

Comment on EFSA draft document on risk assessment of plants developed with Type 1 (SDN-1) and Type 2 (SDN-2) site-directed nucleases and oligonucleotide directed mutagenesis (ODM)

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Introduction

The judgement of the Court of Justice of the European Union (CJEU) in Case C-528/16 on mutagenesis has clarified that Directive 2001/18/EC is applicable to genetically modified organisms (GMOs) obtained by mutagenesis techniques, e.g. the new methods of genetic engineering involving so-called nucleases ('gene scissors'). Directive 2001/18 deals with the environmental risk assessment of genetically engineered organisms.

Against this backdrop, the EU Commission has asked EFSA whether the application of nucleases, including usage of CRISPR/Cas, raises new or similar challenges to risk assessment compared to other methods of genetic engineering such as transgenic plants. The comparison also includes other methods of so-called random mutagenesis (using chemical or physical mutagens). EFSA published its draft at the end of April and is calling for input from the public to be submitted by 27 May 2020.

Nucleases such as CRISPR/Cas can be considered to be so-called biotechnological (biological) mutagens, which were not available prior to genetic engineering. These applications are based on the synthesis of nucleotides (DNA or RNA) which, in the case of nucleases, are combined with technically designed proteins that can cut, or otherwise alter, the genome or epigenome of the plants. Some biotechnological mutagens (so-called zinc finger) have been known for several years, but were only rarely applied. With the rise of the CRISPR/Cas technique (first made available in 2012), the situation has changed. This nuclease is more flexible and easier to apply than most of the previous biotechnological mutagens.

CRISPR/Cas, by cutting DNA at a specific site, impairs specific genetic information that cannot be restored by the cell. If no additional DNA is inserted, these 'gene scissors' are called site-directed nucleases (SDN) Type -1 or -2. These nucleases are the main focus of the EFSA consultation, with CRISPR/Cas being the most relevant application.

It also includes so-called ODM technology (oligonucleotide-directed mutagenesis), which is based on the insertion of short sequences of nucleotides (DNA) into cells. These short cuts are intended to alter specific short sequences of the plant genome in accordance with the inserted DNA. This technology has, so far, rarely been applied and not much data is available on its potentials and risks. The lack of information is further emphasised in the EFSA draft.

Technical potentials and risks of biotechnological mutagens must be fully considered

The EFSA draft document suffers from major technical deficiencies: EFSA did not properly consider the real potential of SDN-1 and SDN-2 (especially if based on CRISPR/Cas) or the technical specificities that go along with the multistep process as applied to the plants. Consequently, the resulting risks and methodological problems in risk assessment are not sufficiently addressed.

SDN applications, and also ODM, are biotechnological mutagens that, contrary to chemical or physical mutagens, can directly and specifically interact with the biological mechanisms in the cell, on the level of the genome and/or epigenome. These approaches have a high potential to penetrate the genome and generate profound alterations in the biological characteristics of plants, without introducing any additional DNA sequences.

The resulting genetic changes will typically lead to biological characteristics, such as changes in plant composition, that exceed the range of characteristics which can be achieved using previous plant breeding methods. The risks associated with release, cultivation and consumption of these plants need to be fully investigated before any conclusions on the safety of the new organisms can be drawn.

Deficiencies in EFSA opinion

EFSA considers the risks of SDN-1 and SDN-2 processes to be similar to those of conventional breeding. However, EFSA did not provide an adequate scientific basis to compare conventional plant breeding (using non-targeted chemical or physical mutagens) to methods of genetic engineering using biotechnological mutagens. For example, EFSA did not consider that the biotechnological mutagens can sidestep mechanisms of natural gene regulation and typically cause specific patterns of genetic change and a new combination of genetic information, which cannot or hardly be achieved by methods of conventional breeding. Therefore, the EFSA methodology reveals fundamental deficiencies, leading to assumptions which are not sufficiently based on science.

Further, there is a wide range of specific unintended on-target and off-target effects associated with SDN-1 and SDN-2 interventions, largely depending on various technical parameters of the specific processes. All these technical details determine the precision as well as the efficiency of an intervention, and also have to be taken into account for risk assessment. However, EFSA did not consider unintended on-target effects at all.

New challenges in risk assessment

According to Testbiotech, the risk assessment of plants developed with Type 1 and Type 2 site-directed nucleases or with oligonucleotide-directed mutagenesis, has to consider; (i) several, distinct steps during the technical processes; (ii) the new combinations of genetic information and the resulting unintended and intended biological characteristics and (iii) on-target and off-target effects caused by biotechnological mutagen activity.

The set of data needed for risk assessment may substantially differ from those needed for hitherto existing transgenic plants. For example, if the newly generated gene combination results in profound changes of the plant metabolism, comparative risk assessment may be challenged to an extent that goes far beyond existing experience with previous transgenic plants. However, EFSA did not see the need to adapt their guidance accordingly by, for example, including methods such as metabolomics.

There are further problems in environmental risk assessment, including changes in the composition of plants that may impact the food web, or plant communication and interaction with the environment. In addition, there are potential changes in the biological characteristics of the plants regarding invasiveness and next generation effects if they persist and propagate in the environment.

Conclusions

The methodology and guidance for risk assessment needs to be improved and adopted accordingly, taking into account the restrictions inherent in the comparative approach and complex challenges posed by SDN-1 and SDN-2 plants for environmental risk assessment. In regard to ODM, many data are missing; therefore no final conclusion can be drawn on risks.

Whatever the case, detailed examination of an organism's genetic and overall biological characteristics, starting with the process that was used to generate the organism, is needed to decide whether the organism is safe. The set of data needed for risk assessment will depend on a case-by-case assessment and cannot generally be limited to criteria such as the insertion of additional genes.

References:

EFSA (2020) Applicability of the EFSA opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis, www.efsa.europa.eu/en/consultations/call/public-consultation-applicability-efsa-opinion-site-directed

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