Improving Design Quality of Early-tier Non-target Arthropod Studies

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Environmental risk assessment of genetically engineered (GE) plants is designed to answer very specific questions about the potential risks of introducing such plants into the environment. Common to almost all regulatory systems that evaluate GE plants for environmental release (i.e., commercial cultivation) is the requirement to assess the potential adverse impacts that arthropod-resistant GE plants, such as the so-called "Bt crops," may have on non-target arthropods (NTAs). The magnitude of risk to NTAs depends on the likelihood and seriousness of harmful effects that may result from cultivation of the crop. Generation of relevant effects and exposure data for such toxins is fundamental for any assessment of impacts on NTAs. A typical risk hypothesis related to the NTA effects of arthropod-resistant GE plants is that the expressed protein is not toxic to valued NTAs at the concentration present in the field[1,2]. This hypothesis is typically addressed following a tiered approach that starts with laboratory studies under highly conservative or "worst-case" exposure conditions[1,2].

Why a tiered approach makes sense

Laboratory or "early tier" studies have a good ability to detect adverse effects on non-target species. If no adverse effects are seen under the worst-case exposure conditions in early-tier laboratory studies, the risk can be characterized as acceptable. Consequently, there may be no need to conduct any further testing because of the minimal probability of adverse effects in the field where NTAs are exposed to much lower concentrations of the arthropod-active protein. Early tier testing thus allows elimination from further consideration risks that are negligible, and allows assessors to focus resources on more significant risks or uncertainties.

If effects are seen under laboratory conditions at high test substance exposure concentrations, the risk can be further characterized in additional laboratory or higher-tier experiments that use more realistic environmental exposure scenarios. Higher-tier studies can include semi-field tests under enclosed (contained) conditions and open field tests, and are sometimes conducted when evaluations across multiple trophic levels are warranted or estimation of population parameters is sought. The studies may involve the use of population and community responses and may consider geographic and temporal variability of exposure to the stressor. Higher-tier tests require skills and resources for their design, execution, and analysis. Furthermore, results that are difficult to interpret often do not contribute additional confidence to the conclusions of the risk assessment. A recent meta-analysis of published studies on non-target effects of Bt crops has confirmed that laboratory studies "…predicted effects that were on average either more conservative than or consistent with effects measured in the field"[3].

Guidance for improved early tier study design

Good study design is critical for early-tier laboratory studies since it contributes to the robustness of, and confidence in, environmental risk assessments of GE plants. While early tier studies should be reproducible and test clearly defined risk hypotheses, this has not always been the case, confounding data interpretation. A recent paper by Romeis et al.[4] seeks to address this issue by providing guidance and recommendations on experimental design for early tier laboratory studies (termed Tier I and/or Tier II studies, depending on the jurisdiction) used to evaluate potential adverse effects of arthropod-resistant GE plants on NTAs. The paper is the outcome of expert panels convened by the West Palaearctic Regional Section of the International Organisation for Biological Control (IOBC/WPRS) and the International Life Sciences Institute (ILSI) Research Foundation.

Protocols developed to assess the impact of chemical plant protection products on NTAs have provided a useful basis for designing similar protocols to assess the potential effects of GE plants on NTAs. They indicate which species may be suitable surrogates for laboratory studies and describe general procedures, including test system description, organism preparation, test diets, experimental design, and suitable measurement endpoints. They also describe quality criteria such as acceptable control mortalities to adequately address the assessment endpoint. Available protocols range between statements of general principles[5,6] and species specific guidance documents[7,8]. Many of these protocols have been modified to consider the oral exposure pathway of plant-expressed arthropod

active proteins, and several protocols of this type have been described in the literature[9-12].

Good study design minimizes the probability of erroneous fieldtest results: false negatives, i.e., failure to detect potentially harmful adverse effects of substances; and false positives, i.e., detection of adverse effects when the substance is unlikely to be harmful. Thus, reliable test systems should adhere to relevant test protocol design criteria to avoid erroneous results (**Box 1**).

Confidence in a conclusion of no adverse effect on a species (i.e., the avoidance of false negatives) and confidence in extrapolating that conclusion to other species depends upon the ability of the study to detect such effects. Adhering to the principles and recommendations outlined by Romeis et al.[4] should increase confidence in the results of early-tier laboratory studies, and thereby reduce data requirements for stressors that pose low risk. If adverse effects are detected in such studies, the results should be easier to interpret, and higher-tier studies for GE crops producing those substances can be designed.

Conclusions

The recommendations and associated guidance described in Romeis et al.[4] provide a sound scientific foundation for experimenters conducting early-tier NTA tests. They will also facilitate study reproducibility and peer review, and will benefit regulatory authorities by enhancing the quality of information generated for use in risk assessments. Furthermore, high confidence in the results of early-tier laboratory studies is a precondition for the acceptance of data across regulatory jurisdictions[13,14] and should encourage agencies to share useful information and thus avoid redundant testing.

Box 1: Criteria for good NTA laboratory study design.

- The test substance must be well characterized and described. This includes the source and purity of the arthropod-active protein, and its stability and homogeneity in the carrier through which it is provided to the test organism
- The test substances must be biochemically and functionally equivalent to the protein or other active ingredient produced in the GE crop
- The bioactivity of the test substances, as provided to the test organisms, must be established (e.g., in sensitive insect bioassays)
- Test organisms should be exposed to high concentrations of the test substance relative to predicted exposures in the field (if possible) or dose-response studies should be performed
- Exposure of the test organisms to the test substance should be confirmed by, for example, use of a positive control and diet analysis to measure the concentration of the test substance
- Endpoints should be measured that are likely to indicate the possibility of adverse effects on the abundance of NTAs or other assessment endpoints. Risk assessors should agree on how to interpret and use these data in the risk assessment. Determination of the measurement endpoint(s) should consider the knowledge about the impact of the arthropod-active protein on the target organisms, knowledge about the biology of the selected NTA species and life-stages, and the availability of reliable test protocols
- The number of replicates in the study should be such that defined effect sizes can be detected with sufficient statistical power
- Negative control treatments must be included to assess the suitability of the test system, the organisms (e.g., health) and the test conditions, and to evaluate potential effects of the matrix or formulation in which the test substance is delivered. Test results from assays with unacceptable high negative control mortality should be discarded
- Positive control treatments should be included, where feasible, to demonstrate that the test system is able to detect treatment effects

Note: Romeis et al.[4] is open access and is available for download at http://www.springerlink.com/content/0962-8819/20/1/.

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