Ecological risk assessment of GM crops

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Summary of ERAs for genetically modified (GM) crops

- Ecological risk is the product of probability and consequence
  - Probability of harmful ecological effects from using a GM crop
  - Seriousness of those harmful ecological effects

- What is harmful is defined in legislation, regulations etc.
  - Science predicts effects, it does not determine their value

- Two routes by which GM crops may cause harm
  - Unintended effects of transformation
  - Side-effects of the trait, usually production of a new protein

- Only the second route is considered in this talk
  - The probability of harmful unintended effects is assessed using composition and agronomy data
  - Low probability because of rigorous selection of events
Overview of ERAs for genetically modified (GM) crops

- ERA need not characterise every possible effect following an activity
  - Good question: What is the probability of this activity causing ecological harm?
  - Bad question: What will happen following this activity?

- We will assume that harm is reduced abundance of valued organisms
  - Organisms that provide ecological functions (e.g., pollinators)
  - Organisms that are valuable in themselves (e.g., of conservation interest)
  - Convenient to call both kinds “non-target organisms” (NTOs)

- ERA compares predicted exposure of NTOs to transgenic proteins with measured effects of those proteins
  - Usually data on effects are required for pesticidal proteins only
  - Non-pesticidal proteins are assessed based on their mode-of-action
Estimating NTO exposure: “developmental expression study”

- Plants grown in the field at multiple locations
- Protein concentration is measured in several tissues at several developmental stages using an immunological method (ELISA)
- Exact study design depends on specific country regulatory requirements
Estimating exposure from protein concentration data

- NTO exposure is called the estimated environmental concentration (EEC)
- Worst-case EEC is the highest protein concentration to which a small population of NTOs (say in a field) may reasonably be expected to be exposed
- Syngenta uses the highest average concentration detected in the relevant tissue
  - Other companies use other methods as the basis for worst-case EEC
  - For example, 95\textsuperscript{th} percentile of results from individual plants
- The relevant tissue for risk assessment varies among NTO groups
  - EEC for pollinators based on concentrations in pollen
  - EEC for birds based on concentrations in seed
  - EEC for predatory insects based on concentrations in leaves
Refining EEC estimates

- Worst-case exposure is unrealistic for several reasons
  - It assumes that the diet of an organism is 100% crop tissue containing the highest average concentration of protein

- Many non-target organisms do not feed directly on crop tissue
  - They feed on organisms that feed on crop tissue

- Organisms that feed directly on crop tissue have other sources of food
  - Pollinators have pollen and nectar from other plants
  - Seed-eating wild birds and mammals have seeds from other plants

- Conservative EEC estimates make allowances for the above factors
  - Allow for dilution of the protein in diet
  - Use overall mean concentrations of protein in relevant plant tissue
Testing the effects of proteins

- The likely consequences of exposure are assessed by laboratory studies.
- Organisms are exposed to the protein at 1 – 10X worst-case EEC.
- Effects are compared with those in a control group.
- The effects measured depend on the organism and study type:
  - Survival
  - Growth
  - Fecundity
  - Reproduction
- If no reduction in these parameters compared with the control, the concentration of protein in the diet is the no observed adverse effect concentration (NOAEC).
  - EEC/NOAEC is used to estimate risk (see later).
Which species are tested?

- Species are chosen according to two principal criteria:
  - How well they represent the likely effects on the group of organisms for which they are a surrogate
  - The availability of a practical protocol to expose the organism to the protein via a realistic route (often dietary)

- Local regulatory requirements vary, but there is a more-or-less standard set of organism groups for which effects data are required:
  - Wild mammals (use toxicology data)
  - Wild birds
  - Foliar predatory and parasitic arthropods
  - Pollinators
  - Soil invertebrates
  - Aquatic invertebrates
  - Fish
Which species are tested?

- The species tested vary among products owing to research into new protocols and changes in regulatory requirements.
- The table below is a guide to current practice.

<table>
<thead>
<tr>
<th>NTO Group</th>
<th>Number of species required</th>
<th>Typical species</th>
<th>Exposure route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild mammals</td>
<td>1</td>
<td>Mouse</td>
<td>Gavage</td>
</tr>
<tr>
<td>Wild birds</td>
<td>1</td>
<td>Bobwhite quail</td>
<td>Gavage</td>
</tr>
<tr>
<td>Foliar arthropods</td>
<td>2 - 3</td>
<td>Predatory bug (<em>Orius</em>); green lacewing (<em>Chrysoperla</em>); ladybirds (<em>Coccinella</em> and <em>Coleomegilla</em>)</td>
<td>Artificial diet (often meat-based)</td>
</tr>
<tr>
<td>Pollinators</td>
<td>1</td>
<td>Honey bee</td>
<td>Sucrose solution or pollen</td>
</tr>
<tr>
<td>Soil invertebrates</td>
<td>2 - 3</td>
<td>Rove beetle (<em>Aleochara</em>); ground beetle (<em>Poecilus</em>); Collembola; earthworm</td>
<td>Artificial diet or soil incorporation</td>
</tr>
<tr>
<td>Aquatic invertebrates</td>
<td>1</td>
<td><em>Daphnia</em>; <em>Gammarus</em></td>
<td>In water or leaf discs</td>
</tr>
<tr>
<td>Fish</td>
<td>1</td>
<td>Rainbow trout; channel catfish</td>
<td>In water or grain incorporated into fish feed</td>
</tr>
</tbody>
</table>
Study design: test substance

- Purified protein has advantages over GE plant tissue
- Can achieve high exposures compared with the field
  - No confounding effects of variation among plants
  - Data may be used for all crops producing the same protein
- Sufficient protein is difficult or impossible to purify from plants
- Protein produced in fermentable microbes is an acceptable alternative
  - Large quantities and high purity
- Must demonstrate equivalence between the plant and microbial proteins
  - Sequence, mass, immuno-reactivity, glycosylation, bioactivity…
- Proteins may be equivalent without being identical
Study design: adaptation of CP protocols

- Studies of the effects of pesticidal proteins use protocols developed for studies of the effects of crop protection chemicals

- The protocols are often adapted
  - Longer exposure times because of continuous production of the protein in the crop
  - Dietary instead of contact exposure
  - Testing of juveniles (e.g., larvae instead of adults) owing to adult pests being insensitive to the proteins that control their larvae

- Availability of a diet that maintains protein bioactivity and allows normal development of the organism can limit use of certain test species
  - Control mortality must remain at 20% or below
  - Frozen aliquots of diet treated with protein – thaw a fresh batch daily
Example of an effects study: *Orius insidiosus* and mCry3A

- *Orius insidiosus* is a predatory bug
  - Pierces prey with sharp mouthparts and sucks its body contents

- Need to mimic prey in the lab
  - Pots of liver, meat, egg, honey paste covered with parafilm
  - *Orius* nymphs pierce the film to feed

- Concern that proteases in diet would degrade mCry3A
  - Diet heat-treated in a microwave oven to denature proteases, and cooled before addition of the test substance

- Three treatments
  - Diet + test substance giving 50\(\mu\)g mCry3A/g diet (*ca.* 10X leaf concentration of mCry3A in MIR604 maize)
  - Diet + deionised water (negative control)
  - Diet + insect-growth regulator (positive control)
Orius test design

- Test started with discrete cohort of 2 day old nymphs
  - One nymph per arena
  - 40 nymphs per treatment

- Treated diet prepared at beginning of test and frozen in aliquots

- Nymphs observed & fed freshly thawed diet aliquots daily
  - Scored as alive, dead, missing, squashed
  - Missing or squashed excluded from data

- Observation until nymphs pupated or at 21 days after treatment

- Valid test if −ve control mortality <25% and +ve control mortality >50%
**Orius test system**

- Orius nymph
- Treated diet in holding vessel and covered with Parafilm®
- Damp cotton wool
- Test arena walls treated with Fluon®

2.5 cm
Orius test results

Table 1. The percentage pre-imaginal mortality of *Orius insidiosus* (*n* = 40 per treatment) fed with treated diet. Corrected mortalities were calculated using Abbott’s formula (Abbott, 1925).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (per g diet)</th>
<th>% pre-imaginal mortality</th>
<th>Corrected % mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>MCRY3A-0102</td>
<td>50 µg mCry3A</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>teflubenzuron</td>
<td>10 µg</td>
<td>98 ***</td>
<td>97</td>
</tr>
</tbody>
</table>

- The test is valid: -ve control mortality <25%; +ve control mortality >50%
- NOAEC = 50 µg mCry3A/g diet (the highest concentration tested)
Orius test: confirmation of exposure

- Need to show active protein present in diet during test
- Aliquots of treated diet kept frozen during test and analysed for mCry3A
- ELISA for quantification
  - 95.6% of the nominal concentration of mCry3A recovered
- Western Blotting shows whether the protein is intact
  - A single band at the predicted molecular weight was observed
- Bioassay
  - 1st instar Colorado potato beetle
    - Mortality in Orius diet treatment same as treatment with nominal concentration of mCry3A
- Conclusion
  - Orius nymphs were exposed to the nominal concentration of bioactive mCry3A via the treated diet
Assessing risk

● Risk is estimated as a hazard quotient (HQ) = EEC/NOAEC

● Lower HQ values = lower estimate of risk

● HQ = 1 is generally considered to indicate acceptable risk
  - Indicates no observed adverse effect at the EEC
  - If the NOAEC is the highest (or only) concentration tested, and EEC is worst case, HQ = 1 indicates very low risk

● HQs are often << 1
  - Particularly for birds and mammals where effects tests use a limit dose not a multiple of the EEC

● If a study is conducted at a concentration below the EEC, and no adverse effect is observed, HQ is greater than 1
  - Does not indicate risk, only that less confidence may be placed in that study than those at or above the EEC
## Ecological risk assessment for MIR604 maize

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Worst-case exposure</th>
<th>Conservative exposure</th>
<th>NOAEC or NOAEL</th>
<th>Worst-case HQ</th>
<th>Conservative HQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coccinella</em></td>
<td>10.14 μg mCry3A/g leaves</td>
<td>2.03 μg mCry3A/g diet</td>
<td>9 μg mCry3A/g</td>
<td>1.127</td>
<td>0.226</td>
</tr>
<tr>
<td><em>Orius</em></td>
<td>10.14 μg mCry3A/g leaves</td>
<td>2.03 μg mCry3A/g diet</td>
<td>50 μg mCry3A/g</td>
<td>0.203</td>
<td>0.041</td>
</tr>
<tr>
<td><em>Poecilus</em></td>
<td>4.55 μg mCry3A/g roots</td>
<td>0.15 μg mCry3A/g soil</td>
<td>12 μg mCry3A/g</td>
<td>0.379</td>
<td>0.013</td>
</tr>
<tr>
<td><em>Aleochara</em></td>
<td>4.55 μg mCry3A/g roots</td>
<td>0.15 μg mCry3A/g soil</td>
<td>50 μg mCry3A/g</td>
<td>0.091</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Earthworm</em></td>
<td>4.55 μg mCry3A/g roots</td>
<td>0.15 μg mCry3A/g soil</td>
<td>250 μg mCry3A/g</td>
<td>0.018</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Honeybee</em></td>
<td>0.21 μg mCry3A /g pollen</td>
<td>0.11 μg mCry3A /g pollen</td>
<td>50 μg mCry3A/g</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>Bobwhite quail</td>
<td>0.54 mg mCry3A/kg bw</td>
<td>0.27 μg mCry3A/g bw</td>
<td>652 mg mCry3A/kg bw</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Mouse</em></td>
<td>0.51 mg mCry3A/kg bw</td>
<td>0.37 μg mCry3A/g bw</td>
<td>2377 mg mCry3A/kg bw</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Rainbow trout</em></td>
<td>0.09 μg mCry3A/g feed</td>
<td>0.013 μg mCry3A/g feed</td>
<td>0.09 μg mCry3A/g diet</td>
<td>1.000</td>
<td>0.144</td>
</tr>
</tbody>
</table>

NOAEC = no observed adverse effect concentration; NOAEL = no observed adverse effect level; bw = body weight

- No adverse effect observed in any study (therefore HQs are maximum values)
- Most HQs well below 1
- Negligible risk to NTOs from cultivation of MIR604 maize
Field studies are usually not required to show safety

Increase in realism  
Reduction in generality

Tendency to false positives  
Tendency to false negatives

Ability to detect effects  
Ability to evaluate relevance of effects

Field studies may be required for regulatory reasons, e.g., public acceptance
Further information

- Overview of ecological risk assessment for GM crops
  - *Nature Biotechnology* **26**, 203-208

- Exposure assessment
  - *Transgenic Research* **20**: 599-611; *Transgenic Research* **21**: 813-842

- Test substance characterisation

- Species selection
  - *Chemosphere* **90**, 901-909

- Design criteria for NTO effects tests
  - *Transgenic Research* **20**, 1-22

- Examples of regulatory risk assessments for commercial crops
  - *Transgenic Research* **19**, 595-609; *Transgenic Research* **20**: 599-611
  - *Journal of Applied Entomology* **131**: 391 – 399