

EU Commission draft proposal for criteria concerning the equivalence of NGT plants to conventional plants

Proposed deregulation goes against science

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Summary

The EU Commission proposal for the “Regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed” establishes a category for plants derived from processes of New GE (new genetic engineering or new genomic techniques, NGTs) that would be exempt from current GMO regulation. It is summarized in Annex 1 of the proposal.

This new category sets out criteria that can be used to define certain NGT plants, and thus allow them to be equated to plants obtained from conventional breeding. NGT plants belonging to this “Category 1” will not be subjected to mandatory risk assessment, they would only require notification.

As shown in this backgrounder, the proposed criteria go against the science. If applied, the criteria would lead to NGT plants that are substantially different from those obtained from conventional breeding being classified as equivalent. The criteria are certainly not adequate to establish whether NGT plants can be exempt from current GMO-regulation.

In the light of the intended and unintended genetic changes that can be caused by the processes of NGT, it has to be concluded that also in future, process-based risk assessment will be a necessity: NGTs can be used to target specific sites in the genome in order to knock out gene functions or insert new functions. However, the consequences of these 'cuts' into the genome are frequently not

predictable and cannot be adequately controlled. NGT processes may result in intended or unintended genetic changes and biological (phenotypical) effects that are significantly different compared to those that can be expected from conventional breeding.

Consequently, without detailed risk assessment, no conclusion can be drawn on whether or not an NGT plant may be considered as safe as a plant obtained from conventional breeding. Therefore, we propose that Category 1 is deleted (in addition to the criteria in Annex 1) from the regulatory proposal.

Introduction

A draft proposal for the “Regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed” was published on 5 July.¹ This proposal comprises Annex 1 with “criteria of equivalence of NGT plants to conventional plants” (‘Category 1’).

The category in Annex 1 aims to establish criteria to help to define which NGT plants can be equated to plants obtained from conventional breeding. NGT plants placed in Category 1 are not meant to be regulated GMOs (with exception of organic agriculture). As a consequence, the plants in Category 1 would not be submitted to mandatory risk assessment, they would only require notification.

Based on the proposed criteria, decisions could be taken to exempt plants from current GMO-regulation without risk assessment, based simply on the formal criteria in Annex 1. The information regarding these criteria would be delivered by the applicant. The criteria seem to only address intended genetic changes at the target site that are introduced to achieve specific plant characteristics.

The criteria are meant to provide the necessary information to decide whether the NGT plants are as safe as conventionally-bred plants, without mandatory risk assessment. Significantly, the same criteria can also be applied to plants not obtained from breeding, i. e. non-domesticated plants such as weeds, trees, mosses and algae.

The criteria could also be applied before experimental field releases take place, thus allowing decisions about notification to be made at the level of member states.

However, as shown below, the criteria are arbitrary and do not adequately protect health or environmental safety, they do not take into account phenotypical effects. They appear to be driven by the political goal of extensively deregulating NGT plants without taking the precautionary principle into account.

Technology, biology and regulation

Current GMO regulation (Directive 2001/18) requires that intended and unintended genetic changes are subjected to molecular risk assessment as well as assessment of the effects (direct and indirect, immediate and delayed or accumulated) they may cause. Therefore, genetic changes introduced by the process of genetic engineering (new genomic techniques) have to be taken into account,

¹ https://food.ec.europa.eu/plants/genetically-modified-organisms/new-techniques-biotechnology_en

especially if they are unlikely to occur with conventional breeding methods (including random mutagenesis).

The identification of relevant genetic changes can, for example, be achieved by assessing the likelihood of mutations occurring at these sites and resulting in the same genetic combination (genotype) as if NGTs had not have been applied. The relevant changes may, for example, comprise deletion of gene functions in many genes or gene copies simultaneously, insertions and inversions, chromothripsis and frameshift mutations (see, for example, Biswas et al., 2020; Braatz et al., 2020; Burgio et al., 2020; Kapahnke et al., 2016, Kapusi et al., 2017; Kawall et al., 2020; Kawall 2021a; Kosicki et al., 2018; Lalonde et al., 2017; Liu et al., 2021; Michno et al., 2020; Samach et al., 2023; Sharpe, 2017; Skryabin et al., 2020; Wolt et al., 2016).

Larger structural genomic changes, such as translocations, deletions, duplications, inversions and scrambling of chromosomal sequences, can occur near the SDN target site (as well as at the SDN target site), which would otherwise be unlikely to occur (see, e. g. Hahn & Nekrasov 2019).

Furthermore, the CRISPR/Cas machinery is known for its potential to confuse target regions with specific off-target regions, and also to cause the unintended insertion of additional genes, the decoupling of genes and other specific genomic alterations (in the categories of inversions, deletions or rearrangements) that are unlikely to result from spontaneous mutations or physical and chemical mutagenesis (see, for example, Biswas et al., 2020 Braatz et al., 2017; Hahn & Nekrasov 2019). Yang et al. (2022) provided an overview of irregular genetic changes and specific unintended effects caused by intrinsic factors inherent to CRISPR/Cas systems in plants. These include off-target DNA cleavage, repetitive unit deletion and indels of various sizes (Chakrabarti et al., 2019; Kapusi et al., 2017; Manghwar et al. 2020; Molla and Yang, 2020; Zhang et al., 2014).

While these types of mutations are well known from conventional breeding (including random mutagenesis), the sites of the mutations and their frequency (at the specific sites) and the resulting genetic combination may differ greatly. The reason: NGTs can cause genetic changes at genomic sites that are otherwise well protected by mechanisms in the cells to maintain or restore gene function. It seems that the frequency of mutations within the genome is not distributed equally (see, for example, Monroe et al., 2022).

There are several mechanisms known in cells that can help to maintain or restore gene functions (such as repair mechanisms and gene copies). This may be because of local differences in genome properties with regard to: i) folding; ii) nucleotide sequence; iii) epigenetic coding or iv) the repair mechanisms. It is very likely these are evolutionary mechanisms that are meant to ‘protect’ certain genomic regions against a high frequency of mutation. However, CRISPR/Cas increases the likelihood of transection within such protected areas. The NGTs can overcome these mechanisms and make the genome available to much greater extent (Kawall 2019). This is one of the reasons why these plants should undergo detailed risk assessment.

Other relevant criteria include resulting biological effects if they are unlikely to occur with non-regulated conventional breeding. In this regard, for example, changes in gene expression may result in extreme traits characterized by changes in composition or altered reactions to environmental conditions to a degree that is unlikely to result from conventional breeding (see, for example, EFSA, 2022 on Zsögön et al., 2018; Morineau et al., 2017; Nonaka et al., 2017).

In general, NGTs can cause genetic changes (‘genotype’) and biological effects (‘phenotype’) that differ from those derived from conventional breeding, even if no additional genes are inserted. The

European Food Authority (EFSA) has so far not adequately considered these factors, mechanisms and effects. However, overlooking resulting genetic changes and effects may have an impact on food, environmental safety and the future of plant breeding, as these may all accumulate in future plant generations.

The proposed criteria

The proposed criteria are mostly based on the number of genetic alterations, e. g. insertions, substitutions, deletions, etc., the type of mutation, and, in part, their size. In addition, a comparison is made to what is called the breeders' gene pool (genotypes from crossable sources). Unfortunately, the criteria are inadequately defined and not sufficiently backed by science.

The EU Commission proposes having an overall threshold of 20 genetic modifications (of each of the types referred to in points 1 to 5, see below) throughout the genome. It seems this proposal does not cover unintended genetic changes caused by the processes of NGTs, as nowhere in the overall regulatory proposal is there an explicit request to assess unintended effects caused by NGT processes.

The genetic modifications 1 to 5 are characterized by

- (1) the number of nucleotides 20 changed in regard to insertions or substitutions. It seems that this criterion is primarily aimed at plants developed with SDN-1;
- (2) any deletions, regardless of size or number;
- (3) targeted insertion or substitution by using gene variants already in the breeders' gene pool. It seems that this criterion is primarily aimed at plants developed with SDN-2 and SDN-3 and resulting in cisgenic plants (but not intragenic or transgenic plants);
- (4) any targeted inversion, regardless of size or number;
- (5) any other targeted genetic changes, on the condition that the resulting DNA sequences already occur (possibly with alterations as defined in criteria 1 & 2 above) in a species in the breeders' gene pool.

General comments

In general, the proposed criteria seem to be arbitrary and unclear, making them difficult to interpret in detail. Furthermore, it does not comprise those which we identified as the most relevant: the site of mutations and the resulting genotypes and biological effects (phenotype) are completely disregarded. Furthermore, criteria such as the overall number of mutations or the number of nucleotides changed (Criterion 1) lack scientific reasoning. For example, by introducing a lower number of genetic changes (and / or lower number of changes in nucleotides), it is possible to generate traits that go beyond what can be expected from conventional breeding. Even though the resulting plants are in fact significantly different to those that are likely to result from conventional breeding, they are nevertheless categorized as equivalent in the proposed criteria.

In addition, there is no scientific justification for criteria that allow any kind of deletion (Criterion 2) or targeted inversion (Criterion 4) to occur. It is known that these genetic interventions can also result in plants that are substantially different compared to those that can be obtained from conventional breeding. Nevertheless, these NGT plants would still be considered equivalent.

Overall, the proposed criteria only seem to address intended changes. For example, Criteria 3 and 5 do not request analysis if non-targeted changes caused by the processes of NGTs can be found in the breeders' gene pool. In addition, the site of insertion is not confined to corresponding sites in the genome, where the DNA sequence would be found naturally. It is known that the site and frequency of intended and unintended genetic changes in NGT plants can differ from those expected with conventional breeding. Nevertheless, these NGT plants would be considered equivalent.

In addition, several expressions, e. g. "sequence similarity", are not well defined or put into the correct context: for example, it is known that similarity in gene sequences can lead to mismatch results with nucleases such as CRISPR/Cas. In this case, the genetic change would not occur in "predictable DNA sequences" since, in many cases, these unintended genetic changes are not predictable. As a result, unintended genetic changes caused by the application of NGTs can largely differ in their site and frequency from those expected from conventional breeding. Nevertheless, these NGT plants would be considered to be equivalent.

Case studies: Application of the criteria in Annex 1 for specific NGT plants

The table below uses two NGT plants as examples to show some of the limitations of the criteria listed in Category 1. These examples are camelina with a change in oil content (Morineau et al., 2017) and tomatoes with an increase in gamma aminobutyric acid (Nonaka et al., 2017). In both cases, it seems the number and size of the genetic changes falls within the new category that is meant to define equivalence to conventional breeding. In fact, the genetic differences are so significant that it would be no problem to develop methods to detect such plants.

Table: Exploration of the potential outcome of Category 1 criteria for two NGT plants

Example	Application of criteria of Category 1	Overall equivalence with conventionally-bred plants	Further comments
In camelina, 3 genes (18 gene sites) were knocked out to alter oil quality and oil concentration for production in agrofuels (Morineau et al., 2017).	<p>The number of genetic changes is below 20. The number of substituted nucleotides at each site is likely to be below 20 (at least at most of the sites, if deletions are set aside).</p> <p>According to the publication, no genes from outside the breeders' gene pool are inserted.</p> <p>Therefore, it cannot be ruled out that equivalence to conventional breeding would simply be assumed.</p> <p>If equivalence is assumed, notification could be accepted before experimental releases are started by any of the EU member states. This</p>	<p>It can be concluded from the Morineau et al., (2017) study that the overall pattern of intended genetic changes and the composition of the oil produced by the plants go beyond that expected from conventional breeding.</p> <p>No conclusions can be drawn on unintended insertions of transgenic elements, off-target effects or other unintended genetic changes, as no data have so far been published.</p> <p>In summary, these plants cannot be considered equivalent to those derived from conventional breeding.</p>	<p>Kawall (2021b) showed that environmental risk assessment of NGT plants that can persist, propagate and spread in the environment is essential.</p> <p>However, if equivalence were to be concluded, no environmental risk assessment would be performed.</p> <p>In addition, this would not require health risk assessment for plants that might be introduced unintentionally into the food chain.</p> <p>Finally, no method for detection would be made available.</p>

Example	Application of criteria of Category 1	Overall equivalence with conventionally-bred plants	Further comments
In tomatoes, 2 genes (4 gene sites) were knocked out to increase the content of gamma aminobutyric acid (GABA), which is supposed to potentially lower blood pressure after consumption (Nonaka et al., 2018).	<p>decision could be sufficient to enable free movement within the EU.</p> <p>The number of genetic changes is below 20. The number of substituted nucleotides at each site is likely to be below 20 (at least at most of the sites, if deletions are set aside).</p> <p>According to the study, no genes from outside of the breeders' gene pool are inserted.</p> <p>Therefore, it cannot be ruled out that equivalence to conventional breeding would be assumed.</p> <p>If equivalence is assumed, notification could be accepted before experimental releases are started by any of the EU member states. This decision can be sufficient to enable free movement within the EU.</p>	<p>It can be concluded from the Nonaka et al., (2018) study that the overall pattern of intended genetic changes and the GABA content go beyond what could be expected from conventional breeding.</p> <p>No conclusions can be drawn on unintended insertions of transgenic elements, off-target effects or other unintended genetic changes, as no data have so far been published. However, several unintended gene insertions were reported at the target site.</p> <p>In summary, these plants cannot be considered equivalent to those derived from conventional breeding.</p>	<p>The increased content in GABA is meant to provide health benefits. Tomatoes with these qualities are formerly unknown so they may be assessed under the novel food (Directive 2015/2283) and health claims regulation (EC No 1924/2006). However, these regulations are not meant to assess unintended genetic changes or related effects, they are focused on the intended effects of the products.</p> <p>Assessment of environmental risks would be not required, even though it is known that the metabolism of GABA plays a role in the response of the plants to environmental stressors.</p>

Conclusions

The criteria as proposed in Annex 1 are:

- not clearly defined overall;
- do not address the most relevant differences between conventional breeding and NGTs in regard to their genotype and phenotype;
- do not address unintended genetic changes caused by NGT processes;
- lacking in adequate scientific reasoning.

If applied, the criteria would lead to NGT plants that are substantially different from those obtained from conventional breeding being classified as equivalent. Therefore, the criteria are not adequate to establish equivalence between NGTs and conventional breeding.

We are well aware of the intended and unintended genetic changes that can be caused by NGT processes and, therefore, conclude that even in future, process-based risk assessment will be a necessity:

NGTs can be used to target specific sites in the genome in order to knock out gene functions or insert new functions. However, the consequences of these 'cuts' into the genome are, in many cases, unpredictable and cannot be sufficiently controlled. NGT processes may result in intended or

unintended genetic changes and biological effects that are significantly different compared to those expected from conventional breeding.

Therefore, decisions on whether NGT plants are ‘equivalent’ or different in comparison to plants obtained from conventional breeding can only be made after full molecular risk assessment. Consequently, without detailed risk assessment, no conclusions can be drawn as to whether an NGT plant may be considered as safe as a plant obtained from conventional breeding.

In summary, process-based risk assessment will in future continue to be necessary for NGT plants. Only after full molecular risk assessment, can reliable conclusions be drawn to inform further steps in risk assessment.

In this context, Category II proposed by the EU Commission will also need further discussion. We do not expect that the risk assessment of all NGT plants will need the same amount of data, nevertheless, all NGT plants need to undergo molecular risk assessment (including the assessment of intended and unintended changes) and cannot simply be exempted from current GMO regulation.

Therefore, we propose that Category 1 and the criteria in Annex 1 are deleted from the regulatory proposal.

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