Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Economic Affairs,
Education and Research EAER

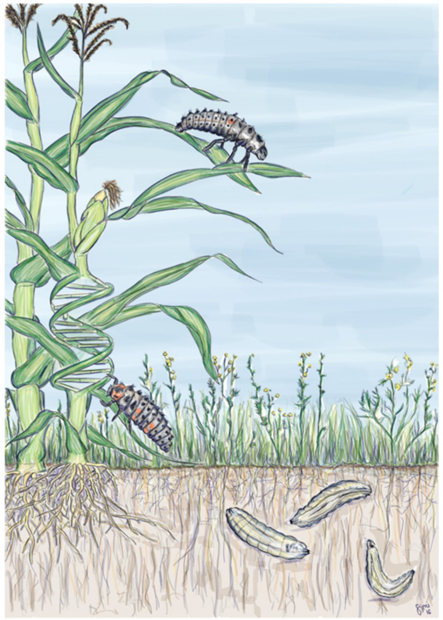
Agroscope

Agroscope

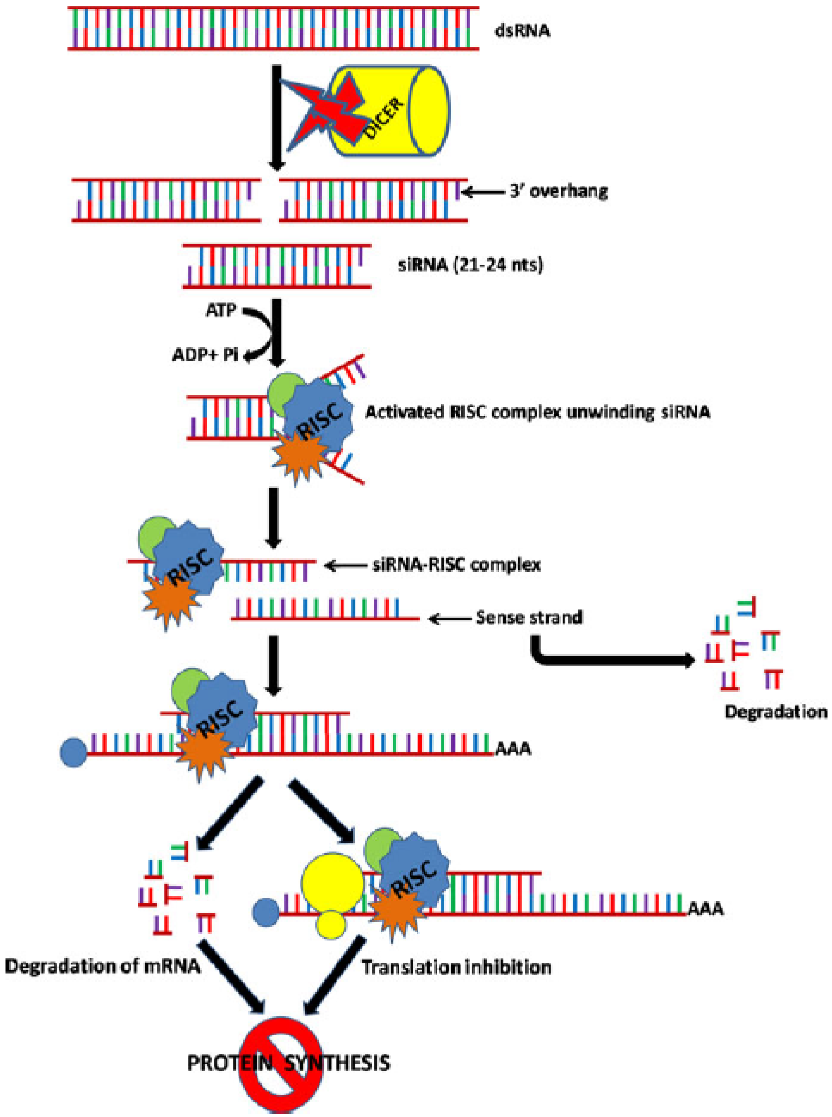
Problem formulation for the ERA of insecticidal RNAi-based genetically modified plants and RNAi-based pesticides: Effects on non-target arthropods

Jörg Romeis

Agroscope, Zurich, Switzerland



RNAi – how does it work ?



Endogenous **sequence-specific gene silencing** mechanism elicited by small RNA molecules

- dsRNA is ingested
- Processed by DICER into siRNA (21-25 bp)
- Incorporated into RISC
- siRNA-RISC complex targets similar sequence in mRNA(degradation or inhibition of translation)
- Protein synthesis is blocked

Jagtap et al. (2011) Naturwissenschaften 98, 473-492

Problem formulation

What do we not want to see harmed? What must be protected?

➡ *Protection goals*

Can we envision a way in which they could be harmed?

➡ *Pathway to harm*

How can we assess whether they are likely to be harmed?

➡ *Development of risk hypotheses and a plan to test them*

Gray (2012) Collection of Biosafety Reviews 6, 10-65

Protection goals

Millennium Ecosystem Assessment

Published online: August 11, 2015

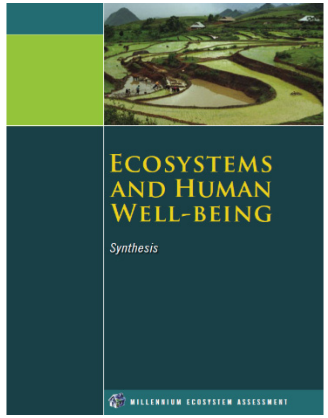
Science & Society

EMBO reports

Optimising environmental risk assessments

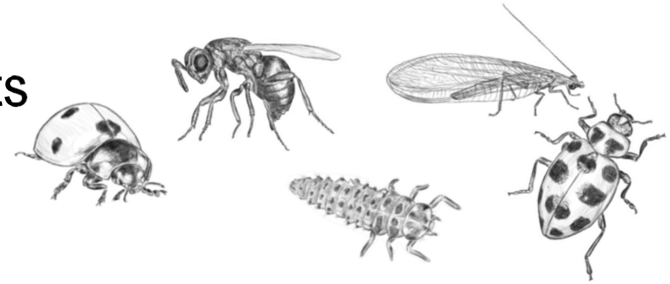
Accounting for ecosystem services helps to translate broad policy protection goals into specific operational ones for environmental risk assessments

Yann Devos[1], Jörg Romeis[2], Robert Luttik[3], Angelo Maggiore[4], Joe N Perry[5], Reinhilde Schoonjans[4], Franz Streissl[6], José V Tarazona[6] & Theo CM Brock[7]



Regulating services

- **Biological control** of arthropod pests
- Pollination



Cultural services

- Protected butterflies

Supporting services

- Nutrient cycling, decomposition

Agroscope

Pathway to harm

Cultivation of dsRNA-producing GM plant

Application of dsRNA-containing spray product

Biological control function is disrupted

Pathway to harm

Cultivation of dsRNA-producing GM plant

dsRNA present in pollen

Herbivores contain dsRNA

Predators consume pollen

Predators consume herbivores

Predators ingest (active) dsRNA

dsRNA causes adverse effects

Population of predators is reduced

Biological control function is disrupted

Pathway to harm

Cultivation of dsRNA-producing GM plant

Application of dsRNA-containing spray product

dsRNA present in pollen

Herbivores contain dsRNA

Predators covered by spray

Predators consume pollen

Predators consume herbivores

Predators groom

Predators ingest (active) dsRNA

dsRNA causes adverse effects

Population of predators is reduced

Biological control function is disrupted

Agroscope

Testable risk hypotheses

Cultivation of dsRNA-producing GM plant

Application of dsRNA-containing spray product

Focus Exposure

- dsRNA molecule is not produced in plant pollen
- Predators do not consume plant pollen
- Predators not covered by spray
- Herbivores do not contain (active) dsRNA

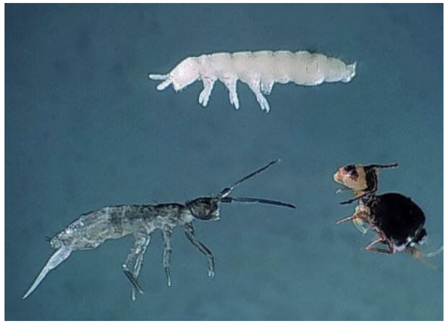
Focus Hazard/ effect

- Predators are not affected by consumed dsRNA
- Effects do not result in population declines
- Population declines do not lead to a disruption of the biological control function

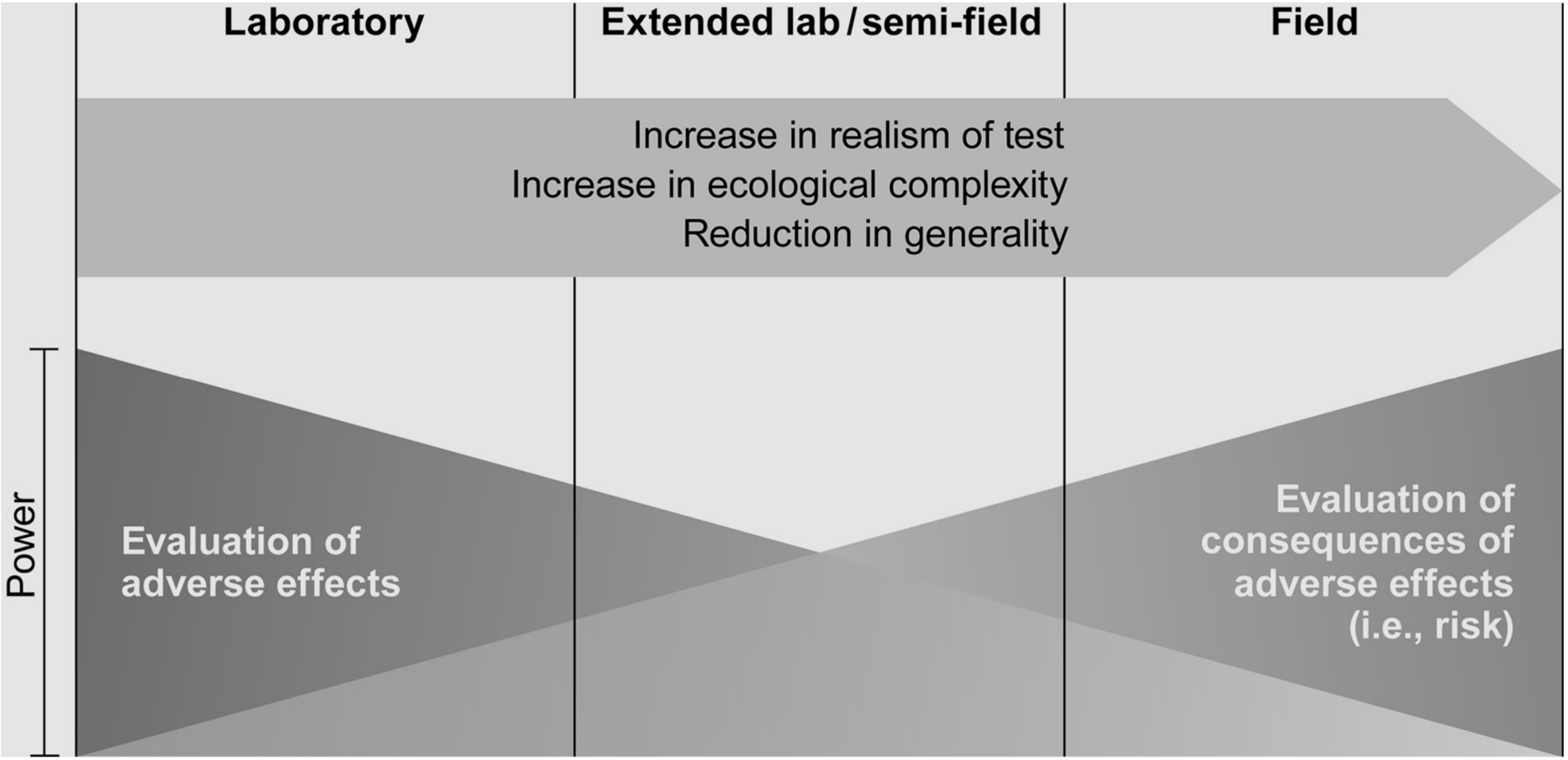
Agroscope

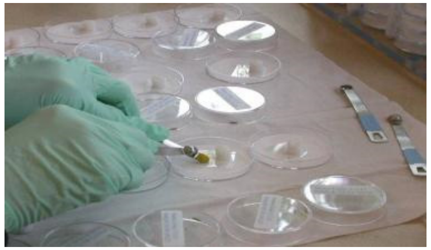
Testable risk hypothesis

"*The dsRNA does not affect valued non-target arthropods at the concentration present in the field* "

Tiered risk assessment





Romeis et al. (2008) Nature Biotechnology 26, 203-208

Ingested dsRNA could cause …

- off-target effects
  - Silencing of any gene in a non-target organism
- general immune stimulation
- saturation of the RNAi machinery as consequence of the ingestion of high doses of dsRNA

> ➢ Conduct feeding studies with dsRNA, similar to insecticidal Cry proteins
>
> ➢ Consider mortality and sublethal endpoints

Lundgren & Duan (2013) BioScience 63, 657-665; Roberts et al. (2015) Front. Plant Sci. 6, 958

Selection of test species for GMP

EFSA (2010) "Considering that not each of these species can be tested, a representative subset of NTO species […] shall be selected, on a case-by-case basis."

- **representative** of **valued taxa** and **functional groups** that are **most likely to be exposed**
- species **most likely to be sensitive** to the test compound *(considering mode of action, known toxicity)*
- **Amenability** and **availability** to testing

Romeis et al. (2013) Chemosphere 90, 901-909

Agroscope

Selection of test species for GMP

NTOs most likely to be sensitive:

- Arthropod orders differ in their sensitivity to dietary RNAi. Coleoptera are more sensitive then other insect orders *(Baum and Roberts, 2014, Adv. Insect Physiol. 47, 249-295)*
- **Pylogenetic relationship** of NTO to target is important *(Bachmann et al., 2013, Transgenic Res. 22, 1207-1222)*
- **Bioinformatic analyses** help predict NTO effects: analyse sequence complementarity between pool of siRNA and genome or (target) gene in NTO *(Roberts et al., 2015, Front. Plant Sci. 6, 958)*

Selection of test species for PPP

- Data requirements are provided in Commission Regulation (EU) No 283/2013 and 284/2013 for the approval of *active substances* and the authorisation of *plant protection products*
- Commission Regulation (EU) 546/2011 and Regulation (EC) 1107/2009 of the European Parliament and of the Council define uniform principles for evaluation and authorisation of plant protection products incl. defined set of test parameters and endpoints!
- SANCO, Draft Guidance Document on Terrestrial Ecotoxicology, 2002
- ESCORT 2, SETAC, Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection products with Non-target Arthropods, 2001

Selection of test species for PPP

Fixed set of NTO test organisms

(birds, mammals, earthworms, fish, aquatic invertebrates)

Terrestrial arthropods

- Honey bees (pollinator; Hymenoptera)
- **Beneficial arthropods other than bees**
  - *Aphidius rhopalosiphi* (parasitoid; Hymenoptera)
  - *Typhlodromus pyri* (predatory mite; Acarina)

Aphidius rhopalosiphi © INRA, Bernard Chaubet

Selection of test species for PPP

European Food Safety Authority

EFSA Journal 2015;13(2):3996

SCIENTIFIC OPINION

Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]

- **Address oral exposure** of NTAs to PPP
- Difficult to relate currently available **endpoints** from tier 1 assessments (glass-plate tests) to realistic exposure
- **Standardised tests** for addressing oral exposure are missing
- Test systems should
  - cover **exposure routes** particular to the active substance
  - allow detection of effects resulting from **specific/ novel modes of action**

Agroscope

Conclusions

- Problem formulation helps to focus the risk assessment
- Regulatory framework established for GMPs works also for plants producing dsRNA
- Case-by-case approach allows to select most appropriate test species
- Test systems for oral exposure are available
- To detect non-target effects lethal and sublethal endpoints should be recorded
- The established NTO test list for PPP is not sufficient to test for non-target effects of dsRNA spray products

Thank you for your attention

Drawing by Simone Haller

joerg.romeis@agroscope.admin.ch

Agroscope