# The freshwater *Daphnia magna* as an integrative model in biosafety testing of GM crops

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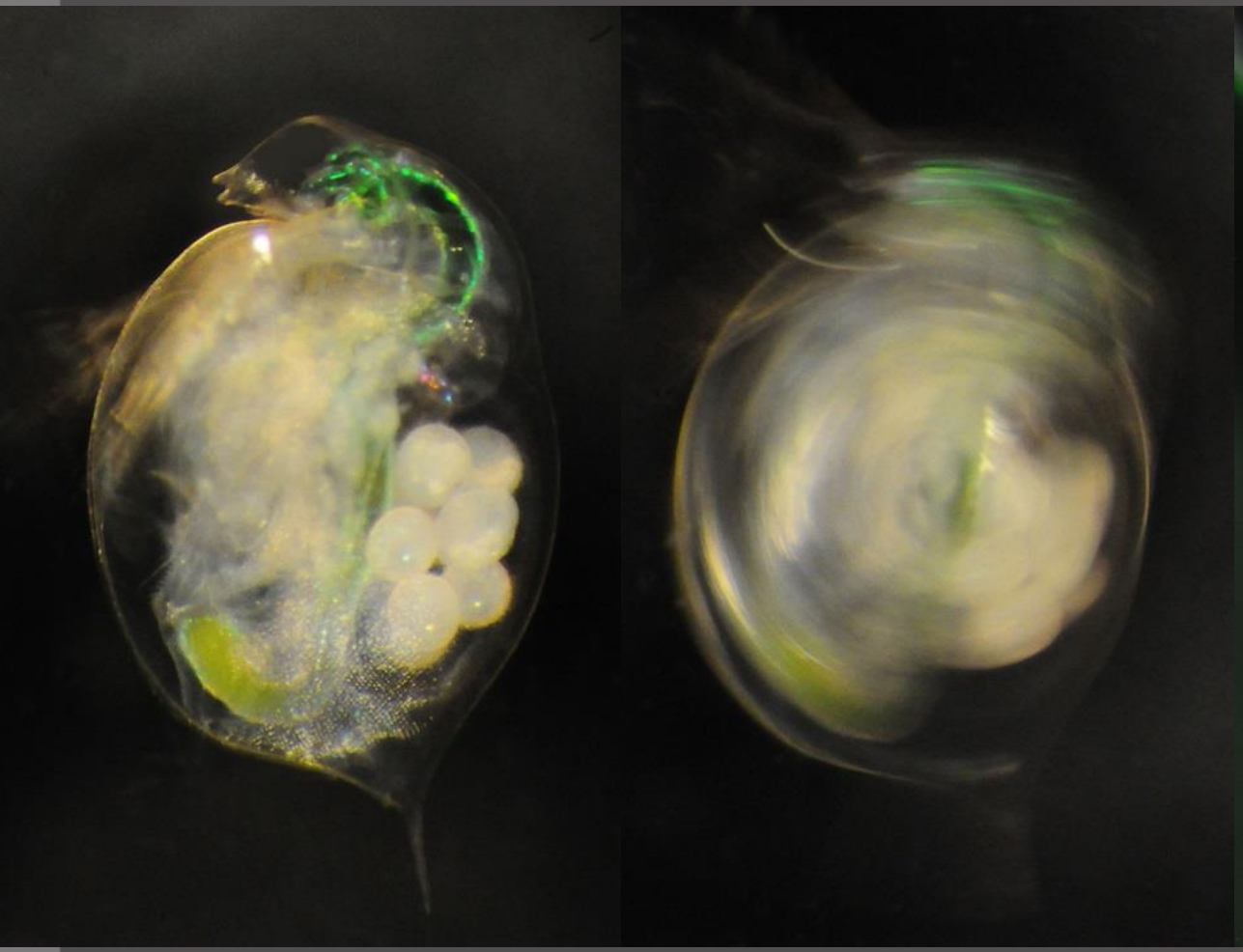
Tromsø, March 15th
“Spring”

*Daphnia magna*

*Daphnia borealis*





# GM crops as of today

- Herbicide tolerant crops (“Roundup-ready”)
- Insect-resistant crops (“Bt-toxins”)
- Combination of these two (“multistack”)

> 90 %

# Important biosafety questions:

1. Toxicity of Bt-toxins
2. Toxicity of co-technology herbicides
3. Open the field of combinatorial toxicology
4. Linking effects in the phenotype and genotype

# Roundup:
# "The environmentally friendly herbicide"

"*Children and pets need NOT be excluded from treated areas*"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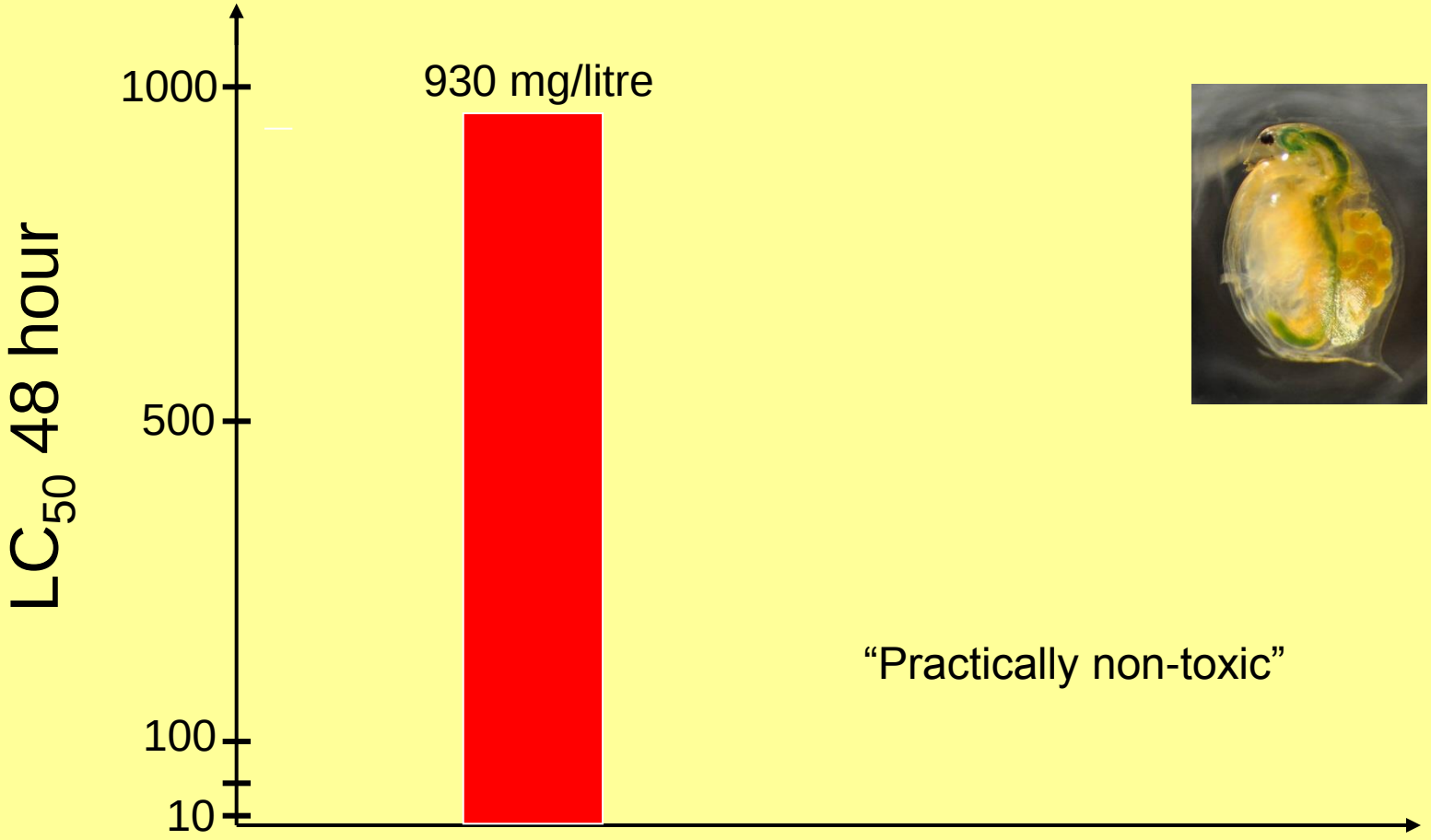
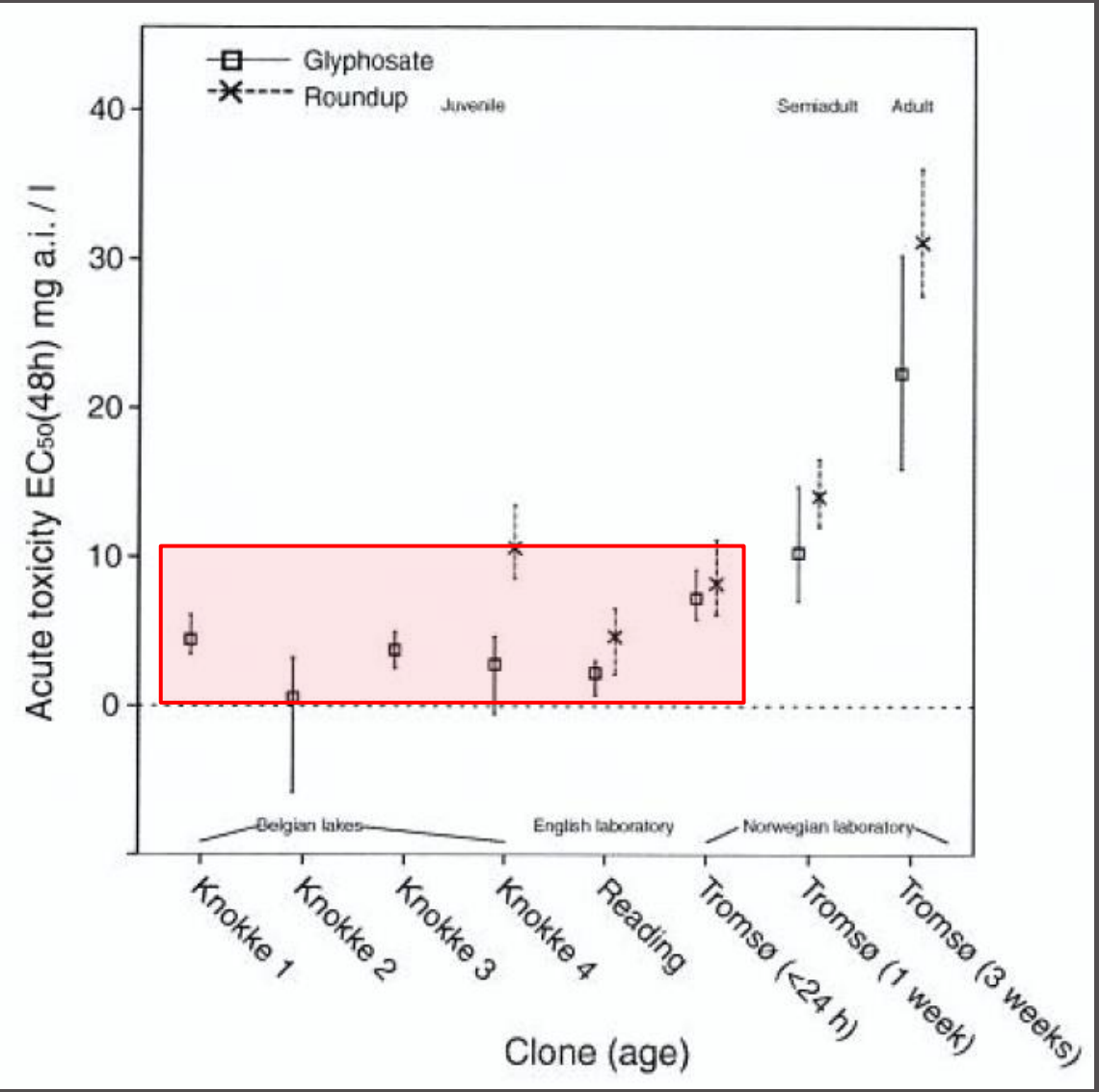
# Acute Toxicity of glyphosate on *Daphnia magna*






Early Monsanto studies for EPA
(McAllister and Forbis 1978)

# Acute toxicity revised




From Cuhra et al. 2013 *Ecotoxicology*


**Chronic** toxicity of Roundup tested

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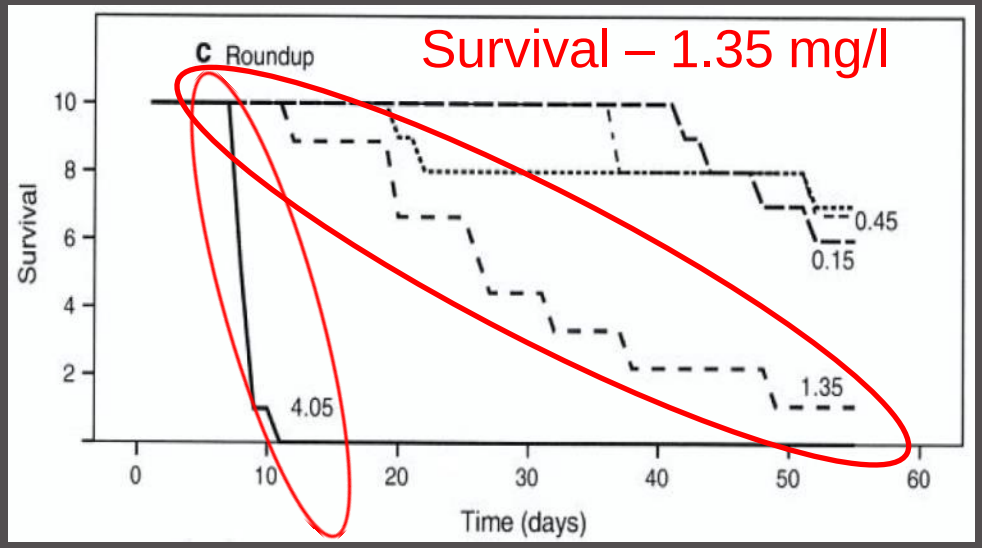
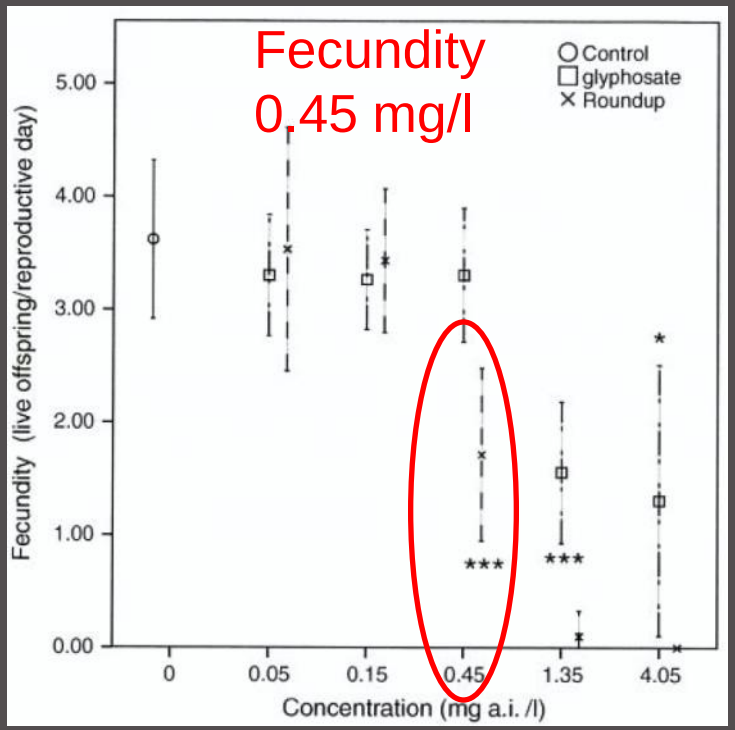
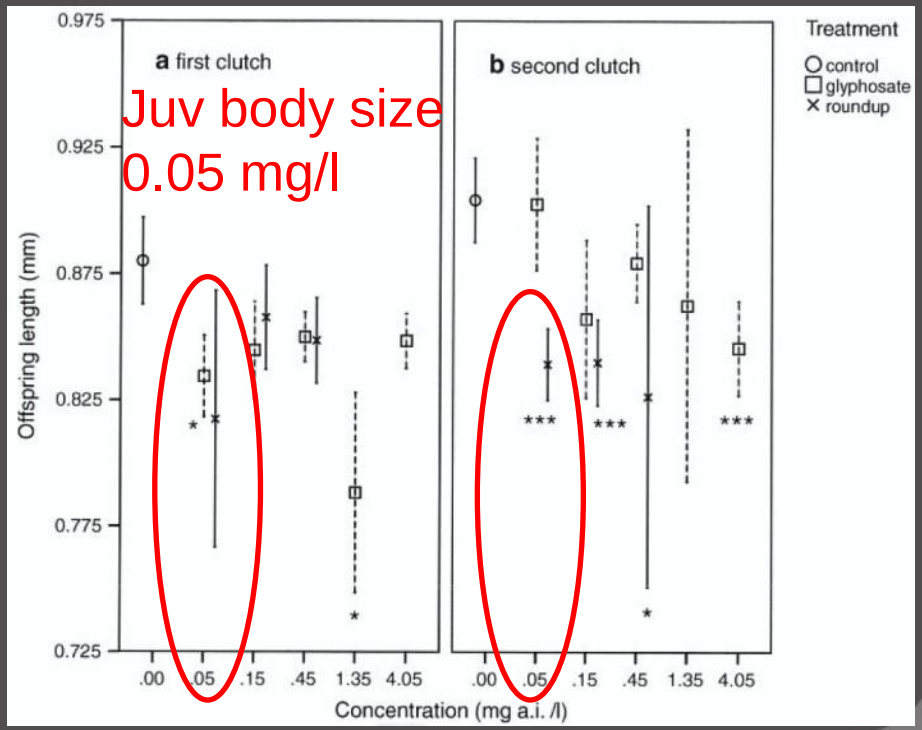
# Chronic toxicity

- Significant effects below environmental concentrations accepted in the US (0.7 mg/l)




From Cuhra et al. 2013 Ecotoxicology

# What does this mean?

- In the *D. magna* model
  - 0.05 mg/l give some negative effects
- In food and feed
  - On average 9.0 mg/kg in GM soybeans from Iowa

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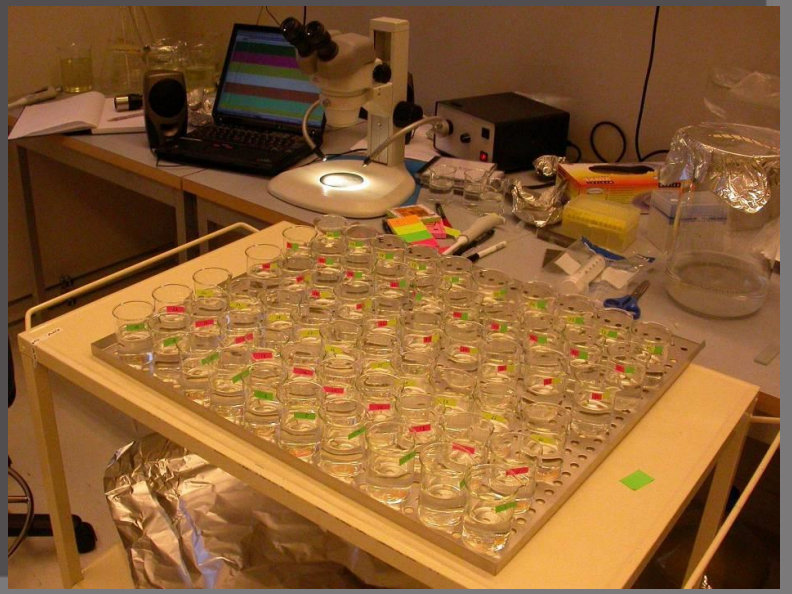
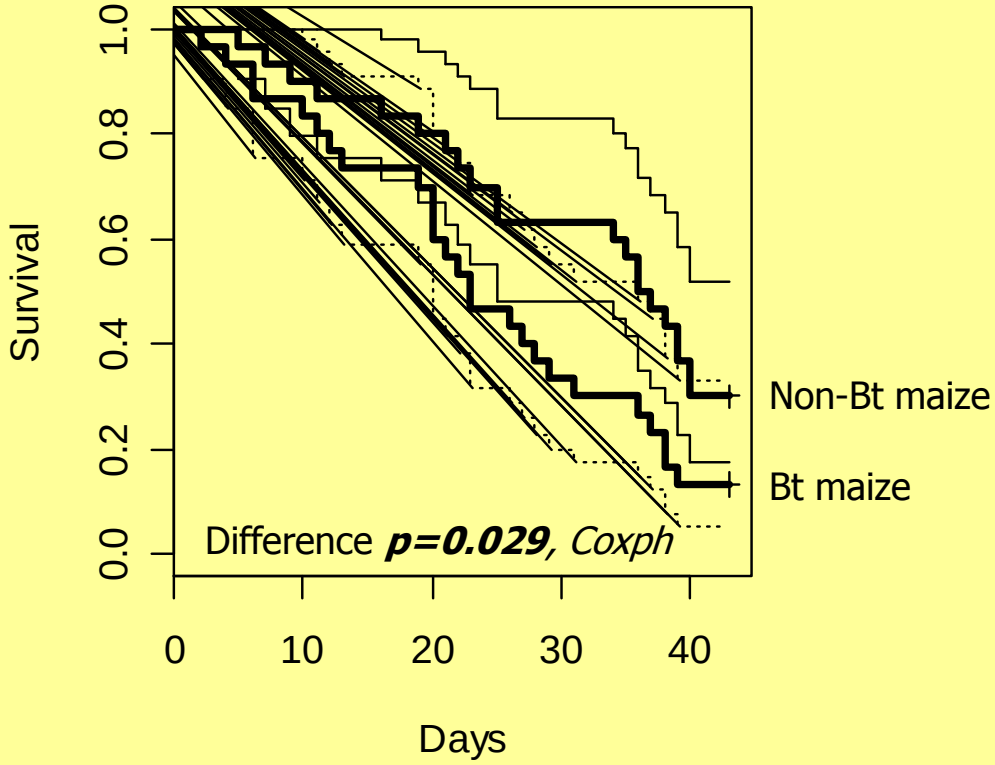
# Testing Bt-transgenic maize (MON810) vs parent line from the Philippines



# Testing Bt-maize in *Daphnia*



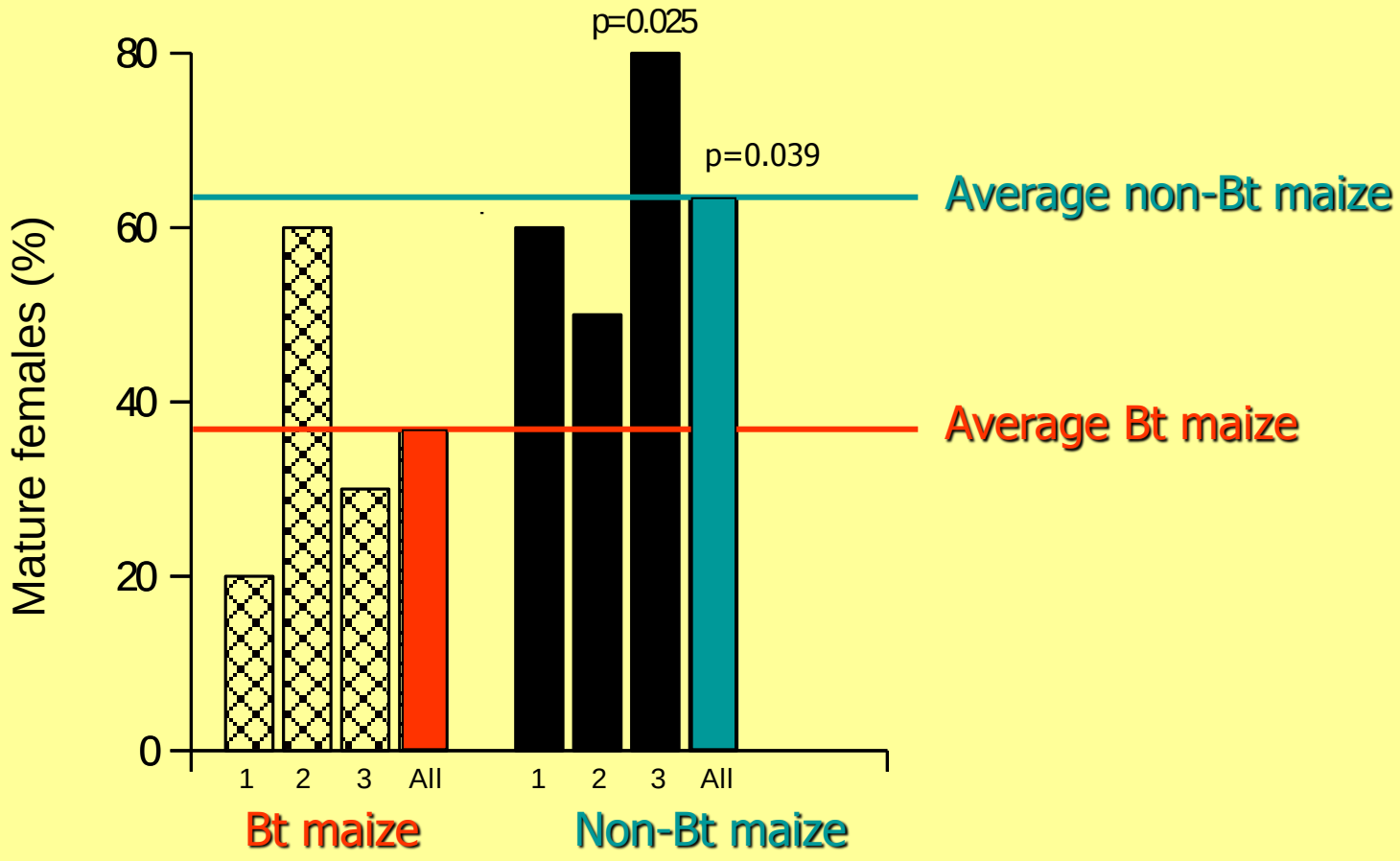

Expected average longevity estimated from model:

45 days for non-Bt maize

28 days for Bt-maize

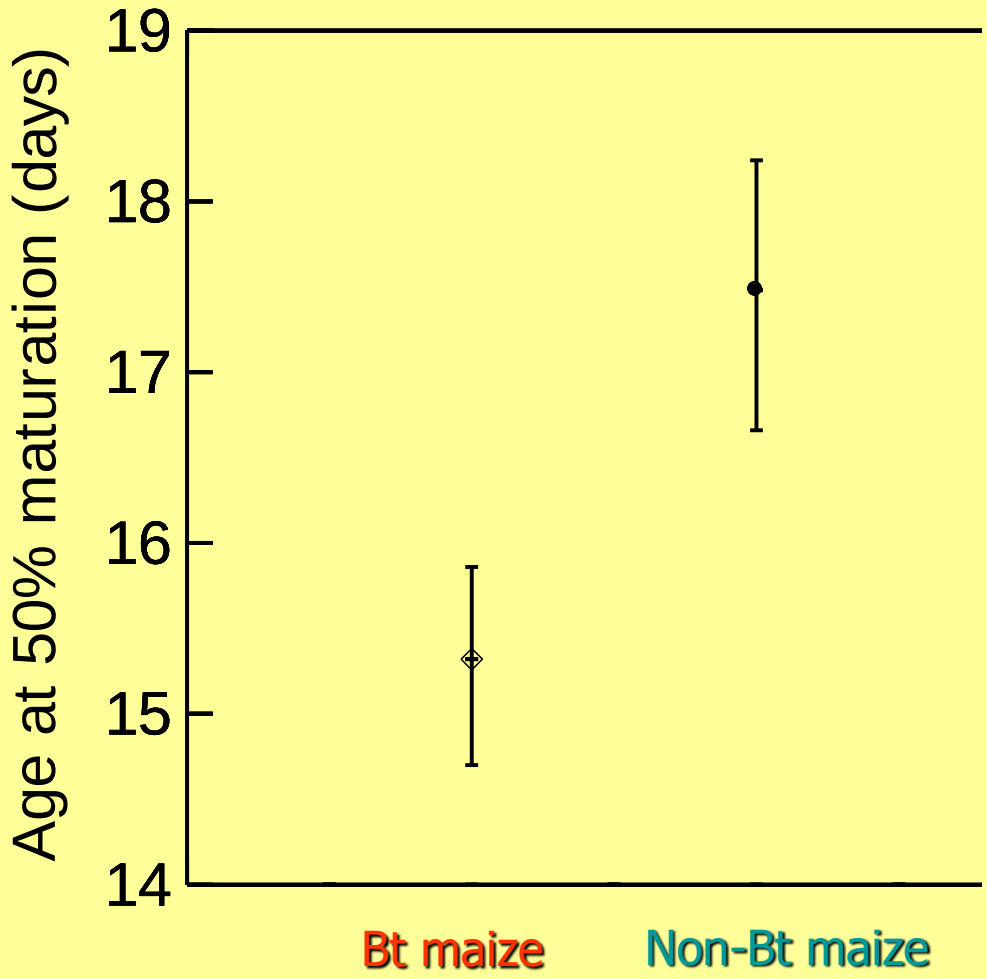
Bøhn et al. 2008 *Arch.Env.Cont.Tox*

# Percentage of females reaching maturity

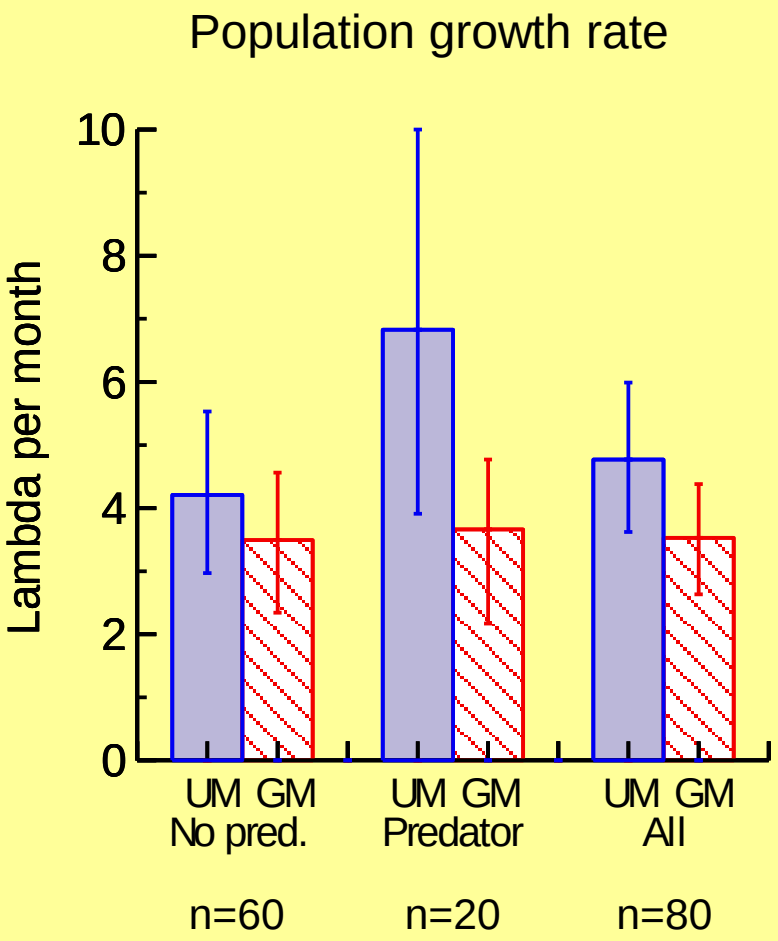


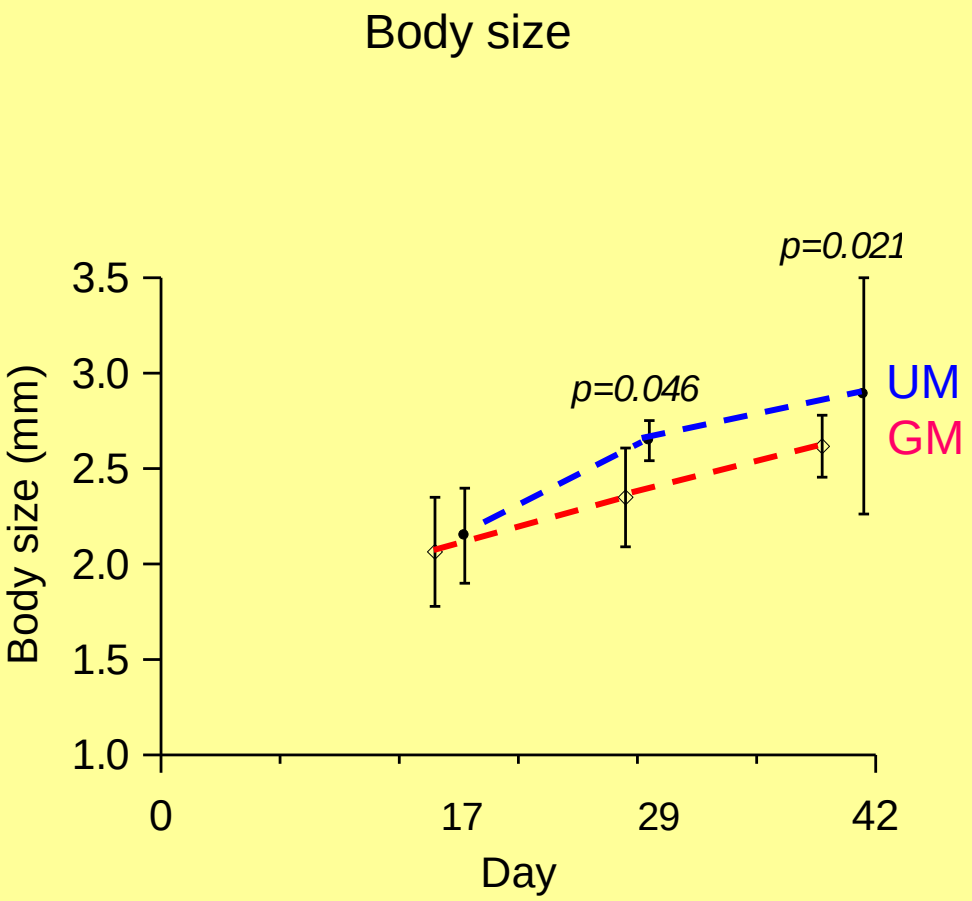

Bøhn et al. 2008

# Fewer offspring and early reproduction = likely response to toxicity



# Exposure to Bt + predator smell




Bøhn et al. 2010 *Ecotoxicology*

# Leslie matrices summarises age-specific vital rates

## Non-Bt-fed *Daphnia* (n=40)

Stage →

Fecundity

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0,088235 | 0,806452 | 0,666667 | 0,615385 | 0,75 | 0,652174 | 0,636364 | 0,45 | 0,866667 | 0,727273 | 0,340657 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0,973684 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0,945946 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0,971429 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0,911765 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0,967742 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,866667 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,923077 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,958333 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,956522 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,909091 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,75 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,733333 | 0,7937 |

Survival probability

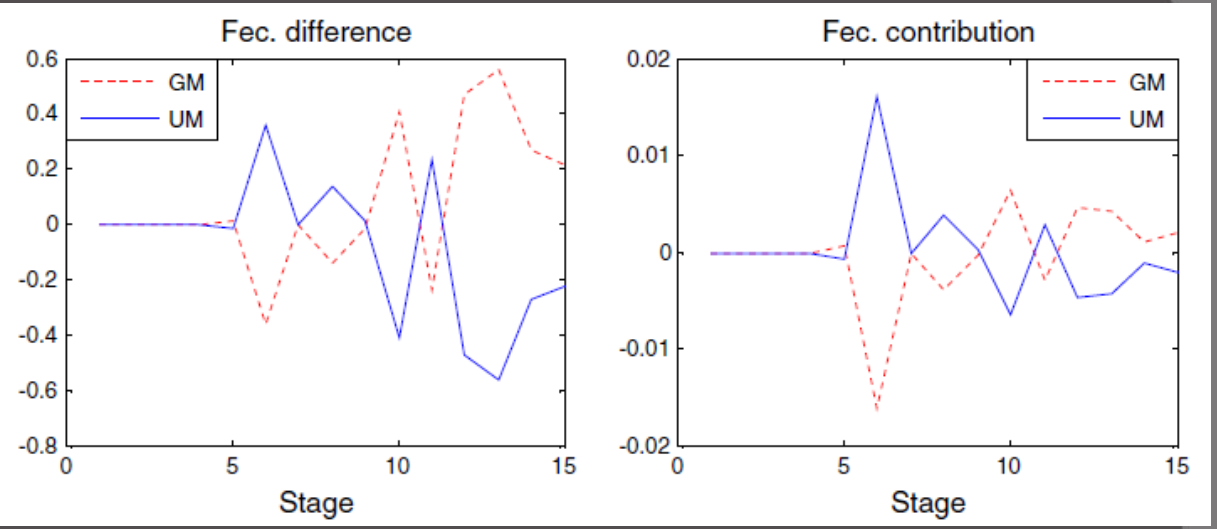
## Bt-fed *Daphnia* (n=40)

Stage →

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0,1 | 0,448276 | 0,666667 | 0,47619 | 0,736842 | 1,058824 | 0,4 | 0,923077 | 1,428571 | 1 | 0,558608 |
| 0,9667 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0,8966 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0,8846 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0,9565 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0,7727 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,8235 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,8571 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,8333 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,9 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,8889 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0,5 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0,7631 |

Bøhn et al. 2010 *Ecotoxicology*

# Sensitivity analysis




# Population effects

**Table 3** Demographic data on *D. magna* fed GM and UM maize

| Stage-classified model | GM-fed | UM-fed |
|---|---|---|
| No. of females in experiments | 40 | 40 |
| Age at first reproduction (days) | 13 | 13 |
| Number of eggs | 109 | 141 |
| Upper 95% confidence interval | 134 | 175 |
| Lower 95% confidence interval | 82 | 109 |
| Average lifetime reproduction ($R_0$) | 2.73 | 3.53 |
| Upper 95% confidence interval | 3.35 | 4.37 |
| Lower 95% confidence interval | 2.05 | 2.73 |
| Generation time | 23.9 | 24.2 |
| Upper 95% confidence interval | 24.6 | 24.7 |
| Lower 95% confidence interval | 22.3 | 23.3 |

Confidence intervals are calculated from bootstrapping. Data from all experiments, with and without predator ($n = 80$)

Bøhn et al. 2010

# Explanation for effects in Bøhn et al. 2008 and 2010

- Result: weak toxicity of the Bt maize feed
  - Not possible (in these studies) to single out cause(s):
    - Toxicity of Bt-toxin present
    - Up- or down-regulation of other genes

# Critique of Bøhn et al. 2008

Transgenic Res (2010) 19:1–12
DOI 10.1007/s11248-009-9297-5

LETTER TO THE EDITOR

## Is the German suspension of MON810 maize cultivation scientifically justified?

Agnès Ricroch · Jean Baptiste Bergé · Marcel Kuntz

Ricroch et al. 2010

# Response to critique

Bøhn *et al. Environmental Sciences Europe* 2012, **24**:22
http://www.enveurope.com/content/24/1/22

Environmental Sciences Europe
a SpringerOpen Journal

**DISCUSSION** **Open Access**

## The German ban on GM maize MON810: scientifically justified or unjustified?

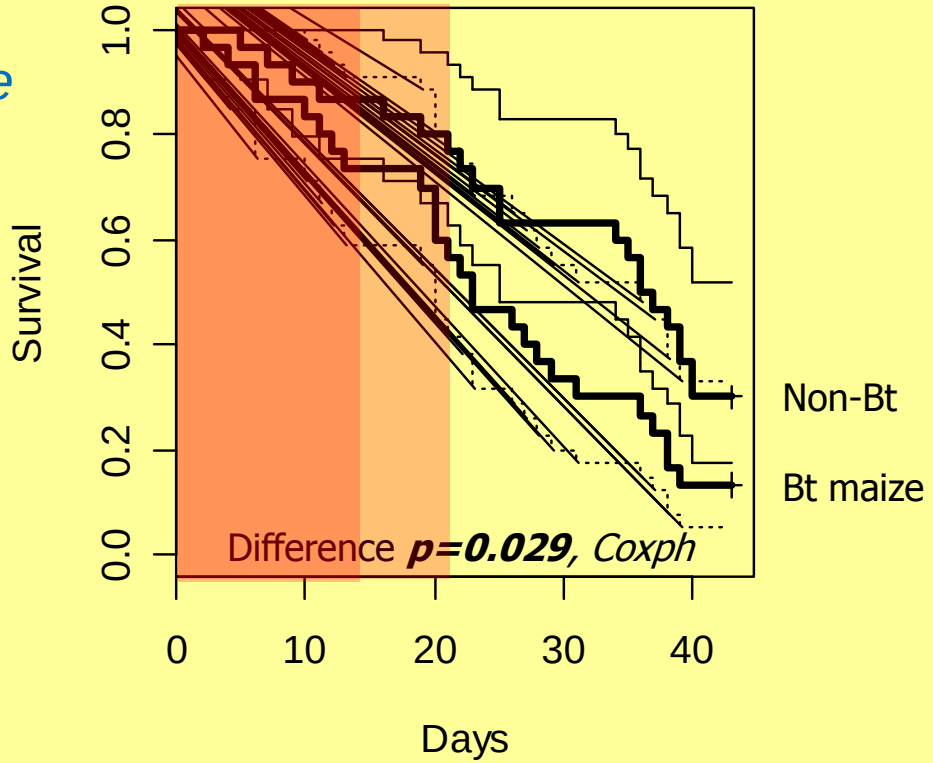
Thomas Bøhn[1,2*], Raul Primicerio[2] and Terje Traavik[1,2]

Bøhn et al. 2012

# Ricroch critique I

- Mortality: *"Reasonable to consider only shorter experimental time points(without high mortality in the control animals) to aim for a biological valid result"*

*"When these time points are considered (up to 14–21 days depending on the experiments), no statistically significant difference in mortality was observed between GM and non-GM feed."*




From Ricroch et al. 2010

# Ricroch critique II

- *"No data were provided on the dose of toxins the daphnia were exposed to (and how much they ingested)"*

**Reduced Fitness of *Daphnia magna* Fed a Bt-Transgenic Maize Variety**

**Thomas Bøhn · Raul Primicerio · Dag O. Hessen · Terje Traavik**

Received: 16 November 2007 / Accepted: 11 February 2008 / Published online: 18 March 2008


**Abstract** Genetically modified (GM) maize expressing the Bt-toxin Cry1Ab (Bt-maize) was tested for effects on survival, growth, and reproduction of the water flea *Daphnia magna*, a crustacean arthropod commonly used as a model organism in ecotoxicological studies. In three repeated experiments, *D. magna* were fed 100% ground maize in suspension, using either GM or isogenic unmodified (UM) maize. *D. magna* fed GM-maize showed a significantly reduced fitness performance: The mortality was higher, a lower proportion of females reached sexual maturation, and the overall egg production was lower compared to *D. magna* fed UM isogenic maize. We conclude that the tested variety of Bt-maize and its UM counterpart do not have the same quality as food sources for this widely used model organism. The combination of a reduced fitness performance combined with earlier onset of reproduction of *D. magna* fed Bt-maize indicates a toxic effect rather than a lower nutritional value of the GM-maize.

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Springer

**Introduction**

A few herbivore insect species are considered important pests of major crop plants. These pests are targeted with genetic engineering by insertion of various *Bacillus thuringiensis* (Bt) *cry* genes. In the plant, these genes express Cry proteins with toxic effects on different target pest insects. The Cry1Ab protein is the most widely used toxin against the order *Lepidoptera*. The most common Cry1Ab transgenic plants, known as Bt-plants, are maize, rice, cotton, and potato. In maize, the most frequent modifications include insect resistance (Bt), herbicide tolerance, or the stacking of genes that provide both of these traits. Insect-resistant Bt-maize contribute to more than 50% of the transgenic maize grown globally, but the use of varieties with stacked genes are increasing (James 2006).

The purpose of Bt-plants is to selectively kill insect pest species, thereby increasing plant yield without affecting other (nontarget) species. For the environment, the purported selectivity and localized effect of Bt-plants might be an improvement over the use of sprayed pesticides. According to the US Environmental Protection Agency, Bt-crops pose no significant risk to the environment or to human health (Mendelson et al. 2003). However, the specificity of Bt-plant defense has yet to be established (Dutton et al. 2003, 2005), especially because the mode of action of Cry proteins, both in target and nontarget organisms, is not fully understood (Brandt et al. 2004; Bravo et al. 2007; Crickmore 2005; Hilbeck and Schmidt 2006).

Many nontarget organisms, including herbivores, pollinators, parasitoids, and predators will directly and indirectly be exposed to transgene products and altered interactions (Andow and Hilbeck 2004; Dutton et al. 2003;

We subsampled a 50-kg bag of GM-maize and analyzed the expression of the *cry1Ab* gene with enzyme-linked immunosorbent assay (ELISA) on 15 samples from the bag (diluted 1:20–1:33), using a commercially available kit (Abraxis; http://www.abraxiskits.com). On average, the GM-maize expressed 67 (±27 SD) ng Cry1Ab toxin per gram of dried grain tissue. All negative controls and the UM-maize showed negative results.

From Ricroch et al. 2010

# Conclusion from Ricroch et al. 2010

- *"…for all stakeholders, raising the objectivity of the debate on GMOs is the most important request."*

# *D. magna* exposed to Vip3A for 10 days (Raybould and Vlachos 2011)



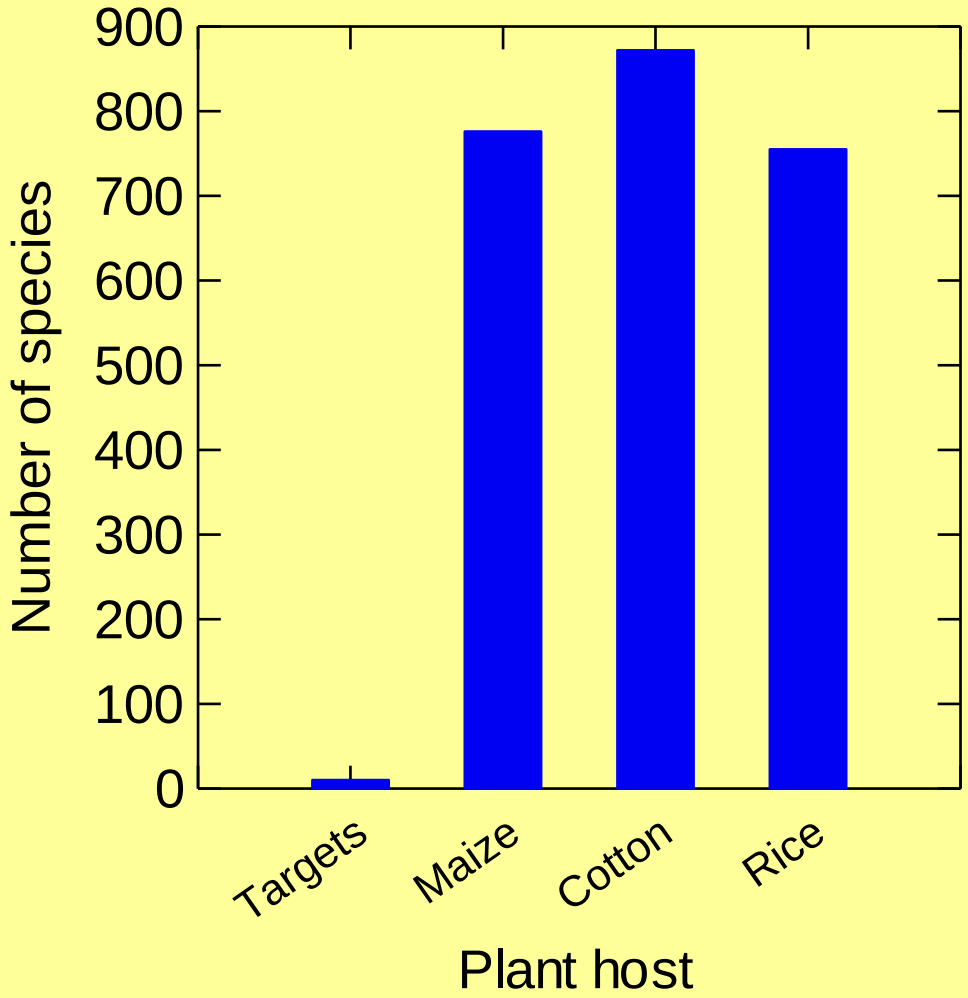
**Table 3** Summary of results of effects tests of Vip3A

| Test organism | Concentration or dose of Vip3A | Diet analysis | Endpoint | Positive control | Control (± S.D.) | Treatment (± S.D.) |
|---|---|---|---|---|---|---|
| *C. septempunctata* | 7250 µg Vip3Aa19/g diet | Nom (A/R) | Mortality | Y[1] | 0% | 5.0% |
| *C. maculata* | 7.24 µg Vip3Aa19/g diet | Nom (P) | Survival | Y[2] | 92 ± 6.9% | 93 ± 2.3% |
| *O. insidiosus* | 7250 µg Vip3Aa19/g diet | Y (EWB) | Mortality | Y[3] | 12.5% | 15.0% |
| *C. carnea* | 7250 µg Vip3Aa19/g diet | Y (EWB) | Mortality | Y[3] | 22.5% | 25.0% |
| *A. bilineata* | 500 µg Vip3Aa20/g diet | Y (EWB) | Fecundity[a] | Y[4] | 614 ± 236 | 528 ± 81 |
| *F. candida* | 43.1 µg Vip3Aa19/g diet | Nom (P) | Survival | Y[5] | 83 ± 15% | 83 ± 5% |
| | | | Fecundity[b] | | 66.2 ± 11.8 | 50.4 ± 13.8 |
| *E. foetida* | 3.60 µg Vip3Aa19/g soil | Nom (P) | Survival | Y[6] | 100% | 100% |
| | | | Weight increase | | +6.1 ± 1.3% | +7.4 ± 3.4% |
| *A. mellifera* | 50 µg Vip3Aa20/g diet | Nom (A/S) | Adult emergence | Y[4] | 86.8 ± 8.2% | 86.1 ± 13.2% |
| | 200 µg Vip3Aa20/g diet | Nom (A/S) | | | 86.8 ± 8.2% | 93.0 ± 1.8% |
| | 500 µg Vip3Aa20/g diet | Nom (A/S) | | | 86.8 ± 8.2% | 87.2 ± 6.9% |
| *C. virginianus* | 400 mg Vip3Aa1/kg body weight | Nom (G) | Mortality | N | 0% | 0% |
| | | | Body weight | | 171.70 ± 14.54 g | 169.70 ± 12.05 g |
| *M. musculus* | 1250 mg Vip3Aa20/kg body weight | Nom (G) | Mortality | N | 0% | 0% |
| | | | Body weight | | ♂ 39.3 ± 3.40 g | ♂ 40.3 ± 1.20 g |
| | | | | | ♀ 35.5 ± 1.60 g | ♀ 36.2 ± 1.00 g |
| *D. magna* | 752.6 µg Vip3Aa20/L | Nom (A/R) | Survival | N | 100% | 100% |
| | | | Fecundity[b] | | 13 ± 6 | 17 ± 9 |
| | | | Length | | 3.72 ± 0.10 mm | 3.46 ± 0.10 mm[c] |
| | | | Body weight | | 0.57 ± 0.10 mg | 0.46 ± 0.10 mg[c] |

"unlikely to harm aquatic invertebrates: the effect is small; it does not adversely affect mortality or fecundity…"

# The order Lepidoptera – a biodiversity perspective

- Target:
  - Single or a few pest species
- Non-target:
  - 180.000 species




(Natural History Museum Lepidopteran Database)

# Are GM crops affecting pollinators?

- Main pollinators:
  - Bees
  - Bats
  - Beetles
  - Birds
  - Butterflies
  - Flies
  - Moths
  - Wasps

- Main *Cry*-genes:

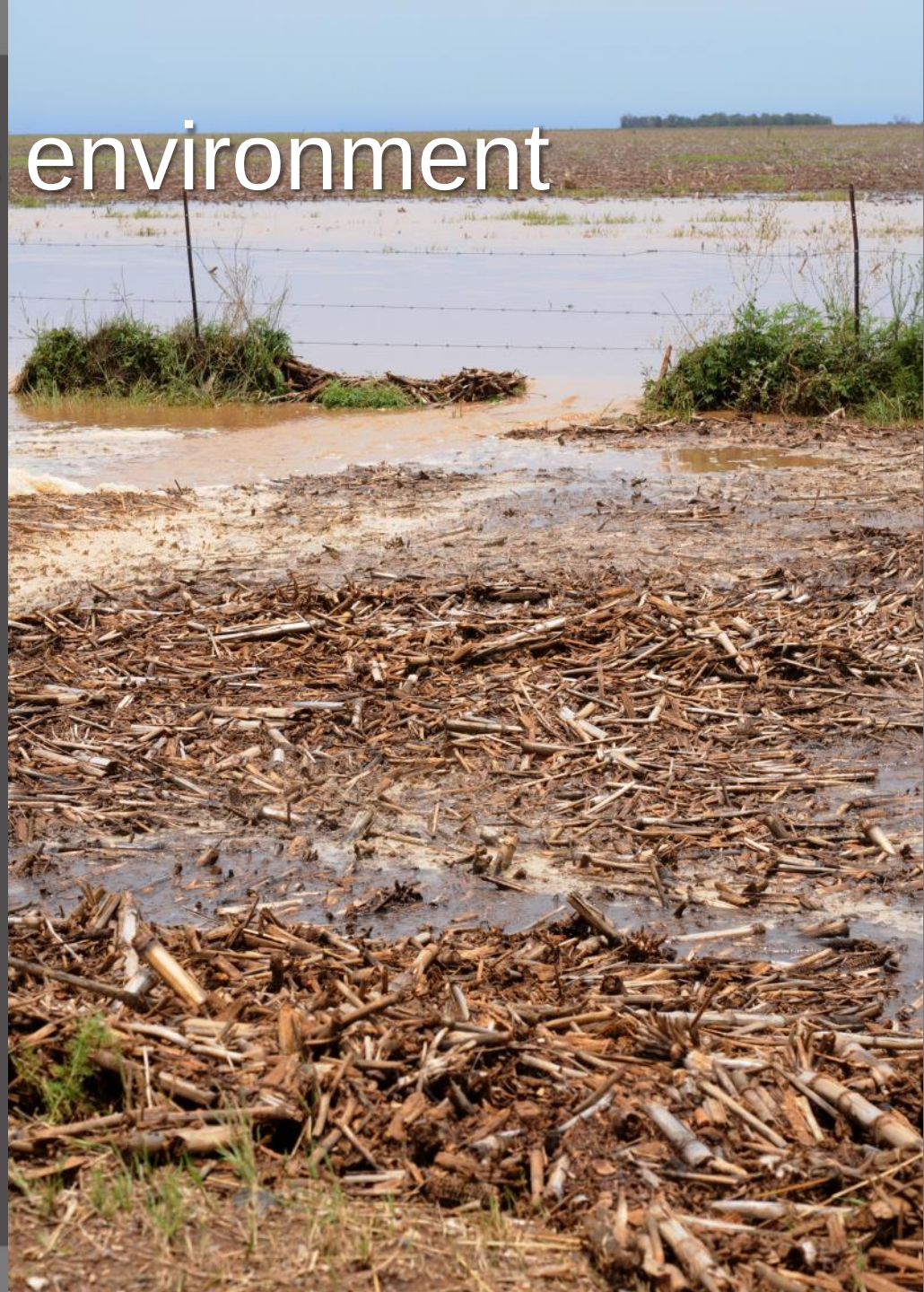
*Cry1Ab / Ac / F / Cry2*
*Cry9*

*Cry3A / 3Bb / 34 / 35*

*Cry4*

# The aquatic environment

- Main input energy to aquatic ecosystems
  - Allochtonous plant parts
  - Including Bt toxin(s)
  - Herbicide residues?
- Drift from spraying
  - Pesticides
  - Herbicides



# Conclusions

- Bt-maize
  - Reduced fitness in *Daphnia magna* fed Bt-maize
  - Meta-analysis: Confirmed negative effects of
    - Pesticides
    - Cry1Ab - not explained
- Evolution of resistance to Cry1Ab
  - The case of South Africa
    - Spraying pesticides on MON810
- Stacking of Bt and herbicide tolerance
- The aquatic ecosystem overlooked
- New research needed in combinatorial toxicology

# Further research in *D. magna*

**Toxicity**

- Roundup
- Dicamba
- 2,4-D
- Combinatorial tox

**Feeding studies to test GM plant quality**

- Soy
- Maize
- Multistack plants

**Data on phenotype**

- Survival
- Growth
- Fecundity
- Population effects

**Data on genotype**

- Transcriptomics
- miRNA
- Pathway analysis
- Link to Birmingham (DGC)

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(GMO)-discussion!


Regards from the Arctic!