Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

NPBT in the European Union:
Experience, regulation and existing guidance

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New plant breeding techniques

- **Breeding and genetic modification techniques evolve at a rapid pace**
- **Techniques that result in plants of in offspring of plants that does not contain 'foreign' DNA**
- **Some of the techniques have an intermediate GMO step or it is not clear whether recDNA is used during the modification**

Establishment EU working group

- **Questions from companies on the regulatory status of these new breeding techniques**
- **On request of some Member States (incl. NL) an EU working group was established in 2007**
- **Regulatory status of techniques?**
- **Regulation has not been changed since 1990**

Techniques discussed

(list from 2007, non exhaustive list)

- ✓ **Zinc finger nuclease technology**
- ✓ **Oligonucleotide-directed mutagenesis**
- ✓ **Cisgenesis/intragenesis**
- ✓ **RNA-dependent DNA methylation**
- ✓ **Grafting**
- ✓ **Reverse breeding**
- ✓ **Agro-infiltration**
- ✓ **Synthetic biology**

Definition of a GMO

Directive 2001/18/EC

GMO/GMM defined as *"an organism/micro-organism… in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination"*

Annexes in Directive:

- ✓ **Non-exhaustive list of techniques that lead to genetic modification**
- ✓ **Techniques not considered to result in genetic modification (exhaustive list)**
- ✓ **Techniques excluded from the scope of the GMO legislation (exhaustive list)**

Art. 2(2) 2001/18/EC

A **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[1]

Annex 1A, Part 2

The techniques listed in Annex 1A, part 2 are **not considered to result in genetic modification**:

in vitro fertilization

natural processes like conjugation, transduction or transformation

polyploidy induction

GMO

Annex 1A, Part1

Techniques of genetic modification are *inter alia*:

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

Techniques involving the direct introduction into an organism of **heritable material** prepared outside the organism including micro-injection, macro-injection ad micro-encapsulations

cell fusion (including protoplast fusion) or hybridization 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

GMO excluded from Directives

Annex 1B

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they **not involve the use of recombinant nucleic acid molecules** or genetically modified organisms other than those produced by one or more of the techniques listed below are:

mutagenesis

cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods

Working Group on New Techniques

- **Mandate**

  Each technique evaluated in the context of:

  - ✓ **the GMO definition**
  - ✓ **the annexes of the directive**
  - ✓ **the most recent available scientific data**

- **Working group consisted of experts of 22 member states of EU**

- **Meetings from 2008-2011 (9 meetings)**

Working Group on New Techniques

Analysis:

- **whether NPBT constitute techniques of genetic modification**
- **If so, whether the resulting organism falls within or outside the scope of the GMO legislation, or to be excluded**
- **Similarity to conventional techniques, to natural processes and suggestions for future status**

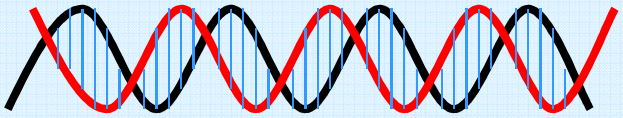
Discussions in working group

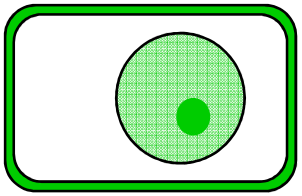
- **Genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination?**
- **Formation of new combinations of genetic material?**
- **Use of recombinant nucleic acid molecules?**
- **Transient presence of recDNA?**
- **Offspring of GM plant or intermediate product?**

NEW PLANT BREEDING TECHNIQUES

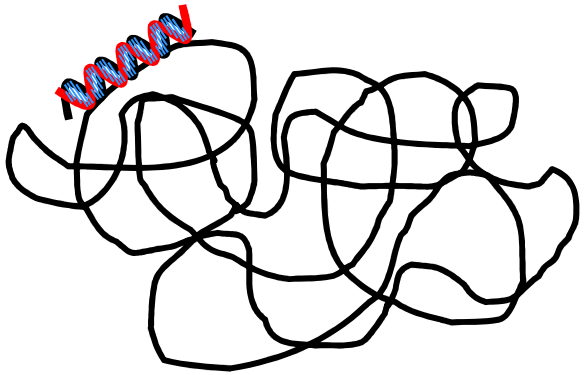
ODM: OLIGONUCLEOTIDE DIRECTED MUTAGENESIS

OLIGONUCLEOTIDES



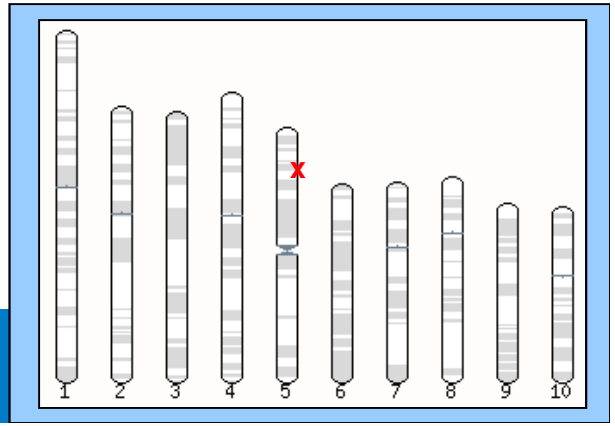


Plant cell



Plant genome

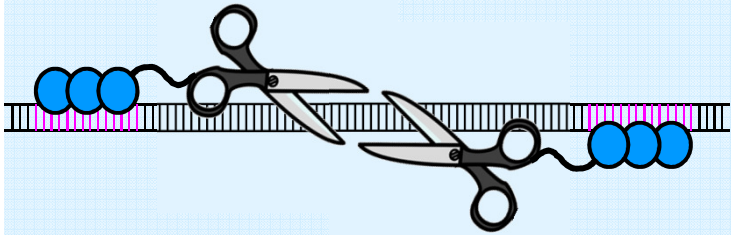
ODM plants

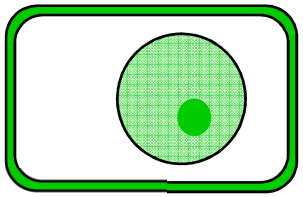


NEW PLANT BREEDING TECHNIQUES

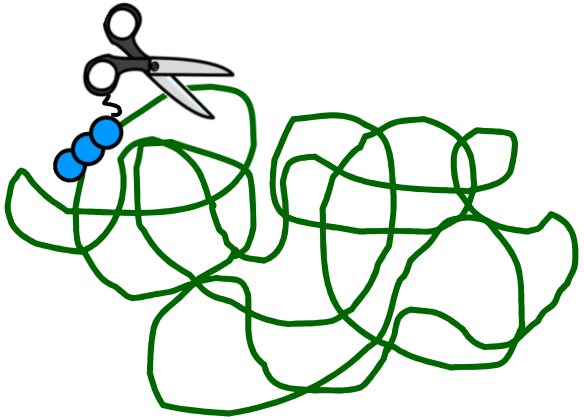
ZFN TECHNOLOGY 1,2

ZINC FINGER NUCLEASE



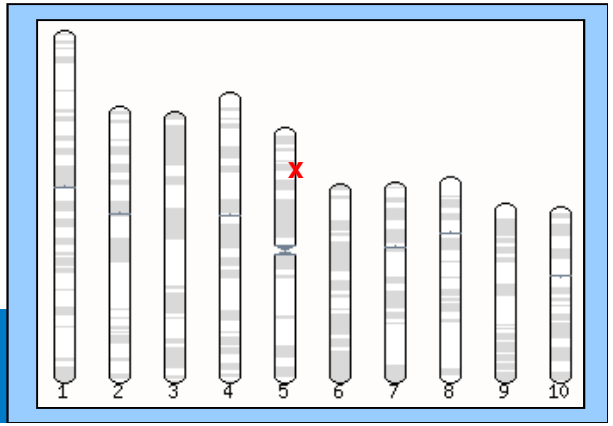


Plant cell



Plant genome

ZFN plants



Report WG

- **Finalized in 2011**
- **Disagreement if all NPBT fall under the definition of GMO or not**
- **Agreement on exclusion NPBT in future**

The views expressed in this report are those of an expert working group and do not necessarily represent those of the European Commission or the Competent Authorities. Only the European Court of Justice can give a binding opinion on EU law.

New Techniques Working Group

FINAL REPORT

1.0 Introduction

EC Mandate to EFSA on NPBT

Q1. Determine whether there is a need for new guidance or whether the existing guidance on risk assessment* should to be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques.

Q2. What are the risks in terms of impact on humans, animals and the environment that the techniques could pose?

- compare plants obtained by these new techniques with plants obtained by conventional plant breeding techniques and secondly with plants obtained with currently used genetic modification techniques.

*Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011) http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010) http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf

EFSA GMO Panel opinions on NPBT

efsa
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]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission requested that the EFSA Panel on Genetically Modified Organisms deliver a scientific opinion related to risk assessment of cisgenic and intragenic plants. The EFSA GMO Panel considers that the *Guidance for risk assessment of food and feed from genetically modified plants* and the *Guidance on the environmental risk assessment of genetically modified plants* are applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and for performing an environmental risk assessment and do not need to be developed further. It can be envisaged that on a case-by-case basis lesser amounts of event-specific data are needed for the risk assessment. The EFSA GMO Panel compared the hazards associated with plants produced by cisgenesis and intragenesis with those obtained either by conventional plant breeding techniques or by transgenesis. The Panel concludes that similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants. The Panel is of the opinion that all of these breeding methods can produce variable frequencies and severities of unintended effects. The frequency of unintended changes may differ between breeding techniques and their occurrence cannot be predicted and needs to be assessed case by case. Independent of the breeding method, undesirable phenotypes are generally removed during selection and testing programmes by breeders. The risks to human and animal health and the environment will depend on exposure factors such as the extent to which the plant is cultivated and consumed.

KEY WORDS

Cisgenic, cisgenesis, intragenic, intragenesis, transgenic, GM plant

- Opinion on *cisgenesis and intragenesis* adopted in the 71st plenary (January 2012)

http://www.efsa.europa.eu/en/efsajournal/doc/2561.pdf

efsa
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

ABSTRACT

The European Commission requested that the EFSA Panel on Genetically Modified Organisms deliver a scientific opinion related to risk assessment of plants developed using the zinc finger nuclease 3 technique (ZFN-3) which allows the integration of gene(s) in a predefined insertion site in the genome of the recipient species. Since other nucleases with a similar function to ZFN are considered in this opinion the term site-directed nuclease 3 (SDN-3) is used to describe the technique rather than ZFN-3 specifically. 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. 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. The EFSA GMO Panel compared the hazards associated with plants produced by the SDN-3 technique with those obtained by conventional plant breeding techniques and by currently used transgenesis. With respect to the genes introduced, the SDN-3 technique does not differ from transgenesis or from the other genetic modification techniques currently used, and can be used to introduce transgenes, intragenes or cisgenes. The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome. Therefore, the SDN-3 technique can minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome. Whilst the SDN-3 technique can induce off-target changes in the genome of the recipient plant these would be fewer than those occurring with most mutagenesis techniques. Furthermore, where such changes occur they would be of the same types as those produced by conventional breeding techniques.

KEY WORDS

TALEN, meganuclease, ZFN, genome editing, gene targeting, transgenic, site-directed nucleases

- Opinion on *Zinc Finger Nucleases–3 (ZFN-3)* adopted in the 75th plenary (October 2012)

http://www.efsa.europa.eu/en/efsajournal/doc/2943.pdf

Potential adverse effects of Cisgenic and Intragenic plants

Identification of characteristics having the potential to cause adverse effects

- Source of genes and safety of gene products
- Alterations to the genome
- Presence of non-plant sequences in the insert
- Modification of gene expression

Source of genes and safety of gene products

History of safe use of the cisgene source and product, possible scenarios :

- the **donor** plant (e.g. variety, landrace, wild relative) **has a history** of cultivation and consumption by humans;
- the **donor** plant has **no history** of consumption by humans, **but** has been **used in conventional breeding**;
- the **donor** plant has **not been exploited** yet for variety development, but there is **knowledge of the gene family** in terms of the structure and functions of the proteins they encode;
- **None of the above**

Source of new genes

- Cisgene inserted
  - Same gene could be bred into the commercial varieties from breeder's gene pool
    - Similar hazards related to cisgene
    - No linkage drag in cisgenesis
- Intragene inserted
  - Intragenesis and transgenesis offers more possibilities
    - No linkage drag in intragenesis

Alterations to the genome

Changes in the genome induced by the insert

- Novel DNA is integrated using plant DNA repair mechanism
- Disruption/deletion/rearrangement of endogenous genes and regulatory sequences possible
- Creation of novel open reading frames at the junction

Changes in the genome not linked to insert

- Somaclonal variation

Presence of non-plant sequences

- No vector backbone can be present in cisgenic plants
- Selectable markers can only be from breeders' gene pool
- Small remnants of the transformation vector
  - Similar sequences present in plants
  - Similar short sequences can be created via insertion of filler DNA

Modification of gene expression

- Promoter functionality
  - Regulatory elements may be missing
- Position effect
  - Impact on surrounding genes
  - Altered expression of cisgene

For cisgenic plants similar levels as in donor might occur, for intragenic plants more options for modifying gene expression

Conclusions on Cisgenesis & Intragenesis (1/2)

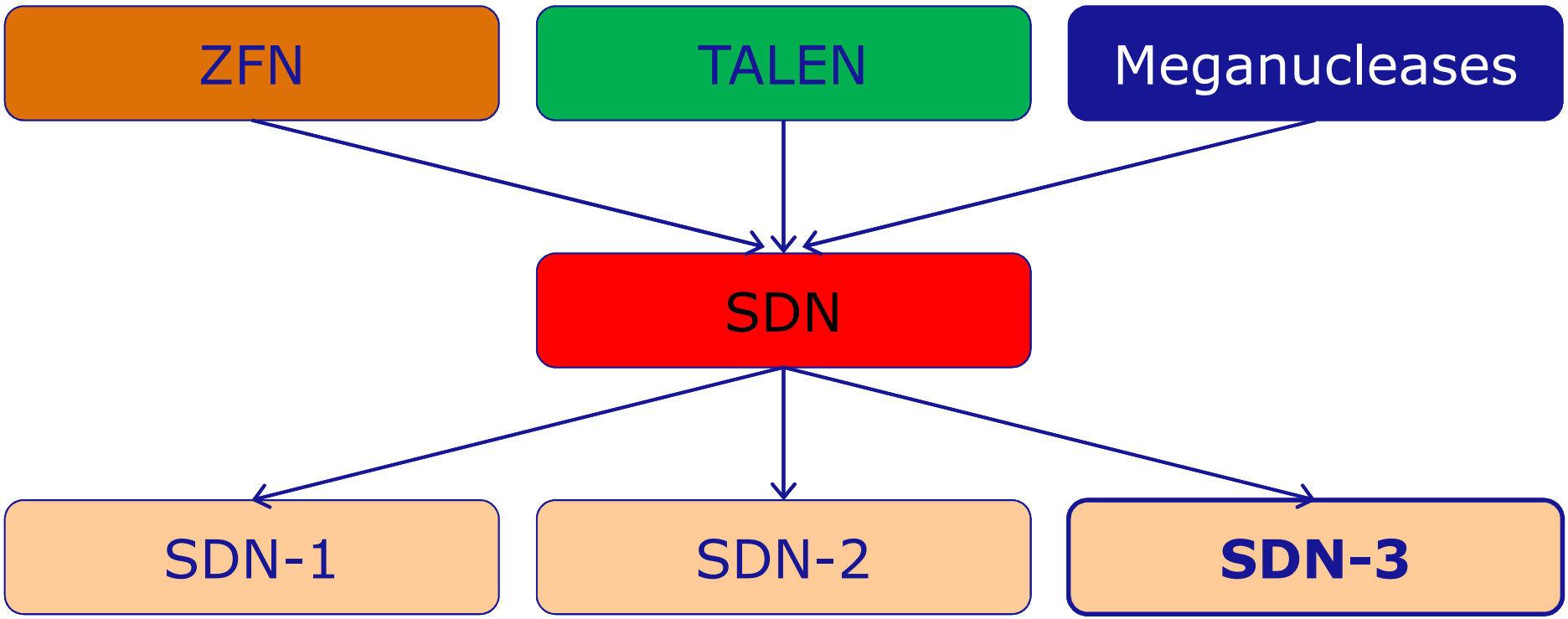
- The EFSA GMO Panel considers that **the Guidance documents** are applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and for performing an environmental risk assessment and **do not need to be further developed**.

- It can be envisaged that on a case-by-case basis **lesser amount of event-specific data are needed** for the risk assessment.

Conclusions on Cisgenesis & Intragenesis (2/2)

- The Panel concludes that **similar hazards can be associated with cisgenic and conventionally bred plants**, while novel hazards can be associated with intragenic and transgenic plants.

Site directed nucleases



SDN-**1** Site specific random point mutation (in/del)

SDN-**2** Site specific gene modification(in/del)

SDN-**3** Site specific insertion of a transgene or cisgene

Potential adverse effects of SDN-3

Identification of characteristics having the potential to cause adverse effects:

- the source of the DNA and the safety of gene products
- alterations to the host genome at the insertion site and elsewhere

  -Alteration at the insertion site

  As DNA is introduced into an exact, pre-defined location in the plant genome during SDN-3, unintended effects can be minimised

  -Alteration elsewhere in the genome

  Due to off-target activity

- the potential presence of non-plant sequences in the insert
- the expression of the trait and its potential wider implications

Applicability of the guidances

The EFSA GMO Panel is of the opinion that the two EFSA GMO Panel guidance documents for GM plants cover all of the elements and approaches that might be required to risk assess plants developed using SDN-3 approaches.

On a case-by-case basis lesser amounts of data are needed

- Examples
  - Where SDN-3 is used for cisgenesis
  - Integration of a well known transgene (e.g. Cry1Ab) into a position in the genome that has been used previously (without indication of unintended effects)

Conclusions on SDN-3

- The Panel concludes that the SDN-3 technique does not differ from transgenesis.
- The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome. Therefore, the **SDN-3 technique can minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome.**

EU approach NPBT (2011)

- **Working Group on New Techniques report**
- **JRC work on New Plant Breeding techniques**
  - ✓ **IPTS study on adoption and economic impact**
  - ✓ **IHCP task force on detection challenges**
- **EFSA opinion- safety aspects NPBT**
- **Legal analysis by European Commission**
- **Decision by EU Competent Authorities**

State of play in EU (2014)

- **WG report finalized in 2011**
- **JRC contribution published in 2011**
- **EFSA opinion on safety of cis/intragenesis and SDN-3**
- **No mandate for other NPBT**
- **Analysis of European Commission: expected at the end of 2014?**

Experience so far with NPBT in the EU

- **Only in field trials, not yet on commercially grown**
- **Experience with cisgenesis, intragenesis, ODM, SDN and RNAi (OECD survey)**
- **Wait for: EU analysis, decision EU member states……**