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EFSA's activities on the risk assessment of RNAi-based GM crops

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Trusted science for safe food

European Food Safety Authority

RISK ASSESSMENT OF GMO IN EUROPE

- EFSA is responsible to perform a pre-market **risk assessment (RA) of GMOs** with regard to **human and animal health** and **the environment**
- RA is performed by the **GMO Panel**, *ad-hoc* **experts**, and **GMO Unit scientists**
- GM CROPS: **soybean, maize, oilseed rape, cotton**
- MAJOR TRAITS (single or stacked): **insect resistance** and **herbicide tolerance**

RNAi IN GM PLANT APPLICATIONS

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RNAi technology application in GM plants:

- is used for down-regulation of specific target genes *in planta* or in insect pests
- dsRNA or artificial miRNA can be used to elicit a silencing response

REVIEW OF EFSA'S ACTIVITIES ON RA OF RNAi-BASED GM CROPS

1. International EFSA WORKSHOP ON RNAi (2014): Academia, research, industry, regulators.

2. EFSA PROCUREMENTS: MC (2017), ERA (2018), FF (2019)

3. GMO PANEL STRATEGY FOR ASSESSMENT OF RNAi OFF-TARGET ANALYSIS (2017)

4. EFSA SCIENTIFIC OPINION ON GM MAIZE RESISTANT TO WESTERN CORN ROOTWORM (WCR) (2018)

EFSA 2014 WORKSHOP ON RNAi AND RA

REPORT: International scientific workshop 'Risk assessment considerations for RNAi-based GM plants' (4–5 June 2014, Brussels, Belgium). EFSA supporting publication 2014:EN-705, 38 pp.

KEY MESSAGES FOR MC

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Off-target effects in the RNAi based GM plant:

- Bioinformatics useful to predict potential off-target effects on plant genes

Processing of ingested RNAi molecules:

- dsRNA produced in plants can be processed upon oral ingestion by some organisms (e.g. insects and worms)

RNAi molecule intake, processing and mode-of-action may differ between different taxonomic groups→ review baseline information (Paces et al., 2017)

KEY MESSAGES FOR FF-1

Ingestion of RNAi-based GM plants by humans and livestock- Hazard identification/characterization:

Uptake of ncRNAs molecules through intestinal barrier

- low absorption of naked or formulated ncRNAs in feeding studies

Uptake of ncRNAs through the respiratory route

- from pollen: too large for systemic exposure, contains low amounts of RNAi
- No evidence of absorption of ncRNAs from the air by inhalation

KEY MESSAGES FOR FF-2

Considerations on the usefulness of animal studies for food/feed risk assessment based on:

- Properties of dsRNA (absorption, stability, degradation)
- Physical barriers
- No evidence for toxicity of RNAi molecules after ingestion

➢ **not supported by scientific knowledge on dsRNA properties and mode of action**

KEY OUTCOMES FOR ERA-1

Off target effects on non-target arthropods of ingested RNAi-based GM material

- Any dsRNA or siRNA molecule with sufficient homology to an arthropod mRNA could trigger an off-target effect

- Substantial overlap in 21 bp regions necessary to trigger an off-target effect, but some mismatches allowed

➢ **Bioinformatics data may provide supplementary information to predict off-target effects and support species selection for RA, but cannot at present replace toxicity studies**

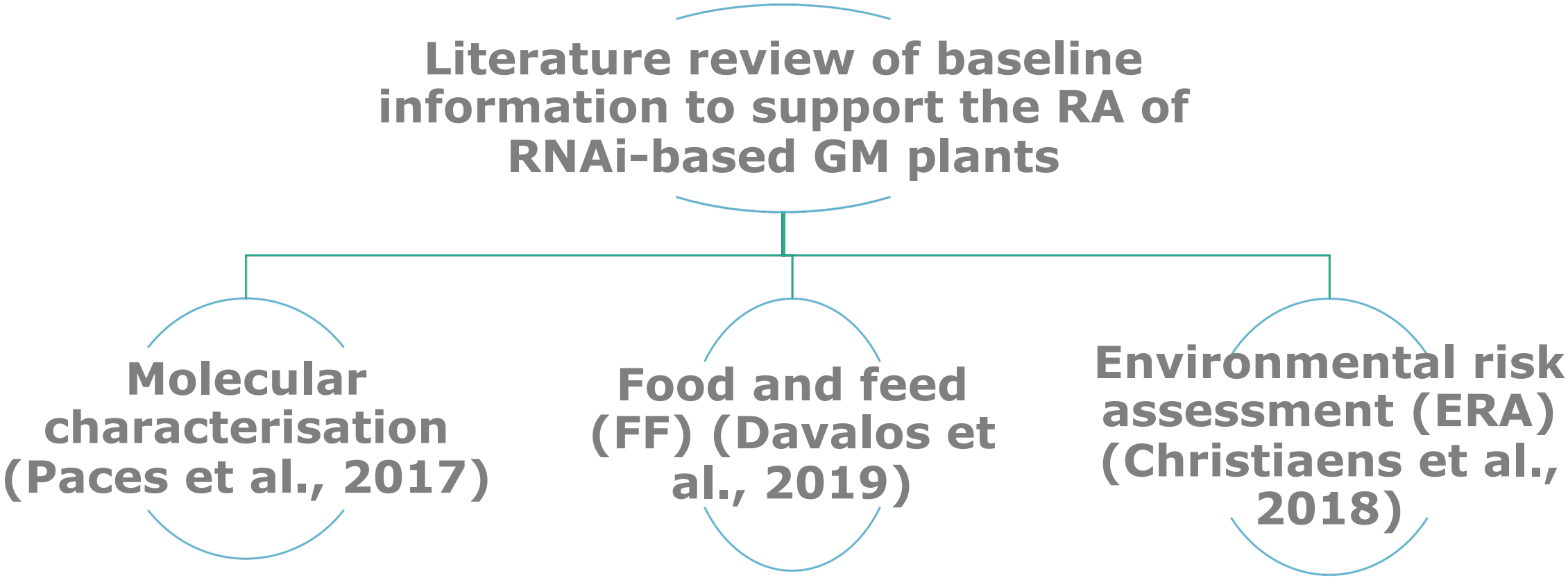
KEY OUTCOMES FOR ERA-2

Considerations on Testing approach and Test Substance

- Ecotoxicological studies:
  - dsRNA has been used as test material (e.g., Bachman et al., 2016), as mostly dsRNA is ingested by arthropods
  - NTO testing with *in planta* material, to capture unanticipated unintended effects

➢ **Tiered approach remains appropriate**

THREE EFSA PROCUREMENTS



PREDICTION OF RNAi OFF-TARGET GENES IN PLANTS

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Internal note on the strategy and technical aspects for small RNA plant off-target bioinformatics studies (Annex II, Minutes of the 118th GMO Panel meeting) - considerations on:

- the search algorithm parameters (e.g. complementarity mismatches)
- the number of different small RNAs showing significant similarity to the same potential off target transcript
- the established or predicted function of the potential off-targets that could impact on the safety of the GM plant and/or derived products as food/feed, or in the environment

The search for small RNA off-targets in the GM plant could have value for the risk assessment of GM plants

RA OF RNAi-BASED GM MAIZE

Assessment of genetically modified maize MON 87411 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2015-124)

- Resistance to western corn rootworms (Diabrotica spp.) by:
  I. the expression of a modified version of the *Bacillus thuringiensis* cry3Bb1 gene, and
  II. a DvSnf7 dsRNA expression cassette;
- Tolerance to glyphosate-containing herbicides by the expression of a cp4 epsps gene.

240nt long dsRNA used to down-regulate the Snf7 gene transcript in WCR via RNAi, leading to pest mortality after consumption

RA OF RNAi -BASED GM MAIZE

MC

- Assessment for off-target genes in plant;
- Measure of DvSnf7 dsRNA levels in different tissues, not considered relevant for RA.

FF

- 28-day tox study provided for maize MON 87411, not assessed: a) usefulness not scientifically justified.

ERA

- No/limited exposure of GM maize expected

CONCLUSIONS

Current framework for GMO RA (guidelines and regulatory data requirements) is sufficient for RA of RNAi-based GM plant RA.

Additional considerations on:

MC:

- Analysis of off-target effects in plant genes adds value to RA
- dsRNA expression levels in the GMP not relevant for the FF safety assessment

FF:

- Animal studies for assessment of dsRNA toxicity in FF RA is not justified based on current scientific knowledge

ERA:

- dsRNA market applications exclude cultivation in Europe; if NT arthropods feed directly on dsRNA-expressing plants, or consumption of plant-fed herbivores is expected, further information for exposure assessment will be needed.
- Bioinformatics to be used in conjunction with agro-pheno compo data

ACKNOWLEDGEMENTS

GMO Panel Experts

EFSA GMO Unit

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