Annex

Request for a scientific opinion on *in vitro* random mutagenesis techniques

Background

The judgment of the Court of Justice of the European Union (CJEU) in Case C-528/16[1] on mutagenesis held that Article 3(1) of Directive 2001/18 on the deliberate release of Genetically Modified Organisms (OGM)[2] must be interpreted as meaning that "*only GMOs obtained by means of techniques/methods of mutagenesis which have conventionally been used in a number of applications and have a long safety record*" are excluded from the scope of that directive. The JCEU in its reasoning referred to the "*application of conventional methods of random mutagenesis*" without distinguishing further between *in vivo* and *in vitro* random mutagenesis and distinguished them from "*new techniques/methods of mutagenesis which have appeared or have been mostly developed since Directive 2001/18 was adopted*" [3].

Following the ruling of the CJEU, the Conseil d'Etat of France issued on 7 February 2020 a judgment on organisms obtained by mutagenesis. In its judgment, the Conseil d'Etat describes conventional or random mutagenesis as a technique triggering random mutations in a DNA sequence through the action of chemical or physical mutagens. The French Conseil d'Etat distinguishes between *in vivo* and *in vitro* random mutagenesis techniques. *In vivo* random mutagenesis would consist in the application of chemical or physical mutagens to whole plants or parts of plants, which would then be subject to selection procedures in order to identify the interesting mutations. *In vitro* random mutagenesis would consist in subjecting plant cells to chemical or physical mutagenic agents. The modified cells would then be subject to techniques of *in vitro* cell culture in order to regenerate the whole plant.

EFSA, in its Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function[4], examines conventional plant breeding techniques relevant for a comparison with Site Directed Nuclease-3 technique. Among these conventional techniques, EFSA describes mutation breeding by chemical and physical mutagenesis. While EFSA explains the various modes of action depending on the chemical mutagens or the type of radiation used, the Authority makes no distinction between the application of the techniques *in vitro* or *in vivo*.

Member States have never made a distinction between *in vitro* and *in vivo* either when implementing the seed legislation, the plant propagating material legislation or the GMO legislation.

It is therefore important to provide a robust scientific understanding of random mutagenesis techniques and a robust scientific analysis as to whether the distinction between *in vitro* and *in vivo* is scientifically justified.

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[1] Case C-528/16, *Confédération paysanne and Others*, Judgment of 25 July 2018, EU:C:2018:583.

[2] Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1), Article 4.

[3] Case C-528/16, *Confédération paysanne and Others*, Judgment of 25 July 2018, EU:C:2018:583, points 48 et 51.

[4] 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. Available online: www.efsa.europa.eu/efsajournal

Terms of reference

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

- To provide a more detailed description of random mutagenesis techniques as applied *in vivo* and *in vitro*.
- To assess whether the types of genetic modification induced by random mutagenesis techniques are different depending on whether the technique is applied *in vivo* or *in vitro*.
- To assess whether the molecular mechanism underlying random mutagenesis techniques is different if the techniques are applied *in vivo* or *in vitro.*
- To assess whether *in vitro* random mutagenesis techniques are to be considered as different techniques compared to *in vivo* random mutagenesis techniques or on the contrary, if they are to be considered as a continuum.