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
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
Introduction

– 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
  - Potato beetle
  - Southern green stink bug in soya beans
  - Western corn root worm
  - Repression of glyphosate resistance
  - Tomato spotted wilt virus in tomato
  - Fusarium infections in barely
  - ........

1Different presentations by Monsanto, Bio Direct
2Koch et al. 2016 Plos pathogen
3Syngenta [https://www.syngenta.be/file/11716/download?token=vMNDSg4h](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
Challenge formulation

• 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 not inactivated
  - ds-RNA plus the biomass debris and spent fermentation medium, microorganisms inactivated
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
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
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)

• 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
Summary

– 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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