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

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RNAi – how does it work?

- 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

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

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

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

Cultural services
- Protected butterflies

Supporting services
- Nutrient cycling, decomposition
Pathway to harm

Cultivation of dsRNA-producing GM plant

Application of dsRNA-containing spray product

Biological control function is disrupted
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

- dsRNA present in pollen
- Predators consume pollen

Herbivores contain dsRNA

- Predators consume herbivores
- Predators covered by spray
- Predators groom

Predators ingest (active) dsRNA

- dsRNA causes adverse effects

Population of predators is reduced

Biological control function is disrupted
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
"The dsRNA does not affect valued non-target arthropods at the concentration present in the field"
Tiered risk assessment

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Extended lab/semi-field</th>
<th>Field</th>
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<tbody>
<tr>
<td></td>
<td>Increase in realism of test</td>
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<td>Increase in ecological complexity</td>
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<td>Reduction in generality</td>
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<tr>
<td>Evaluation of adverse effects</td>
<td>Evaluation of consequences of adverse effects (i.e., risk)</td>
<td></td>
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</tbody>
</table>

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

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
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 than 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.


• SANCO, Draft Guidance Document on Terrestrial Ecotoxicology, 2002

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)
Selection of test species for PPP

- **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**
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