Assessment of effects on Non-Target Organisms in EFSA's guidance

Salvatore Arpaia, EFSA GMO Panel

Italy

"EFSA and GMO risk assessment for Human and animal Health and the Environment" Brussels, 14-15 September 2009

“Non target” organisms

Non target organisms are defined as

all those species directly and/or indirectly exposed to the GM plant, and which are not the targets of the newly expressed metabolite(s) in these plants

Environmental Exposure to GM Plants

- AIR: Transgene escape **(via pollen) and its consequences on biodiversity**
- PLANTS: Effects on non target organisms
  - **Arthropods (herbivores, natural enemies, pollinators)**
  - **The wider environment:, Mollusca, rodents, birds, mammals**
- SOIL: Toxin production and transgene escape
  - **Horizontal gene transfer**
  - **Effects on soil organisms**

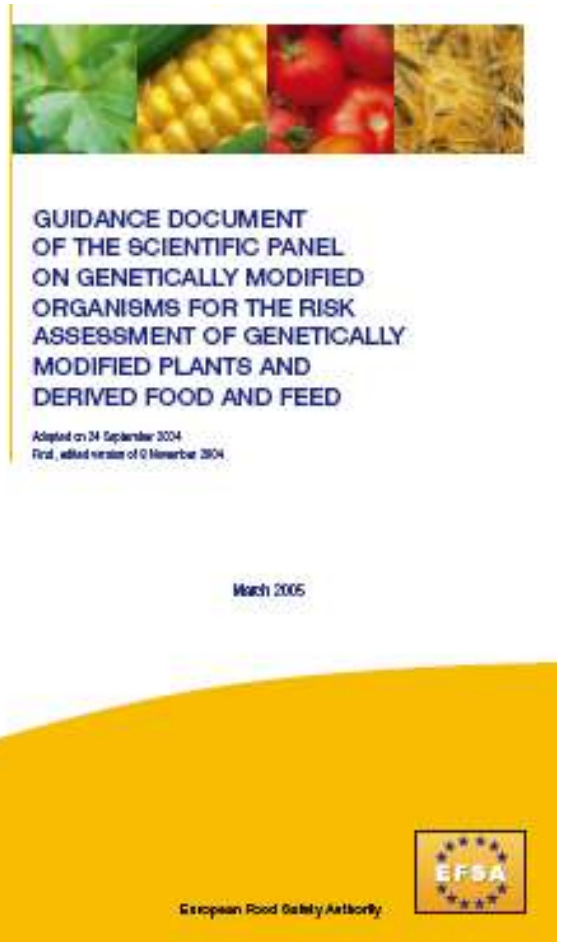
Agricultural Effects of GM Plants

- Gene escape /invasiveness
- Indesiderable effects on non-target organisms: damage to the ”ecosystem services” (Daily,1997):
  - Natural biological control
  - Pollination
  - Decomposition
- Soil functioning

Directly or through changes in agricultural practices

Guidance Document, EFSA 2006

- ✓ *Comparative assessment approach*
- ✓ *Intended and unintended effects, immediate and delayed effects*
- ✓ *Hazard identification (including trophic layer effects), Exposure Studies , Monitoring*
- ✓ *Case by case approach*



Case study MON810

- **26 pages of references, 322 documents were reviewed**

- **6.1.4. Interactions between the GM plant and non-target organis**
- 6.1.4.1. Natural enemies: predators and parasitoids
- 6.1.4.2. Non-target Lepidoptera
- 6.1.4.3. Pollinating insects: honeybees
- 6.1.4.4. Water-dwelling organisms
- 6.1.4.5. Soil organisms: earthworms
- 6.1.4.6. Soil organisms: enchytraeid worms
- 6.1.4.7. Soil organisms: nematodes
- 6.1.4.8. Soil organisms: isopods
- 6.1.4.9. Soil organisms: collembolans
- 6.1.4.10. Soil organisms: diplopods

- **125 pages of comments by Member States**
- **Safeguard Clauses from Austria, France, Germany, Greece, Hungary, Luxembourg**

***The EFSA Journal* (2009) 1149, 1-85**

ERA of the GM maize MON810

Questions of the EFSA GMO Panel

24/04/2008: 1st round questions from EFSA GMO Panel

- Molecular characterisation aspects

12/11/2008: 2d round questions from EFSA GMO Panel

- Food-feed aspects (lack litterature review)
- Considering European non-target Lepidoptera species likely to be found in and around maize crops, request to provide a comprehensive risk assessment including the levels of exposure to Cry1Ab toxin and potential impacts on populations.

11/02/2009: 3d round questions from EFSA GMO Panel

- Food-feed aspects (updates bioinformatics-supported studies)

ERA of the GM maize MON810

Delegation to the Spanish Competent Authority

09/05/2008: 1st round questions from Spanish CA

- IRM
- lack recent publications about i) the likelihoods of the occurrence of the potential adverse effects, ii) laboratories and field studies in European countries or with European organisms
- lack of references to PMEM in different European countries

18/07/2008: 2d round questions from Spanish CA

- further information on the assessment of risks to NT Lepidoptera in representative EU maize growing regions
- further analysis of studies on potential effects on NTOs + organised by exposure/effect assessment on NTOs/ecological prossese (predators, decomposers, pollinators)
- Revision of the PMEM plan proposed

Hazard characterization

- NATURAL ENEMIES: In general, invertebrate parasitoids appear to be more sensitive than predators. Mechanisms direct/indirect effects
- NON TARGET HERBIVORES: Larvae of a range of lepidopteran species are susceptible to the Cry1Ab toxin
- WATER-DWELLING ORGANISMS: Trichoptera might be susceptible to the Cry1Ab toxin
- POLLINATORS: no toxic effects of Cry toxins on the health of honeybees and bumblebees

Exposure

- NATURAL ENEMIES
  - Expected abundance of non-target invertebrates:

    Near-isogenic control fields>Bt>Insecticides
- POLLINATORS: In most cases, the proportion of maize pollen as a total of all pollen collected during a summer will be low. Moreover, due to the low concentration of Cry1Ab in MON810 pollen, honeybees will only be exposed to very low concentrations of the toxin
- WATER-DWELLING ORGANISMS: due to the low level of Cry proteins in aquatic systems, exposure of Trichopterans in aquatic ecosystems is likely to be very low.
- SOIL ORGANISMS GM Plants may induce changes in species assemblages, but these usually follow within ranges of natural variability (due to e.g. plant variety, soil type, chemical composition, etc.)

Exposure: Non target herbivores

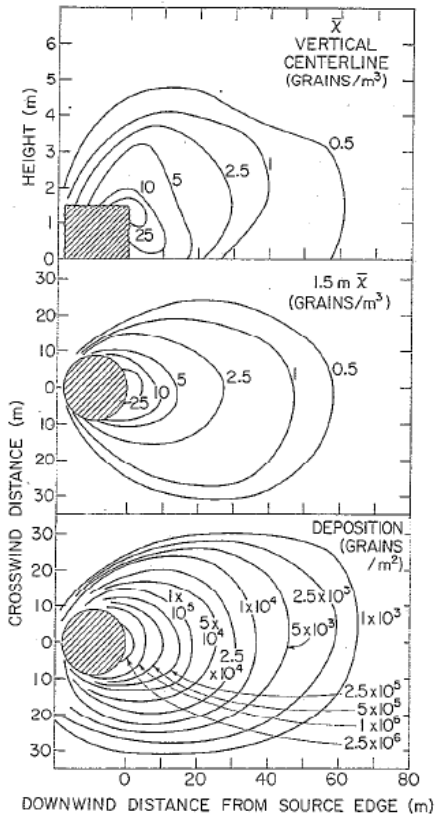


Fig. 1. Typical concentration ($\overline{\chi}$) and deposition patterns from a corn pollen area source represented by the shaded figures. Concentrations are shown in the horizontal at a height of 1.5 m and in the vertical along the plume centerline.

Losey et al., 1999 Nature Corn pollen lethal to monarch larvae

P.N.A.S., October 2001

Hellmich et al. Monarch larvae sensitivity to Bacillus thuringiensis-purified proteins and pollen PNAS 98: 11925-11930

Oberhauser et al Temporal and spatial overlap between monarch larvae and corn pollen PNAS 98: 11913-11918

Pleasants et al Corn pollen deposition on milkweeds in and near cornfields PNAS 98: 11919-11924

Model of exposure for three European species of Lepidoptera (Perry et al., submitted)

Full model predicts proportion suffering mortality is:

M = yzvxa (25e√Ch + mfg) / (25e√C + fD)

The model was run with parameter estimates submitted by the EFSA GMO Panel Environment Working Group.

100% exposure is reduced successively by multiplying by proportions representing various effects

7 parameters largely specific to the particular area / host-plant combination being modelled

4 parameters specific to the species modelled.

Regions and species considered in the model

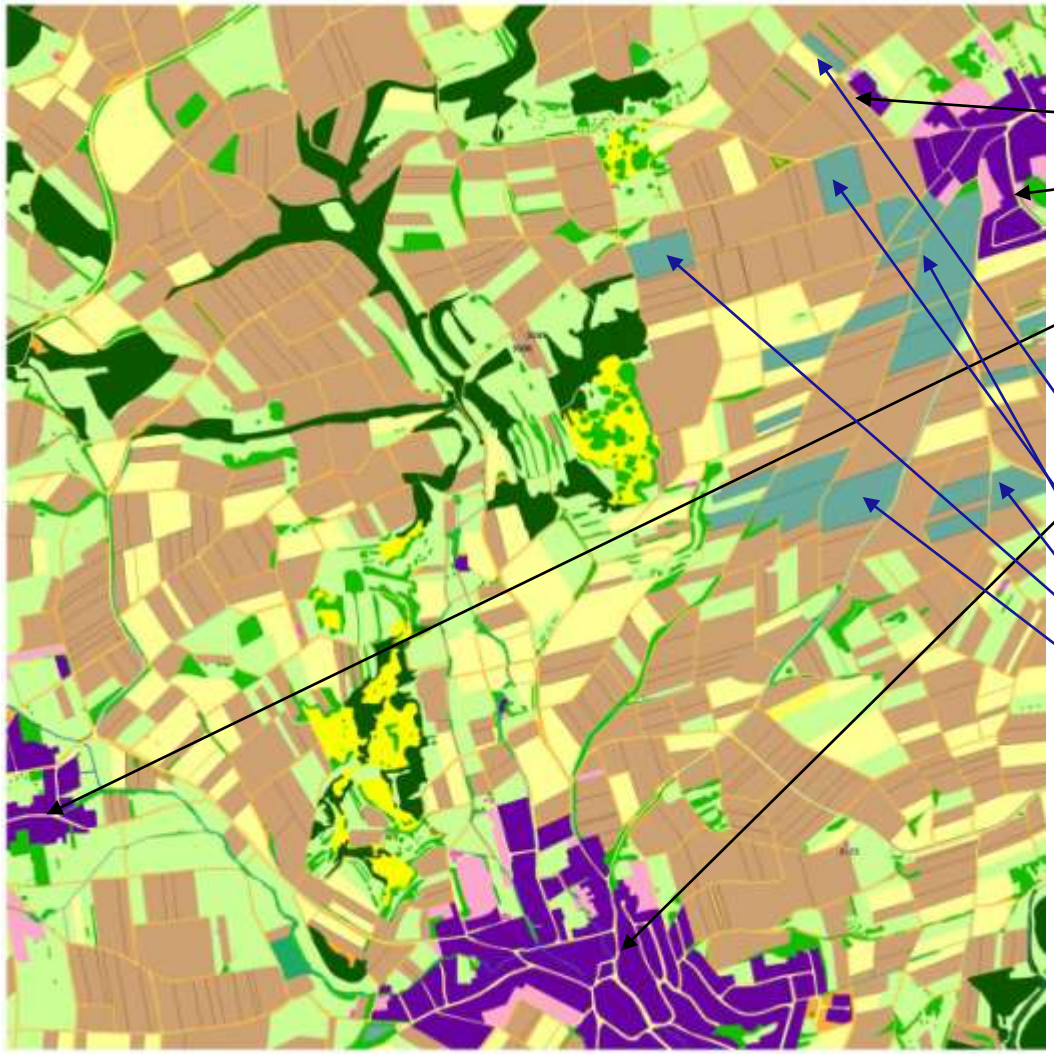
Inachis io

Vanessa atalanta

Plutella xylostella

Germany	Oderbruch, Achen, Grebbin, Berkatal, Upper Rhine Valley
Italy	Po Valley
Hungary	Tolna County
Spain	Madrid, Catalunya

Area-specific parameters



proportion of arable fields in maize, *z*

proportion of maize that is *Bt*, the 'uptake' parameter, *v*

proportion of the host-plant found within arable fields and their margins, *y*

Area-specific parameters

Average extent of field margin is *D* metres from edge of field

2 metre band, for *Inachis,* within which there is *some* mortality from *Bt* pollen

Maize field *C* hectares

density of host-plant = *e* per square metre within field

density of host-plant = *f* per square metre in field margin

Species-specific parameters

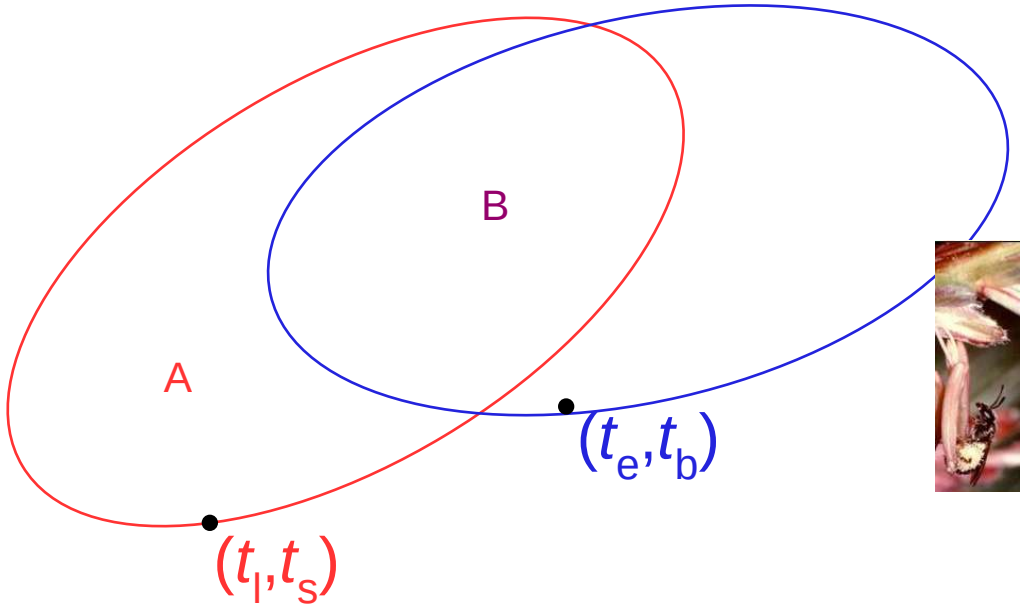
Time of start of period

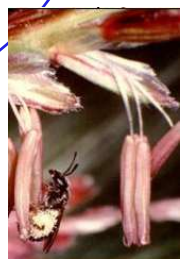
Any point in area A is entirely within the ellipsoid for instar development, but outside that of maize pollen shed. Any point in area B is within both ellipsoids.

The larger the proportion B/(A+B) the larger is the overlap, and vice-versa – this proportion is

the temporal overlap parameter, a

susceptible instar development period





Time of end of period

Species-specific parameters

physical effects parameter,

X,

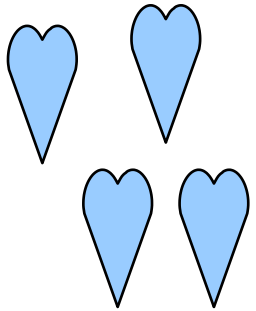
proportion of larvae remaining exposed after allowance for:

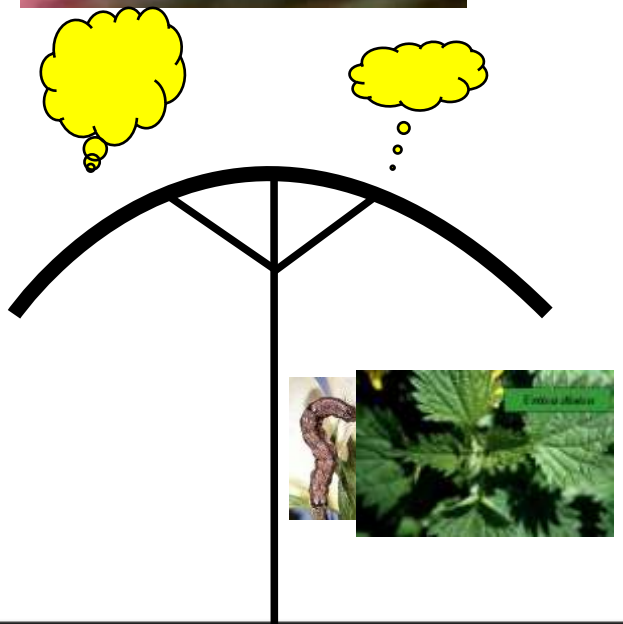
larvae feeding on underside of leaves,

'shading' of lower leaves by upper,

rain washing pollen off leaves,

etc.,





Death in the field and margins

proportion of larvae suffering mortality in the field –

worst-case scenario, h

proportion of larvae suffering mortality in the 2m marginal band –

worst-case scenario, g

Conclusions

Variability in estimated mortality and sublethality results from (i) natural variation between areas; (ii) differences between experts' estimates; (iii) uncertainties arising from variation between (the limited number of) datasets.

For the majority of areas for *I. io* and *V. atalanta*, the best estimate for mortality was less than one individual in every 1800, and of sub-lethality was less than one individual in 550.

For the majority of areas for *P. xylostella*, the best estimate for mortality was less than one individual in every 300, and of sub-lethality was less than one individual in 100,

Under worst-case scenario of maximum uptake of MON810 maize by growers (80%).

The amounts of MON810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae. (0,5% additional mortality for *P. xylostella*)

The GMO Panel is aware that all modelling exercises are subject to uncertainties and further data are required to reduce the variability of the estimates reported here.

General Conclusions on NTOs

1. The EFSA GMO Panel is of the opinion that maize MON810 will not cause reductions to natural enemies that are **significantly greater than those caused by pesticides used to control corn borers**.
2. The amounts of MON810 pollen grains found in and around maize fields are unlikely to adversely affect a **significant proportion of non-target lepidopteran larvae**
3. The likelihood of adverse effects on bees is expected to be negligible.
4. There is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on soil organisms.

Recommendations

- The EFSA GMO Panel considers it advisable that, especially in areas of abundance of non-target Lepidoptera populations, **the adoption of the cultivation of maize MON810 be accompanied by management measures in order to mitigate the possible exposure** of these species to MON810 pollen.
- As an example, the planting of border rows of non-Bt-maize adjacent to uncultivated field margins of maize MON810 fields, could limit the exposure to those individuals feeding on weeds present within maize field borders and also could contribute to the required percentage of non-Bt-maize necessary to constitute refuge areas for lepidopteran target pests in the framework of resistance management plans.

***The EFSA Journal* (2009) 1149, 1-85**

Conclusions

- The development of resistance of the corn borers *O. nubilalis* and *Sesamia* spp. has been identified as a risk
  ⇒ IRM under case-specific monitoring is recommended
- Mitigation measures for non-target Lepidoptera
- No specific measures for other taxa

- The development of resistance of the corn borers *O. nubilalis* and *Sesamia* spp. has been identified as a risk
  => IRM under case-specific monitoring is recommended
- For non-target Lepidoptera, effects have to be considered more deeply in Monitoring plans.
- No specific measures for other taxa

Transgene Product	Number of cases (% in parentheses)				
	Negative significant	Negative n.s.	Neutral	Positive n.s.	Positive significant
Predators					
Cry1Ab/c/2A	69 (21)	67 (21)	166 (51)	12 (4)	10 (3)
C. carnea only	55 (30)	35 (19)	89 (49)	4 (2)	0 (0)
Without *C. carnea*	14 (10)	32 (23)	77 (55)	8 (6)	10 (7)
Cry 3A/Bb	3 (4)	13 (18)	45 (62)	11 (15)	1 (1)
GNA/CpTI, OCI *	19 (26)	10 (14)	28 (38)	9 (12)	7 (10)
Parasitoids					
Cry1Ab/c	61 (37)	17 (10)	78 (47)	7 (4)	2 (1)
Cry1Ab/c+CpTI *	22 (44)	12 (24)	15 (30)	1 (2)	0 (0)
Other Cry toxins	8 (47)	3 (18)	5 (29)	0 (0)	1 (6)
GNA/CpTI, OCI *	66 (23)	57 (20)	134 (46)	22 (8)	12 (4)

Lovei, Andow & Arpaia, 2009. Environ. Entomol: 38(2): 293-306

How Many Species have been tested?

Arpaia, in press.

efsa
European Food Safety Authority

Functional group	Order	Family	No. of species
Predators	Heteroptera	Anthocoridae	4
Predators	Heteroptera	Nabidae	1
Predators	Heteroptera	Geocoridae	2
Predators	Heteroptera	Miridae	2
Predators	Heteroptera	Reduvidae	1
Predators	Heteroptera	Pentatomidae	1
Predators	Coleoptera	Coccinellidae	9
Predators	Coleoptera	Carabidae	17
Predators	Neuroptera	Chrysopidae	1
Predators	Araneae	Araneidae	2
Predators	Acarina	Phytoseidae	1
Parasitoids	Hymenoptera	Braconidae	8
Parasitoids	Hymenoptera	Ichneumonidae	3
Parasitoids	Hymenoptera	Eulophydae	1
Parasitoids	Hymenoptera	Aphelynidae	1
Parasitoids	Hymenoptera	Encyrtidae	1
Parasitoids	Hymenoptera	Trichogrammatidae	1
Pollinators	Hymenoptera	Apidae	5

Need to update (Tabiano Colloquium, 2007)

- EFSA established a self-tasking working group on NTO with the aim of:

(1) producing a scientific review of the current guidance document of the EFSA GMO Panel for Environmental Risk Assessment, focusing on the potential impacts of GM plants on NTOs;

(2) proposing criteria for NTO selection; and

(3) advising on standardised testing methodologies.

- ✓ General update of the GMO Panel ERA Guidance Document
- ✓ Non-target organisms
- ✓ Long-term effects
- ✓ Receiving environments
- ✓ Farming practices
- ✓ Field trials
- ✓ General discussion

Self-tasking Working Group on environmental impacts of GM plants on Non-Target Organisms (NTOs)

- At the end of its 2-year mandate, the self-tasking working group will prepare a scientific opinion as well as specific NTO guidelines to update the overall ERA guidance document
- Focus on arthropods and some invertebrates

Deadline	Deliverable
March 2009	Intermediate report
May 2009	Review by referees
October 2009	Adoption by GMO Panel
December-January	Public Consultation
March 2010	Final document

Species selection

- Not everything can be tested
- Not every species/process is equally important
- Finite resources are available for biosafety testing
- Current practice for selecting test organisms (surrogate species):
  - What is available (parasitoids: *Cotesia flavipes*), already used in ecotoxicological tests (*Daphnia magna*, springtail *Folsomia* candida), team has experience with organism (green lacewing, *Chrysoperla* spp.). Abundance/widespread distribution (Cowgill & al. 2003)

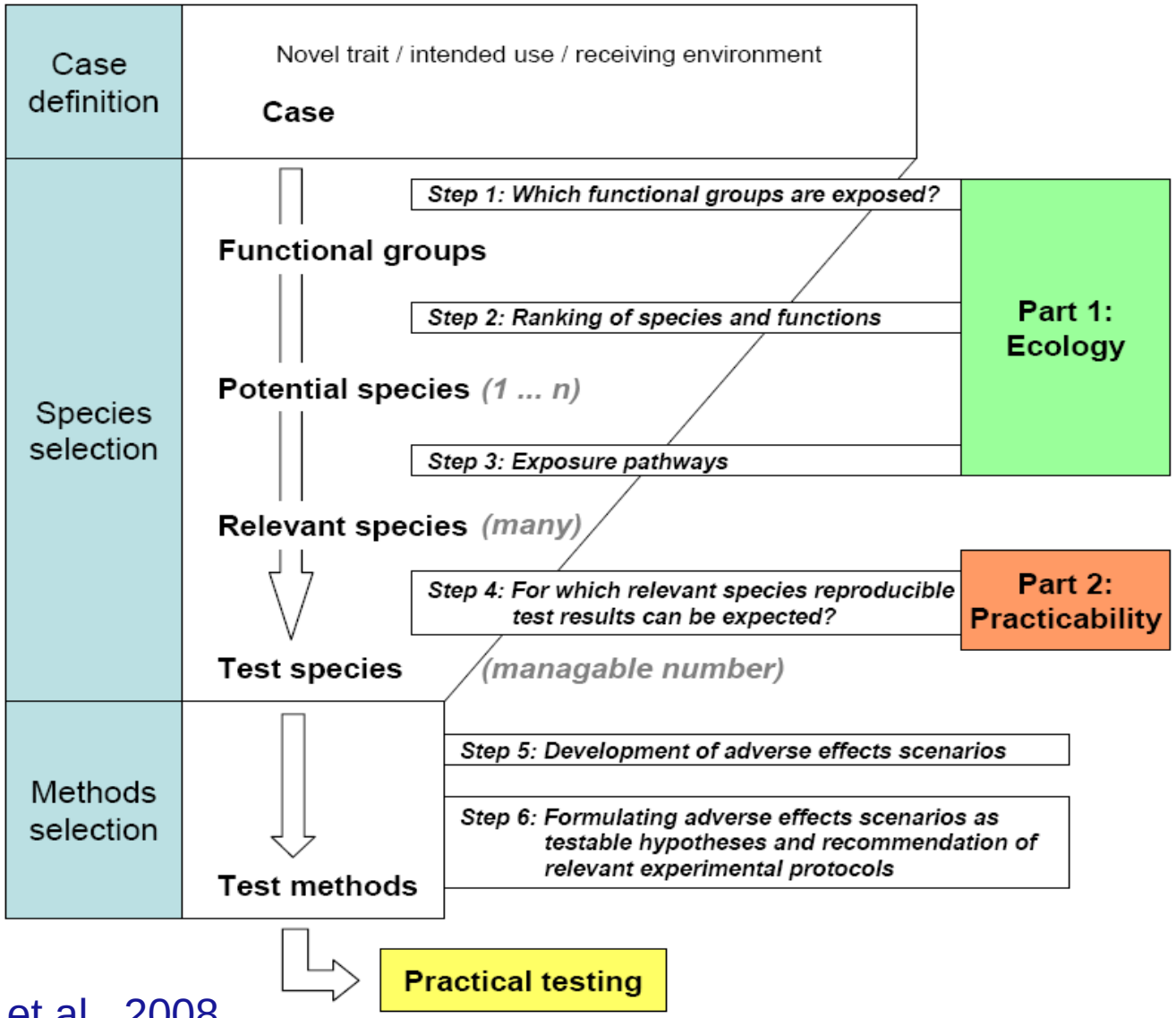
Selection of "**focal species**"

European Food Safety Authority

Preserving the functional biodiversity

- ✓ Herbivores
- ✓ Predators
- ✓ Parasitoids
- ✓ Pollinators, pollen feeders
- ✓ Decomposers
- ✓ Species of conservation/cultural concern

Selection of “focal species”

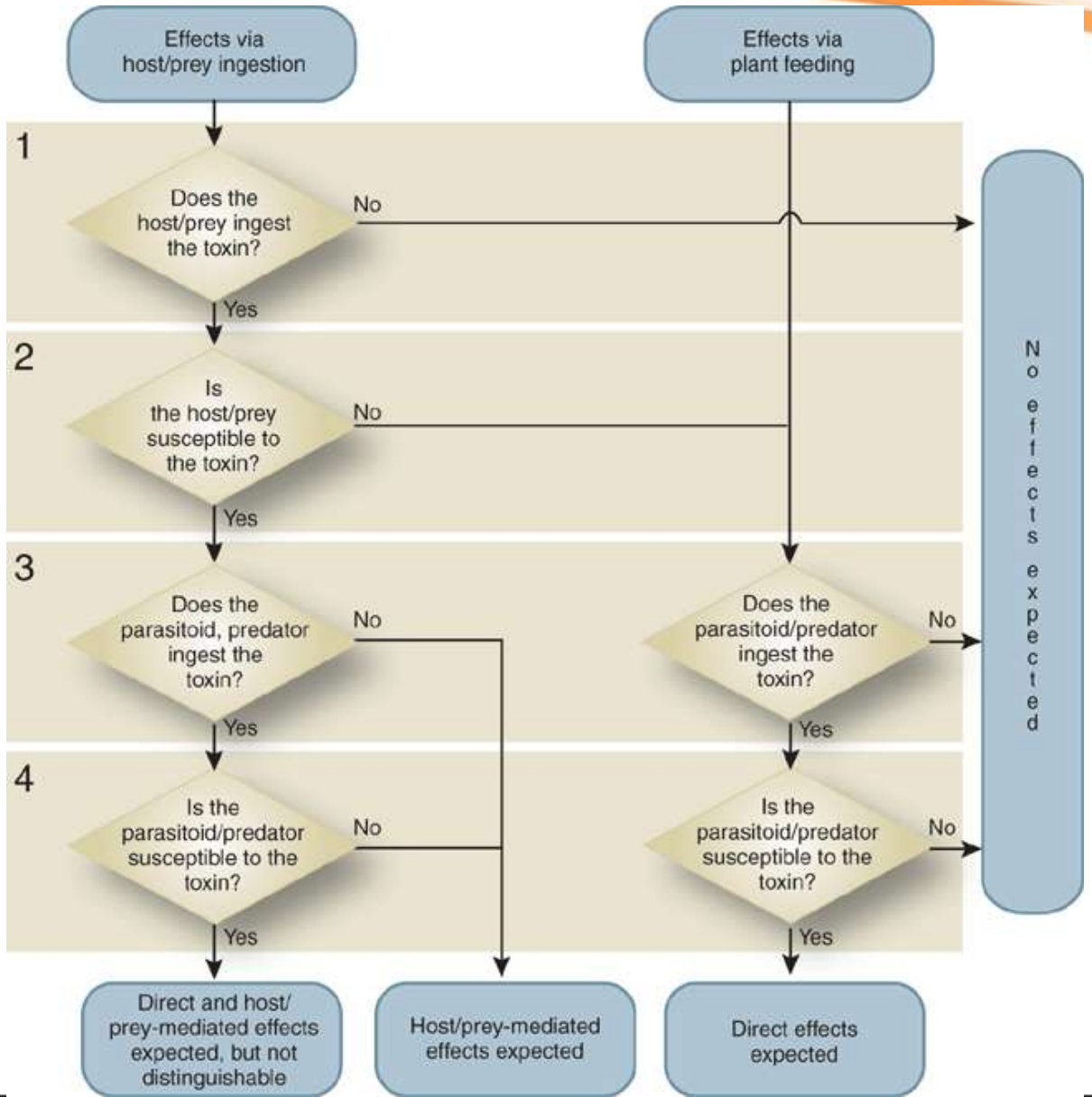


after Hilbeck et al., 2008

Prioritization Criteria

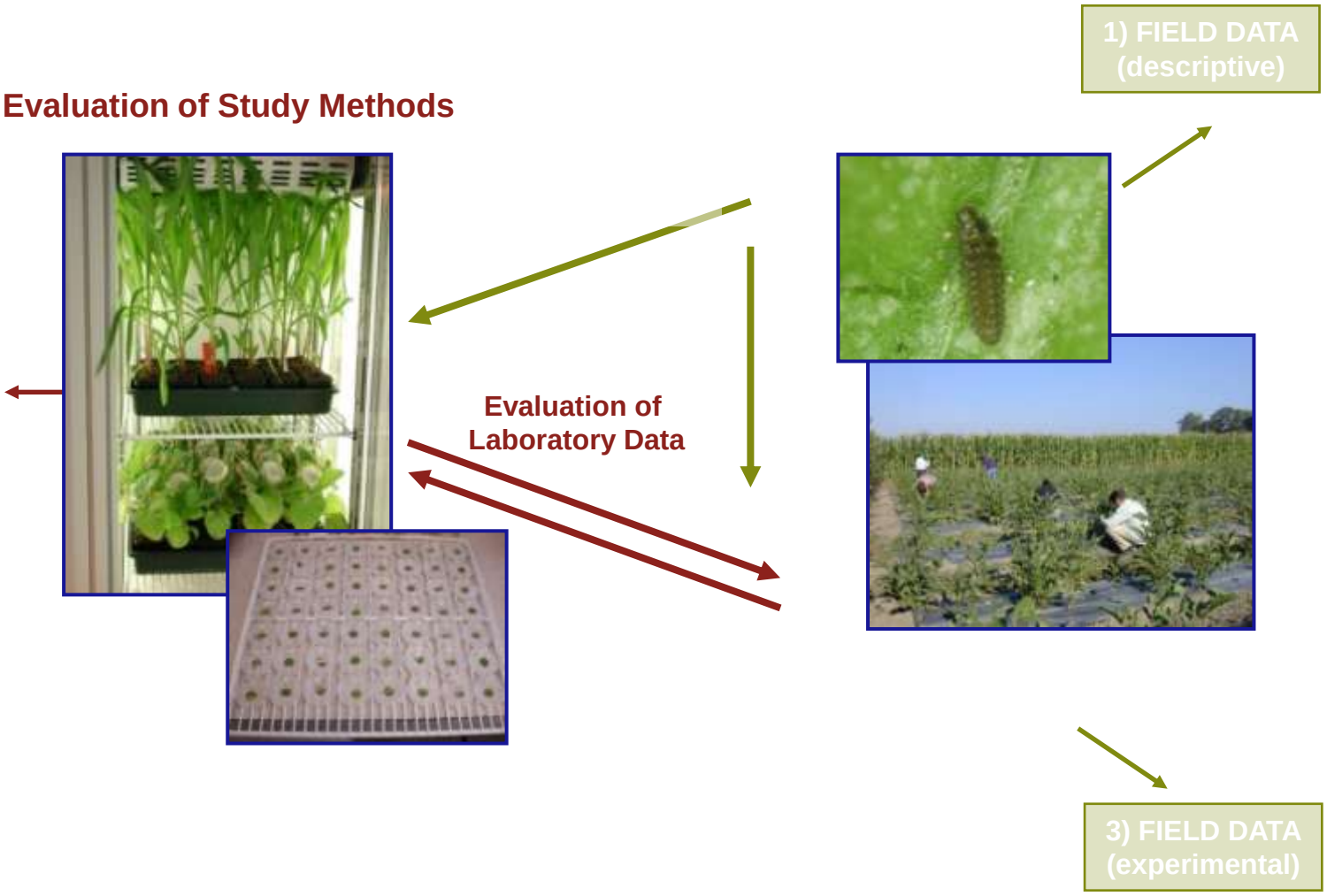
- ✓ **Diet regimes** (e.g. larvae vs. adults, mixed feeding by certain carabids, coccinellids, *Orius* spp, etc.).
- ✓ The **occurrence/presence** of NTOs/arthropods (considering specifically exposed life stages) during the most likely period of exposure;
- ✓ **Ecological significance** of the species;
- ✓ **Abundance** of the species;
- ✓ **Susceptibility** of NTOs (i.e. are certain populations already threatened and thus more sensitive to additional pressures?);

Tiered Approach (Romeis et al., 2006)

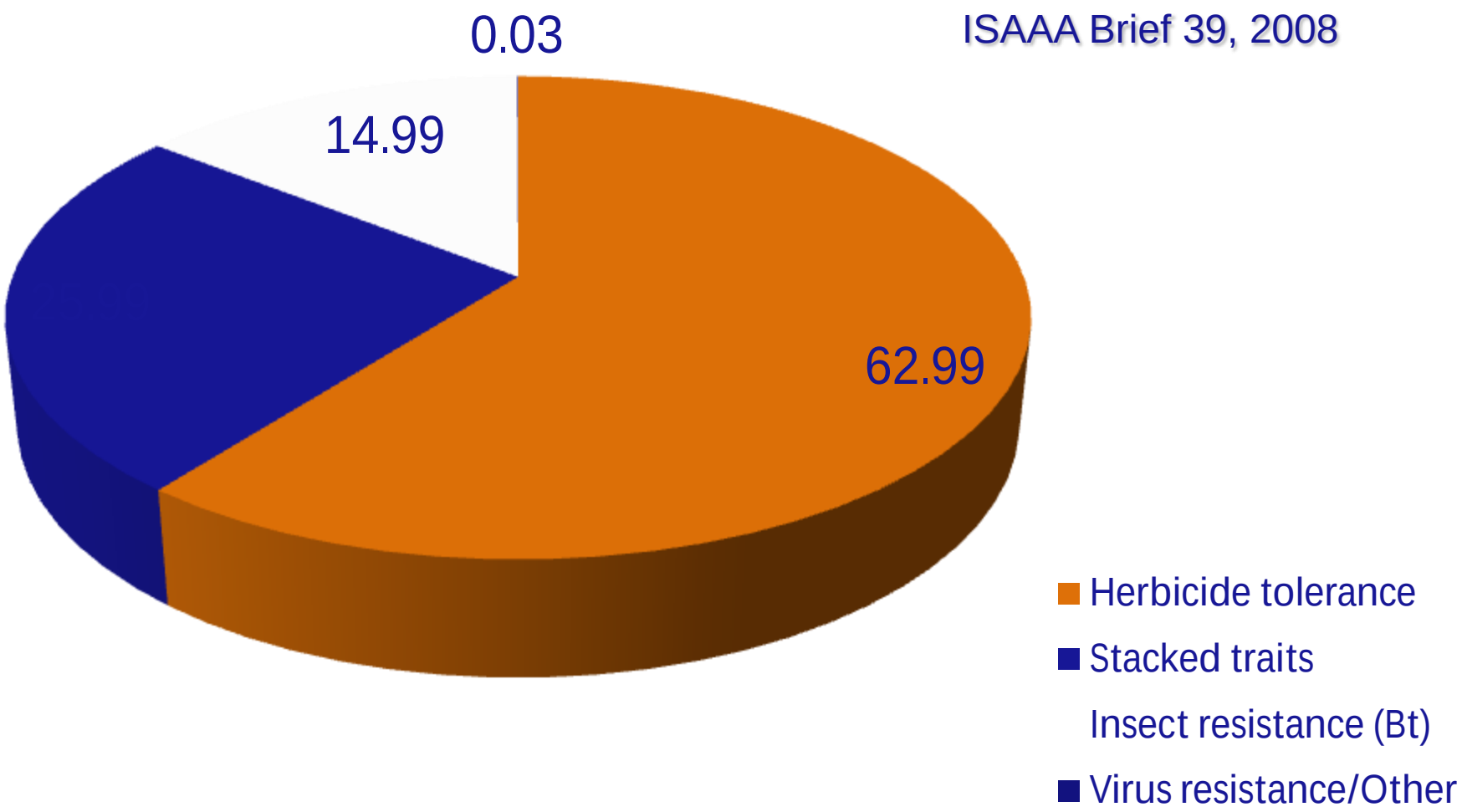


Ecological approach

Andow & Hilbeck, 2004



Adoption of GM Crops by trait (%) - 2008



Hypothesis driven

- **Specific hypothesis-driven investigation**
  - Tiered approach including “in-planta” tests, tritrophic exposure and measurement endpoints for sub-lethal effects
- **General hypothesis-driven investigation (possible impacts on ecosystem functions)**
  - the optimal standard would be field trials under the design requirements defined in the updated GD. Semi-field trials, extended compositional analysis, modelling, and any additional information might be considered case by case (Case studies are currently being examined).

Thank you for your attention

efsa
European Food Safety Authority

Salvatore & Co.