GMO Ecology & Risk Assessment

Bt-Plants and non-target effects with special reference to honey bees

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Content of presentation for workshop

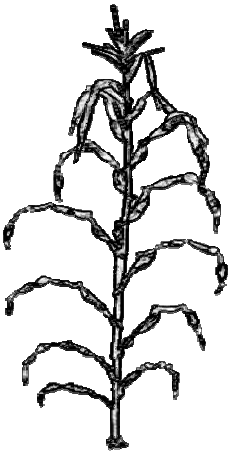
Bacillus thuringiensis proteins in GM plants: what are they, how do they work?

What is known on GMOs and honeybees? An industry view

Bt-Maize produces one or two (or more?) toxin from a bacteria (*Bacillus thuringiensis* = Bt) that kill the LARVAE of a number of caterpillar and beetle species (Cry1, Cry2 and Cry3)

here: European corn borer (*Ostrinia nubilalis*)

For example: insecticidal Bt-plants



Constitutive Bt-expression

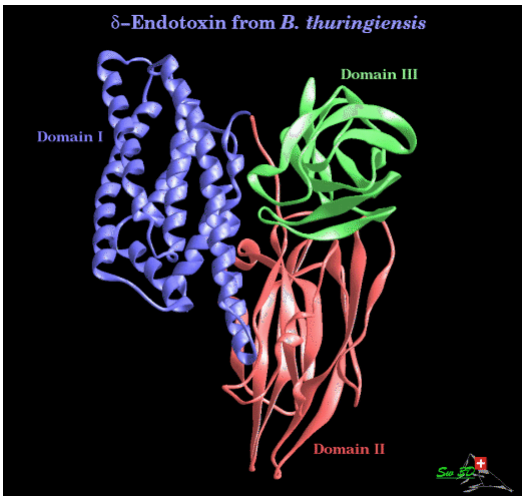
- → **all plant parts**
- → **most plant fluids, except perhaps phloem/xylem**
- → **season-long**

Molecular weights of expressed transgene product (=Bt-toxin) 65, 69 and 91 kDa

Other fragments <50, 40 kDa due to in-plant processing

- → **Unclear bioactivity**

Molecular structure of Bt-toxins



Gene Crystal shape Protein size(kDa) Insect activity

cry I [several subgroups: A(a), A(b), A(c), B, C, D, E, F, G] **bipyramidal** 130-138 lepidoptera larvae - inactive

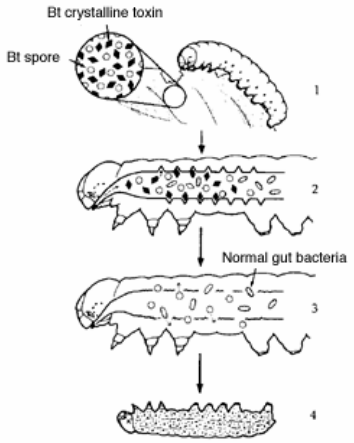
cry II [subgroups A, B, C] **cuboidal** 69-71 lepidoptera and diptera active

cry III [subgroups A, B, C] **flat/irregular** 73-74 coleoptera cry IV [subgroups A, B, C, D] bipyramidal 73-134 diptera cry V-IX various 35-129 various - active

Some commercially available Bt varieties and target pests:

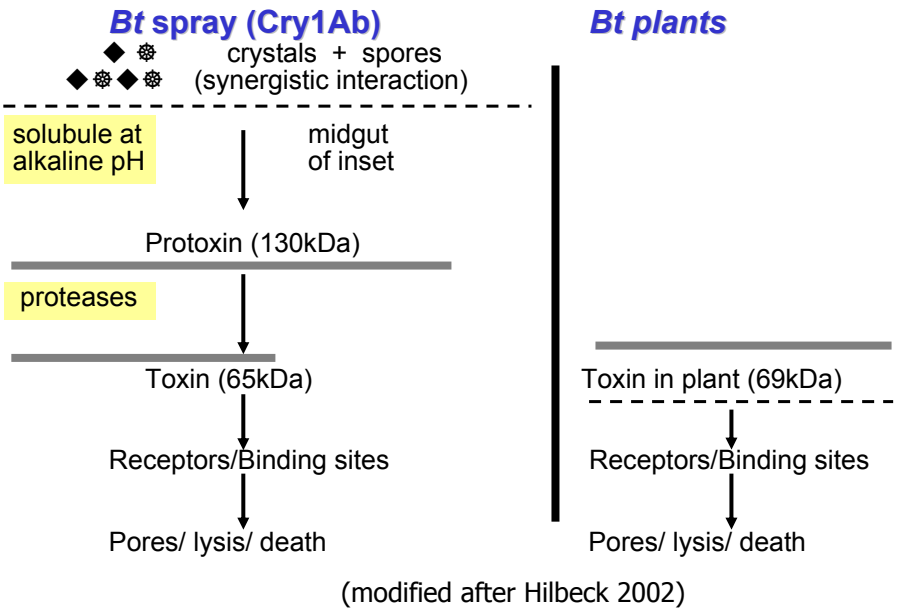
- *Bacillus thuringiensis*
  - var. *tenebrionis* - Colorado potato beetle and elm leaf beetle larvae
  - var. *kurstaki* - caterpillars
  - var. *israelensis* - mosquito, black fly, and fungus gnat larvae
  - var. *aizawai* - wax moth larvae and various caterpillars, especially the diamondback moth caterpillar

Action of *Bacillus thuringiensis* var. *kurstaki* on caterpillars



1) Caterpillar consumes foliage treated with Bt (spores and crystalline toxin).
2) Within minutes, the toxin binds to specific receptors in the gut wall, and the caterpillar stops feeding.
3) Within hours, the gut wall breaks down, allowing spores and normal gut bacteria to enter the body cavity; the toxin dissolves.
4) In 1-2 days, the caterpillar dies from septicemia as spores and gut bacteria proliferate in its blood.

Differences between *Bt* spray and *Bt plants*



(modified after Hilbeck 2002)

Definition of susceptibility

‚**Economic definition**' = acute effect, i.e. quick kill of target insect with small doses

‚**Ecological definition**' = all effects including and beyond acute toxic responses, i.e. sublethal effects, longterm effects, etc.

New results on Bt mode of action

Broderick et al. 2006 PNAS September

Midgut bacteria required for Bt insecticidal activity

If target insects are treated with antibiotics Bt toxins do NOT work! Unclear why but apparently certain (which?) bacteria are necessary to induce mode of action.

Might explain all kinds of ‚strange' non-target effects but would require concerted efforts in research.

Went astoundingly unnoticed by Bt-scientists

Reported non-target effects

Review by Hilbeck & Schmidt. 2006. Another view on Bt proteins – How specific are they and what else might they do? Biopesticides International – download at www.gmo-guidelines.info

In 27 (50%) of reviewed 54 studies reported negative effects on one or more of the tested parameters.

Positive effects were rare

The observed effects were in terms of degree and type of impact often unpredictable.

Reported non-target effects

„The mode of action of Cry1 toxins in non-target Lepidotera is presumed to be similar to that in target Lepidoptera. However, additional studies seem to be necessary to confirm this, in particular for non-target lepidoptera that exhibited only sublethal effects. Most notably, Deml et al. (1999) who conducted an extensive study with native Bt toxins found that also the coleopteran-active Cry3A toxins can have adverse effects on non-target Lepidoptera.

Similarly, Hussein et al. (2005) and Hussein et al. (2006) reported deleterious effects on polyphagous moth *Spodoptera littoralis* when caterpillars were fed Cry3A-expressing potato foliage.“

Observed effects in our lab studies:

- Adverse effects on ladybird larvae and green lacewing larvae (tri-trophic and bi-trophic)

- **No adverse effects on some bug predators**

- **Preference of spider mites for Bt-egg plants and preference of predatory spiders for non-Bt fed spider mites**

Feeding habit: Inject enzyms in prey, liquefied prey contents are sucked and ingested.

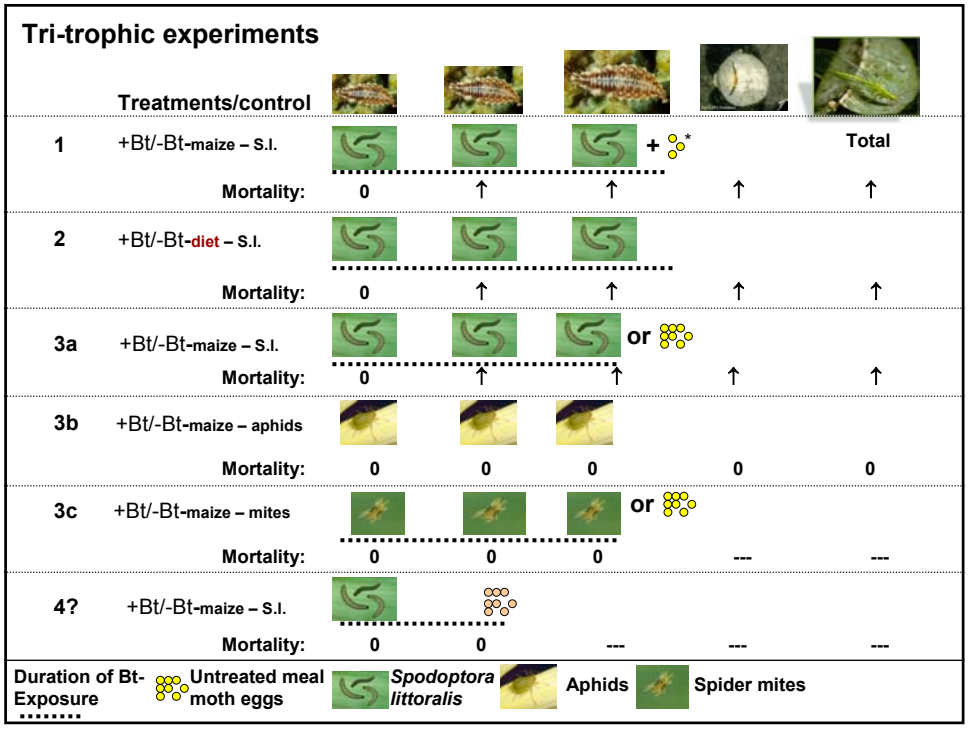
Prey: Larvae eat many other insects incl. fellow chrysopids. Preference for aphids if present. Optimal prey are small lepidoptera eggs.

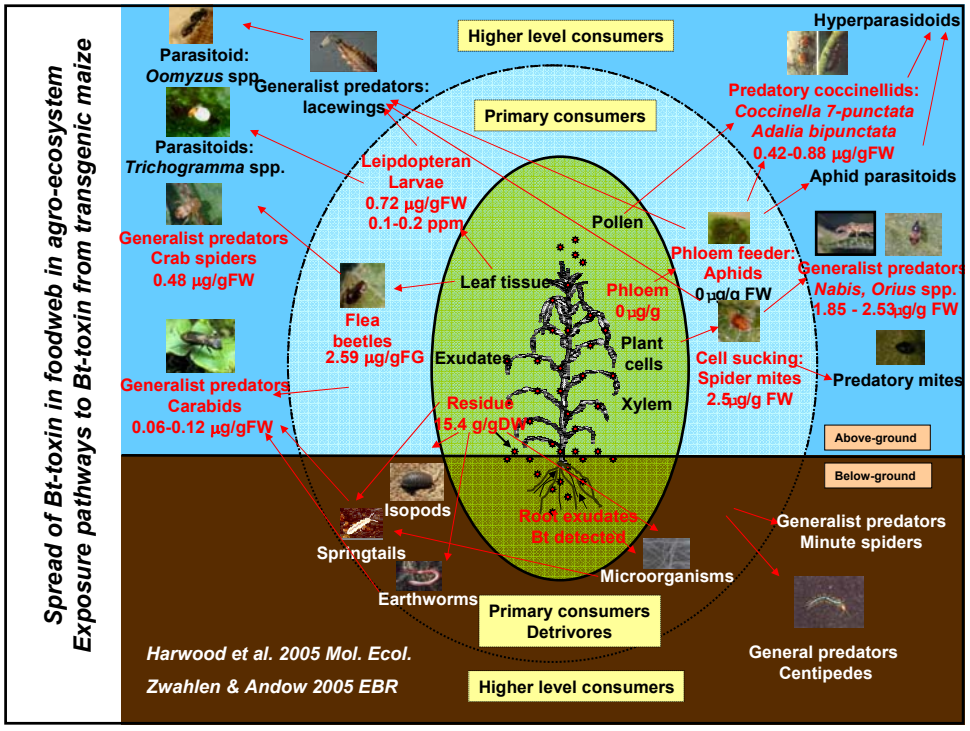
Ecotox testing approach: 'Bi-trophic' feeding studies

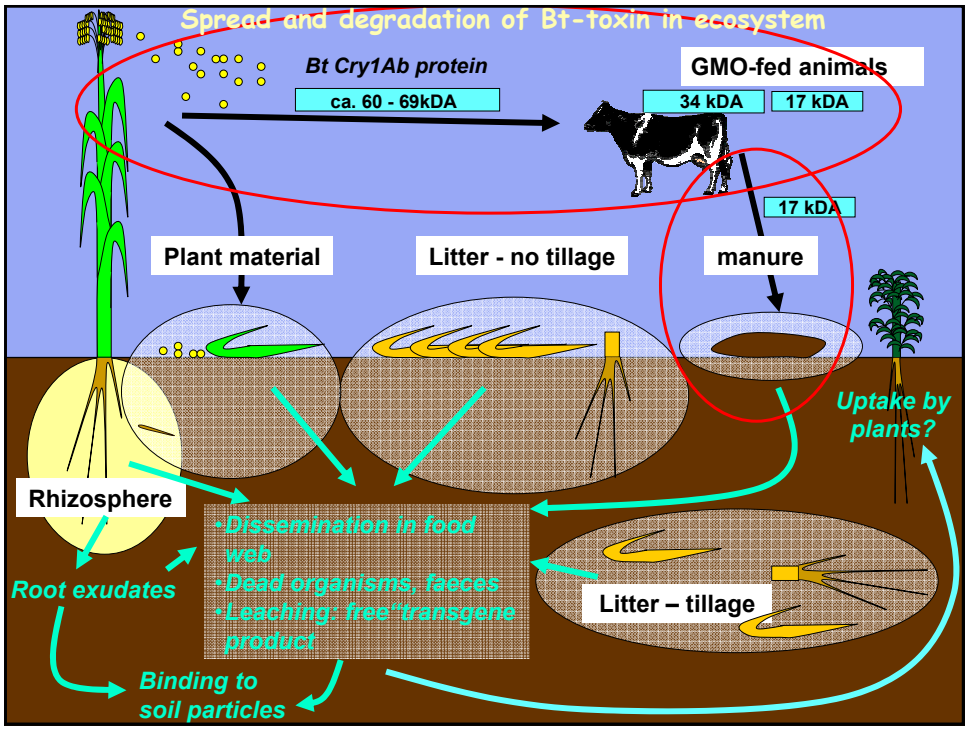
Bi-trophic experiments

	Treatments/control					Total
1a	+Bt/-Bt – continual development					
	Mortality:	=	↑	↑	↑	↑
1b	+Bt/-Bt – continual development					
	Mortality:	=	↑	↑	↑	↑
1c	Negative control					
	Mortality:	=	=	=	=	=
2a	+Bt/-Bt – arrested development		All test insects die			
	Time to death:	=	---	---	---	---
2b	+Bt/-Bt – arrested dev. Followd by recovery					
	Mortality:	=	=	=	---	---
3	+Bt/-Bt			Exposure still needs to be demonstrated		
	Mortality:	=	=	---	---	---

Duration of Bt-Exposure ..... | Artifical lacewing diet w/wo Bt toxin | Untreated meal moth eggs | Water drops w/wo Bt toxin | Coated meal moth eggs w/wo Bt toxin


Tri-trophic experiments
Treatments/control
1
+Bt/-Bt-maize – S.I.
Total
Mortality:
0
2
+Bt/-Bt-diet – S.I.
3a
+Bt/-Bt-maize – S.I.
or
3b
+Bt/-Bt-maize – aphids
3c
+Bt/-Bt-maize – mites
4?
+Bt/-Bt-maize – S.I.
Duration of Bt-Exposure
Untreated meal moth eggs
Spodoptora littoralis
Aphids
Spider mites


Spread of Bt-toxin in foodweb in agro-ecosystem
Exposure pathways to Bt-toxin from transgenic maize
Higher level consumers
Primary consumers
Parasitoid:
Oomyzus spp.
Generalist predators:
lacewings
Parasitoids:
Trichogramma spp.
Generalist predators
Crab spiders
0.48 µg/gFW
Generalist predators
Carabids
0.06-0.12 µg/gFW
Leipdopteran
Larvae
0.72 µg/gFW
0.1-0.2 ppm
Flea
beetles
2.59 µg/gFG
Leaf tissue
Pollen
Phloem
0 µg/g
Plant
cells
Exudates
Xylem
Residue
15.4 g/gDW
Root exudates
Bt detected
Hyperparasidoids
Predatory coccinellids:
Coccinella 7-punctata
Adalia bipunctata
0.42-0.88 µg/gFW
Aphid parasitoids
Phloem feeder:
Aphids
0 µg/g FW
Generalist predators
Nabis, Orius spp.
1.85 - 2.53µg/g FW
Cell sucking:
Spider mites
2.5µg/g FW
Predatory mites
Above-ground
Below-ground
Isopods
Springtails
Earthworms
Microorganisms
Generalist predators
Minute spiders
General predators
Centipedes
Primary consumers
Detrivores
Higher level consumers
Harwood et al. 2005 Mol. Ecol.
Zwahlen & Andow 2005 EBR



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GMOs and Honey Bees

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GMO Ecology & Risk Assessment

Focus on trait = e.g. transgene product means applying ‚pesticide model' (ecotoxicological testing)

Strategy: Expose single species (of standard set) to single chemicals in a hierarchical tiered system

- Tests commence with simple inexpensive range-finding tests on single species

- Measure acute toxicological response to a chemical stressor

- Proceed to more expensive higher tiered tests (incl. some chronic toxicity tests), only if first tier experiments yield results of concern

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Standard non-target organisms tested according ‚pesticide paradigm'

Water fleas (*Daphnia magna*) – acute, 48 hrs static renewal with pollen
Springtail (*Folsomia candida*) – chronic, 28 days, yeast + test material
Earthworm (*Eisenia foetida*) – 14 days, soil + test material
Honey bee (*Apis mellifera*) – acute, 45 minutes, undigested pollen + water

Predatory/parasitoids insects
***Hippodamia convergens* - adults tested, bitrophic**
***Nasonia vitripennis* – adults tested; pupal parasitoid of house flies, minor ecological relevance, bitrophic**
***Chrysoperla carnea* – larvae, bitrophic, coated meal-moth eggs, ca. 1 week**

Testmaterial used:
- Lyophilized leaf protein as dietary test material
- Microbially produced, activated Bt-toxin

Test duration:
- short time, acute

Test endpoints: toxicological parameters

Table 1: Some standardized guidelines for ecotoxicological testing of pesticides and GMOs (OECD 1998)[1]

Testorganisms	Test method	Duration	OECD Guideline No
Water fleas (*Daphnia* spp.)	Acute immobilization Acute toxicity	24 – 96 hours	202
Fish spp. (e.g. rainbow trout)	Acute toxicity	24 – 96 hours	203
Fish spp.	toxicity of juvenile life stages	4 – 12 weeks	210
Compost worm (*Eisenia foetida*)	Acute toxicity	7 – 14 days	207
Bobwhite quail and mallards duck	Acute toxicity	14 – 21 days (few days treatment)	205
Honey bees	Acute toxicity (oral) Acute toxicity (contact)	4 – 24 hours	New (1998) 213 214

[1] http://ecb.jr.it/testing-methods/
http://www.oecd.org/dataoecd/9/11/33663321.pdf

This is not sufficient!

GM plants and their novel transgene products resemble plants rather than chemicals!

‚Scientifically sound' testing must account for that!

Sounds trivial but really is not:

Since late 90ies.....'an undeliverable message'

AgBioview

„There is extensive information on the lack of non-target effects to diverse groups of beneficial insects including honey bees and other pollinators from Bt microbial preparations that contain Bt proteins.

--Bt proteins are ideal for use in organic production and in Bt crops because they are extremely selective and are toxic only to specific pests.... they bind specifically to receptors on the mid-gut of sensitive caterpillar pests and have no deleterious effect on beneficial/non-target insects under the conditions of use, including predators and parasitoids of targeted caterpillar pests and honeybees.“

AgBioview

„ .... and binding of the partially digested "activated" protein to specific high-affinity receptors on the surface of the midgut epithelium of target insects.

Bt proteins are ideal for use in organic production and in Bt crops because they bind specifically to receptors on the mid-gut of sensitive caterpillar pests and have no deleterious effect on beneficial/non-target insects, under the conditions of use, including predators and parasitoids of targeted caterpillar pests and honeybee (Apis mellifera) . .“

Cont'd AgBioview

„- Scientists perform extensive honeybee safety assessments on all insect-protected crops, including Bt corn and Bt cotton. The Bt proteins in these crops have been shown to have no adverse effect on the honeybee.

- EPA risk assessments have demonstrated that Bt proteins expressed in Bt crops do not exhibit detrimental effects to non-target organisms in populations exposed to the levels of Bt proteins produced in plant tissues."

Cont'd AgBioview

„- Specific studies involving Cry1Ab provide strong evidence of the safety of MON 810 Bt corn to the honeybee (similar studies have been conducted with other Bt proteins in genetically modified crops).

- The EPA concluded that based on the weight of evidence there are no unreasonable adverse effects of the Cry1Ab protein expressed in MON 810 Bt corn to non-target wildlife or beneficial invertebrates."

Industry funded study:

Methodology:

Honey bee larvae were exposed to Cry1Ab protein in their natural diet by including a maximum hazard dose (20 parts per million in distilled water mixed with honey) in developing brood cells. This maximum nominal concentration of 20 ppm was approximately 100 times greater than the maximum expected Cry1Ab protein level in MON 810 pollen. In addition to this treatment group, a negative control group was treated with distilled water. Another control group was treated with heat-attenuated (inactivated) Cry1Ab protein (20 ppm), and one set of larvae received no treatment (untreated control). At least 50 bees (1 to 4 days old) were in each replicate, and there were three replicates for each group. The treatments were administered to each larval cell through an electronic micro-applicator, which delivered 5 microliters of the test diet.

Industry funded study:

Cont'd methodology:

Once the first bee emerged on day 15, daily counting of emerged bees was performed and emerged bees were removed to an adult holding cage. The test diet was renewed daily and the study was terminated 48 hours after the last bee had emerged on day 19.

Results of honey bee larvae trials

There were no statistically significant ($P>0.05$) differences in honeybee larval survival to adult emergence among the four treatment groups. The mean adult survival rates after emergence ranged from 91.7% to 96.0% across all groups, including the controls and Cry1Ab-treated groups. This study demonstrates that honeybee larvae were not adversely affected after being exposed to Cry1Ab protein at a concentration of 20 ppm in their diet.

Industry funded study:

Methodology for adult bee trial:

.... mixing the appropriate amount of the insecticidally-active Cry1Ab protein with a honey-water (50-50) syrup to a concentration of 20 parts per million (microgram protein/g diet; ppm). The negative control group was fed the same diet with the exception that no Cry1Ab protein was added to the honey-water mixture. A second control group was fed heat-attenuated (inactivated) Cry1Ab protein at the same concentration (20 ppm) as the treatment group.

Results of adult experiment:

Adult honeybees exposed to the Cry1Ab protein in a honey-water solution for 9 days at a concentration of 20 ppm showed no signs of treatment-related mortality or toxicity. At the end of the testing period, the mortality percentage was calculated for each group. Mortality in the treatment and the negative control groups was 16.20% and 22.28%, respectively. The heat-attenuated control group mortality was 32.59%. Mortality showed a sharp increase in all three groups from days 6 through 9. At the termination of the test, the highest mortality was observed in the group that was fed the heat-attenuated Cry1Ab protein diet, while the lowest mortality was observed in the group that was fed the Cry1Ab protein diet.

The mortalities in the treatment group are not considered to be treatment-related because the two control groups showed a higher percentage of mortality over the same time interval. There was no significant statistical difference (P>0.05) in mortality patterns between any of the groups.

The EPA concluded that based on the weight of evidence there are no unreasonable adverse effects of the Cry1Ab protein expressed in MON 810 Bt corn to non-target wildlife or beneficial invertebrates . They reported no measurable deleterious effects were observed in submitted studies of the Cry1Ab protein administered to honey bee larvae, honey bee adults, parasitic wasps, Ladybird beetles, green lacewings, Collembola (springtails), and Daphnia.

Problems:

- only microbially produced proteins used at one arbitrary concentration (20 ppm = 20 microgram per gram diet

For Mon810 – low levels of Bt-toxin is reported

For other events much higher concentrations are reported. Extremely high concentrations exceeding 20 ppm by far are reported for Mon863 – against the corn root borer! Experiments?

- short term: 9 – 15/19 days

- questionable statistics and experimental designs with low statistical power to detect differences in treatments

GMO Ecology & Risk Assessment

Thank you!!

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