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European Food Safety Authority

Genetically Modified Organisms UNIT

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**Subject: Request to the European Food Safety Authority to provide technical assistance on issues related to the legal analysis of new plant breeding techniques**

*Ref. Your letter dated 28 August 2015 Incoming N° 115421*

Dear Ms André,

I thank you for your letter dated 28 August 2015 requesting EFSA to provide technical assistance on issues related to the legal analysis of new plant breeding techniques (NPBTs).

In your letter, you request EFSA's opinion on four questions that should help your legal analysis on whether organisms produced through these technologies fall under the scope of Directives 2001/18/EC and 2009/41/EC.

**Question 1: Definition of the term "recombinant nucleic acid molecule"**

**DG SANTE question:** *In the light of previous discussions with you and your staff, would EFSA agree with the following definition?*

*Recombinant nucleic acid molecule: molecules which are generated by joining nucleic acid molecules and which can replicate in a living cell.*

*Please consider that in Directive 2001/18/EC the term is used in Annex IA, Part 2 and Annex 1B as follows: "... on the condition that they (the techniques) do not involve the use of recombinant nucleic acid molecules...". In this context the term does not encompass natural recombination.*

**EFSA answer:** The concept of "recombinant nucleic acid molecule" has been introduced in the 1970s to describe the possibility to generate new combinations of nucleic acid molecules joining nucleic acid stretches that were not possible to combine before the development of the recombinant nucleic acid technology (for a review see Itakura and Riggs, 1980).

In this context and in the broad sense, a recombinant nucleic acid molecule can be defined as a molecule that is generated by joining two or more nucleic acid molecules.

This definition reflects the first part of the definition proposed by DG SANTE ("molecules which are generated by joining nucleic acid molecules"), but does not include the second part of the definition proposed by DG SANTE, referring to replication in a living cell.

However, it is recognised by the EFSA GMO unit that frequently, following the generation of a recombinant nucleic acid molecule, this recombinant nucleic acid molecule is transferred into a living cell/organism to enable its replication.

EFSA notes that the definition proposed by DG SANTE is very similar to that proposed in the guideline of the National Institute of Health (NIH). In this guideline (NIH, 2013), targeted to clinical and basic research, recombinant nucleic acid molecules are defined as "*molecules constructed by joining nucleic acid molecules and that can replicate in a living cell*".

**Question 2: Mutagenesis**

**DG SANTE question:** *Does EFSA consider oligonucleotide directed mutagenesis, ZFN 1 and ZFN 2 (and similar site directed nucleases techniques) as a form of mutagenesis? Could EFSA provide the rationale behind its opinion in this regard?*

**EFSA answer:** The EFSA GMO Unit considers that the currently available oligonucleotide directed mutagenesis (ODM), ZFN-1 and ZFN-2 and similar site directed nuclease (SDN) techniques create point mutations similar to those introduced via natural or induced mutagenesis, and can thus be considered a form of mutagenesis.

**Rationale:** Natural and induced mutagenesis can introduce different classes of mutations, one of which is the point mutation. A point mutation results in a change of one (or few) base pairs (bp) in the nucleotide sequence. Point mutations include substitutions (transitions and transversions) and insertions and deletions (defined as *frameshift* mutations). The EFSA GMO Unit considers that the modifications that can be obtained with ODM, ZFN-1, ZFN-2 and similar SDN are comparable in type and extent to point mutations which can be obtained via natural or induced mutagenesis (for further details see Section 3.2 EFSA GMO Panel, 2012). Point mutations introduced with ODM, ZFN-1, ZFN-2 and similar SDN are indistinguishable from point mutations introduced by natural or induced mutagenesis.

- The types of modification which can be introduced with the ODM, ZFN-1, ZFN-2 and similar SDN techniques are in fact substitutions, insertions and deletions.
- The extent of the mutation introduced with the aforementioned techniques is limited in length. Currently, in plants, ODM is used to obtain a single bp mutation and rarely to introduce 2 bp mutations into a target sequence (Dong et al., 2006; Breyer et al., 2009). In case of ZFN-1, ZFN-2 and SDN, by definition, a point mutation is introduced (DG SANCO Ares(2011)201516 – 23/02/2011; Section 1 of EFSA GMO Panel, 2012; Lusser et al., 2012; Podevin et al., 2013).

In case the above presented 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 in order to address question 2.

**Question 3: Definition of the term "genetic material"**

**DG SANTE question:** *Could EFSA provide a definition of "genetic material"?*

**EFSA answer:** The classical studies of Griffiths (1928) and Avery, MacLeod and McCarty (1944) demonstrated that nucleic acids are the genetic material responsible for the transfer of traits (see for example Krebs et al., 2014).

Against this background, a definition of genetic material can be: the genetic material is the nucleic acid molecule as defined by its nucleic acid sequence which contains the genetic information.

The EFSA GMO Unit, in the definition provided above, did not consider scenarios which include non-sequence-related aspects, for example those involving the modulation of the gene expression. The provided definition of genetic material is based on the classical studies.

**Question 4: RNA dependent DNA methylation**

**DG SANTE question:** *Considering that the DNA methylation is an epigenetic modification, would EFSA nonetheless regard it as an alteration of the genetic material? Or would "an alteration of the genetic material" be restricted to the cases where the nucleotide sequence is modified?*

**EFSA answer:** In line with the definition provided in the previous answer, the term "alteration of the genetic material" could be restricted to situations where the nucleotide sequence is modified.

**Rationale:** DNA methylation is one type of epigenetic regulation. It occurs at the level of nitrogenous base(s) of the nucleic acid molecule (mainly on cytosine residues) and can impact gene expression. However, the nucleotide sequence is not altered by DNA methylation, and the modification is reversible.

**Remark**

The EFSA GMO Unit is aware that NPBTs evolve extremely rapidly and some of the considerations mentioned above might require revision in the light of new methodological developments.

With this letter, I hope to have responded to your questions.

Yours sincerely,

p.o. Claudia Paoletti

Elisabeth Waigmann

Head of the GMO Unit

cc: Ms Pelsser, Ms Ciabatti – DG SANTE

Ms Kleiner, Ms Waigmann, Ms Paoletti, Ms Gomes, Mr Divéki, Mr Ramon, Mr Gennaro – EFSA

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