerns that a drought tolerant maize product is likely to confer a "fitness" advantage (Snow et al., 2005). Raybould (2006) points out that measuring increased fitness does not necessarily address the goal of protecting nontarget plant communities. Having more precise information on the yield benefit (efficacy) of a drought-tolerant maize compared to conventional maize under water stress would provide no better information to the risk assessor beyond knowing that there is a comparative yield advantage (increased fecundity) under stress. Conversely, having data showing that the drought-tolerant maize plant's ability to survive outside of cultivation is not different compared to conventional maize could better answer this concern than having more mechanistic and efficacy data.

An analysis plan, like the one depicted in Figure 4, would be conservative in accordance with the principles in a first tier evaluation recommending detailed characterization and certain confirmatory studies. Because this example is hypothetical, it lacks sufficient detailed information on the gene to make a more complete analysis of potential impacts to microorganisms and animals. A product-specific problem formulation would deal with these elements more extensively.

However, based on the genes described in the literature (Hu et al., 2006; Nelson et al., 2007), direct toxic effects on microorganisms and animals is highly unlikely. In addition, this example does not assume any tissue specificity or level of expression of the introduced gene that may be a factor for a specific product. It is conceivable that based on the nature of the introduced gene and its expression in tissues, no reasonable risk hypothesis for increased adverse effects to nontarget organism from the expressed protein would be formulated. Nevertheless, the analysis plan in the problem formulation outlined in Figure 4 is conservative because it includes confirmatory tests with animals (e.g. bird and mammal). Similar studies are frequently prescriptive requirements of regulatory systems and, as such, they are conducted on all GM crops.

CONCLUSION

Crops with tolerance to abiotic stress are now being developed using the tools of modern biotechnology (Vij and Tyagi, 2007). Because these crops are developed using genetic modification techniques they will be subject to a detailed environmental risk assessment prior to commercial use. As technology providers develop these crops, they will need to understand how to approach the risk assessment starting with a proper problem formulation. Over the years, much counsel has been given on the importance of understanding ecological processes and principles in the context of protecting the environment from the risks posed by GM crops (Snow et al., 2005). Some of these scientists call for an ecological approach to environmental risk assessment on the basis that it is more rigorous and even protective. However, this approach has been shown to be inappropriate when compared to the ecotoxicological model described by Raybould (2007). This article describes an approach beginning with problem formulation that is based on established risk assessment principles that have been applied successfully to both chemical products and GM crops (Raybould, 2007). Problem formulation integrates knowledge in a systematic and organized manner to help risk assessors develop conceptual models and analysis plans that will provide information relevant to protecting valued environmental entities. Importantly, a well-developed problem formulation increases the efficiency of the environmental risk assessment and the certainty of its conclusions. This approach is based on a proven conceptual standard that can be applied on a case-by-case basis to GM crops with improved tolerance to abiotic stress.

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