Environmental risk assessment of RNAi-based plant protection

A literature review

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Introduction

- RNA interference (RNAi) means gene silencing in organisms caused by specific double-stranded RNA (dsRNA) or other small RNA molecules
- RNAi is a promising approach for crop protection, due to expected low environmental persistence of RNA molecules and potentially high specificity
- While genetically modified plants using RNAi against insect pests have not applied for cultivation in the EU, first RNAi plant protection products are expected to seek approval in the EU in the near future

Objectives

- Summarize methods to analyse environmental fate and hazards of small RNA molecules used for RNAi
- Compare ERA frameworks for GMP and PPP
- Identify knowledge gaps

Literature review

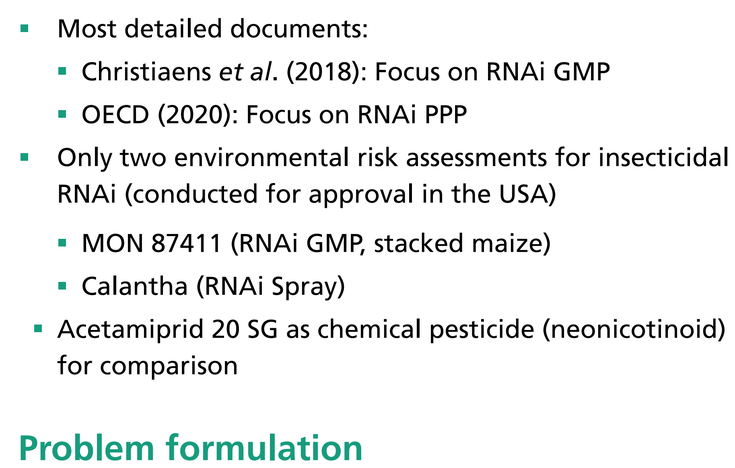
- Search in WebofScience and Pubmed plus inverse snowballing
- Many reviews but only limited comprehensive data sets on fate, exposure and hazard
- Most detailed documents:
  - Christiaens *et al*. (2018): Focus on RNAi GMP
  - OECD (2020): Focus on RNAi PPP
- Only two environmental risk assessments for insecticidal RNAi (conducted for approval in the USA)
  - MON 87411 (RNAi GMP, stacked maize)
  - Calantha (RNAi Spray)
- Acetamiprid 20 SG as chemical pesticide (neonicotinoid) for comparison

Problem formulation

- '*Pathway to harm*' (adapted from Roberts *et al*. 2015)
  1. Introduction into environment
  2. Uptake by non-target organism
  3. No degradation after consumption
  4. Activation of RNAi machinery
  5. Sequence dependent (gene silencing) or sequence independent effect
  6. Effects on viability of the organism (surv, dev, repro)
  7. Effects relevant for Specific Protection Goals

  1–2: Environm. fate, exposure routes
  3–4: Physiological barriers

Conceptual diagram of the 'pathway to harm'



Comparison of Environmental Risk Assessments for an RNAi GMP, an RNAi spray and a typical chemical insecticide

	RNAi GMP, e.g. MON87411	RNAi spray, e.g. Calantha	Chemical insecticide, e.g. Acetamiprid 20 SG
Fate	dsRNA produced by crop Conc. in tissues? Long-term availability	Short-term availability on surfaces Uptake by crop?	Substance and formulation specific fate and exposure
	Low off-field exposure (debris, pollen)	Relevance of drift, run-off and drainage to soil, off-field areas and surface waters	
	Very low environmental stability of dsRNA		
		Formulations can increase ds persistence	Fate depends on molecule and formulation
Exposure	Almost only via diet		Via contact (overspray, surfaces, soil, water) and diet
Mode of action	Gene silencing => only one target pest, high specificity Sequence independent effects less relevant		E.g. neurotoxic often several target pests low specificity
Hazard	Bioinformatics to identify low and high sensitive taxa		
	Focussed bioassays Species selection, protocol modifications		Standard bioassays / data requirements
	Field tests		Higher tier tests
Risk	Case specific, e.g. levels of concern		Uniform principles using risk quotients or similar approaches

Conclusions

- RNAi approaches can provide alternatives to chemical PPP due to often lower persistence of the active substances (short ~~ds~~RNA molecules) and higher specificity
- RNAi PPP most likely to enter EU market
- EU regulation and frameworks for chemical PPP also applicable to RNAi PPP but some adaptations are needed
- Bioinformatics supports selection of test species but bioassays are still needed

Recommendations

- Consider 'pseudo-persistence' of RNA produced by GMP
- Develop refined exposure models, considering e.g. release of dsRNA from crop and food chain transfer
- Consider PPP formulation effects on fate of RNA
- Follow the 'need to know' approach of the EU commission in problem formulation for low risk PPP (e.g. check need of vertebrate testing)
- Adapt test protocols (dietary exposure, prolonged duration, sublethal effects, additional test species)

References

Christiaens et al. (2020). Double-Stranded RNA Technology to Control Insect Pests: Current Status and Challenges. Frontiers in Plant Science, 11, 451. https://doi.org/10.3389/fpls.2020.00451

OECD (2020). Considerations for the Environmental Risk Assessment of the Application of Sprayed or Externally Applied ds-RNA-Based Pesticides. Series on Pesticides and Biocides. OECD. https://doi.org/10.1787/576d9ebb-en

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