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

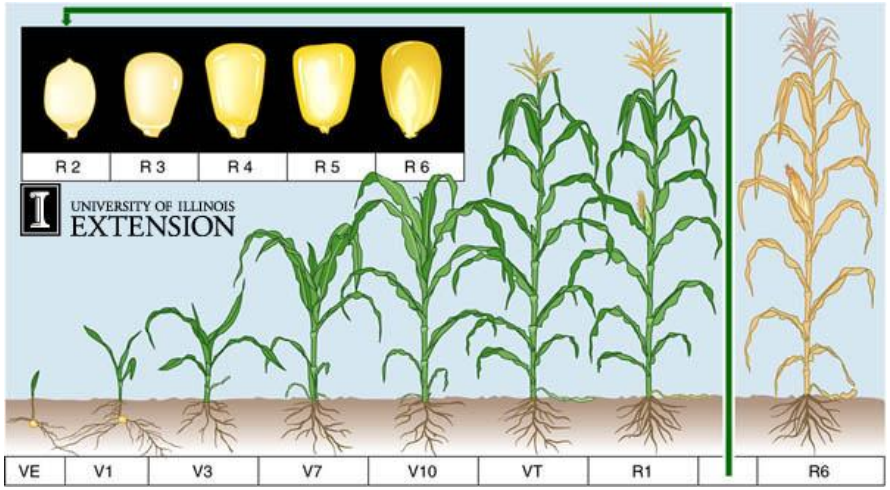
- ERA need not characterise every possible effect following an activity
  - Good question: What is the probability of this activity causing ecological harm?
  - Bad question: What will happen following this activity?

- 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



	Developmental Stage			
Tissue	V9 –V12	Anthesis (R1)	Seed maturity (R6)	Senescence
Leaf	✓	✓	✓	✓
Root	✓	✓	✓	✓
Pith		✓	✓	
Kernel			✓	
Silk		✓		
Pollen		✓		
Whole plant	✓	✓	✓	✓

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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, 95th 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

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Refining EEC estimates

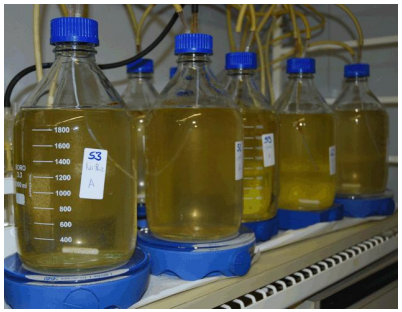
- Worst-case exposure is unrealistic for several reasons
  - It assumes that the diet of an organism is 100% crop tissue containing the highest average concentration of protein
- Many non-target organisms do not feed directly on crop tissue
  - They feed on organisms that feed on crop tissue
- Organisms 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

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

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

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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

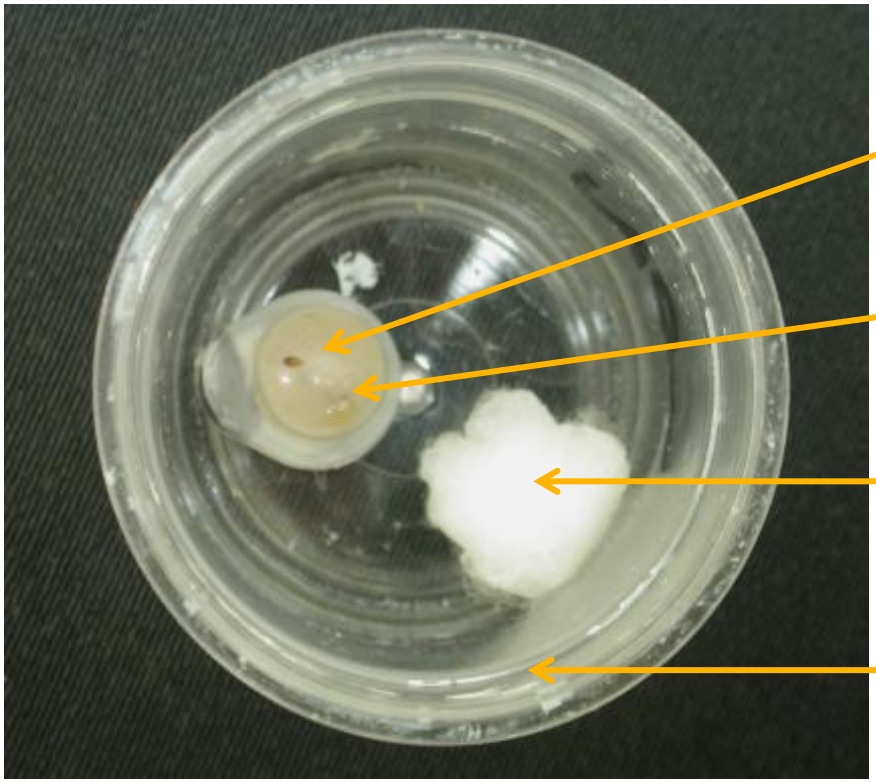
Example of an effects study: *Orius insidiosus* and mCry3A

- *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 50μg 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 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).

Treatment	Rate (per g diet)	% pre-imaginal mortality	Corrected % mortality
Control		23	-
MCRY3A-0102	50 µg mCry3A	18	0
teflubenzuron	10 µg	98 ***	97

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

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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

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

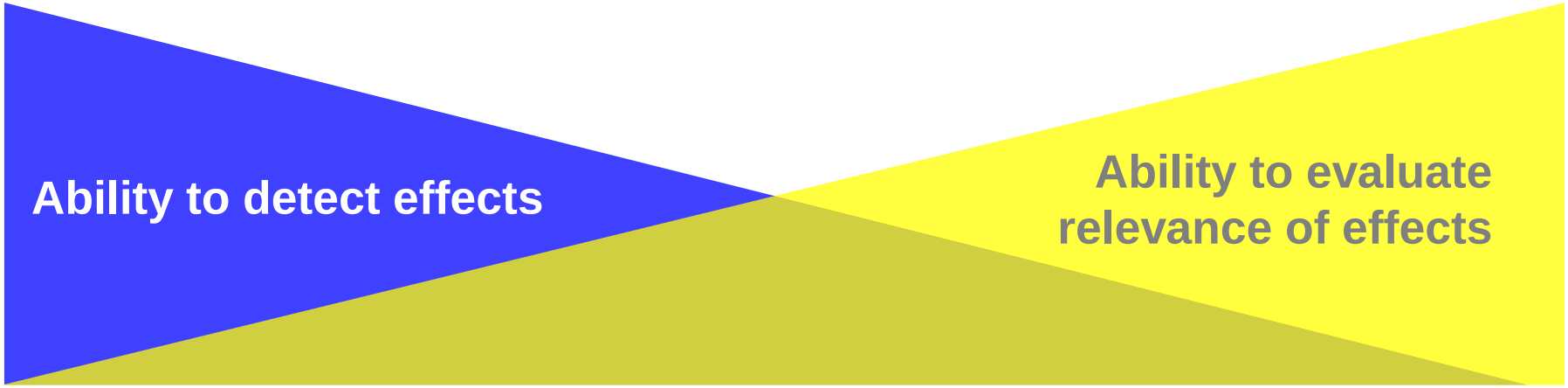
NOAEC = no observed adverse effect concentration; NOAEL = no observed adverse effect level; bw = body weight

- 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



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

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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
  - *Transgenic Research* **22**, 445-460
- 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