

Assessment of risk of insect-resistant transgenic crops to nontarget arthropods

Jörg Romeis¹, Detlef Bartsch², Franz Bigler¹, Marco P Candolfi^{3,19}, Marco M C Gielkens⁴, Susan E Hartley⁵, Richard L Hellmich⁶, Joseph E Huesing⁷, Paul C Jepson⁸, Raymond Layton⁹, Hector Quemada¹⁰, Alan Raybould¹¹, Robyn I Rose¹², Joachim Schiemann¹³, Mark K Sears¹⁴, Anthony M Shelton¹⁵, Jeremy Sweet¹⁶, Zigfridas Vaituzis¹⁷ & Jeffrey D Wolt¹⁸

An international initiative is developing a scientifically rigorous approach to evaluate the potential risks to nontarget arthropods (NTAs) posed by insect-resistant, genetically modified (IRGM) crops. It adapts the tiered approach to risk assessment that is used internationally within regulatory toxicology and environmental sciences. The approach focuses 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). It is intended to provide guidance to regulatory agencies that are currently developing their own NTA risk assessment guidelines for IRGM crops and to help harmonize regulatory requirements between different countries and different regions of the world.

IRGM crops that express Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) have been grown in several countries on a steadily increasing acreage since their introduction in 1996. In 2006, transgenic varieties of cotton and maize that express *Bt* proteins were grown on 32.1 million hectares worldwide¹. Several crops expressing novel insecticidal proteins are also under development and these are expected to be commercialized in the near future. (Although insecticidal traits associated with commercialized genetically modified (GM) crops have all been proteins, we recognize that future traits might not necessarily be restricted to this class of molecule.) In common with conventional agricultural pest control products (which include synthetic and organic insecticides, biological control agents and host-plant resistance developed by conventional breeding), one of the risks associated with the growing of IRGM crops is their potential to adversely affect nontarget organisms. These include a range of arthropod species that fulfill important ecological functions such as biological control, pollination and decomposition. The potential for adverse effects of IRGM crops on these NTAs has been evaluated as part of the environmental risk assessment (ERA) process that takes place before the decision to cultivate these crops commercially^{2,3}. The relative novelty of IRGM crops and the complexity and sophistication of ERA procedures present regulatory authorities with a challenge when they are required to develop appropriate risk assessment methodologies. This is a particularly difficult task in the developing world, where the regulatory infrastructure is still being established.

General guidance for conducting an ERA for genetically modified (GM) plants exists⁴⁻⁹. There remains, however, a need for detailed descriptions for NTA risk assessment procedures, including selection criteria for the NTA test species and test methods that can apply to different regions if these general guidelines are to be adapted for specific crops in specific agriculture ecosystems. To address this need and to formulate the underlying rationale of the existing ERA approaches, an initiative was launched within the GM organisms working group of the West Palaearctic Regional Section (WPRS) of the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (<http://www.iobc-wprs.org/>)¹⁰. The group consists of European and North American scientists from public research institutes, regulatory agencies, the agricultural biotechnology industry and a commercial testing laboratory. The current focus of this group is the development of an IRGM-specific rationale for a tiered toxicological testing system which, when integrated with exposure, will enable poten-

¹Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstr. 191, 8046 Zurich, Switzerland. ²Federal Office of Consumer Protection and Food Safety (BVL), Mauerstrasse 39-42, 10117 Berlin, Germany. ³RCC Laboratories India Private Ltd., Genome Valley, Turkapally, Shameerpet, Ranga Reddy District, Hyderabad 500078, India. ⁴National Institute for Public Health and the Environment, Expertise Centre for Substances, PO Box 1, 3720 BA Bilthoven, The Netherlands. ⁵Department of Biology and Environmental Science, University of Sussex, Falmer, Brighton BN1 9QG, UK. ⁶USDA-ARS, Corn Insects and Crop Genetics Research Unit and Department of Entomology, Iowa State University, 110 Genetics Laboratory c/o Insectary, Ames, Iowa 50011, USA. ⁷Regulatory Sciences, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Missouri 63167, USA. ⁸Integrated Plant Protection Center and Department of Environmental and Molecular Toxicology, Oregon State University, Cordley Hall, 2040, Corvallis, Oregon 97331-2907, USA. ⁹Regulatory Sciences, DuPont Crop Genetics, 7250 NW 62nd Ave., PO Box 552, Johnston, Iowa 50131-0052, USA. ¹⁰Program for Biosafety Systems, Department of Biology, Calvin College, 1726 Knollcrest Circle, S.E., Grand Rapids, Michigan 49546-4403, USA. ¹¹Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK. ¹²USDA-APHIS Biotechnology Regulatory Services, 4700 River Rd., Unit 146, Riverdale, Maryland 20737, USA. ¹³Julius Kuehn Institute, Federal Research Centre for Cultivated Plants (JKI), Institute for Biosafety of Genetically Modified Plants, Messeweg 11/12, 38104 Braunschweig, Germany. ¹⁴Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada. ¹⁵Department of Entomology, Cornell University/NYSAES, 630 W. North Street, Geneva, New York 14456, USA. ¹⁶Environmental Consultant, The Green, Willingham, Cambridge CB4 5JA, UK. ¹⁷Biopesticides and Pollution Prevention Division, USEPA 7511P, 1200 Pennsylvania Ave. NW, Washington, DC 20460, USA. ¹⁸Biosafety Institute for Genetically Modified Agricultural Products and Department of Agronomy, Iowa State University, 164 Seed Science Center, Ames, Iowa 50011, USA. ¹⁹Present address: BASF SE, APD/EE, LI 425, 67117 Limburgerhof, Germany. Correspondence should be addressed to J.R. (joerg.romeis@art.admin.ch).

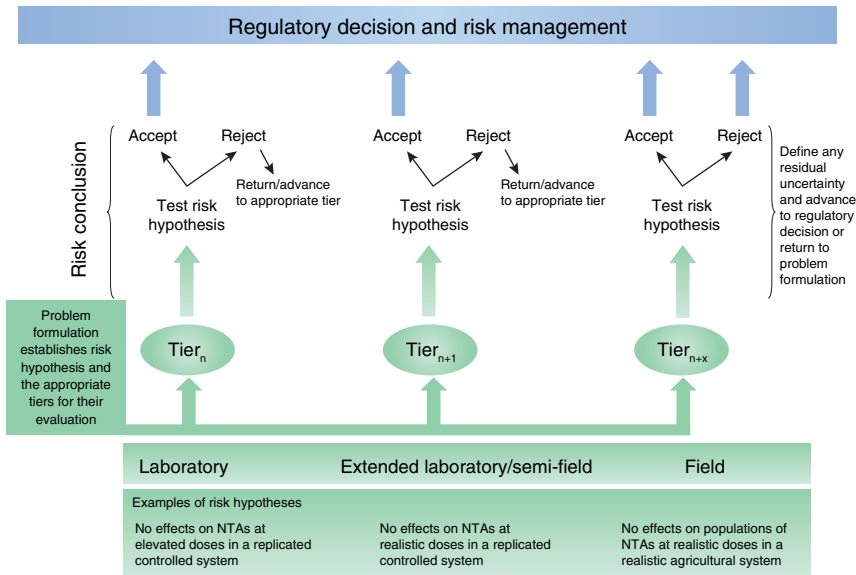


Figure 1 Assessment continuum within a tiered scheme of ecological risk assessment. The decision to reject the risk hypothesis includes consideration of residual uncertainties. With increasing tiers, the assessment becomes more complex and realistic, with conclusions that are more specific. The assessment can stop at any stage during the process as soon as sufficient information has been compiled to address the risk hypothesis. Thus collection of data irrelevant to the risk assessment is minimized. N, level of risk assessment tier; NTA, nontarget arthropod.

tial risks to NTAs to be defined and assessed. This is intended to provide regulators with a scientific rationale for the risk assessment decisions that they make. Ultimately, the risk management decision that regulators make also takes into account relevant social and political considerations^{11,12}.

In the following article, we outline the basic principles and rationale of the tiered approach. We also describe the ways in which this approach has been refined by scientists from public, industrial and regulatory sectors, who have extensive experience with IRGM crops.

Problem formulation

In our approach, the problem formulation stage is designed to identify the areas of greatest concern or uncertainty concerning ecological risks, and to define the scope of the risk assessment by generating testable scientific hypotheses that are subsequently addressed in the analytical phase of the risk assessment^{8,13,14}. The information that is considered during problem formulation takes many forms, including published scientific literature, expert opinion, stakeholder deliberations and data developed by the registrants and submitted to the regulatory authority as part of the registration dossier. This information establishes the level of ‘familiarity’ (that is, the similarities in ecologically relevant characteristics) between the IRGM crop and nontransformed crop^{15–17} and, together with the related concept for food of ‘substantial equivalence’, serves as a starting point to focus the ERA process on potential stressors of concern^{18,19}. If substantial equivalence and familiarity are established, the ERA can proceed with emphasis on narrowly defined, stressor-mediated effects that arise from the expressed trait in the IRGM crop (e.g., a *Bt* protein)^{14,20}. In cases where substantial differences other than those directly related to the expressed trait are detected, these characteristics become additional potential stressors that also need to be evaluated, following the same tiered approach that we outline. Thus, the greater the extent of familiarity between the IRGM crop and the nontransformed crop, the more specific and focused the risk hypotheses will be. The feedback inherent in the process described increases the efficiency of ERA for familiar crops and focuses resources on less familiar commodities as they arise.

The data commonly requested by authorities to satisfy regulatory assessments are particularly important for the establishment of familiarity and typically include a description of the host crop, the source and molecular characterization of the introduced genetic elements, the nature and stability of protein expression, the spectrum of protein activity, macro- and micronutrient composition, the content of important toxicants and antinutrients, and morphological and agronomic plant characteristics. Regulatory agencies may also request the results of field trials conducted at several locations that are representative of where the crop will be grown. In all cases, descriptions of plant characteristics, which are made with reference to plants that are generally regarded as environmentally ‘acceptable’, are used to identify meaningful differences that may need to be addressed in the risk assessment.^{2,8,15,16,18} A ‘meaningful difference’, in this context, refers to a substance or another attribute previously associated with effects that may be of environmental concern (e.g., an unintended increase in alkaloid levels in GM cotton plants modified to express a Cry protein could affect NTAs). It is evident that the degree of familiarity with a given crop and IRGM approach and its

conventional comparators will increase over time and with experience.

The problem formulation may result in recommendations that a narrowly defined set of experimental evaluations should be undertaken for the risk assessment of some IRGM crops with well-known characteristics. This could result in criticism that the risk assessment is superficial and not likely to detect potential risks. However, the process explicitly considers the specifics of the stressor’s mode of action, spectrum of activity and levels of exposure of NTAs to the stressor. Data to support the problem formulation may derive, for example, from the tests carried out during IRGM plant development. The pest activity spectrum is commonly explored during development by testing the activity of the insecticidal protein against a range of pest species belonging to different orders. Additional data may also exist in the literature concerning the spectrum of activity of some proteins (e.g., certain Cry proteins from *Bt*) that have been studied by public sector scientists^{21,22}. The information base that is inherent to the concept of familiarity is therefore considerable; thus, we argue that risk assessment for specific applications associated with familiar crops/traits can be both thorough and accurate without requiring extensive additional testing.

Problem formulation identifies scientifically analyzable endpoints that reflect management or protection goals that are set by public policy. For example, if ‘protection of biodiversity’ is the management goal, a typical assessment endpoint would be the abundance and species richness of certain groups of NTAs, such as those that fulfill important ecological functions. Different regulatory agencies may define different assessment endpoints or even have different management objectives. These must all be considered explicitly in the problem formulation stage so that the risk hypotheses can generate data that address the goals of the regulator and the requirements of the policy. The problem formulation should culminate in a conceptual model and analysis plan that is consistent with the risk hypotheses and that establishes the relationship between the stressor and the ecological impacts of concern (the assessment endpoints). The conceptual model should take into account ecological considerations that might affect the nature and extent of possible environmental impacts, including the intended scale of cultivation of the IRGM crop.

The ERA framework and moving through it

Scientific assessment of risks from IRGM plants is conceptually similar to the assessment of risks from traditional synthetic insecticides even though insecticidal proteins expressed by GM plants may differ from synthetic insecticides (e.g., by mode of action, specificity, exposure route)²³. The tiered process of toxicity testing is generally used to assess the nontarget effects posed by traditional insecticides²⁴ because it is suitable for assisting the decision-making process in an effective and rigorous way. We argue that it is also the most rigorous approach, from both scientific and regulatory standpoints, for determining the potential of IRGM plants to adversely affect NTAs. Versions of this approach are also in current use in established regulatory systems for GM crops^{4,8}.

A typical risk hypothesis that emerges from problem formulation may be that the stressor (that is, the insecticidal protein) does not harm NTAs at the concentration expressed in the field. The testing of this hypothesis frequently leads to toxicity tests on select arthropod species. These tests are conducted within experimental ‘tiers’ that are initiated with elevated dose exposure tests (e.g., at ten times the expected environmental exposure), often using laboratory procedures with purified protein in artificial diets and proceeding to more realistic scenarios of exposure with IRGM plants if impacts exceed certain specified threshold values (Fig. 1).

Examples of the risk hypotheses that are addressed at the different tiers are provided in Figure 1. The conceptual pathway leads from relatively simple and controllable lower tier assessments to increasingly complex higher tier assessments. The conclusion regarding risk drawn at each tier will lead either to a regulatory decision after the residual uncertainty of the assessment has been defined or to additional investigations. These need to be conducted at the appropriate tier, which could

be any of the lower, current or higher tiers of evaluation. Throughout the assessment, the risk assessor needs to confirm continually that the problem being addressed is still appropriate and, if necessary, revisit the problem formulation.

Lower tier tests serve to identify potential hazards, if they exist, and are typically conducted in controlled laboratory conditions (Supplementary Note online). Lower-tier tests are designed to measure a specific endpoint (or set of endpoints) under controlled conditions using protein concentrations that are usually several times higher than those present in the field. Such studies provide a powerful means to detect hazards because the biological impacts of the insecticidal protein can be isolated²⁵. The tests are not meant to reflect real-world exposures but to increase the likelihood that a hazard will be detected should one be present, and so provide confidence of minimal risk should no adverse effect be detected. The sequence of testing continues after the initial elevated-dose or dose-response tests if potential hazards were detected (that is, the ‘no-effect’ hypothesis had to be rejected) or if unacceptable uncertainties about possible hazards remain (Fig. 1). For example, conducting further lower tier tests in the laboratory can refine the hazard assessment by increasing the taxonomic breadth or local relevance of test species. In cases where lower tier tests detect a potential hazard (that is, the ‘no-effect’ hypothesis is rejected), higher tier tests, which include more complex semi-field (that is, under containment using live GM plant material) or open field tests, can then serve to confirm whether an effect can still be detected under more realistic rates and routes of exposure to the protein (Fig. 1). In cases where uncertainty about the risk remains after higher tier studies, one can always return to lower tiers to conduct additional studies, for example, by including additional test species (Fig. 1). In exceptional cases, higher-tier studies or studies

Box 1 Evaluation path for *Bt* maize expressing Cry1Ab for nontarget arthropods

In the evaluation of *Bt* maize expressing Cry1Ab, entities of concern included biological control organisms belonging to, for example, the orders of Coleoptera (lady beetles), Neuroptera (lacewings) and Hymenoptera (parasitoid wasps), as well as pollinators such as bees (also Hymenoptera), decomposers such as soil arthropods (for example, springtails) and nontarget Lepidoptera. The problem formulation identified several risk hypotheses that were subsequently addressed in the analytical phase of the risk assessment (Fig. 2). Because analysis of the available precursor information revealed with sufficient certainty that the only meaningful difference between *Bt* maize and its nontransformed comparators was the expression of the

Cry1Ab protein, early tier (worst-case) studies were conducted using elevated doses of purified protein or plant tissue. These studies confirmed existing knowledge (precursor information) that these proteins are not likely to affect nonlepidopteran insects (risk hypotheses 1–3)^{22,42}. Testing could thus be terminated at this early tier. The potential hazard to nontarget Lepidoptera (risk hypothesis 4) was recognized initially but it was concluded that the risk is negligible⁴³. Additional studies under more realistic exposure conditions were triggered once a note⁴⁴ and a more comprehensive study⁴⁵ had revealed a hazard of Cry1Ab to larvae of the monarch butterfly. Studies were conducted under semi-field conditions. These studies concluded that the risk of Cry1Ab maize to monarch populations is negligible because larval exposure to Cry1Ab toxin under field conditions is low^{46–49} confirming the initial risk assessment⁴³.

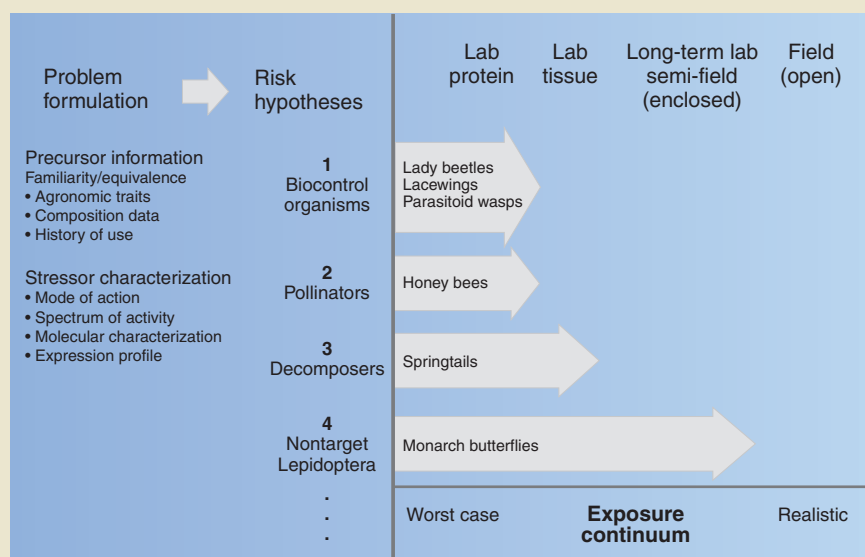


Figure 2 Reconstruction of NTA risk assessment for *Bt* maize expressing Cry1Ab showing that different risk hypotheses require different types of data and synthesis at different tiers.

using alternative designs may be conducted at the initial stage of the risk assessment process when lower tier tests are not possible. Lower tier tests are, however, extremely valuable to the ERA process because they are highly controlled, and may provide data that are broadly applicable within various risk assessments. It should be noted that field observations on *Bt* crops have failed to find any adverse NTA impacts that could not have been predicted from laboratory or small-scale field studies^{22,26}.

Movement between tiers takes place either because the available information is insufficient to accept the risk hypothesis of 'no effect' or because this hypothesis has been rejected. 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 undertake additional testing (Fig. 1). The iterative and flexible tiered testing scheme described herein is designed to provide the information to support a regulatory decision as efficiently and rigorously as possible. In cases where the risk hypothesis is rejected at the highest tier (Fig. 1, Tier_{n+x}), an adverse impact on NTAs can occur. As a consequence, the GM variety may either not be authorized or the regulatory agency may require monitoring or risk mitigation. Alternatively, a new problem formulation may be required in cases where a potentially adverse outcome is found.

A key principle of the tiered process is that particular studies are conducted only when they serve to reduce uncertainty in the risk assessment. Where no hazard or risk is detected, effective tiered processes prevent costly and unnecessary testing (see **Supplementary Note**; the lacewing case). Consequently, the assessment of different risk hypotheses will follow different evaluation paths (Box 1). The process is designed to optimize the expenditure of resources by identifying and defining sources of potential risk, thereby minimizing the collection of data that are irrelevant to the risk assessment.

Box 2 Nontarget species selection

The number and type of NTA species that need to be tested depend on the risk hypotheses generated during the problem formulation. The species selection will also depend on how much information already exists to test the hypotheses: the level and quality of information on the plant, the specific stressor (that is, the insecticidal protein) expressed (e.g., spectrum of activity, mode of action, expression level) together with information about the feeding habits of the NTAs. Increasing the level and quality of information reduces the number of risk hypotheses that will require testing.

Example 1: novel *Bt* maize event expressing Cry1Ab. There is a high probability, based upon an extensive research history, that the protein would be active only against Lepidoptera and that harm to NTAs in other taxonomic orders would be negligible. Additionally, there is a high level of familiarity with maize, the trait and the toxin from experience with other Cry1Ab-expressing maize events. (Event indicates a unique transformation of a plant by insertion of a particular transgene into its genome). Further testing for this novel event is not scientifically justified provided that problem formulation shows the expression of the Cry1Ab protein is the only relevant difference compared with closely related, nontransformed maize varieties and that expression levels are similar to those in previously evaluated events.

Example 2: novel *Bt* maize event expressing a Cry3 protein. Because the protein is targeting corn rootworms (*Diabrotica* spp.) and has a known specificity to Coleoptera, the risk assessment should focus on nontarget coleopteran species rather than species belonging to other taxonomic groups.

Example 3: novel *Bt* maize event without toxin expression in the pollen. Honeybees are exposed only to insecticidal proteins expressed by GM maize varieties when these are present in the pollen. If the proteins are not expressed in the pollen, there is no scientific justification for conducting feeding studies with bees or other pollen-feeding arthropods.

Species selection

For practical reasons, only a small fraction of potentially exposed terrestrial arthropods can be considered for regulatory testing. It is therefore necessary to select appropriate species to serve as surrogates that can be tested effectively under laboratory conditions^{27–29}. For regulatory testing of IRGM plants, surrogate species should be representatives of ecologically and economically important NTA taxa in the crop and represent different ecological functions including predation and parasitism of pest organisms, pollination and decomposition of plant material^{27,30}. Key species or guilds that are representative of different functional groups are known in most systems and appropriate surrogates can therefore be selected. Despite recognized limitations³¹, the application of the surrogate concept is widely applied in related fields including regulatory toxicity testing^{28,29,32,33} and environmental monitoring^{34–36}. In addition, the risk assessment may consider species with special aesthetic or cultural value or species classified as threatened or endangered. These species are regionally specific and can be evaluated within the ERA independent of their ecological function.

The most effective surrogate taxa, for example honeybees (*Apis mellifera*), are representatives of NTA taxa that are found in many different crops or regions. More specific, crop-associated species may be selected that represent an important genus (e.g., *Orius* spp.), and other taxa may be selected that are broadly representative of whole families (e.g., parasitic wasps of the Ichneumonidae) or orders (e.g., Coleoptera) that are known to be important. Even the nontarget pest species that are screened for their sensitivity to the insecticidal protein during product development can serve as surrogates for NTAs.

Information on the stressor (e.g., protein specificity, and the pattern and level of expression in the plant), together with information on the feeding habits of the test species, which is accumulated during problem formulation, must be considered during the selection of appropriate surrogates (Box 2). In general, nontarget species that are related taxonomically to the target pests are most likely to be affected by the protein; thus selection of these taxa increases the likelihood of detecting a hazard if one exists. Species that are not exposed to the insecticidal protein do not need to be tested to draw a negligible-risk conclusion. Some additional, practical considerations include the ease of working with a species, the potential for unambiguous taxonomic recognition, the ability to rear the species in captivity, the availability of permanent source colonies and validated and accepted test methods.

The purpose of using IRGM plants is the same as for any other pest management tactic; that is, to reduce pest populations below economic injury levels. As a result of applying the tactic, the abundance of pest insects should be significantly reduced and this will have corresponding implications for those organisms that exploit these pests as prey and hosts. Thus, the potential for these indirect ecological effects on biological control organisms should not be regarded as a unique ecological risk associated with the IRGM crop^{8,18,22}. Large reductions, however, should be expected if the pest management strategy is effective. Because IRGM crops are often grown in vicinity with non-GM

crops to prevent resistance build-up by the target pest(s)³⁷, specialist antagonists can persist in these 'refuges', in other crops and in non-crop habitats and retain the potential for recolonization of the IRGM crop area. On the basis of these considerations, regulatory testing of the specialist predators and parasitoids of target pests may not be necessary.

Study design

Once the surrogate species are selected, they should be evaluated in scientifically designed and validated studies to test the risk hypotheses. Historically, protocols developed to assess the impact of pesticides on NTAs^{38,39} formed the basis for the tests used to evaluate IRGM plants. These protocols were modified to account for the oral exposure pathway and the potential for extended exposure time to plant-expressed insecticidal proteins⁴⁰. New protocols are being developed to address the specific research or regulatory needs associated with IRGM risk assessment. The new or modified tests often span a significant portion of the test insect lifespan, which is appropriate for the period of exposure to the toxin in the field, and may include a number of measurement endpoints in addition to mortality (see **Supplementary Note** for examples using these new protocols).

Conclusions

The tiered NTA testing approach presented here provides the 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. It is 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 help to facilitate risk assessment data acceptability and provide greater scope for comparison of ecological effects data internationally.

The approach presented here ensures rigorous testing of clearly stated and relevant risk hypotheses that are linked to defined assessment endpoints while optimizing data requirements. The risk hypotheses are developed from current knowledge about the biology of the crop, the introduced trait, the receiving environment and the interactions of all three. It therefore makes maximum use of the existing data and aims to minimize collection of data that are irrelevant to the risk assessment. The process is intended to be efficient and rigorous, focusing the resources to address potential risks or uncertainties and eliminating from further consideration the risks that are negligible. Potential hazards are evaluated with representative surrogate/indicator species that are selected case by case for their suitability and amenability to test relevant risk hypotheses.

The general approach we outline here has evolved based upon current events and insecticidal proteins and is flexible and adaptable to new IRGM products. Several aspects of the approach may, however, have to be further developed to take account of new traits and potential risks.

First, threshold values need to be defined that trigger the advance to higher tiers as has been done for environmental risk assessments of conventional pesticides^{32,33}. If these trigger values are not exceeded, the testing stops and the regulatory decision follows. The specific triggers applied in a given case of an ERA for IRGM plants are informed by expert opinion and require deliberation among risk assessors and risk managers, who consider the problem being evaluated and the effects regarded as 'acceptable'. We recognize, however, that defining the trigger values is not solely a scientific question but also depends on whether policy-makers are concerned about under- or overestimating risks.

Second, a list of surrogate species needs to be compiled that can serve as a basis for selecting the most appropriate species for laboratory testing.

The species tested should provide the most rigorous tests of the risk hypotheses for a particular IRGM plant in a specific agricultural and environmental setting.

And third, more standardized, validated test protocols for surrogate test species may need to be developed. These are needed to ensure data comparability and facilitate international regulatory acceptance.

We believe that the tiered NTA testing approach presented above minimizes the likelihood of false negatives, which could result in the release of IRGM plants with undesirable effects on NTAs and, at the same time, should reverse the trend of increased delays for introducing products that may be environmentally more benign than existing methods of pest control⁴¹.

Note: Supplementary information is available on the Nature Biotechnology website.

DISCLAIMER

In addition to scientists that work within the public sector, the IOBC/WPRS working group includes scientists that work within regulatory agencies, the commercial biotech industry and commercial contract laboratories. Although these organizations have an interest in the final outcome of the working group program, members of the working group participate as individuals, not as representatives of these organizations. The publications of the working group reflect a consensus that has developed as a result of its open meetings and discussions and the opinions expressed by individuals or in working group publications may not necessarily, therefore, represent the policies of their organizations. Any mention of a proprietary product in meetings or publications does not constitute an endorsement or a recommendation by the working group. There is no commercial sponsorship or endorsement for the working group or its members, beyond the support provided to individual participants by their organizations to attend meetings.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturebiotechnology/>.

Published online at <http://www.nature.com/naturebiotechnology/>

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions/>

- James, C. Global status of commercialized biotech/GM crops: 2006. ISAAA Briefs No. 35 (International Service for the Acquisition of Agric.-Biotech Applications, Ithaca, NY, USA, 2005).
- Nap, J.P., Metz, P.L.J., Escaler, M. & Conner, A.J. The release of genetically modified crops into the environment - Part I. Overview of current status and regulations. *Plant J.* **33**, 1–18 (2003).
- Conner, A.J., Glare, T.R. & Nap, J.P. The release of genetically modified crops into the environment - Part II. Overview of ecological risk assessment. *Plant J.* **33**, 19–46 (2003).
- Rose, R.I. (ed.) 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, 2007). <<http://www.epa.gov/pesticides/biopesticides/pips/non-target-arthropods.pdf>>
- Canadian Food Inspection Agency. Assessment criteria for determining environmental safety of plants with novel traits. Directive 94–08 (Canadian Food Inspection Agency, Government of Canada, Ottawa, Ontario, 2004).
- European Community (EC). Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Off. J. Eur. Commun. L* **106**, 1–39 (2001). <http://ec.europa.eu/environment/biotechnology/pdf/dir2001_18.pdf>
- European Commission (EC). Commission Decision (2002/623/EC) of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council of the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Off. J. Eur. Commun. L* **200**, 22–33 (2002). <http://ec.europa.eu/environment/biotechnology/pdf/dec2002_623.pdf>
- European Food Safety Authority (EFSA). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. *EFSA J.* **99**, 1–100 (2006). <http://www.efsa.europa.eu/EFSA/Scientific_Document/gmo_guidance_gm_plants_en,0.pdf>
- Secretariat of the Convention on Biological Diversity. Cartagena Protocol on Biosafety to the Convention on Biological Diversity: Text and Annexes (Secretariat of the Convention on Biological Diversity, Montreal, Canada, 2000).
- Romeis, J. Non-target risk assessment of GM crops and regulation. *IOBC WPRS Bull.* **29**(5), 197–200 (2006).

11. Wolt, J.D. & Peterson, K.D. Agricultural biotechnology and societal decision-making: the role of risk analysis. *AgBioForum* **3**, 39–46 (2000). <<http://www.agbioforum.org/v3n1/v3n1a06-wolt.htm>>
12. Johnson, K.L., Raboult, A.F., Hudson, M.D. & Poppy, G.M. How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends Plant Sci.* **12**, 1–5 (2007).
13. US Environmental Protection Agency (USEPA). Guidelines for ecological risk assessment. EPA 630/R-95-002F (Environmental Protection Agency, Washington, DC, USA, 1998).
14. Raybould, A. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environ. Biosafety Res.* **5**, 119–125 (2006).
15. National Research Council (NRC). Field testing genetically modified organisms: framework for decisions (NRC Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment, National Academy Press, Washington, DC, 1989).
16. Tiedje, J.M. *et al.* The planned introduction of genetically modified organisms: ecological considerations and recommendations. *Ecology* **70**, 298–315 (1989).
17. Hokanson, K. *et al.* The concept of familiarity and pest resistant plants. in *Proc., Ecological Effects of Pest Resistance Genes in Managed Ecosystems*, January 31–February 3, 1999, Bethesda, Maryland (eds. Trayner, P.L. & Westwood, J.H.), 15–19 (Information Systems for Biotechnology, Blacksburg, Virginia, USA, 1999).
18. Organisation for Economic Cooperation and Development (OECD). Safety considerations for biotechnology: scale-up of crop plants (Organisation for Economic Cooperation and Development, Paris, 1993). <<http://www.oecd.org/dataoecd/26/26/1958527.pdf?channelId=34537&homeChannelId=33703&fileTitle=Safety+Considerations+for+Biotechnology+Scale-up+of+Crop+Plants>>
19. Kuiper, H.A., Kleter, G.A., Noteborn, H.P.J.M. & Kok, E.J. Assessment of the food safety issues related to genetically modified foods. *Plant J.* **27**, 503–528 (2001).
20. Bradford, K.J., Van Deynze, A., Gutterson, N., Parrott, W. & Strauss, S.H. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nat. Biotechnol.* **23**, 439–444 (2005).
21. Glare, T.R. & O'Callaghan, M. *Bacillus thuringiensis: Biology, Ecology and Safety* (John Wiley & Sons Ltd, Chichester, UK, 2000).
22. Romeis, J., Meissle, M. & Bigler, F. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol.* **24**, 63–71 (2006).
23. Hill, R.A. & Sendashonga, C. General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. *Environ. Biosafety Res.* **2**, 81–88 (2003).
24. Touart, L.W. & Maciorowski, A.F. Information needs for pesticide registration in the United States. *Ecol. Appl.* **7**, 1086–1093 (1997).
25. Peters, R.H. *A Critique for Ecology* (Cambridge University Press, Cambridge, UK, 1991).
26. Marvier, M., McCreedy, C., Regetz, J. & Kareiva, P. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* **316**, 1475–1477 (2007).
27. Dutton, A., Romeis, J. & Bigler, F. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* **48**, 611–636 (2003).
28. Barrett, K.L., Grandy, N., Harrison, E.G., Hassan, S. & Oomen, P. (eds.) Guidance document on regulatory testing procedures for pesticides with non-target arthropods. From the ESCORT workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing) (Society of Environmental Toxicology and Chemistry Europe, Brussels, Belgium, 1994).
29. American Society for Testing and Materials (ASTM). *Standard guide for selection of resident species as test organisms for aquatic and sediment toxicity tests. Document E 1850* (American Society for Testing and Materials ASTM International, West Conshohocken, Pennsylvania, USA 2004).
30. Scholte, E.J. & Dicke, M. Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies. A literature-based study, proposing an ecologically based first step to select non-target organisms. *COGEM report CGM 2005–06* (2005). <<http://www.cogem.net/ContentFiles/CGM%202005-06.pdf>>
31. Niemi, G.J. & McDonald, M.E. Application of ecological indicators. *Annu. Rev. Ecol. Evol. Syst.* **35**, 89–111 (2004).
32. Candolfi, M. *et al.* Principles for regulatory testing and interpretation of semi-field and field studies with non-target arthropods. *J. Pest Sci.* **73**, 141–147 (2000).
33. Candolfi, M.P. *et al.* (eds.) Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing), a joint BART, EPPO/CoE, OECD and IOBC Workshop, organized in conjunction with SETAC Europe and EC (Society of Environmental Toxicology and Chemistry Office, Pensacola, Florida, USA, 2001).
34. Levin, S.A., Harwell, M.A., Kelly, J.R. & Kimball, K.D. Ecotoxicology problems and approaches. in *Ecotoxicology: Problems and Approaches*. (ed. Levin, S.A.) 3–8 (Springer, New York, USA, 1989).
35. Caro, T.M. & O'Doherty, G. On the use of surrogate species in conservation biology. *Conserv. Biol.* **13**, 805–814 (1999).
36. Duelli, P. & Obrist, M.K. Biodiversity indicators: the choice of values and measures. *Agric. Ecosyst. Environ.* **98**, 87–98 (2003).
37. Bates, S.L., Zhao, J.-Z., Roush, R.T. & Shelton, A.M. Insect resistance management in GM crops: past, present and future. *Nat. Biotechnol.* **23**, 57–62 (2005).
38. Candolfi, M.P. *et al.* (eds.) Guidelines to evaluate side-effects of plant protection products to non-target arthropods (IOBC/WPRS, Gent, Belgium, 2000).
39. US Environmental Protection Agency (USEPA). Harmonized test guidelines, series 885 microbial pesticide test guidelines OPPTS (Office of Prevention, Pesticides and Toxic Substances Washington, DC, USA, February 1996 <http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/index.html>
40. Raybould, A. *et al.* Non-target organisms risk assessment of MIR604 maize expressing mCry3A for control of corn rootworms. *J. Appl. Entomol.* **131**, 391–399 (2007).
41. Jaffe, G. Regulatory slowdown on GM crop decisions. *Nat. Biotechnol.* **24**, 748–749 (2006).
42. US Environmental Protection Agency (USEPA). Biopesticide registration action document. *Bacillus thuringiensis* (Bt) plant-incorporated protectants. 15 October 2001. <http://www.epa.gov/oppbpd1/biopesticides/pips/bt_brad.htm>
43. US Environmental Protection Agency (USEPA). Pesticide fact sheet for *Bacillus thuringiensis* ssp. *kurstaki* Cry1(A)b delta-endotoxin and the genetic material necessary for the production (plasmid vector pCIB4431) in corn. EPA publication no. EPA 731-F-95-004 (Environmental Protection Agency, Washington, DC, USA, 1995).
44. Losey, J.E., Rayor, L.S. & Carter, M.E. Transgenic pollen harms monarch larvae. *Nature* **399**, 214 (1999).
45. Hellmich, R.L. *et al.* Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proc. Natl. Acad. Sci. USA* **98**, 11925–11930 (2001).
46. Dively, G.P. *et al.* Effects on Monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environ. Entomol.* **33**, 1116–1125 (2004).
47. Prasifka, J.R., Hellmich, R.L., Dively, G.P. & Lewis, L.C. Assessing the effects of pest management on nontarget arthropods: The influence of plot size and isolation. *Environ. Entomol.* **34**, 1181–1192 (2005).
48. Sears, M. *et al.* Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. USA* **98**, 11937–11942 (2001).
49. Wolt, J.D., Peterson, R.K.D., Bystrak, P. & Meade, T. A screening level approach for nontarget insect risk assessment: transgenic Bt corn pollen and the monarch butterfly (Lepidoptera: Danaidae). *Environ. Entomol.* **32**, 237–246 (2003).

Supplementary Information

Romeis et al. (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nature Biotechnology* 26, 203–208.

The lacewing case

The predatory green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae) has been intensively studied with respect to the potential for *Bt* (Cry1Ab) maize effects¹. Higher tier, tritrophic studies involving *Bt* maize and lepidopteran larvae as prey revealed that alterations in prey-quality were an important confounding factor². Use of a formal process of problem formulation would have shown that the environmental exposure level of the *C. carnea* larvae to Cry1Ab is low³. Furthermore, consideration of binding studies⁴ in the problem formulation and/or Cry1Ab protein feeding studies⁵ in lower tiers of analysis would have shown the negligible sensitivity of this species to the *Bt* toxin and would therefore have been sufficient to draw a risk conclusion without the need for additional higher tier testing.

Study design

Hazard assessment tests (generally referred to as tier-1 tests) are usually conducted using elevated protein doses in the laboratory, following standardized and validated testing protocols. These tests should follow the principles of Good Laboratory Practices (GLP)⁶ whenever possible to ensure the integrity and reconstructability of the study, and to render the data acceptable to regulatory authorities worldwide. This assures study repeatability, interpretability and quality, and thus ensures a high level of confidence in the reported data. Consequently, test results should be transportable among regulatory authorities, making them applicable for ERAs of novel IRGM plants that express similar insecticidal proteins, if the taxa are relevant and appropriate. Test protocols that account for the oral exposure pathway of plant-expressed insecticidal proteins are continuously being developed to address the specific research or regulatory needs associated with IRGM risk assessment⁷⁻¹⁵.

Prior to testing, the objectives of the individual studies need to be defined, and specific measurement endpoints described that address the risk hypotheses. Appropriate measurement endpoints include life-cycle parameters such as mortality or fecundity, which can easily be evaluated and related to assessment endpoints such as population size of a certain NTA. Other endpoints such as body mass or behavioral effects may be used; however, risk assessors should agree how to interpret these data if they have a less direct connection with population statistics.

For testing protocols to be effectively employed, testing personnel must know the species' morphological and biological characteristics (e.g., lifespan, size of individuals, distinction between sexes and stages, characters for unambiguous species recognition) and it must be possible to define the life-stages that permit evaluation of the selected endpoints. Obtaining test animals that meet test quality criteria usually necessitates that the animals be available and reared in either public sector or commercial rearing facilities.

Lower-tier tests are designed to measure a specific endpoint (or set of endpoints) at protein concentrations that are usually several times higher than those present in the field. These tests are not meant to reflect real-world exposures but provide high dose exposure scenarios. The insecticidal protein provided to test organisms may be derived from IRGM plants or more typically from recombinant bacteria. A number of analyses (termed bridging and equivalence studies) are used to confirm that the bacterially-produced protein is biochemically, structurally and functionally equivalent to the one expressed by the plant¹⁶⁻¹⁸. In most cases the purified protein is provided to the test species in artificial diets. In situations where the study organism cannot be fed on an artificial diet or when no purified protein is available, plant material containing the protein can be used as the hazard source although the use of plant tissue may prevent high dose hazard testing. In the case of the monarch butterfly for example, larval development was selected as the measurement endpoint but larvae could not be reared reliably on an artificial diet through to the adult stage. Thus the studies were conducted using leaf discs dusted with varying doses of *Bt* maize pollen grains to estimate pollen/protein effects for use in the hazard assessment^{7,19}.

All tests should adopt quality control criteria that help to validate the test system. For example, for lower tier tests these may include: (i) a requirement for low negative control mortality, (ii) the use of a positive toxic control to confirm that the test system is working effectively, (iii) homogeneity of the test material to ensure uniformity of exposure, (iv) stability of the insecticidal compound throughout the bioassay period, and (v) sufficient statistical power for the testing of risk hypotheses. Retrospectively, some of the studies that have been conducted to date have not been designed to deliver adequate levels of statistical power²⁰. It is recognized that trade-offs may exist between the duration of the test, the number of life-stages that can be monitored, control mortality and the power of the test system. For example, longer-term, multi-life stage tests that increasingly demonstrate mortality within control arthropod groups would require high levels of replication in order to retain statistical power. The flexibility within our approach to expand the range or number of lower tier tests may compensate for some of these constraints. For example, a range of tests using different life-stages of a test organism might substitute for full life-cycle tests.

Higher tier tests, using live plant material, include semi-field tests that are conducted under enclosed conditions, or open field tests, and are sometimes conducted when multi-trophic evaluations are warranted, or population parameters are sought. These risk assessment studies involve the use of population and community responses and they may incorporate sources of geographic and temporal variability in exposure to the expressed protein if they are conducted at multiple sites. These tests are highly complex and may be difficult to interpret. Higher-tier tests therefore place high

demands on skills and resources in design, execution and data analysis and are often subject to the problem of low statistical power²¹⁻³². These tests therefore should 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.

1. Romeis J, Meissle M & Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol.* **24**, 63–71 (2006).
2. Dutton, A., Klein, H., Romeis, J. & Bigler, F. Uptake of *Bt*-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecol. Entomol.* **27**, 441–447 (2002).
3. Dutton, A., Romeis, J. & Bigler, F. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: *Bt*-maize expressing Cry1Ab as a case study. *BioControl* **48**, 611–636 (2003).
4. Rodrigo-Simón, A. *et al.* Lack of detrimental effects of *Bacillus thuringiensis* Cry toxins on the insect predator *Chrysoperla carnea*: a toxicological, histopathological, and biochemical approach. *Appl. Environ. Microbiol.* **72**, 1595–1603 (2006).
5. Romeis, J., Dutton, A. & Bigler, F. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *J. Insect Physiol.* **50**, 175–183 (2004).
6. Organisation for Economic Cooperation and Development (OECD). Good Laboratory Practice. OECD Principles and Guidance for Compliance Monitoring (OECD Publishing, 2006).
7. Hellmich, R.L. *et al.* Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proc. Natl. Acad. Sci. USA* **98**, 11925–11930 (2001).
8. Lundgren, J.G. & Wiedenmann, R.N. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). *Environ. Entomol.* **31**, 1213–1218 (2002).
9. Duan, J.J. *et al.* Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. *Entomol. Exp. Appl.* **104**, 271–280 (2002).
10. Morandin, L.A. & Winston, M.L. Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ. Entomol.* **32**, 555–563 (2003).
11. Stacey, D., Graser, G., Mead-Briggs, M. & Raybould, A. Testing the impact on non-target organisms of insecticidal proteins expressed in transgenic crops. *IOBC/WPRS Bull.* **29**(5), 165–172 (2006).
12. Duan, J.J. *et al.* Assessing nontarget impacts of *Bt* corn resistant to corn rootworms: Tier-1 testing with larvae of *Poecilus chalcites* (Coleoptera: Carabidae). *Environ. Entomol.* **35**, 135–142 (2006).
13. Duan, J.J., Huesing J. & Teixeira, D. Development of Tier-I toxicity assays for *Orius insidiosus* (Heteroptera: Anthracoridae) for assessing the risk of plant-incorporated protectants to nontarget heteropterans. *Environ. Entomol.* **36**, 982–988 (2007).
14. Rose, R., Dively, G.P. & Pettis, J. Effects of *Bt* corn pollen on honey bees: emphasis on protocol development. *Apidologie* **38**, 368–377 (2007).
15. Babendreier, D., Reichhard, B., Romeis, J. & Bigler, F. Impact of insecticidal proteins expressed in transgenic plants on bumble bee micro-colonies. *Entomol. Exp. Appl.*, in press (2008).
16. United States Environmental Protection Agency (USEPA). Biopesticide registration action document. *Bacillus thuringiensis* (*Bt*) plant-incorporated protectants. 15 October 2001. http://www.epa.gov/oppbpd1/biopesticides/pips/bt_brad.htm.
17. Scientific Advisory Panel (SAP). Report of the FIFRA Scientific Advisory Panel Meeting, “Sets of scientific issues being considered by the Environmental Protection Agency regarding: section II – mammalian toxicity assessment guidelines for protein plant pesticides”, June 6-7, 2000, Arlington, USA (<http://www.epa.gov/scipoly/sap>, 2000).
18. Codex Alimentarius Commission (CAC) Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003. (http://www.who.int/foodsafety/biotech/en/codex_guidelines_plants.pdf).
19. Dively, G.P. *et al.* Effects on Monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environ. Entomol.* **33**, 1116–1125 (2004).
20. Marvier M. Improving risk assessment for nontarget safety of transgenic crops. *Ecol. Appl.* **12**, 1119–1124 (2002).
21. Stanley-Horn, D.E. *et al.* Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proc. Natl. Acad. Sci. USA* **98**, 11931–11936 (2001).
22. Candolfi, M.P., Brown, K., Grimm, C., Reber, B. & Schmidli, H. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. *Biocontr. Sci. Technol.* **14**, 129–170 (2004).
23. Prasifka, J.R., Hellmich, R.L., Dively, G.P. & Lewis, L.C. Assessing the effects of pest management on nontarget arthropods: The influence of plot size and isolation. *Environ. Entomol.* **34**, 1181–1192 (2005).
24. Naranjo, S.E. Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of non-target arthropod natural enemies. *Environ. Entomol.* **34**, 1193–1210 (2005).
25. Naranjo, S.E. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. *Environ. Entomol.* **34**, 1211–1223 (2005).
26. Whitehouse, M.E.A., Wilson, L.J. & Fitt, G.P. A comparison of arthropod communities in transgenic *Bt* and conventional cotton in Australia. *Environ. Entomol.* **34**, 1224–1241 (2005).
27. Dively, G.P. Impact of transgenic VIP3A X Cry1Ab lepidopteran-resistat field corn on the nontarget arthropod community. *Environ. Entomol.* **34**, 1267–1291 (2005).
28. Bhatti, M.A. *et al.* Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected *Bt* corn on ground-dwelling invertebrates. *Environ. Entomol.* **34**, 1325–1335 (2005).
29. Bhatti, M.A. *et al.* Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected *Bt* corn on foliage-dwelling arthropods. *Environ. Entomol.* **34**, 1336–1345 (2005).
30. Duan, J.J. *et al.* Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified *Bacillus thuringiensis* corn. *Ecol. Entomol.* **31**, 521–531 (2006).
31. Gathmann, A., Wirooks, L., Hothorn, L., Bartsch, D. & Schuphan, I. Impact of *Bt*-maize pollen (MON810) on lepidopteran larvae living on accompanying weeds. *Mol. Ecol.* **15**, 2677–2685 (2006).
32. Rose, R. & Dively, G.P. Effects of insecticide-treated and lepidopteran-active *Bt* transgenic sweet corn on the abundance and diversity of arthropods. *Environ. Entomol.* **36**, 1254–1268 (2007).