Proceedings of the 9th International Symposium on the Biosafety of Genetically Modified Organisms, September 24-29, 2006, Jeju Island, Korea, pp. 62-67 (2006)

Moving Through the Tiered and Methodological Framework for Non-Target Arthropod Risk Assessment of Transgenic Insecticidal Crops

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Abstract

Transgenic insecticidal crops have the potential to pose risks to non-target organisms. These risks need to be addressed as part of the environmental risk assessment that precedes the commercialization of any novel transgenic crop. An international initiative has been launched to develop a scientifically-sound, generic, and pragmatic approach to assess the risks to terrestrial non-target arthropods. The basis for this work is the widely-established and effective tiered testing approach from regulatory toxicology. The basic principles of this approach are described. These may provide guidance to countries that are currently developing their own non-target risk assessment guidelines and help to harmonize regulatory requirements in different regions.

Keywords

Non-target arthropods, risk assessment, study design, species selection, tiered approach, transgenic insecticidal crops

Introduction

Transgenic insecticidal crops that express Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) have been grown on a steadily increasing area worldwide since their first introduction in 1996. A number of crops expressing novel insecticidal proteins are also under development and expected to reach the market stage in the near future. Like conventional agricultural pest control products, one of the risks associated with the growing of transgenic insecticidal crops is their potential impact on non-target organisms including a range of arthropod

species that fulfill important ecological functions such as biological control, pollination and decomposition. Potential non-target risks need to be assessed as part of the environmental risk assessment (ERA), prior to the cultivation of any transgenic crop.

Regulations and guidelines exist in the USA (Rose 2006), the European Union (EC 2002; EFSA 2004) and internationally (SCBD 2000). These provide general guidance on conducting an ERA of transgenic plants. However, there is still a need for detailed descriptions of non-target risk assessment procedures, for development of rigorous criteria for the selection of non-target species that need to be tested, and for establishment of test methods that apply to different regions.

The "West Palaearctic Regional Section" (WPRS) of the "International Organisation for Biological and Integrated Control of Noxious Animals and Plants" (IOBC) (http://www.iobc-wprs.org/) has a long history of assessing side-effects of plant protection products. A special initiative was launched in 2005, under the umbrella of the IOBC/WPRS working group "GMOs in Integrated Plant Production", with the aim of establishing generic ERA guidelines for transgenic insecticidal crops with particular emphasis on terrestrial non-target arthropods (NTAs) (Romeis 2006). This initiative involves scientists from diverse institutions including public research institutes, the agricultural biotech industry, representatives from regulatory agencies, and a commercial testing laboratory. The group has experience in the application of tiered risk assessment from a research and regulatory perspective. The final aim of this initiative is to propose a scientifically-sound, generic, and pragmatic NTA risk assessment method that can be adopted by different countries after adaptation to their specific regulatory needs and local circumstances.

A framework for assessing risk

A conceptual framework is critical in risk assessment and risk management. It can provide common understanding for regulators, registrants and scientists. It can also provide a predictable pathway for requesting, acquiring, organizing and evaluating data. Such a framework consists of four steps: (1) evaluation of need, (2) problem formulation, (3) information gathering, and (4) overall assessment. The initial evaluation of need determines whether a risk assessment is required for a specific case. Clearly defining the need as it meets the expectations of the final audience will help to design the overall risk assessment and determine how the information will be used and communicated. Common reasons for conducting an ERA include regulatory requirements, scientific inquiry, and scientific responses to public concerns. The main focus here is the ERA that is triggered by regulatory requirements. Once the need for the ERA has been clearly defined, the risk assessment moves forward to the problem formulation phase.

Problem formulation

The ERA is initiated with problem formulation (USEPA 1998; EFSA 2004). Problem formulation is used to define the scope of the risk assessment through generation of relevant risk hypotheses. For the ERA to go forward, a body of precursor information must determine that, other than for the expression of the trait of interest, the transgenic plant is equivalent to non-transformed comparators (see for example EuropaBio 2003). Once equivalence has been established on the basis of the transgenic plant characterization, the ERA can proceed with emphasis on stressor-mediated effects, where the potential stressor is the expressed trait, e.g., a Bt protein. The problem formulation considers the specifics of the stressor mode of action, the spectrum of activity and susceptibility, mode of expression, and relevant exposure profiles.

Additionally, it must also take into account ecological considerations that might affect the nature and extent of possible environmental impacts. One of the most significant factors in this regard is the intended scale of cultivation since ecological consequences of NTA impacts are likely to be positively correlated with scale. On this basis, the problem formulation then identifies assessment endpoints reflecting management goals and the scale and nature of the receiving ecosystem that is being considered. It should culminate in a conceptual model and analysis plan that is consistent with the risk hypotheses and establishes the relationship of the stressor to ecological impacts of concern. It also identifies possible surrogate test species and outlines an exposure analysis that accounts for the intended use and nature of the deployment of the transgenic plant.

Regardless of where in the world the ERA is conducted, the problem formulation approach should be very similar, using similar information that is modified by local cropping system information. The ERA process underlies the locally relevant tiered testing scheme, which should also reflect the basic design principles outlined below. The overall process may reflect additional national and regional regulatory needs and it must be achievable within the specific capacities and capabilities of the agency conducting the ERA.

The framework and progressing through it

A tiered risk assessment is recognized as being the most appropriate and rigorous approach to assess non-target affects from both scientific and regulatory standpoints. Both hazard and exposure can be evaluated within different levels or “tiers” that progress from worst-case hazard and exposure to more realistic scenarios. Lower tier tests serve to identify potential hazards, and they are conducted in the laboratory to provide high levels of replication and study control which increase the statistical power to test hypotheses. Where potential hazards are detected in these early tier tests, additional information is required. In these cases, higher tier tests can serve to confirm whether an effect might still be detected at more realistic rates and routes of exposure. Higher tier studies including semi-field or field-based tests offer greater environmental realism, but they may have lower statistical power. These tests are only triggered when early tier studies in the laboratory indicate potential hazards at environmentally relevant levels of exposure. In exceptional cases, higher tier studies may be conducted at the initial stage when early tier tests are not possible, for example plant tissue might be used because purified toxin is not available. Higher levels of replication or repetition may be needed to enhance statistical power in these circumstances.

In cases where a potential hazard is detected in a lower tier test, the tiered approach provides the flexibility to undertake further lower tier tests in the laboratory to increase the taxonomic breadth or local relevance of test species, thus avoiding the costs and uncertainties of high tier testing. Depending on the nature of the effect, one may also progress to higher tier testing, particularly in cases where there is no previous experience with the crop or toxin under investigation. The various tiered approaches that have been described for non-target risk assessment (e.g. Dutton et al. 2003; EuropaBio, 2004; Rose 2006) differ in their specific definitions of individual tiers, but they all follow the same underlying principles.

Movement between tiers during information gathering is based on the sufficiency of information that is available. If sufficient data and experience from toxicological testing and exposure analyses are available to characterize the potential risk as being acceptable, then there is no need to untertake additional testing. The process is designed to optimize the use of resources and to identify and define sources of potential risk. Where no reasonable hazard is detected, effective tiered processes prevent costly and unnecessary testing from taking place.

Species selection

For practical reasons, only a small fraction of all possible terrestrial arthropods can be considered for regulatory testing. It is therefore necessary to select appropriate species to serve as surrogates for ecologically and economically important NTAs that can be tested under worst-case conditions in the laboratory (Barrett et al. 1994). Species should be chosen to represent different ecological functions such as herbivory, pollination of cultivated and wild plants, predation and parasitism of pest organisms and decomposition in the soil. In order to reflect biogeographical variation, it is crucial to determine what relevant species are likely to occur in the cropping systems where the transgenic plant is expected to be grown. Another important source of information that serves as a basis for selecting relevant species is the information on the stressor (specificity, mode of expression and exposure profile) that is accumulated during problem formulation. The information collected in these previous steps will direct the selection of representative NTAs from a proposed set of species that capture key ecological functions. Criteria such as amenability to testing, availability of test methods that respect the standards of Good Laboratory Practices (GLP) and unambiguous taxonomic recognition are crucial for non-target testing. Based on these criteria, a list of NTA species that represent those living in the crop and in adjacent non-crop habitats is proposed. As a result of this process, test protocols for species that are of high relevance in particular regions may need to be developed.

The application of the surrogate species concept enhances the transferability of data from lower tier tests to a wide range of regions and to both annual and perennial crops. If higher tier studies are required, tests should be done using appropriate surrogates for the species potentially at risk. Appropriate surrogates may be the species used in the lower tier studies (Candolfi et al. 2000a); however it is not essential to use those species if the risk can be refined more effectively using others.

Study design

Once the surrogate test species are selected, they are evaluated in properly designed tests that fulfill established quality control standards, e.g., GLP. Experience has shown that early tier tests conducted under worst-case conditions in the laboratory (generally referred to as Tier 1 tests) can be well standardized. This is important to assure study repeatability, interpretability and quality, and thus to ensure a high level of confidence in the reported data. This process also facilitates the transportability of the test results among laboratories, countries and across crops, where this is appropriate. Protocols developed to assess the impact of pesticides (e.g., Candolfi et al. 2000b; OPPTS Series 885.4340, see http://www.epa.gov/opptsfrs/home/guidelin.htm) have historically formed the basis for the standard protocols used for the assessment of the potential effects of transgenic insecticidal crops on NTAs. Many of these protocols have been modified to consider the oral exposure pathway of plant-expressed insecticidal proteins and a number of new protocols have been developed.

Before entering into testing, the objectives of the individual studies need to be defined, and specific measurement endpoints described. Appropriate endpoints for risk assessment studies include life-table parameters such as mortality or fecundity, because they can easily be evaluated and the data can be related to measurable effects in the field. Other endpoints (e.g., weight, development and behavior) are possible, however, risk assessors should agree beforehand how to interpret these data. Because possible effects of insecticidal compounds expressed by transgenic crops may be delayed, multiple life-stage testing is recommended when possible. The life stages

that are selected should be chosen based on exposure, sensitivity and the amenability of the test system available for the selected arthropod.

Early tier tests usually entail a simple, well-defined test system designed to measure a specific endpoint (or set of endpoints) at concentrations that are several times higher than those that will be seen in the field. Elevated doses are applied since these tests use a small number of surrogate arthropods and because higher dose limit tests can add additional certainty to the safety assessment. All tests should adopt quality control parameters that help validate the test system which may include: (i) low negative control mortality, (ii) use of a positive control, (iii) homogeneity of test material, (iv) stability of the insecticidal compound, and (v) sufficient statistical power. It is recognized that there is a trade-off between the duration of the test, the number of life-stages that can be monitored, control mortality and thus the power of the test system. Flexibility to expand the range or number of lower tier tests may compensate for some of these constraints

Higher tier tests usually involve semi-field or field tests and sometimes are conducted when life-cycle (especially reproduction parameters) or tri-trophic evaluations are warranted. In general, these tests are problematic because of their complexity and high intrinsic uncertainty. Higher tier tests place high demands on skills in design, execution and data analysis and as a consequence they are subject to problems of low statistical power. These tests should therefore only be conducted when they can further reduce uncertainty in the risk assessment, and only when justified by detection of unacceptable risk at the lower tiers of testing.

Overall risk assessment

An ERA is a necessary step in the deregulation or regulatory approval of transgenic crops. It is comprised of the risk hypothesis, conceptual model, the characterization of hazard and exposure, and the results obtained from testing. The study quality, dosing levels, and the certainty levels associated with hazard tests should also be described. The test results should be placed in context and the following questions should be considered. Were any effects detected that were direct or indirect in nature? Were they restricted to one species or were they broad in taxonomic spectrum? Critical uncertainties should be identified and the temporal and spatial variability understood and explained at appropriate levels of detail. Once this information has been summarized, the predicted hazard is compared with the predicted exposure. Simple and powerful risk characterizations are based on the ratio between hazard and exposure values. Higher tiered, but more realistic, risk assessments involve the use of population and community responses which may include sources of geographic and temporal variability in exposure.

Two key factors should be kept in mind when completing a risk assessment. First, the risk assessment should be science-driven. Social and political concerns are important, but they are taken into account in risk management or in decision making that lies outside the risk assessment framework. Second, the risk assessment does not constitute a decision in itself, but represents a source of information for decision makers to use.

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