Risk estimation of genome editing techniques in the view of a GMO Panel member of EFSA

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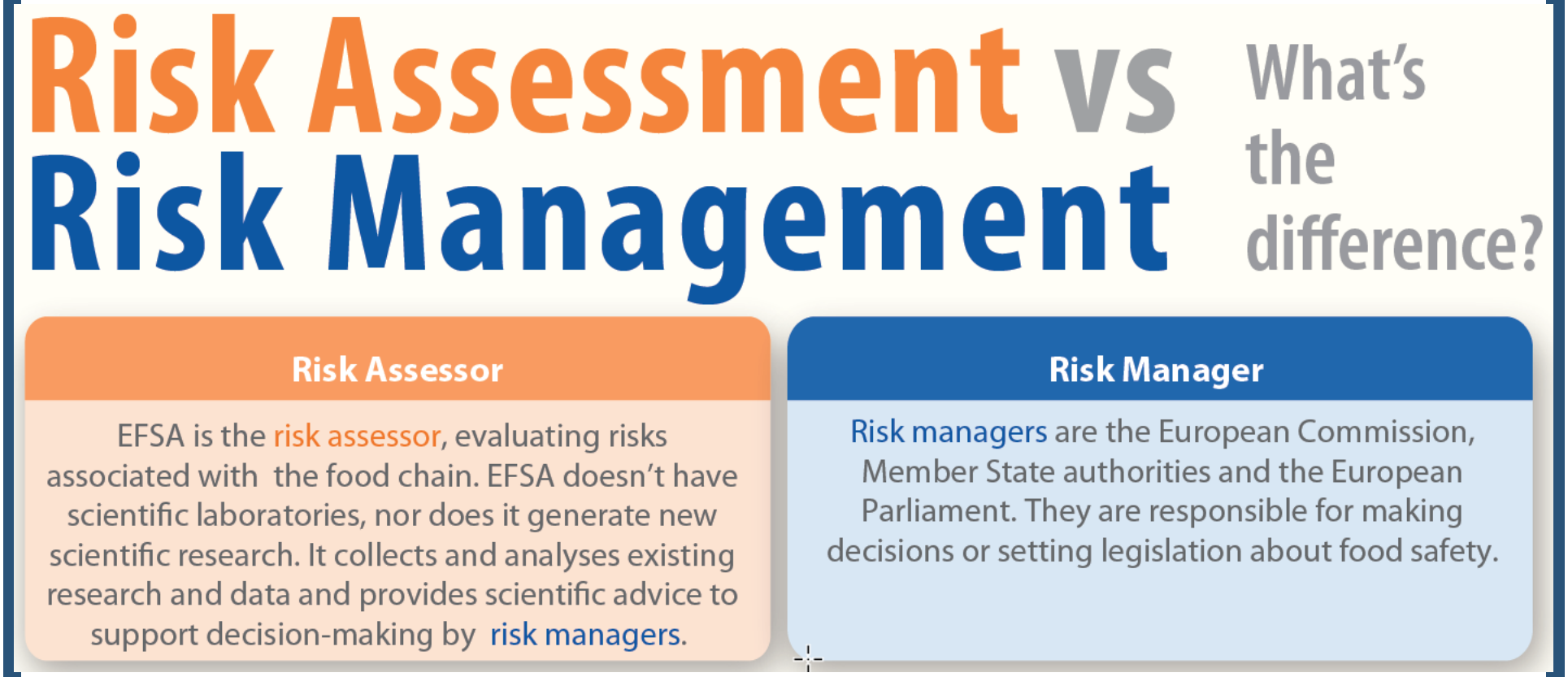
Outline

- Roles of risk managers and risk assessors in GMO risk assessment in the EU
- New plant breeding techniques
- EFSA opinions on cisgenesis and site-directed nucleases 3 (SDN-3)
- The implications of the European Court of Justice case
- If some NPBTs are to be considered as GMO, what are the implications for the EFSA GMO Panel?

It is not in the EFSA remits to decide, if plants obtained through NPBTs should be considered as GMO or not

EC and EFSA



https://www.efsa.europa.eu/en/press/news/140416M

New breeding techniques

- Oligonucleotide Directed Mutagenesis (ODM)
- Zinc Finger Nuclease Technology (ZFN) comprising ZFN-1, ZFN-2 and ZFN-3
- Cisgenesis and Intragenesis
- Grafting
- Agro-infiltration
- RNA-dependent DNA methylation (RdDM)
- Reverse breeding
- Synthetic genomics

As defined by the EC Working Group on New breeding techniques

https://ec.europa.eu/food/plant/gmo/legislation/plant_breeding_en

EFSA opinions

European Food Safety Authority

EFSA Journal 2012;10(2):2561

SCIENTIFIC OPINION

Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

Europea

European Food Safety Authority

EFSA Journal 2012;10(10):2943

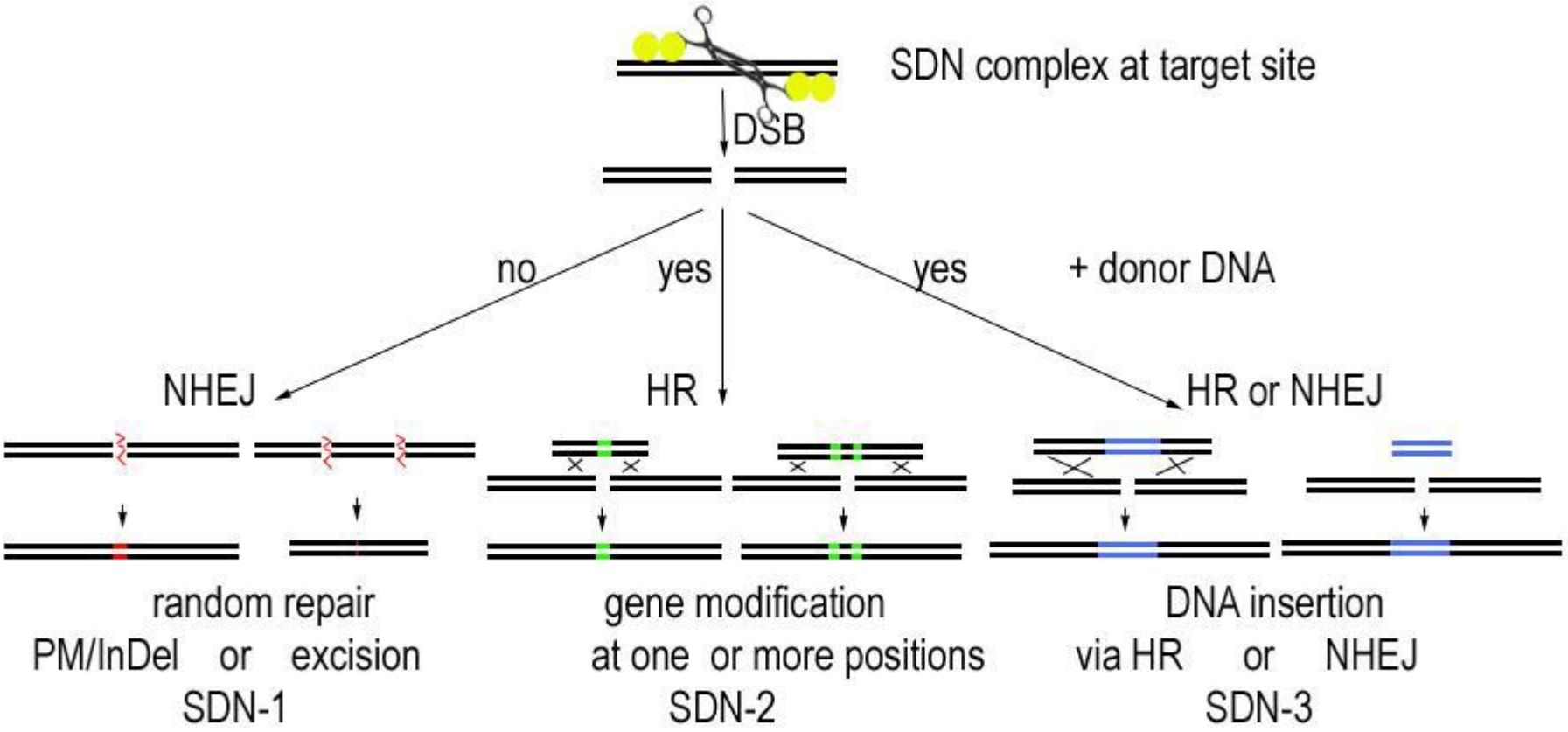
SCIENTIFIC OPINION

Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Site-directed nucleases



EFSA Panel on Genetically modified organisms (GMO); Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA Journal 2012;10(10):2943. [31 pp.] doi:10.2903/j.efsa.2012.2943

Implications for RA

- The EFSA GMO Panel considers that the *Guidance for risk assessment of food and feed …* and the *Guidance on the environmental risk assessment …* are applicable for the evaluation of food and feed products derived from **cisgenic and intragenic** plants and for performing an environmental risk assessment … on a case-by-case basis lesser amounts of event specific data are needed for the risk assessment….
- The EFSA GMO Panel considers that its guidance documents are applicable for the evaluation of food and feed products derived from plants developed using the **SDN-3 technique** and for performing an environmental risk assessment. …on a case-by-case basis lesser amounts of event specific data may be needed for the risk assessment …

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Letter from CIBUS to six Competent Authorities in Europe (Ireland, Finland, Germany, Spain, Sweden, UK)

February 2014
Letter from the Finnish Board of Gene Technology to the European Commission

July 2014
Letter from CIBUS to BVL

2015

14th February
Legal opinion of the BVL classifying products deriving from RTDS as non-GMO

9th March
Objection by several NGOs

3th June
BVL rejects the objection

15th June
Letter from the European Commission; clarifying legal opinion announced

August
Letter from the European Commission to EFSA asking for technical assistance

September
Letter from EFSA to European Commission
Letter from BVL to European Commission

September
Legal opinion on behalf of NGOs

October
Legal opinion on behalf of BFN

December
Legal opinion by BVL

2016

February
Legal Interpretation by European Commission still pending

Sprink et al. (2016) Regulatory hurdles for genome editing: process- vs. product-based approaches in different regulatory contexts. Plant Cell Reports *35*, 1493-1506.

Positions on NPBTs

	BVL[1]	ZKBS[2]	NTWG[3]	EFSA[4,5]	NGOs[6]	BFN[7]
SDN-1	Non GMO	Non GMO	Non GMO	Non GMO	GMO	GMO
SDN-2	Non GMO	Non GMO	Non GMO	Non GMO	GMO	GMO
SDN-3	GMO	GMO	GMO	GMO[b]	GMO	GMO
ODM	Non GMO[a]	Non GMO	Non GMO	Non GMO	GMO	GMO
RdDM	n.d	Non GMO	Non GMO	Non GMO	n.d	GMO
Interpretation	Process/product	n.d	n.d	n.d	Process	Process

The classification refers to plants generated by using these techniques without stable integration of recombinant DNA

SDN site-directed nucleases, *ODM* oligonucleotide-directed mutagenesis, *RdDM* RNA-dependent DNA methylation, *n.d* no opinion given, *GMO* genetically modified organism, *BVL* German Federal Agency for Consumer Protection and Food Safety, *ZKBS* Zentrale Komission für biologische Sicherheit, *NTWG* New technology working group, *EFSA* European Food Safety Authority. *1* BVL 2015d, *2* ZKBS 2012, *3* Lusser et al. 2011, *4* EFSA 2012, *5* EFSA GMO unit 2015, *6* Krämer 2015, *7* Spranger 2015

[a] Serial steps should be considered separately

[b] Due to the known target site of the transgene lesser amounts of event-specific data might be necessary for the risk assessment

Sprink et al. (2016) Regulatory hurdles for genome editing: process- vs. product-based approaches in different regulatory contexts. Plant Cell Reports *35*, 1493-1506.

Request from EC

In September 2015 EFSA received a request from EC to provide scientific advice to support the legal interpretation of the Dir 2001/18. EC requested to provide clarifications on:

1. definition of the term “recombinant nucleic acid molecule”
2. **if ODM and ZFN-1 and ZFN-2 can be considered a form of mutagenesis**
3. definition of the term “genetic material”
4. if the epigenetic modification produced by RdDM can be considered an alteration of the genetic material

http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2015-00525

EFSA response to EC

- The EFSA GMO Unit considers that the currently available ODM, ZFN-1 and ZFN-2 and similar SDN techniques create point mutations similar to those introduced via natural or induced mutagenesis, and can thus be considered a form of mutagenesis
- In case the … rationale would not be applicable anymore (e.g. due to technological advancement of the techniques leading to modifications that go beyond the creation of point mutations) further analysis may be needed…

European Parliament briefing

New plant-breeding techniques. Applicability of GM rules

«*The Commission is currently working on a legal interpretation of the regulatory status of products generated by new plant-breeding techniques, which should be published in the course of 2016. The Commission has highlighted that its legal interpretation is intended to give guidance to national authorities on the scope of GMO legislation, but that it is the sole prerogative of the European Court of Justice to render a final and binding opinion on the interpretation of EU law*.»

http://www.europarl.europa.eu/thinktank/lv/document.html?reference=EPRS_BRI(2016)582018

GM – non-GM

European Court of Justice case on plants produced by the new breeding techniques

GMO

GMO legislation applies
EFSA assessment required
Risk assessment based on:

- molecular characterization;
- comparative assessment (agro, pheno, compo);
- food and feed safety assessment (toxicology, allergenicity, nutrition);
- environmental risk assessment

few cultivation dossiers expected

non-GMO

GMO legislation does not apply
EFSA assessment not required
Regular plant variety procedures and free cultivation
However, there may be some novel traits (HT, reduced phytate, changes in fatty acid or starch composition), which would require some consideration

Consequences of ruling

EFSA has already provided assessment that some of the NPBT do not involve genetic modification, but can indeed be considered as a form of mutagenesis, which is specifically exempt from the EU legislation on GMO

However, we are not risk managers or policy makers and we will comply with the ECJ ruling

Following slides are for the case, if the ruling is «GMO»

Implications for EFSA

If the ECJ rules that plants produced by NPBT fall under the GMO legislation, EFSA will need to assess the applications for authorization

EFSA is in position to do so, according to requirements of IR 503/2013 and using the existing guidance documents

EFSA has the capacity and the expertise to develop new assessment strategies, if needed

Intended and unintended changes

1. Intended changes in plants produced by NPBTs
2. Potential unintended changes in plants produced by NPBTs

Intended changes

- Nucleotide substitutions resulting in amino acid changes (affecting active sites of enzymes, overall protein structure, protein – protein and protein – nucleic acid interactions)
- Nucleotide insertions or deletions resulting in frameshift mutations and, potentially, in non-functional proteins
- Mutations in splice sites resulting in alternative splicing and, potentially, in non-functional proteins
- Mutations in regulatory regions resulting in modified gene expression

All these changes can be assessed within the existing framework, but in most cases less information would be required

Less information for intended changes

For MC:

- No inserted sequences
- No unintended disruption of endogenous genes
- Intended changes are simple and are likely to have predicatable consequences (modified protein sequences, non-functional proteins, modified expression)
- Protein expression will need to be assessed

Unintended changes I

- Potential off target mutations caused by site-directed nucleases and internal DNA repair mechanisms
- Indistinguishable from natural genetic variation or radiation/chemical-induced mutations and can occur anywhere in the genome
- GWAS have indicated that most of SNPs that affect traits are not in coding regions – so nothing short of high quality full genome sequencing will allow to catalogue all unintended changes (not practical!)

- *Approaches for DSB detection, such as, http://www.nature.com/nbt/journal/v33/n2/abs/nbt.3101.html, could allow to estimate off target potential for certain engineered nucleases and sgRNAs*

Unintended changes II

- Molecular characterization of unintended off target mutations is not practical
- EFSA already require agronomic, phenotypic, compositional and nutritional data, as well as toxicity and allergenicity assessment of whole food/feed to assess GMO safety
- Comparative data would allow us to conclude on safety of plants produced by NPBTs, if that would become required by EC

Other EFSA challenges

- IR 503/2015 on stacks «…applications for genetically modified food and feed from segregating crops should include **all subcombinations independently of their origin** and not yet authorised…»
- IR 503/2015 «In the case of … stacked transformation events, the safety of potential **interactions between any unintended modifications** at each insertion site shall be assessed»
- Assessment of genetic backgrounds *vs.* events

Acknowledgments

- Federal Institute for Risk Assessment for possibility to share the experience
- EFSA for experience in EFSA GMO panel and MC WG
- University of Latvia for tolerating my frequent travel to Parma