



Harmonizing the Non-target Risk Assessment for GM Crops

Jörg Romeis

Since the commercialization of Bt maize in 1996, the global area planted to maize or cotton varieties expressing Cry proteins derived from *Bacillus thuringiensis* (Bt) has steadily increased and reached a total of more than 42 million hectares in 2007¹. Several crops expressing novel insecticidal proteins derived from Bt or other sources are currently under development and will be commercialized in the near future.

Similar to conventional agricultural pest control technologies, one risk associated with growing insect-resistant, genetically modified (IRGM) crops is their potential to adversely affect non-target organisms, which include a range of arthropod species that fulfill important ecological functions such as biological control. The potential for adverse effects of IRGM crops on non-target arthropods (NTAs) thus has to be evaluated as part of the environmental risk assessment (ERA) process that takes place prior to the decision to cultivate these crops commercially. The relative novelty of GM crops and the complexity of ERA procedures present regulatory authorities with a challenge in developing appropriate risk assessment methodologies. This is a particularly difficult task in the developing world, where regulatory infrastructure is still being established.

Environmental risk assessment

Various countries and international organizations (e.g., Appendix III of the Cartagena Protocol) provide general guidance for conducting an ERA for GM plants. There remains, however, a need for detailed descriptions for NTA risk assessment procedures, including selection criteria for appropriate NTA test species and test methods. To address this need, an initiative was launched within the GMO working group of the 'West Palaearctic Regional Section' (WPRS) of the 'International Organization for Biological and Integrated Control of Noxious Animals and Plants' (IOBC)². IOBC/WPRS is an independent scientific organization that encourages collaboration in promoting feasible and environmentally safe methods of pest and pathogen control within an integrated pest management context. Among others, it elaborates guidelines for integrated production of agricultural crops and develops and standardizes methods of testing the effects of pesticides on beneficial species (<http://www.iobc-wprs.org>).

An expert group was established that consists of European and North American scientists from public, industry, and regulatory sectors who have extensive experience with GM crops. The group identified the most valuable elements from within a variety of guidance documents, formulated the underlying rationale of existing ERA approaches, and distilled lessons accumulated from the institutional experience of the working group members. The outcome of this initiative was published in February 2008 in *Nature Biotechnology*³. The approach intended to provide regulators with a scientific rationale for the risk assessment decisions that they make and should help to harmonize the NTA risk assessment of IRGM crops worldwide.

The approach consists of an adaptation of the tiered approach to risk assessment that is accepted internationally within regulatory toxicology and environmental sciences, and versions of it are already in use in established and effective regulatory systems for GM crops.⁴ The approach has a strong focus on the formulation and testing of clearly stated risk hypotheses, making maximum use of available data, and using formal decision guidelines to progress between testing stages (or tiers).

Problem formulation

During the problem formulation stage, meaningful differences between IRGM plants and their non-GM counterparts are identified in order to focus the ERA on areas of greatest concern or uncertainty⁵. This includes establishing the similarities in ecologically relevant characteristics between the IRGM crop and the non-transformed crop. Additionally, it must also take into account ecological considerations that might affect the nature and extent of possible environmental impacts. In all cases, descriptions of plant characteristics (e.g., macro- and micro-nutrient composition, content of important toxicants and anti-nutrients, and morphological and agronomic plant characteristics) are made with reference to familiar comparators, i.e., plants that are generally regarded as environmentally 'acceptable', to identify meaningful differences that may need to be addressed in the risk assessment.

This assessment (generally referred to as the concepts of 'familiarity' and 'substantial equivalence') serves as a starting point to focus the ERA process on potential stressors of concern^{6,7}. If a lack of significant differences between the IRGM plant and its comparators is established, the ERA can emphasize the effects of the insecticidal protein. The problem formulation furthermore considers known specifics of the mode of action of the expressed insecticidal protein, the spectrum of activity and susceptibility, mode of expression, and relevant spatial and temporal exposure profiles. This knowledge allows a narrowing of the risk hypotheses that need addressed in the analytical phase of the risk assessment. For example, knowledge on the spectrum of activity of the Cry proteins expressed in today's Bt-transgenic crops is substantial^{8,9} and should be considered in future risk



assessments.

During the problem formulation stage, assessment endpoints are identified that reflect management goals set by regulatory policies. A typical management goal is “protection of biodiversity.” This goal is, however, difficult (if not impossible) to be addressed scientifically. A typical assessment endpoint that is scientifically analyzable is the abundance or species richness of certain groups of NTAs, such as those important for biological control. Finally, the problem formulation will culminate in a conceptual model and analysis plan that is consistent with the risk hypotheses and that establishes the relationship between the stressor of concern (i.e., the insecticidal protein) and changes in the assessment endpoints.

Regardless of where in the world the ERA is conducted, the problem formulation approach should be very similar, using similar data modified by local cropping system information.

The framework

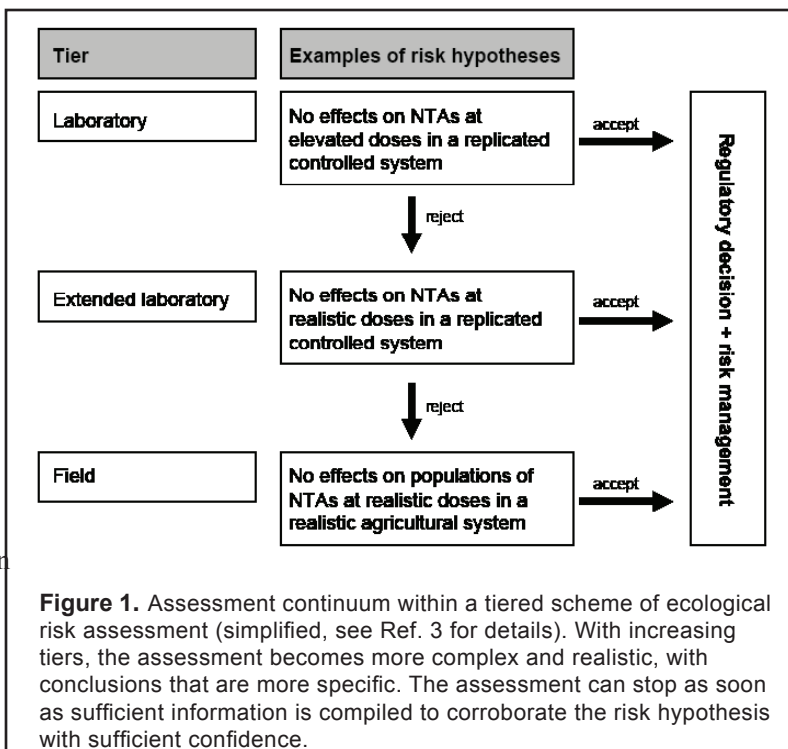
Scientific assessment of risk from GM crops is conceptually similar to the assessment of traditional pesticides in which the tiered process of toxicity testing is generally used because it is suitable for assisting the decision making process in an effective and rigorous way¹⁰.

A typical risk hypothesis resulting from the problem formulation phase may be that the insecticidal protein does not cause any harm to NTAs at the concentration expressed in the field. Both hazard and exposure can be evaluated within different levels or “tiers” that progress from worst-case hazard and exposure to more realistic scenarios (Fig. 1)^{4,11}. Lower tier tests serve to identify potential hazards, and they are generally conducted in the laboratory to provide high levels of replication and study control, which increase the statistical power to test hypotheses. When potential hazards are detected in these early tier tests, additional information is required. In these cases, higher tier tests can 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 often have lower statistical power. These tests are thus 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. When a potential hazard is detected in a lower tier test, the tiered approach also provides the flexibility to undertake further lower tier tests in the laboratory to increase the taxonomic breadth or local relevance of test species.

Movement between tiers is based on the sufficiency of information (Fig. 1). If sufficient data and experience from toxicological testing and exposure analyses are available to characterize the potential risk as acceptable, then there is no need to undertake additional testing. The process is thus designed to optimize resources and to identify and define potential risk with high scientific rigour.

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. Species should be chosen to represent different ecological functions, such as predation and parasitism of pest organisms, pollination of cultivated and wild plants, and decomposition. In order to reflect biogeographical variation, it is crucial to determine what taxa are likely to occur in the cropping systems where the transgenic plant will be grown. Another important basis for selecting relevant surrogate species is information on insecticidal protein (specificity, mode of expression and exposure profile) that accumulates 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, are amenable to testing, and for which standardized testing protocols exist.

If, in the case of an IRGM maize plant for example, the insecticidal protein is not expressed in pollen, a honeybee study may not be required. Since feeding on pollen is the only way honeybees can ingest the insecticidal protein, exposure does not occur. In the case of an IRGM maize plant that expresses a Cry3 protein to control beetle pests such as corn rootworms (*Diabrotica* spp.), risk assessment would focus on other beetle species, since these are most likely to be affected.

Generally, species should be selected for testing that provide the most rigorous test of the risk hypotheses for a particular IRGM plant in a specific agricultural and environmental setting. Application of the surrogate species concept enhances transferability of data from lower tier tests to a wide range of regions and crops.

Study design

Hazard assessment tests (generally referred to as tier-1 tests) are usually conducted using elevated protein doses in the laboratory, using standardized testing protocols. Prior to testing, the objectives of individual studies are defined, and specific measurement endpoints are described that address the risk hypotheses. Testing protein concentrations several times higher than those seen in the field increases the likelihood that a potential hazard will be detected, adding further certainty to the risk assessment. All tests should adopt quality control criteria that help validate the test system. For example, for lower tier tests these may include: (i) a requirement for low negative control mortality; (ii) use of a positive toxic control to confirm that the test system is working effectively; (iii) homogeneity of test material to ensure uniformity of exposure; (iv) stability of the insecticidal compound throughout the bioassay period; and (v) sufficient statistical power for testing the risk hypotheses.

The integrity and repeatability of these studies, together with a high power to detect potential hazards, assures a high level of confidence in the conclusions drawn from the data, transportability and acceptability among regulatory authorities, and applicability for further ERAs.

Higher tier tests that are, for example, conducted in the field are more realistic but highly complex. They have a high intrinsic uncertainty for showing hazards but more certainty for showing whether hazards pose a risk. Higher tier studies should thus only be conducted when they can further reduce uncertainty in the risk assessment, and only when justified by detection of potentially adverse effects in the lower tiers of testing.

Strength of our approach

The approach described above provides a scientific rationale for the ERA of IRGM crops to assist regulatory decision-making. The framework provides a well defined and predictable pathway for requesting, acquiring, organizing, and evaluating data and is designed to support effective regulatory decision-making. The specific benefits of our approach are as follows.

- Tiered evaluation of potential hazards using representative surrogate species and conservative exposure estimates provides a rigorous and effective basis for estimating risk.
- This approach minimizes the likelihood of false negatives, which could result in the release of IRGM plants with undesirable effects on NTAs.
- The tiered approach ensures testing of clearly stated relevant hypotheses.
- The process focuses resources to address potential significant risks or uncertainties and eliminates from further consideration risks that are negligible.
- It thereby minimizes the collection of data irrelevant to risk assessment.
- Decisions about acceptable risk can be made in a reasonable period of time.

Our document is intended to provide a framework for regulatory agencies that are currently developing their own NTA risk assessment guidelines for GM crops. It represents the consensus of a diverse group of stakeholders and therefore provides a basis for improving harmonization of international risk assessment guidelines. Harmonized procedures in ERA facilitate risk assessment data acceptability and provide a greater scope for comparing data on ecological effects internationally.

Acknowledgements

I would like to thank the following colleagues for their continuous hard work and stimulating discussions within this IOBC/WPRS activity that has resulted in the described risk assessment approach: Detlef Bartsch, Franz Bigler, Marco Candolfi, Marco Gielkens, Sue Hartley, Rick Hellmich, Joe Huesing, Paul Jepson, Ray Layton, Hector Quemada, Alan Raybould, Robyn Rose, Joachim Schiemann, Mark Sears, Tony Shelton, Jeremy Sweet, Zig Vaituzis and Jeff Wolt.



References

1. James C. (2007) Global status of commercialized biotech/GM crops: 2007. *ISAAA Brief* No. 37, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, USA
2. Romeis J. (2006) Non-target risk assessment of GM crops and regulation. *IOBC/WPRS Bulletin* **29**(5), 197-200
3. Romeis J, et al. (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nature Biotechnology* **26**, 203-208
4. Rose RI (Ed.) (2007) *White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target invertebrates for regulatory risk assessment*. USDA-APHIS and US Environmental Protection Agency, Washington, DC, USA <http://www.epa.gov/pesticides/biopesticides/pips/non-target-arthropods.pdf>
5. Raybould A. (2006) Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environmental Biosafety Research* **5**, 119-125
6. Organisation for Economic Cooperation and Development (OECD) (1993) Safety considerations for biotechnology: scale-up of crop plants. Organisation for Economic Cooperation and Development, Paris. <http://www.oecd.org/dataoecd/26/26/1958527.pdf?channelId=34537&homeChannelId=33703&fileTitle=Safety+Considerations+for+Biotechnology+Scale-up+of+Crop+Plants>
7. European Food Safety Authority (EFSA) (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. *EFSA Journal* **99**, 1-100. http://www.efsa.europa.eu/EFSA/Scientific_Document/gmo_guidance_gm_plants_en,0.pdf
8. United States Environmental Protection Agency (USEPA) (2001) *Biopesticide registration action document. Bacillus thuringiensis (Bt) plant-incorporated protectants*. 15 October 2001. http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm
9. Romeis J, Meissle M, Bigler F. (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology* **24**, 63-71
10. Hill RA, Sendashonga C. (2003) General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. *Environmental Biosafety Research* **2**, 81-88
11. Garcia-Alonso M, et al. (2006) A tiered system for assessing the risk of genetically modified plants to non-target organisms. *Environmental Biosafety Research* **5**, 57-65

Jörg Romeis
Agroscope Reckenholz-Tänikon Research Station ART
Reckenholzstr 191, 8046 Zurich, Switzerland
joerg.romeis@art.admin.ch