



Bundesamt für  
Verbraucherschutz und  
Lebensmittelsicherheit

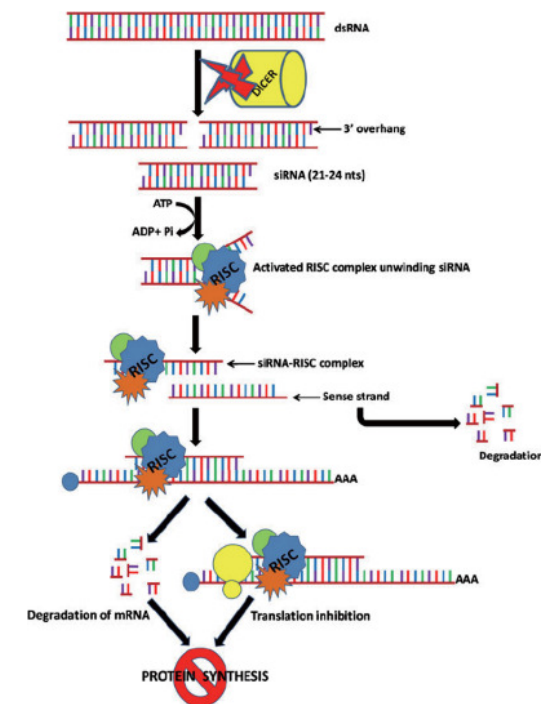


# ds-RNA based plant protection products as novel strategy in pest control: Challenges for risk assessment and risk management

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- ds-RNA: new active ingredient in sprayable PPP
- Mode of action
- What's new? - Challenges for the current risk assessment and risk management
- Legal considerations

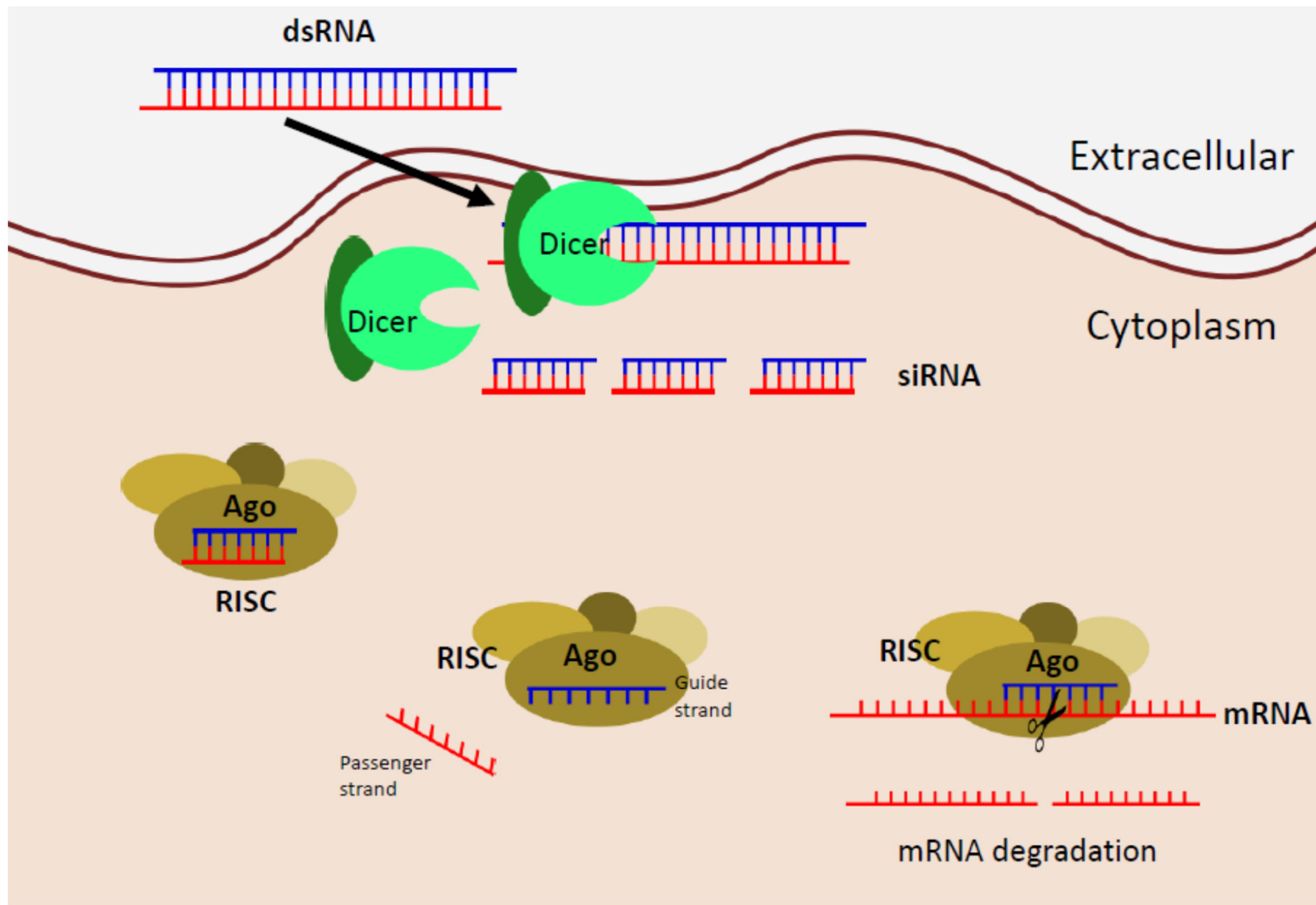


Jagtap et al. 2011 Science  
of Nature 98(6):473-92

- Some companies intensively developing plant protection products with ds-RNA as a.i.
- No sprayable dsRNA-based plant protection product is authorised worldwide yet
- Some experiences with GMO using the same mode of action
- ds-RNA products are also developed for veterinary medicine products e.g. for control of Varoa mites in honey bees

- Mode of action is interfering RNA (RNAi, siRNA)
  - Post-transcriptional gene silencing
  - mRNA degradation to prevent formation of protein
  
- Cellular functions of the process
  - Protection against viruses
  - Internal gene regulation
  - Protection against ‘jumping genes’

# Mode of action – cellular pathway



- A targeting repertoire of a small RNA is largely determined by its seed
  - 2-8 nucleotides
  - not absolute rule
  - allows some predictability, especially for conserved targets
- Targeting efficiency is determined by
  - small RNA abundance (stoichiometry)
  - target site accessibility
  - Complementarity with the target
- Vertebrates have lack of systemic RNAi and corresponding amplification system
- Different pathways make ds-RNA inefficient in vertebrates

# GMOs using gene silencing mechanisms

- **History of save use of RNAi silencing**
  - Many GMOs using the gene silencing mechanism like
    - blue carnation,
    - not browning apples
    - `Flavr Savr` tomato
    - compositionally-modified soybeans and alfalfa (Lucerne),
    - virus-resistant papaya, squash, plums, potatoes and beans
    - bruise-resistant potatoes

Target is the metabolism of the plant (but none of them are authorised in the EU for cultivation)

- **New products with target outside of the plant**
  - MON87411 against corn root worm (construct DvSnf7; assessed for import and processing)
  - Sprayable PPP

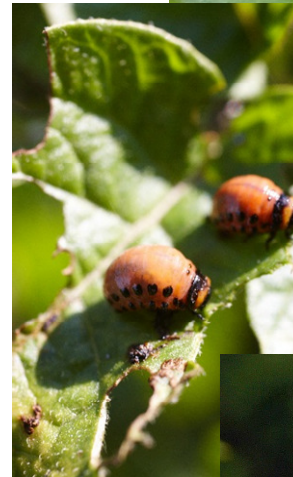
# Targets of sprayable ds-RNA PPP

- Some examples:
  - Flea beetles oil oil seed rape<sup>1</sup>
  - Potato beetle
  - Southern green stink bug in soya beans<sup>3</sup>
  - Western corn root worm<sup>3</sup>
  - Repression of glyphosate resistance<sup>1</sup>
  - Tomato spotted wilt virus in tomato<sup>1</sup>
  - Fusarium infections in barely<sup>2</sup>
  - .....

<sup>1</sup>Different presentations by Monsanto, Bio Direct

<sup>2</sup>Koch et al. 2016 Plos pathogen

<sup>3</sup>Syngenta <https://www.syngenta.be/file/11716/download?token=vMNDsg4h>





# Why RNAi products might be useful?

- New mode of action – high value for resistance management
- Potential for safe products
  - High specificity – minor effects on NTO
  - Low stability in the environment – low environmental load in soil, surface and ground water
  - Low risk for vertebrates due to existing physiological barriers and missing cellular pathways
  - Similar expectations for human health
  - Residues in food and feed might not be relevant

**But** it might depend on the formulation of the product

**But** some data gaps must be filled

- **For efficient application RNAi must be protected from degradation (RNase, UV light)**
  - Possibly new approaches for stabilising RNAi
- **Improving RNAi efficiency**
  - delivery/uptake of intact RNAi into cells
- **Methods in discussion**
  - Polymeric nanoparticles e.g. chitosan (Zhang et al. 2010)
  - Liposomes (Whyard et al. 2009)
  - Chemical modifications e.g. adding methylgroups on specific parts of the RNAi
  - ds-RNA plus the biomass debris and spent fermentation medium
  - .....

# Production of ds-RNA

- Synthesis from nucleotides
- **Microbial production system with GM-bacteria/viruses**
  - Purified ds-RNA
  - ds-RNA plus the biomass debris and spent fermentation medium, microorganisms inactivated
  - ds-RNA plus the biomass debris and spent fermentation medium, microorganisms not inactivated

## Legal classification

- dsRNA-base products has to be considered as any other plant protection product at the moment
- Therefore the whole data package is needed for authorisation as active substance and later on for plant protection products.
- Open questions:
  - Is the current testing scheme appropriate?
  - Are there possibilities to adopt the testing scheme?



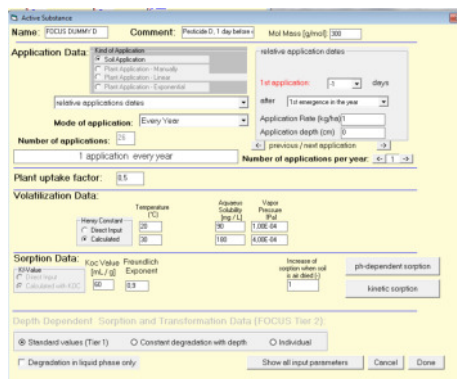
# Example terrestrial arthropods

- Terrestrial arthropods
  - Bees
  - *Aphidius rhopalosiphi*
  - *Typhlodromus pyri*
- Most test use contact toxicity
- ds-RNA need oral exposure



# Challenges – adoption of risk assessment methods

- **Methods in risk assessment may need adoption**
  - Adaption of ecotoxicological testing schemes?
  - Are models used in risk assessment appropriate to model exposure in soil, surface and ground water?
  - Do we need additional information to support risk assessment e.g. bioinformatics?
- **Risk management**
  - Mechanisms of resistance evolution



The screenshot shows the 'Active Substance' window in the Pelmo software. It contains several sections for data entry:

- Application Data:** Includes fields for Name (FOCUS DUMMY D), Comment (Pesticide D, 1 day before), and Mul Mass (g/m<sup>2</sup>) (100). It also has a 'relative application dates' section with '1st application' set to 1 day.
- Volatilization Data:** Includes a table for Henry Constant, Vapor Pressure, and Vapor Solubility at different temperatures (25, 50, 100 °C).
- Sorption Data:** Includes fields for K<sub>oc</sub> Value, Freundlich Exponent, and a checkbox for 'kinetic sorption'.

Screen copy Pelmo

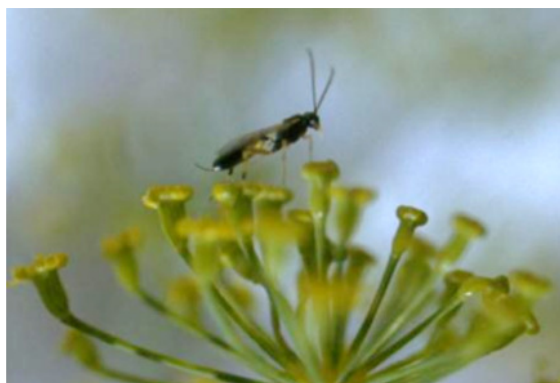


Photo: „Folienserie“ BVL

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TCACACCTATTGACGTAAATCTGCAATTTATTATGTAG
```

# Challenges – procedural issues

- **Product chemistry**

- Does each difference in sequence lead to a new active substance?
- Sequence differences, but matches to the same binding site?
- Criteria for identification of the product?
- No experience about purity: Have all ds-RNAs in a product the same sequence?
- How much variability in RNA sequence is acceptable in a product?
- If microbial production system will be used (GMO)
- Inactivation of microorganisms might be guaranteed

## Article 77 (EC/1107/2009)

### Guidance documents

The Commission may, in accordance with the advisory procedure referred to in Article 79(2), **adopt or amend technical and other guidance documents such as explanatory notes or guidance documents on the content of the application concerning micro-organisms, pheromones and biological products**, for the implementation of this Regulation. The Commission may ask the Authority to prepare or to contribute to such guidance documents.





## Adopted requirements for according Article 77

- Microorganisms
  - Guidance Document for the assessment of the equivalence of technical grade active ingredients for identical microbial strains or isolates approved under regulation (EC) no 1107/2009 (SANCO/12823/2012 –rev. 4,12 December 2014)
- Pheromones/Straight Chain Lepidopteran Pheromones (SCLPs)
  - Guidance Document on semiochemical active substances and plant protection products (SANTE/12815/2014 rev. 5.2 May 2016)
  - Guidance Document on the assessment of new substances falling into the group of Straight Chain Lepidopteran Pheromones (SCLPs) included in Annex I of Council Directive 91/414/EEC (SANCO/5272/2009 rev. 3 28 October 2010)
- Plant extracts/Botanicals
  - Guidance document on botanical active substances used in plant protection products (SANCO/11470/2012– rev. 8 20 March 2014)
- However for dsRNA-based products such guidance documents are not available
- There is no initiative on preparation of such GD at the moment

Reference for documents: [https://ec.europa.eu/food/plant/pesticides/approval\\_active\\_substances/guidance\\_documents\\_en](https://ec.europa.eu/food/plant/pesticides/approval_active_substances/guidance_documents_en)

- New product class with new mode of action and possibly lower environmental harm
- Risk assessment of PPP is established but might need regulatory and methodological adoptions
- Limited experiences with this new class of products
- Data gaps need to be filled
- Some unknowns such as formulation strategies
- Some procedural aspects need to be clarified

# Thank you for your attention!

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