

## Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis

EFSA published a Statement on criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis, and [related FAQs](#) in October 2022. Following this we received additional requests for information and clarifications on the risk assessment of new genomic techniques (NGTs) from the public. To make our replies accessible to everyone interested in this topic we have collected and made available these additional FAQs.

### **Will EFSA assess the 'speed or rapidity of certain developments' of these plants?**

No. EFSA's risk assessment is focused on the properties of a GM plant, and how it was generated. The time required to generate a GM plant does not impact on its properties and does not itself create a hazard. EFSA does not therefore consider the duration of development in its risk assessment.

### **What does 'breeder's gene pool' mean for the risk assessment of NGTs?**

The breeder's gene pool definition was provided in the EFSA scientific opinion on cisgenesis and intragenesis (EFSA GMO Panel, 2012). The same definition is maintained in the EFSA statement on 'Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis' (EFSA GMO Panel, 2022a). The concept of breeder's gene pool demarcates transgenic plants from plants that may be obtained through conventional breeding methods. The concept is one criterion, amongst others, used to determine the relevance of different assessment questions for the risk assessment.

### **What exactly is meant by 'safe harbour'?**

Genomic safe harbours are regions of the genome that can guarantee the expression of an inserted DNA sequence without disrupting any (endogenous) plant genes, allow for a predictable/stable expression, and avoid the generation of functional hazardous open reading frames at the insertion junction sites.

Targeted insertions in such regions are characterised by a significantly reduced potential for unintended effects compared to random insertions or conventional breeding (EFSA GMO Panel, 2022a).

In the 2022 EFSA statement this consideration is embedded in criterion 3, which differentiates between random integration and targeting to a safe-harbour (mediated by approaches such as site-directed nucleases (SDN)-3 discussed in EFSA GMO Panel, [2012b](#)).

### **Can EFSA provide additional clarifications on the evaluations of History of Safe Use and environmental familiarity?**

[FAQs](#) published by EFSA in October 2022 explain what 'history of use' refers to and how it can be evaluated. To further clarify, the History of Safe Use (HoSU) is a proposed criterion for a proportionate risk assessment of plants obtained by cisgenesis, intragenesis and targeted mutagenesis as the newly modified DNA sequence and associated trait may already be present in nature (EFSA GMO Panel, 2022). The demonstration of a HoSU is based on evidence that some or all parts of a plant have been consumed in the diet (food and/or feed and derived products) for a considerable time with no evidence of harmful effects for the consumer, and that exposure from a new use will be within the range of that of the 'historic' use. The concept is already established in the assessment of GMOs obtained with techniques developed before



2001. A similar approach, called environmental familiarity, is used in assessing risks to the environment.

If the History of Safe Use of the newly modified DNA sequence and associated trait cannot be sufficiently demonstrated, it is proposed in criterion 6 to assess its structure and function. As indicated in the 2022 EFSA statement on 'Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis', an operational definition of HoSU should be developed in the near future to support the risk assessment in the context of this statement and other areas where the concept is utilised.

### **Was EFSA requested to assess unintended effects caused by NGTs in the recent opinions?**

Assessment of unintended effects in GM plants is part of the risk assessment under Directive 2001/18/EC, Regulation (EC) 1829/2003, Regulation No 503/2013 and related EFSA guidance. In the recent EFSA opinions on NGTs, the GMO Panel addressed specific terms of reference requested by the European Commission to identify potential hazards and risks that plants obtained by targeted mutagenesis (EFSA GMO Panel, [2020](#)), and cisgenic and intragenic approaches (EFSA GMO Panel, [2022a](#)) or synthetic biology approaches (EFSA GMO Panel, [2021](#)) could pose for humans, animals and the environment. These hazards and risks include those possibly associated with unintended effects caused by the genetic modification, including unintended genetic changes and unintended effects to human and animal health and the environment. Unintended effects potentially caused by the use of NGTs were therefore part of the mandate and considered by EFSA.

### **How did EFSA assess unintended effects caused by NGTs in the recent EFSA opinions?**

As part of the data and methodology to develop these outputs, the GMO Panel experts considered all relevant scientific literature. The scientific literature analysed by EFSA in the opinion on site-directed nucleases (SDN) amounted to more than 150 articles (EFSA GMO Panel, [2020](#)). On synthetic biology, an independent literature screening and experts' horizon mapping including genome editing case studies, were performed (EFSA GMO Panel, [2021](#)). For the 2022 cisgenesis and intragenesis opinion, that also included the use of SDN technologies, EFSA screened 650 publications. In addition, our scientists carried out a patent search, applying the criteria listed in a specific protocol. This information is publicly available in two detailed annexes published by EFSA together with the scientific opinion (EFSA GMO Panel 2022b).

### **How was the literature proposed by respondents to the public consultations considered in EFSA's work and specifically in the development of the EFSA statement (which did not undergo a public consultation)?**

The 2022 EFSA statement is based on previous EFSA scientific opinions. Public consultations were held for all these EFSA opinions. EFSA's GMO Panel reviewed the comments and assessed the literature indicated in the public comments. Responses were provided to each comment in each consultation report. The GMO Panel always considers each comment and literature paper received in the public consultation and amends the draft scientific opinion if deemed necessary. If no new evidence is reported that impacts the Panel's conclusions in relation to the Terms of Reference of each assessment, the proposed citations are not included in the reference list of the output.

A Statement of EFSA is prepared as advice or a factual statement for consideration by the European Commission, and because of its nature (i.e. being based on previous opinions) a public consultation was not held, but the statement was discussed publicly in a dedicated [Webinar](#) where feedback was received from different stakeholders and at the [14th meeting](#) of the Network on Risk Assessment of GMOs.



### **Do EFSA's assessment criteria take into account the possibility of multiplexing with NGTs?**

Simultaneous modification of multiple genomic loci can be achieved not only by NGTs but also by transgenic and conventional breeding approaches (for example, gene stacking). The risk assessment of plants with complex traits obtained by multiplexing is discussed in the 2022 EFSA statement on 'Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis' and opinions on Synthetic biology (EFSA GMO Panel, 2021; 2022a). No new hazard was identified for plants obtained by multiplexing approaches compared to transgenesis and conventional breeding. The GMO Panel did, however, highlight certain challenges that the risk assessment could bring up when assessing such products according to the current methodologies.

### **Are NGT modifications also possible in genomic locations which are not accessible through conventional breeding?**

A recent study provided evidence that genetic mutations do not occur randomly and that there are genomic regions which appear to be less prone to accumulate mutations than others (Monroe J.G. et al., 2022). However, current available data do not indicate the existence of genomic regions in which mutations cannot occur at all. Rather, some regions show a reduction in mutation frequency compared to others (Monroe J.G. et al., 2022). Therefore, conventional breeding (including random mutagenesis) may still be able to achieve the same outcome as NGTs although it may require more effort and time to generate and select a specific genetic modification.

### **Is it therefore correct that the modification of the genome with NGTs goes beyond what is done with conventional breeding?**

No. The claim that due to multiplexing and genome accessibility the genetic modifications achievable by NGTs go beyond what is possible with conventional breeding is not correct. As indicated in the answer to the *previous question*, multiplexing can also be achieved by conventional breeding (for example, by gene stacking) and conventional breeding (including random mutagenesis) may be able to achieve the same outcome as NGTs, although it may require more effort and time to generate and select a specific genetic modification.

## **References:**

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