Development of risk assessment of genome-edited organisms

Dr. Nils Rostoks, Novi Sad, 06.11.2019.

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ANNO 1919

Outline of the talk

- GMO risk assessment framework
- EFSA and risk assessment of plants developed with site-directed nucleases (SDNs)
- EC mandate for risk assessment of SDN-1/2 plants
- Considerations for risk assessment of genome-edited plants

GMO legal framework in the EU

- Directive 2001/18/EC on the deliberate release of GMOs into the environment
- Regulation (EC) 1829/2003 on genetically modified food and feed
- Commission Implementing Regulation (EU) No 503/2013 on applications for authorisation GM food and feed in accordance with Regulation (EC) No 1829/2003
- Directive (EU) 2015/412 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of GMOs in their territory
- Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC, concerning the environmental risk assessment (ERA) of GMOs

GMO definition - Directive 2001/18/EC

- Article 2 of the Directive
- (2) genetically modified organism (GMO) means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;

- Within the terms of this definition:
- (a) genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1;
- (b) the techniques listed in Annex I A, part 2, are not considered to result in genetic modification;

Techniques of genetic modification

- (1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur, but in which they are capable of continued propagation;

- (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

- (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally

Exemptions I

- Annex IA, part 2
- Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:
- (1) in vitro fertilisation,
- (2) natural processes such as: conjugation, transduction, transformation,
- (3) polyploidy induction.

Exemptions II

- Annex IB
- Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:
- (1) **mutagenesis**,
- (2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through
- traditional breeding methods.

GENOME – EDITED ORGANISMS ARE GMO

Press and Information

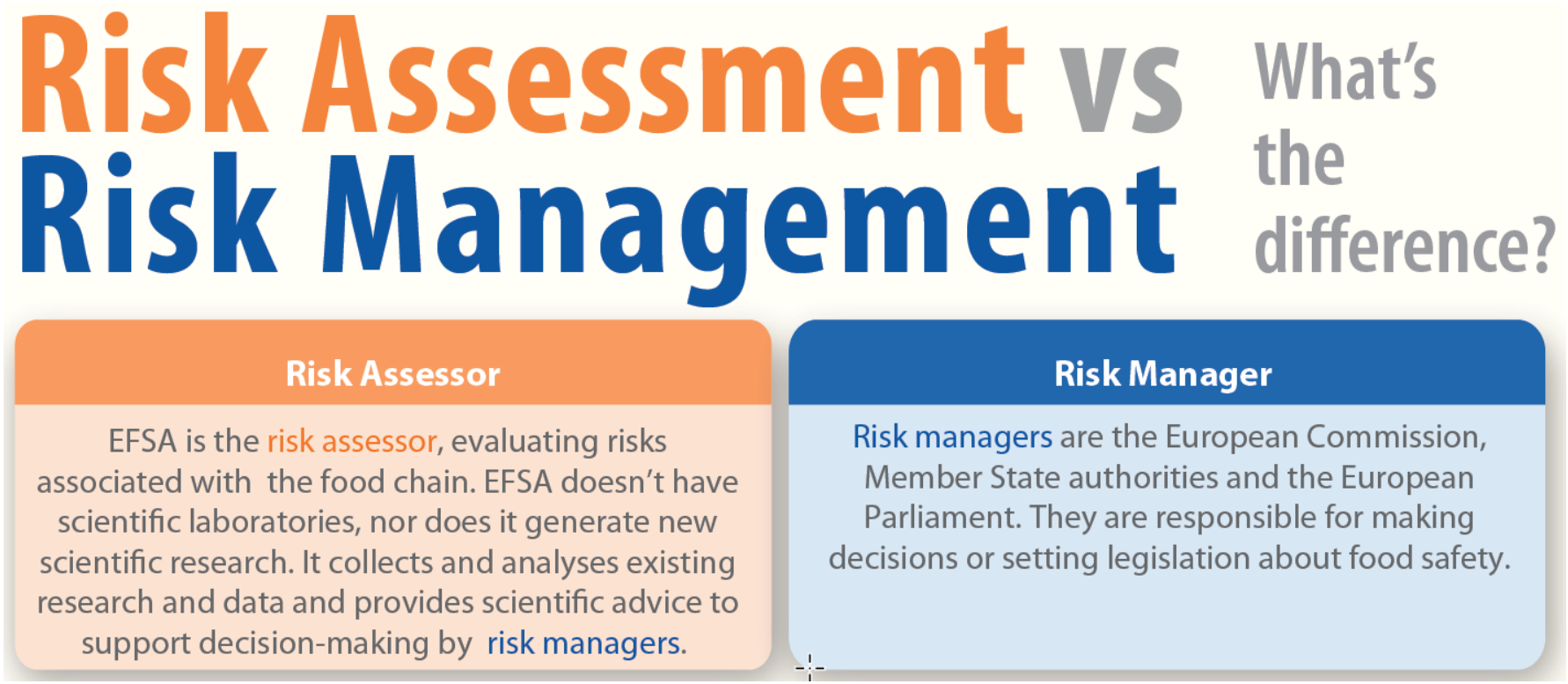
Court of Justice of the European Union
PRESS RELEASE No 111/18
Luxembourg, 25 July 2018

Judgment in Case C-528/16
Confédération paysanne and Others v Premier ministre and Ministre de l'Agriculture, de l'Agroalimentaire et de la Forêt

Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive

However, organisms obtained by mutagenesis techniques which have conventionally been used in a number of applications and have a long safety record are exempt from those obligations, on the understanding that the Member States are free to subject them, in compliance with EU law, to the obligations laid down by the directive or to other obligations

EFSA and EC



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

EFSA opinion on SDN-3

European Food Safety Authority

EFSA Journal 2012;10(10):2943

SCIENTIFIC OPINION

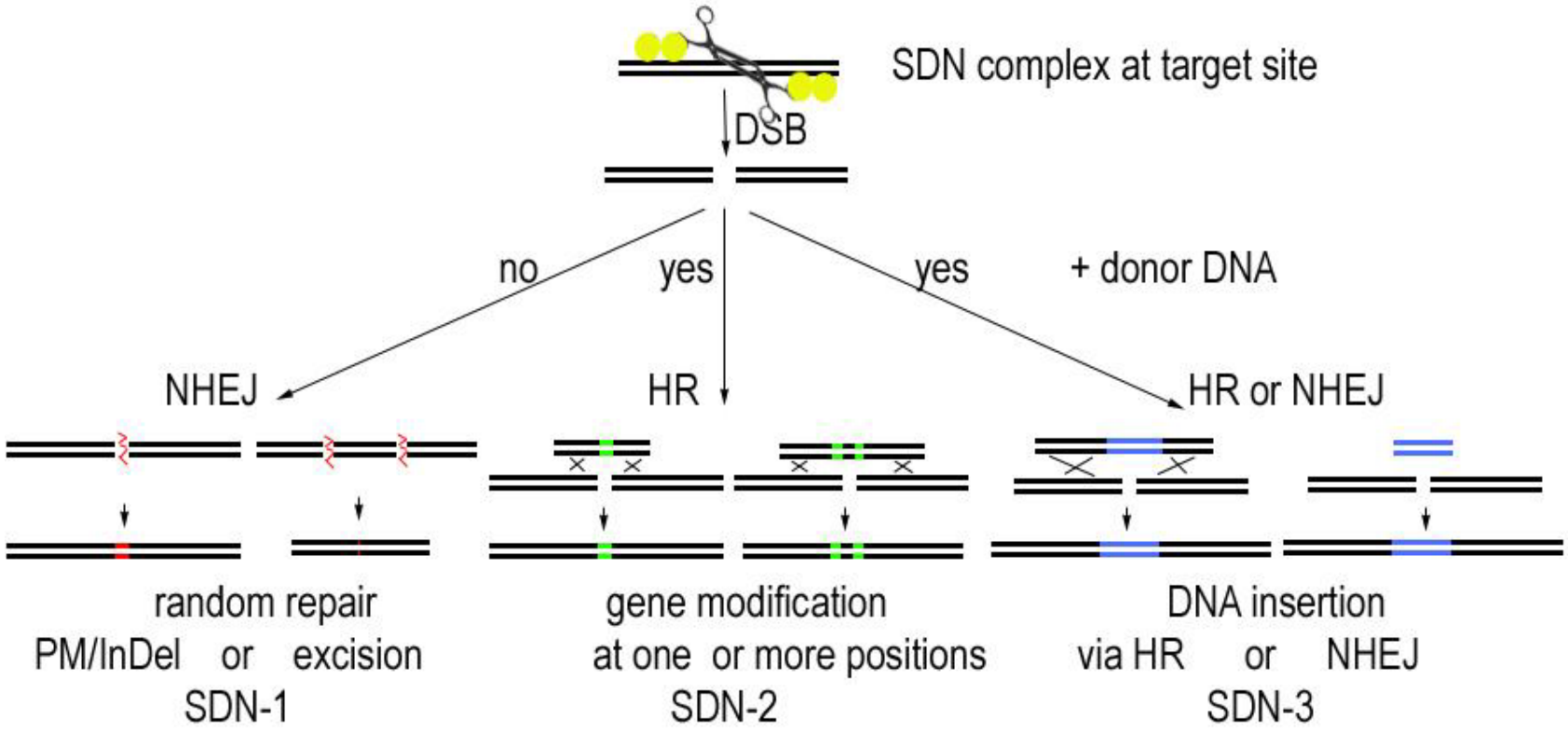
Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function[1]

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

European Food Safety Authority (EFSA), Parma, Italy

Site-directed nucleases and scenarios for genome modification

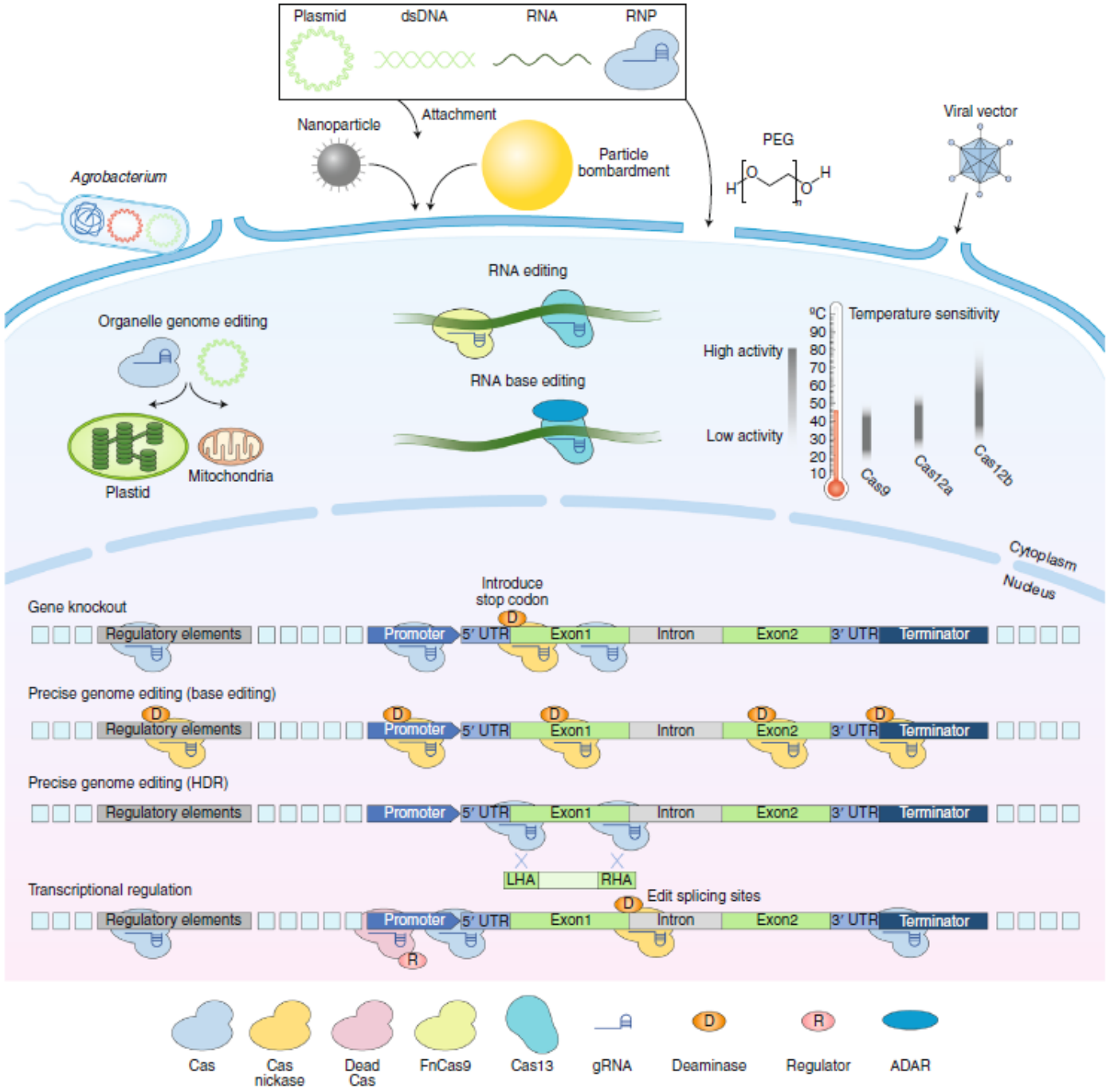
EFSA GMO Panel (2012) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA J, 10:2943. doi:10.2903/j.efsa.2012.2943



Conclusions of SDN-3 opinion

- 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*** …

CRISPR/Cas applications



Zhang et al. (2019) The emerging and uncultivated potential of CRISPR technology in plant science. Nature Plants.

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Prime editing of target sites

MENU nature

and all legal disclaimers apply.

Search-and-replace genome editing without double-strand breaks or donor DNA

Andrew V. Anzalone, Peyton B. Randolph, Jessie R. Davis, Alexander A. Sousa, Luke W. Koblan, Jonathan M. Levy, Peter J. Chen, Christopher Wilson, Gregory A. Newby, Aditya Raguram & David R. Liu

Nature (2019) | Cite this article

Off-target RNA editing

LETTER

https://doi.org/10.1038/s41586-019-1161-z

Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors

Julian Grünewald[1,2,3,4], Ronghao Zhou[1,2,3], Sara P. Garcia[1,6], Sowmya Iyer[1,6], Cale
J. Keith Joung[1,2,3,4]*

CRISPR–Cas base-editor technology enables targeted nucleotide alterations, and is being increasingly used for research and potential therapeutic applications[1,2]. The most widely used cytosine base editors (CBEs) induce deamination of DNA cytosines using the rat APOBEC1 enzyme, which is targeted by a linked Cas protein–guide RNA complex[3,4]. Previous studies of the specificity of CBEs have identified off-target DNA edits in mammalian cells[5,6]. Here we show that a CBE with rat APOBEC1 can cause extensive transcriptome-wide deamination of RNA cytosines in human cells, inducing tens of thousands of C-to-U edits with frequencies ranging from 0.07% to 100% in 38–58% of expressed genes. CBE-induced

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SCIENCE ADVANCES | RESEARCH ARTICLE

GENETICS

Analysis and minimization of cellular RNA editing by DNA adenine base editors

Holly A. Rees[1,2,3]*, Christopher Wilson[1,2,3], Jordan L. Doman[1,2,3], David R. Liu[1,2,3]†

Adenine base editors (ABEs) enable precise and efficient conversion of target A•T base pairs to G·C base pairs in genomic DNA with a minimum of by-products. While ABEs have been reported to exhibit minimal off-target DNA editing, off-target editing of cellular RNA by ABEs has not been examined in depth. Here, we demonstrate that a current ABE generates low but detectable levels of widespread adenosine-to-inosine editing in cellular RNAs. Using structure-guided principles to design mutations in both deaminase domains, we developed new ABE variants that retain their ability to edit DNA efficiently but show greatly reduced RNA editing activity, as well as lower off-target DNA editing activity and reduced indel by-product formation, in three mammalian cell lines. By decoupling DNA and RNA editing activities, these ABE variants increase the precision of adenine base editing by minimizing both RNA and DNA off-target editing activity.

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EC mandate M-2019-0095 on plants developed using SDN1/2 and ODM

Ref. Ares(2019)2488590 - 09/04/2019

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Food and feed safety, innovation
The Director

Brussels,
SANTE E3/IC/gk (2019)2681389

Dear Dr Url,

Subject: **Request for a scientific opinion on plants developed using type 1 and type 2 Site-Directed Nucleases and Oligonucleotide Directed Mutagenesis**

Please find herewith the background and terms of reference for a request to EFSA for a scientific opinion on plants developed using type 1 and type 2 Site-Directed Nucleases and Oligonucleotide Directed Mutagenesis.

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EC mandate M-2019-0095 on risk assessment of SDN1/2 and ODM

Terms of reference

Against this background, the European Commission asks EFSA, in accordance with Article 29 of Regulation (EC) No 178/2002:

1. To advice whether the assessment methodology described in section four of the EFSA scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function, may be applicable, in whole or in part, to plants developed with type 1 and type 2 Site-Directed Nucleases and with oligonucleotide directed mutagenesis.

In case the advice in 1) is affirmative, the Commission would ask EFSA, in accordance with Article 29 of Regulation (EC) No 178/2002:

2. To advice whether the conclusions of the EFSA 2012 scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function are valid, in whole or in part, to plants developed with type 1 and type 2 Site-Directed Nucleases and with oligonucleotide directed mutagenesis.

Risk assessment methodology for SDN-3 (EFSA, 2012)

Hazard identification involves:

- Source of genes and safety of gene products
- Alterations to the genome at target site (gene interruption, changes in genome, new ORFs etc.)
- Alterations to the genome at off-target sites
- Expression of the trait

Risk assessment conclusions for SDN-3

Conclusions:

- In SDN-3, insertion is targeted to a predefined region of the genome. Therefore, the SDN-3 technique can optimise the genomic environment for gene expression and minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome
- SDN-3 technique can induce off-target changes, but these would be fewer than those occurring with most mutagenesis techniques. Where they do occur, the changes would be the same types as those produced by conventional breeding techniques
- The EFSA GMO Panel considers that the Guidance for risk assessment of food and feed from GMP (EFSA, 2011) and the Guidance on the ERA of GMP (EFSA, 2010) are applicable for the evaluation of food and feed products derived from plants developed using the SDN-3 technique and for performing an ERA. However, on a ***case-by-case basis lesser amounts of event-specific data may be needed for the risk assessment of plants developed using the SDN-3 technique. There is therefore a need for flexibility in the data requirements for risk assessments***

How does this relate to SDN-1/2 and ODM?

- If no external genes are introduced, then RA of genes/NEPs is not needed. However, if SDN-1/2 constructs are present, then the organism is assessed as GMO
- Because endogenous genes are modified in a targeted, predicted way, alterations to genomes can be easily characterized, e.g., impact of changes in aa sequence or creation of new ORFs. Moreover, some of these changes may mimic natural or induced variation already present in breeders' genepool; therefore, concept of History of Safe Use may be applicable
- Similarly to SDN-3, off-target changes can be introduced, but those would be fewer and similar to natural/induced variants

Conclusions

- Complicating factor for risk assessment of genome-edited organisms may be linked to diversity of technological solutions which create different uncertainties
- If nuclease constructs used for genome editing are present in the genome, the resulting organism is risk assessed as a conventional GMO
- If no foreign DNA is present in genome-edited organism, then significantly less information may be necessary for risk assessment on a case-by-case basis, because only changes to endogenous genes need to be assessed
- Risk assessment of the intended change should take into account, if such change is already present in breeders' gene pool
- Off-target changes are possible, but they may be fewer and of the same type as natural/induced variation

Acknowledgements

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