Adopted: 19 June 2024

DOI: 10.2903/j.efsa.2024.8894

efsa JOURNAL

SCIENTIFIC OPINION

Scientific opinion on the ANSES analysis of Annex I of the EC proposal COM (2023) 411 (EFSA-Q-2024-00178)

EFSA Panel on Genetically Modified Organisms (GMO) | Ewen Mullins | Jean-Louis Bresson | Tamas Dalmay | Ian Crawford Dewhurst | Michelle M. Epstein | Leslie George Firbank | Philippe Guerche | Jan Hejatko | Francisco Javier Moreno | Hanspeter Naegeli | Fabien Nogué | Nils Rostoks | Jose Juan Sanchez Serrano | Giovanni Savoini | Eve Veromann | Fabio Veronesi | Josep Casacuberta | Ana Afonso | Paolo Lenzi | Nikoletta Papadopoulou | Tommaso Raffaello

Correspondence: nif@efsa.europa.eu

Abstract

EFSA was asked by the European Parliament to provide a scientific opinion on the analysis by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) of Annex I of the European Commission proposal for a regulation 'on plants obtained by certain new genomic techniques (NGTs) and their food and feed, and amending regulation (EU) 2017/625'. The Panel on genetically modified organisms (GMO) assessed the opinion published by ANSES, which focuses on (i) the need to clarify the definitions and scope, (ii) the scientific basis for the equivalence criteria and (iii) the need to take potential risks from category 1 NGT plants into account. The EFSA GMO Panel considered the ANSES analysis and comments on various terms used in the criteria in Annex I of the European Commission proposal and discussed definitions based on previous EFSA GMO Panel opinions. The EFSA GMO Panel concluded that the available scientific literature shows that plants containing the types and numbers of genetic modifications used as criteria to identify category 1 NGT plants in the European Commission proposal do exist as the result of spontaneous mutations or random mutagenesis. Therefore, it is scientifically justified to consider category 1 NGT plants as equivalent to conventionally bred plants with respect to the similarity of genetic modifications and the similarity of potential risks. The EFSA GMO Panel did not identify any additional hazards and risks associated with the use of NGTs compared to conventional breeding techniques in its previous Opinions.

KEYWORDS

genetically modified plants, genome editing, new genomic techniques, risk assessment

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1 | INTRODUCTION

1.1 | Background

The European Commission legislative proposal (COM(2023) 411) adopted on 5 July 2023[1] (hereafter, European Commission proposal) distinguishes between two categories of plants developed by new genomic techniques (NGT plants):

1. NGT plants that could occur naturally or be produced by conventional breeding techniques,[2] including random mutagenesis using chemical and/or physical mutagenic agents ('category 1 NGT plants'). Such plants would be treated similar to conventional plants and would only require a verification procedure to confirm that they are equivalent to plants obtained by conventional breeding.
2. NGT plants characterised by more complex sets of genetic modifications and that should remain subject to the requirements of the EU GMO legislation ('category 2 NGT plants').

To ensure legal certainty, criteria to ascertain whether an NGT plant is equivalent to naturally occurring or conventionally bred plants (i.e. category 1 NGT plants) were proposed by the European Commission in Annex I of the proposal. Those criteria include the characteristics and thresholds for size and number of genetic modifications that are allowed in the genome of category 1 NGT plants.

An opinion of the French National Agency for Food, Environmental and Occupational Health & Safety (ANSES) was published on 24 January 2024 (ANSES, 2023) providing an analysis of Annex I of the European Commission legislative proposal adopted on 5 July 2023, which sets criteria to determine the equivalence of category 1 NGT plants to plants obtained via conventional breeding.

1.2 | Mandate from the European Parliament to EFSA

Following a request on 1 February 2024 from the Chair of the European Parliament's Committee on Environment, Public Health and Food Safety (ENVI), the European Parliament formally requested on 22 February 2024 the European Food Safety Authority (EFSA) in accordance with Article 29 of Regulation 178/2002 '*to deliver a scientific opinion on the analysis by the French Agency for Food, Environmental and Occupational Health Safety (ANSES)*[3] *on Annex I of the Commission proposal for a regulation on plants obtained by certain new genomic techniques and their food and feed, and amending regulation (EU) 2017/625*[4] *(NGTs plants proposal). The proposal which establishes a specific regulatory framework for NGT plants and their products* (lex specialis), *introduces specific criteria to determine whether a NGT plant is to be considered equivalent to conventional breeding and therefore be exempted from the European Union GMOs Legal Framework. Those criteria are listed in Annex I of the proposed Regulation*'. EFSA was specifically requested '*to draw up a scientific opinion on the analysis by ANSES, which examines in particular the criteria set in Annex I.*' (M-2024-00031).

The European Parliament requested EFSA to deliver this scientific opinion by the end of July 2024.

2 | DATA AND METHODOLOGIES

The GMO Panel assessed the opinion that was published online by ANSES on 24 January 2024. The opinion provides conclusions and recommendations regarding the principles of equivalence for certain types of NGT plants in relation to conventionally bred plants, as well as on the criteria to distinguish 'category 1 NGT plants' from 'category 2 NGT plants' proposed in the EC proposal adopted on 5 July 2023.

The GMO Panel leveraged the relevant scientific considerations already published in the detailed EFSA Opinions on targeted mutagenesis (including site-directed nuclease (SDN) type 1, 2 and 3, and oligonucleotide directed mutagenesis), cisgenesis and intragenesis (EFSA GMO Panel, 2012a, 2012b, 2020, 2022) to support the development of this Scientific Opinion.

Since this Opinion includes scientific considerations that were already discussed in previous EFSA Scientific Opinions, and that were subject to open consultation and extensive public scrutiny, a protocol to plan the scientific assessment methodologies was deemed unnecessary for this mandate. EFSA consulted with ANSES[5] to facilitate its work, while maintaining the independence of the scientific process and deliberations of its GMO Panel.

[1]Proposal for a Regulation of the European Parliament and of the Council of 5 July 2023 on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625.

[2]For a description of 'conventional breeding techniques' see: EFSA Panel on Genetically Modified Organisms (GMO). (2012b).

[3]https://www.anses.fr/fr/system/files/BIOT2023AUTO0189.pdf.

[4]https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0625&from=EN.

[5]8th meeting of the GMO Cross-cutting Working Group (minutes: https://www.efsa.europa.eu/sites/default/files/2024-04/applications-cross-cutting.pdf).

3 | ASSESSMENT

The ANSES opinion focuses on (i) the need to clarify the definitions and scope, (ii) the scientific basis for the equivalence criteria and (iii) the need to take potential risks from NGT plants under category 1 into account. As requested by the European Parliament, the EFSA GMO Panel considered ANSES's analysis, conclusions and questions, and deemed the following clarifications necessary:

3.1 | Terms used in the European Commission proposal and in Annex I of the European Commission proposal that ANSES considers as requiring clarification

a. *Definition of cisgenesis/intragenesis, exclusion of intragenesis, definition of genetic material:* the EFSA updated scientific opinion on plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2022) defines cisgenesis and intragenesis as the *'genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species*'. Cisgenesis and intragenesis are defined in the European Commission proposal in the 'Context of the proposal' section as '*Insertion of genetic material (e.g. a gene) into a recipient organism from a donor that is sexually compatible (crossable). The exogenous genetic material can be introduced without (cisgenesis) or with modifications/rearrangements (intragenesis)*' (footnote 4 of the European Commission proposal) and in Article 3 ('Definitions') as '*"cisgenesis" means techniques of genetic modification resulting in the insertion, in the genome of an organism, of genetic material already present in the breeders' gene pool*'. Therefore, in line with these definitions, the European Commission proposal (under 3a and 3b of Annex I) excludes intragenesis from NGT category 1 since Annex I makes explicit reference to a '*contiguous DNA sequence*'. EFSA interprets the term 'contiguous' as a continuous DNA sequence already present in the breeders' gene pool, without rearrangements or modifications. Moreover, the term 'genetic material' is present in the cisgenesis/intragenesis definition described in the EFSA GMO Panel updated scientific opinion on plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2022) to indicate any DNA sequence that could potentially be transferred, irrespective of the specific function (i.e. promoters, terminators, introns, signal peptides, etc.); this term broadens the spectrum provided by the definition given in the EFSA GMO Panel (2012a) opinion, which considered the gene as the only sequence that could be transferred in a cisgenic/intragenic plant.
b. *Exclusion of non-targeted cisgenesis*: the European Commission proposal and Annex I (under points 3, 4, 5) clearly refer to targeted modifications (insertions or substitutions), thus excluding, by definition, non-targeted cisgenesis.
c. *Clarification of the term 'genetic information' in the definition of 'breeders' gene pool'*: the term 'genetic information' is used by the European Commission in the definition of breeders' gene pool as the information in the plant genome that defines the cisgene; i.e. this can be any sequence from the crossable species in the breeders' gene pool. The term 'genetic material' is used in the EFSA updated opinion on cisgenesis and intragenesis (EFSA GMO Panel, 2022) and the European Commission proposal (Article 3; Definitions[6]). The GMO Panel considers the term 'genetic material' to be interchangeable with 'genetic information' within the context of the 'breeders' gene pool' definition.
d. *The need to define the 'targeted site':* EFSA has not yet developed a definition of a targeted site. The term 'targeted site' can be interpreted as the site recognised by the nuclease or the locus intended to be modified. EFSA agrees with ANSES that the term requires clarification. EFSA interprets this term mentioned in Annex I of the European Commission proposal as the specific sequence that has been targeted by the nuclease (e.g. the sequence of the guide RNA in CRISPR-Cas). Nevertheless, this definition could be revised in the legal text and/or when guidelines will be developed by EFSA (European Commission proposal; Article 29; page 43; paragraph 1).
e. *Off-target and unintended changes in the plant genome*: The European Commission proposal requests the identification of potential mutations in sequences showing similarity to the targeted site. For mutations occurring at other locations in the genome than the targeted site, the GMO Panel has concluded that these would be fewer than those occurring with random mutagenesis techniques and that, where they do occur, these changes would also be of the same types as those derived by conventional breeding techniques and could not be distinguished from spontaneous mutations (EFSA GMO Panel, 2020).
f. Regarding the definition of conventionally bred plants which are relevant for the comparison with plants obtained via NGT, EFSA refers to the Opinion on site-directed nucleases where several conventional breeding techniques are described (EFSA GMO Panel, 2012b). Within the context of that Opinion, conventional plant breeding is defined as methods that are used by plant breeders for the improvement of commercial varieties and where the resulting plants/varieties are not considered as GMOs according to Directive 2001/18/EC, or that are excluded by this Directive.

[6]EC Proposal 5 July 2023: 'NGT plant' means a genetically modified plant obtained by targeted mutagenesis or cisgenesis, or a combination thereof, on the condition that it does not contain any genetic material originating from outside the breeders' gene pool that temporarily may have been inserted during the development of the NGT plant.

3.2 | The scientific basis for the equivalence criteria included in Annex I of the European Commission proposal

ANSES requests clarification on the scientific basis for the equivalence criteria included in Annex I of the European Commission proposal. The European Commission proposes a criterion of no more than 20 genetic modifications of defined types (points 1–5 in Annex I) for category 1 NGT plants, which is based on the evidence gathered and summarised in the European Commission Technical Paper on the rationale for the equivalence criteria in Annex I (European Commission services, 2023). The average number of spontaneous mutations per generation, according to the references cited by ANSES, is 10^{-8} to 10^{-10}, which for a genome such as maize would result in 20–30 mutations for every single progeny. This number is 1000–10,000 times higher when random mutagenesis is used according to the references cited by ANSES. Therefore, it is scientifically justified to consider that a plant showing 20 modifications or less compared to its parental could be the result of spontaneous mutations. While the proposed limit of 20 modifications for an NGT plant to be considered a category 1 NGT is a risk management decision, the EFSA GMO Panel considers this number conservative given the data available in the scientific literature.

EFSA reviewed the analysis of the equivalence criteria carried out by the ANSES working group on biotechnology and provides additional considerations for criteria 1, 2 and 4. Criteria 3 and 5 have already been addressed in Section 3.1 above.

Criterion 1: Substitution or insertion of up to 20 nucleotides

ANSES considers that the size of the modification alone does not provide information on its functional consequences. The EFSA GMO Panel agrees with the comment and highlights that this is also the case for mutations arising from conventionally bred plants. Annex I defines criteria to classify NGT plants as 'category 1 NGT plants', equivalent to conventional plants, or NGT-2, not equivalent to conventional plants. These criteria are not meant to define levels of risk but allow certain NGT plants to be classified as equivalent to conventionally bred plants.

Criteria 2 and 4: *Deletions and inversions of any number of nucleotides*

ANSES claims that the available scientific literature shows that genomic structural variations have a size distribution of the order of a kilobase or less. However, the occurrence of larger deletions during plant evolution is well documented, even for cultivated crops generated by conventional breeding. Examples of such deletions or inversions can be found in the literature (Bolon et al., 2014; Li et al., 2001, 2016; Liu et al., 2023; Morita et al., 2009; Seah et al., 2004). As an example of the complexity of genomic rearrangements that can be observed in cultivated plants, the EFSA GMO Panel Working Group on Molecular Characterisation[7] previously discussed the cases of genomic inversions of tandemly arrayed genes as observed in the article by Liu et al. (2023). Such changes can be induced spontaneously as reported by Seah et al. (2004) in tomato. All the above-mentioned examples demonstrate how genomic inversions and deletions are not specific to NGT plants but can be found in plants produced by classical breeding approaches (EFSA GMO Panel, 2020, 2012b).

3.3 | Potential risks from NGT plants under category 1

ANSES concluded that the equivalence criteria do not take into account potential risks and functional consequences derived from the modifications listed in Annex I of the European Commission proposal.

The proposed European Commission criteria to define category 1 NGT plants intend to determine whether a given NGT plant could be considered as equivalent to conventionally bred plants (including plants obtained by random mutagenesis) with respect to the type and number of genetic modifications. These equivalence criteria are not meant to define levels of risk but to allow certain NGT plants to be classified as equivalent to conventionally bred plants (Recital 14, European Commission Proposal). Moreover, with respect to the potential risks from NGT plants, the EFSA GMO Panel did not identify any additional hazard associated with the use of NGTs compared to conventional breeding techniques, which include random mutagenesis using physical or chemical agents (EFSA GMO Panel, 2020, 2022).

4 | CONCLUSIONS

The opinion published by ANSES analyses the criteria included in the Annex I of the European Commission proposal. The criteria are used to ascertain whether an NGT plant is equivalent to naturally occurring or conventionally bred plants (category 1 NGT plants) and focuses on (i) the need to clarify the definitions and scope in the European Commission proposal, (ii) the scientific basis for these equivalence criteria and (iii) the need to take potential risks from category 1 NGT plants into account.

[7]148th meeting of the Working Group on Molecular Characterisation (https://www.efsa.europa.eu/sites/default/files/wgs/gmo/wg-applications-molecular-characterisation-2018-2021.pdf).

I) The EFSA GMO Panel considered the ANSES analysis and comments on various terms used in the criteria included in Annex I of the European Commission proposal and discussed definitions based on previous EFSA GMO Panel opinions. With regard to the definition of the *targeted site*, EFSA agrees with ANSES that the term requires clarification. This definition could be revised in the legal text and/or when guidelines will be developed by EFSA.

II) The European Commission proposes a threshold of 20 genetic modifications of defined types (points 1–5 in Annex I). While the specific number is based on a risk management decision, the EFSA GMO Panel considers this number conservative given the data available in the scientific literature. With respect to all the equivalence criteria, the EFSA GMO Panel considers that the available scientific literature shows that plants containing the types and numbers of genetic modifications used as criteria to identify category 1 NGT plants do exist as the result of spontaneous mutations or random mutagenesis. Therefore, it is scientifically justified to consider these plants as equivalent to conventionally bred plants.

III) The equivalence criteria described in the European Commission proposal for category 1 NGT plants allow certain NGT plants to be classified as equivalent to conventionally bred plants with respect to the similarity of genetic modifications and the similarity of potential risks. The EFSA GMO Panel did not identify any additional hazards and risks associated with the use of NGTs compared to conventional breeding techniques in its previous Opinions.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Mandate from the European Commission (EC) received on 26 February 2024 concerning a request for a scientific opinion on recent studies on the proposal for a regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017 / 625.

Mandate accepted by EFSA on 18 March 2024.

ABBREVIATIONS

ANSES	Agency for Food, Environmental and Occupational Health & Safety
ENVI	Environment, Public Health and Food Safety
GMO	Genetically modified organisms
NGTs	New genomic techniques

ACKNOWLEDGEMENTS

The Panel wishes to thank the members of the Cross-cutting Working Group and the Working Group on Molecular Characterisation for the preparatory work on this scientific output and EFSA staff members Franco Maria Neri and Maria Tsirika for the support provided to this scientific output.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Parliament

QUESTION NUMBER

EFSA-Q-2024-00178

PANEL MEMBERS

Ewen Mullins, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Hanspeter Naegeli, Fabien Nogué, Nils Rostoks, Jose Juan Sanchez Serrano, Giovanni Savoini, Eve Veromann, and Fabio Veronesi.

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How to cite this article: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Rostoks, N., Sanchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Casacuberta, J., Afonso, A., … Raffaello, T. (2024). Scientific opinion on the ANSES analysis of Annex I of the EC proposal COM (2023) 411 (EFSA-Q-2024-00178). *EFSA Journal*, *22*(7), e8894. https://doi.org/10.2903/j.efsa.2024.8894