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The Director General

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OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the scientific analysis of Annex I of the European Commission's Proposal for a Regulation of 5 July 2023 on new genomic techniques (NGTs) – Review of the proposed equivalence criteria for defining category 1 NGT plants

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Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 29 November 2023 shall prevail.

On 6 November 2023, ANSES issued an internal request to conduct an expert appraisal on the following subject: scientific analysis of Annex I of the European Commission's Proposal for a Regulation of 5 July 2023 on new genomic techniques (NGTs) – Review of the proposed equivalence criteria for defining category 1 NGT plants.

1. BACKGROUND AND PURPOSE OF THE INTERNAL REQUEST

Regulatory background

Since the adoption of Directive 2001/18/EC[1] on the deliberate release into the environment of genetically modified organisms (GMOs), scientific and technical progress has led to the development of new genetic modification techniques. These new techniques have been the subject of legal action to determine whether the products derived from them fall within the definition of GMOs and the scope of this Directive.

[1] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, pp. 1-38.

The judgment of the Court of Justice of the European Union in Case C-528/16 (Judgment of 25 July 2018) concluded that new mutagenesis techniques fell within the scope of this Directive, while raising practical issues of applicability. Following this judgment, the European Council invited the European Commission to carry out a study regarding the status of new genomic techniques (NGTs) under Union law (Council Decision (EU) 2019/1904 of 8 November 2019).

In the study published on 29 April 2021, the Commission concluded that current European Union (EU) legislation on GMOs was not appropriate for some of these NGTs, which were formally defined for the first time as being "techniques that are capable of altering the genetic material of an organism and that have emerged or have been developed since 2001". In particular, this study mentioned that the risk assessment provided for in the current legislation had been deemed sometimes inadequate or disproportionate, and that the detection requirements were often inapplicable for plants and plant products obtained through targeted mutagenesis[2] or cisgenesis techniques.

In this context, on 5 July 2023, the European Commission adopted a Proposal for a Regulation[3], one of whose aims was to provide a response to the issues raised by the emergence of these new techniques.

The scope of this Proposal for a Regulation is limited to plants and plant products modified through certain NGTs, as part of requests for release into the environment for purposes other than placing on the market (field trials), and applications for marketing authorisation (MA) for all purposes. The Proposal for a Regulation is intended to constitute *lex specialis* with regard to EU GMO legislation. The rules set out in the Regulation are meant to take precedence over existing legislation, in particular Directive 2001/18/EC and Regulation (EC) No 1829/2003[4] as regards the placing on the market of plants and products or parts of products derived from such plants for use in food and/or feed.

What does the Proposal for a Regulation cover? The Proposal distinguishes between two categories of NGT plants. Category 1 plants, mentioned as being equivalent to naturally occurring or conventionally bred plants, are defined by criteria for equivalence to conventional plants set out in Annex I to the Proposal for a Regulation. Once their category 1 status is established, these plants will no longer be subject to EU legislation on GMOs. On the other hand, NGT plants that are not category 1 are category 2 and will remain covered by GMO legislation, subject to specific provisions and derogations.

Purpose of the internal request

The Regulation is due to be adopted under the ordinary legislative procedure, involving debates and voting in the European Parliament and Council. At this stage of the proposal, this Opinion is intended to inform the preparation of the public decision-making process. In light of time constraints, the internal request focused on a review of the equivalence criteria for defining category 1 NGT plants as mentioned in Annex I to the Proposal for a Regulation. This point is considered sensitive within the legislative proposal because it establishes a new category of genetically modified plants that would be exempted from the requirements of EU GMO

[2] Also known as site-directed mutagenesis.

[3] 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.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268 of 18.10.2003, pp. 1-23.

legislation, in the same way as plants derived from conventional crossing or random mutagenesis techniques under the current framework.

Scope and limitations of the expert appraisal

This internal request focused on the analysis of the proposed criteria for concluding whether certain NGT plants are equivalent to conventional plants.

These criteria, set out in Annex I to the Proposal for a Regulation, were analysed together with the definition of the NGT plants in question, based mainly on the technical paper[5] of the European Commission services dated 16 October 2023 (hereinafter referred to as the "technical paper"), with the aim of answering the following key question: to what extent can plants defined in this way actually be considered equivalent to conventional plants?

To this end, for the dual purpose of clarification and scientific analysis, this work focused on:

- reviewing the proposed equivalence criteria for defining category 1 NGT plants,
- highlighting potential questions and limitations in the definition of these criteria, by reviewing their scientific basis and considering the usefulness of additional criteria.

The issue of the risk assessment framework for NGT plants gave rise to another formal request (No 2021-SA-0019), for which work is already being carried out.

2. ORGANISATION OF THE EXPERT APPRAISAL

This expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

As this expert appraisal was a scientific analysis of equivalence criteria, it did not constitute an expert appraisal in risk assessment. It was entrusted to the Working Group (WG) on "Biotechnology", which met on 22 November 2023 based on initial reports written by three expert rapporteurs. It was conducted based on the Proposal for a Regulation on NGTs adopted by the European Commission on 5 July 2023, its Annex I, and the European Commission's technical paper presenting the rationale for the criteria set out in this Annex; it also considered additional information deemed necessary by the experts in the WG on "Biotechnology".

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public on the following website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE WG

The criteria of equivalence between NGT plants and conventional plants, set out in Annex I to the Proposal for a Regulation to define those plants that would be exempted from the

[5] European Commission services, Regulation on new genomic techniques (NGT) – Technical paper on the rationale for the equivalence criteria in Annex I, 16 Oct. 2023.

requirements of EU GMO legislation, were reviewed with the dual purpose of clarifying and scientifically analysing these proposals.

3.1. Clarification of the objective of Annex I on "Criteria of equivalence of NGT plants to conventional plants" – definition of the terms, scope and techniques considered

Analysing the equivalence criteria set out in Annex I to the Proposal for a Regulation required a preliminary phase of clarifying the objective of these criteria, by defining the terms used and then the scope of the objects being compared, based on the rest of the text of the Proposal for a Regulation and the European Commission's technical paper on the rationale for the criteria used, published on 16 October 2023.

3.1.1. "Equivalence criteria"

Recital 14 of the Proposal for a Regulation states that the objective of the "equivalence criteria" is to identify a subset of plants obtained through NGTs that could have occurred naturally or been produced through conventional breeding techniques. The aims are therefore to compare, within these plants, genetic modifications induced by NGTs with genetic variations or modifications that could occur naturally or result from conventional breeding techniques, and to define objective and scientifically based criteria that would ensure that the NGT plants meeting these criteria could indeed have occurred naturally or been produced using conventional breeding techniques.

3.1.2. The "NGT plants" considered in the Proposal for a Regulation

As a reminder, **NGTs** are defined in the explanatory memorandum of the Proposal for a Regulation as being "a variety of techniques that can alter the genetic material of an organism and that have emerged or have been developed since 2001, when the Union legislation on genetically modified organisms (GMOs) was adopted".

The term **"NGT plant"** used in the Proposal for a Regulation refers to a subset of plants obtained through these NGTs. In Article 3 of the Proposal for a Regulation, an "NGT plant" is defined as 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".

This definition refers to a set of breeding techniques and an exclusion criterion, respectively described and analysed in the following sections.

3.1.2.1. Breeding techniques concerned and associated genetic modifications

The terms "targeted mutagenesis" and "cisgenesis" are defined in Article 3 of the Proposal for a Regulation as follows:

Targeted mutagenesis: "mutagenesis techniques resulting in modification(s) of the DNA sequence at precise locations in the genome of an organism";

Cisgenesis: “techniques of genetic modification resulting in the insertion, in the genome of an organism, of genetic material already present in the breeders’ gene pool”;

where “breeders’ gene pool” is defined in Article 3 as meaning the “total genetic information available in one species and other taxonomic species with which it can be cross-bred, including by using advanced techniques such as embryo rescue, induced polyploidy and bridge crosses”.

The WG on “Biotechnology” stresses that the definition of “breeders’ gene pool” is unclear; this is particularly true for the term “genetic information”, which should be explained. Other texts, in particular the EFSA opinion on cisgenesis (EFSA GMO Panel, 2012), were consulted to determine that reference was being made to the genetic resources available to breeders for the plant breeding of a given species and not just to a gene pool, i.e. a set of coding genes. In other words, this appears to encompass genetic material from all the genomes of the plants with which the plant in question can be cross-bred, whether naturally or by means of more or less advanced conventional breeding techniques, outside the scope of Directive 2001/18/EC.

Therefore, the Proposal for a Regulation refers not to defined genetic modification techniques but to the characteristics of their final products, in which specific genetic modifications have been induced, i.e.

- targeted mutations (limited to changes to the DNA sequence at specific sites in the genome, excluding changes to the epigenome or RNA);
- the insertion of genetic material from the breeders’ gene pool.

To analyse the relevance of the criteria set out in Annex I, the WG on “Biotechnology” considered it necessary to identify the techniques capable of generating these genetic modifications, in order to be able to take into account all of the genetic modifications that they may induce, whether intentional or unintentional.

3.1.2.1.1. Targeted (or site-directed) mutagenesis techniques

Targeted mutagenesis refers to a set of techniques that emerged in the late 1990s, have been further developed since the early 2000s, and are constantly improving in terms of ease of implementation, the efficacy of the precision of the desired modifications, and the minimisation of unintended effects in a growing number of plant species.

It should therefore be noted that the analysis conducted was based solely on the techniques available today, bearing in mind that scientific and technical progress will have to be taken into account.

According to the state-of-the-art review published by the European Commission’s Joint Research Centre (JRC) in 2021 (Broothaerts *et al*., 2021), which proposed a new classification where NGTs were divided into four groups, targeted mutagenesis techniques within the meaning of the Proposal for a Regulation, i.e. techniques capable of inducing modifications of the DNA sequence at precise locations in the genome include:

- Group 1 techniques, based on various DNA repair mechanisms following the targeted induction of a double-strand break. These include all techniques using site-directed

nucleases (SDNs) (with targeted mutagenesis, they are limited to SDN-1 and SDN-2[6] applications) as well as other more recent techniques using site-specific recombinases (SSRs);

- Group 2 techniques, based on DNA repair following the induction of a single-strand break or another mechanism not involving any DNA break. These techniques use SDNs where a nuclease has been modified (base editing, prime editing) or are based on the use of an oligonucleotide (oligonucleotide-directed mutagenesis (ODM)), which can be made more effective if Group 1 SDNs are used concomitantly.

The Group 3 and 4 techniques described by the JRC involve epigenome and RNA modifications, respectively, and therefore fall outside the scope of the definition of targeted mutagenesis used in the Proposal for a Regulation.

Via various mechanisms, described in detail in the JRC report, these techniques can be used to intentionally induce different types of targeted mutations, such as substitutions of one or a few bases, small insertions or deletions (indels), or larger deletions (using targets at the ends flanking the sequence to be deleted) (Broothaerts *et al.*, 2021) (Table 1).

Their implementation is liable to concomitantly induce unintended on-target and off-target modifications of different types and at different frequencies depending on the technique used (Broothaerts *et al.*, 2021) (Table 1).

In a review on the unintended effects of techniques using CRISPR-Cas[7] editing in particular, unintended modifications were reported at the site specifically targeted (small substitutions or indels) or outside the site but within the targeted gene (essentially deletions of various sizes) (Sturme *et al.*, 2022) (Table 1).

Unintended off-target genetic modifications can be induced due to a lack of specificity in sites with some degree of homology to the target. This same review reported small indels and nucleotide substitutions in sites homologous to the target (Sturme *et al*., 2022) (Table 1).

Other unintended off-target genetic modifications have been reported elsewhere in the genome, for example with residual transgenic elements at the site of temporary insertion of the effectors of the targeted modifications (see below), and elements of transformation vector sequences (Sturme *et al*., 2022) (Table 1).

3.1.2.1.2. Cisgenesis techniques (as defined in the Regulation)

The definition of cisgenesis given in Article 3 of the Proposal for a Regulation needs to be further clarified. Indeed, the explanatory memorandum states that cisgenesis as considered in the Proposal for a Regulation includes intragenesis:

[Cisgenesis]: “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)”.

This is not the case in the definition of cisgenesis considered by default by EFSA (EFSA GMO Panel, 2022) and the JRC (Broothaerts *et al*., 2021).

[6] Specific application conditions for SDNs, resulting in point mutations or insertions/deletions of a few nucleotides, without any insertion of exogenous DNA. These targeted mutations can be either random and not pre-determined (SDN-1), or pre-determined, with the addition of a repair matrix (SDN-2).
[7] CRISPR: Clustered regularly interspaced short palindromic repeats. Cas: CRISPR-associated protein.

Recital 2 also states that “cisgenesis techniques result in the insertion, in the genome of an organism, of genetic material already present in the breeders’ gene pool. Intragenesis is a subset of cisgenesis resulting in the insertion in the genome of a rearranged copy of genetic material composed of two or more DNA sequences already present in the breeders’ gene pool”.

The WG on “Biotechnology” considers that the inclusion of intragenesis in cisgenesis should appear in the definition of cisgenesis given in point 5 of Article 3 of the Regulation. Furthermore, the WG underlines that referring to the whole and one of its parts in the same way may lead to confusion.

The WG on “Biotechnology” also notes that the definition of cisgenesis considers the insertion of “genetic material” and not of a gene or a complete expression unit coding for a protein; this change in definition has also been taken into account by EFSA (EFSA GMO Panel, 2012, 2022).

Moreover, it should be noted that the Proposal for a Regulation, as drafted, applies to all cisgenesis products, whether or not they have been produced through targeting.

The WG on “Biotechnology” notes that in the absence of targeting, the JRC does not consider cisgenesis/intragenesis techniques to be NGTs (Broothaerts *et al.*, 2021).

Therefore, the techniques capable of cisgenesis as defined in the Proposal for a Regulation include:

- all established genetic transformation techniques capable of randomly inserting exogenous genetic material into the genome (transformation techniques using *Agrobacterium tumefaciens* or *rhizogenes*, biolistic transformation, etc.), as long as this genetic material comes from the breeders’ gene pool, whether it is inserted identically (cisgenic sequence) or with rearrangements (intragenic sequence),
- genetic transformation techniques enabling the targeted insertion of a cisgenic or intragenic sequence, i.e.
    - standard transformation techniques using *Agrobacterium*, applied to a plant in which a predetermined landing site has been inserted, characterised and selected,
    - Group 1 NGTs using SDNs for an SDN-3 application, i.e. enabling an exogenous sequence to be inserted into the genome, or based on other mechanisms, such as SSRs,
    - prime editing which, according to EFSA, is the only Group 2 NGT that can be used to insert relatively long sequences (but not exceeding 100 bp) that may induce cisgenesis. A variant of this technique (TwinPE), which was recently developed, enables even longer sequences to be inserted (Anzalone *et al.*, 2022; EFSA GMO Panel, 2022).

Furthermore, these Group 1 and 2 NGTs can be used to define the target of cisgenesis so that the cisgenic sequence is inserted at the site of the orthologous gene, possibly by substituting the new allele for the original allele if applicable.

These are the techniques considered by EFSA in its updated opinion on plants developed through cisgenesis and intragenesis (EFSA GMO Panel, 2022).

In light of criterion 5, it would seem that other techniques, such as SDN-2 targeted mutagenesis and the broader insertion/substitution of gene elements, could also be considered as inducing cisgenesis within the meaning of the Regulation, in that they could be used to modify or convert

an endogenous sequence into another sequence from the breeders' gene pool, regardless of the type of modification carried out (see the analysis of criteria).

As with targeted mutagenesis, some of these techniques may require that effectors be inserted into the genome as an intermediate step. It will be necessary to ensure that these have been completely removed once the desired modification has been made (see exclusion criterion 3.1.2.2.).

These various techniques are listed in Table 1 according to their potential for cisgenesis and their possible unintended effects.

Table 1. Summary table of NGTs capable of targeted mutagenesis and cisgenesis, with the genetic modifications that they can induce intentionally and are liable to induce unintentionally.

	Intended genetic modifications[1] considered in the proposed Regulation						Possible unintended genetic modifications[2] (occasionally observed, at various frequencies, in the use of these techniques)			
	Targeted mutagenesis			...	Cisgenesis		Unintended on-target modifications	Unintended off-target modifications		
				(Intermediate between targeted mutagenesis and cisgenesis)			at the targeted site or in the targeted gene	at sites homologous to the target	Presence of transgenic elements	Other modifications
	Substitutions of one or a few bases	Small and medium-sized insertions/deletions	Large deletions (flanked by 2 targeted mutagenesis sites)	Modifications within a gene resulting in a cisgene	Substitution of a cisgene for an endogenous gene	Insertion outside an endogenous gene (targeted or non-targeted)	Substitutions of one or a few bases, small and medium-sized insertions/deletions, larger deletions	Substitutions of one or a few bases, small and medium-sized insertions/deletions, larger deletions	Residual elements of effectors of targeted modifications, transformation vector elements, markers, etc.	Substitutions of one or a few bases, small and medium-sized insertions/deletions, structural variants
SDN-1	(not predetermined) 1 bp (2-4 bp)	(not predetermined) 1-4 bp (<100)	Yes	No	No	No	reported	reported	reported	reported
SDN-2	1 bp 2-4 bp >4 bp	1-4 bp >4 bp	No	Yes	No	No	reported	reported	reported	reported
ODM	1 bp 2-4 bp	1-4 bp	No	Yes	No	No	(not analysed)[3]	(not analysed)	not applicable (no transformation)	(not analysed)
Base editing	1 bp	No	No	Yes	No	No	not reported[4]	reported	not reported	not reported
Prime editing	1 bp 2-4 bp	1-4 bp <100	Yes[5]	Yes[5,6]	Yes[5,6]	Yes[5,6] (targeted)	(not analysed)	(not analysed)	(not analysed)	(not analysed)
Site-specific recombination (SSR)	No	No	Yes	Yes	Yes	Yes (targeted)	(not analysed)	(not analysed)	(not analysed)	(not analysed)
SDN-3	No	No	No	Yes	Yes	Yes (targeted)	potential[6]	potential[6]	potential[6]	potential[6]
Transformation by *Agrobacterium* at a predetermined landing site	No	No	No	No	No	Yes[6] (targeted)	potential[6]	not applicable (*a priori* no other sequences similar to the target)	potential[6]	potential[6]
Standard transformation techniques	No	No	No	No	No	Yes[6] (not targeted)	not applicable (no target)	not applicable (no target)	potential[6]	potential[6]

1. Information primarily taken from the JRC's state-of-the-art review on NGTs (Broothaerts *et al*., 2021); 2. Information primarily taken from the systematic literature review on the unintended effects of targeted mutagenesis by CRISPR/Cas (Sturme *et al*., 2022); 3. Means that the information, outside the scope of the publications consulted, was not analysed by the WG on "Biotechnology"; 4. Modifications not reported in the analysis by Sturme *et al*. (2022); 5. Anzalone *et al*. (2022); 6. EFSA GMO Panel (2022).

SDNs: site-directed nucleases, including zinc-finger nucleases (ZFNs), meganucleases (MNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats with associated protein (CRISPR/Cas); SDN-1 and SDN-2: specific application conditions for SDNs, resulting in point mutations or insertions/deletions of a few nucleotides, without any insertion of exogenous DNA. These targeted mutations can be either not predetermined (SDN-1) or predetermined (SDN-2); SDN-3: specific application conditions for SDNs, resulting in the insertion of an exogenous sequence; ODM: oligonucleotide-directed mutagenesis.

3.1.2.1.3. The issue of standard transgenesis techniques

The WG on “Biotechnology” underlines that even though standard transgenesis techniques are not mentioned among the breeding techniques considered in the definition of NGT plants, they are still commonly used as an intermediate step of most NGT applications in plants to enable the expression of the effectors involved in the targeted modifications (e.g. CRISPR-Cas cassettes).

Inserts must be removed once the targeted modification has been carried out for a number of reasons, in particular (1) to increase the specificity of the technique, as their prolonged expression has been associated with an increased likelihood of off-target modifications, (2) to reduce the risks potentially associated with the random insertion of foreign genetic material into the genome, and (3) because from a regulatory perspective, the presence of transgenic elements in a plant makes it a transgenic plant, subject to the requirements of EU GMO legislation.

These inserts can be removed by genetic segregation and selection, in the progeny, of plants free of these inserts or, when cross-breeding is not possible (with vegetatively propagated plants) or advantageous (with plants with particularly long reproductive cycles), by other means such as excision techniques (e.g. the Cre-Lox system).

Methods other than the temporary insertion of effectors can be implemented, without a transgenesis step (e.g. using ribonucleoprotein (RNP) complexes) (He *et al*., 2021).

3.1.2.2. Criterion for excluding foreign genetic material from the whole genome

The definition of NGT plants contains this criterion for excluding foreign genetic material from the whole genome: “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”.

This criterion may concern the following:

- residual transgenic elements of NGT effectors inserted as an intermediate step in the targeted modification,
- residual transgenic elements of markers that may have been used as an intermediate step in cisgenesis or intragenesis,
- transgenic elements from the transformation vector (used in an intermediate-step transformation with NGTs or in a standard transformation with non-targeted cisgenesis), which were never intended to be inserted in the first place.

The WG on “Biotechnology” considers that if this genetic material has indeed been inserted “temporarily”, it should no longer be present in the NGT plant. As this adds nothing to the condition presented, it recommends deleting the end of the sentence, after “pool”.

Although it is not mentioned in Annex I, this criterion is one of the points that the requester must demonstrate in the procedure for verifying the status of category 1 NGT plants (Article 6 of the Proposal for a Regulation).

3.1.3. "Conventional plants" according to the Proposal for a Regulation

No definition of "conventional plants" is given in the Proposal for a Regulation. However, in its presentation of the equivalence criteria, Recital 14 refers to "naturally occurring or conventionally bred plants", which could be what the Proposal for a Regulation means by "conventional plants" in Annex I.

As natural variation is the primary source of genetic variability used in plant breeding, the WG considers it makes sense for it to be considered in conjunction with conventional breeding techniques. However, the Proposal for a Regulation does not provide a definition of the conventional breeding techniques considered either.

The WG notes, however, that random mutagenesis is one of these techniques, defined in the impact assessment report[8] as "an umbrella term used to describe conventional breeding techniques based on mutagenesis that have been used since the 1950s; they involve irradiation or treatment with chemicals in order to produce random mutations in a genome, and typically involve screening of a large number of mutants to select one with desirable properties".

The WG also notes that the analysis of the scientific literature on naturally occurring mutations and mutations obtained through conventional breeding techniques presented in the technical paper as rationale for the equivalence criteria in Annex I is largely based on mutations caused by random mutagenesis techniques. The WG stresses that, according to Annex IB of Directive 2001/18/EC, (random) mutagenesis is a genetic modification technique producing organisms excluded from the scope of this Directive.

Given that the system for categorising NGTs in this Proposal for a Regulation is based on a comparison with "conventional plants", the WG considers it absolutely essential that these be explicitly defined.

3.1.3.1. Various sources of genetic variability in plant breeding

Plant breeding is one of the breeding techniques considered as being conventional. It largely depends on the genetic variability available. Mutations contribute to the creation of this genetic diversity via point or structural changes in nuclear or cytoplasmic DNA sequences.

Spontaneous mutations are caused by DNA replication errors during cell division, by irradiation (ultra-violet, cosmic radiation), chemical or physical stress, or even parasitic attacks (Quiroz *et al.*, 2023). The rate of spontaneous mutation varies depending on the type of mutation. For substitutions and small indels, the mutation rate is between 10^{-10} and 10^{-8} in plants (Quiroz *et al.*, 2023), which corresponds to around one mutation every 100 million base pairs in each generation (although transitions are more frequent than transversions).

Indels of microsatellite-type repeated motifs occur at a much higher frequency, of around 10^{-5} to 10^{-3} per generation. Conversely, large indels and other types of structural variants (inversions, duplications, transpositions) appear to be much less frequent, but it is still difficult to estimate their rate of occurrence (Quiroz *et al.*, 2023). In plants, many of these structural variants are caused by the activity of transposable elements. In some plants, such as maize, these elements can account for a major portion of the genome (Gill *et al.*, 2021).

[8] Impact assessment report of 5 July 2023 accompanying the document 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) 2017/265.

The amount of genetic variation that is maintained in natural populations is the result of a balance between processes that tend to increase variation (mutations and migrations between different populations) and those that tend to decrease it (genetic drift and natural selection) (Charlesworth and Charlesworth, 2010).

The genetic diversity present in a selected species is therefore the result of the combined action of mutation, genetic drift, selection and migration phenomena. Breeders have partial access to this genetic diversity via international genetic resource centres, which bring together accessions of cultivated varieties and wild ecotypes. They can also seek to introduce genetic diversity from related sexually compatible species through interspecific hybridisation.

When the available genetic diversity is not sufficient in a species or its relatives to select a given trait, additional genetic diversity can be induced via mutagenesis. The aim is to sharply increase the frequency of mutations controlling the expression of the trait compared with the frequency of spontaneous mutations (a 1,000- to 10,000-fold increase), which facilitates the selection of the desired traits by greatly reducing the population to be used (HCB, 2020).

3.1.3.2. Conventional breeding techniques and associated genetic variability

It can be considered that conventional breeding methods and techniques have been used since the early days of plant domestication, when humans began to cultivate some of the plants they harvested for food and a selection process different from natural selection emerged, leading to the fixation of so-called “domestication traits”. Various methods have since been developed and combined to a greater or lesser extent (phenotypic selection, the use of controlled crosses, the transfer of genes of major effect by backcrossing or interspecific hybridisation, marker-assisted selection, and genomic selection. Other methods include ploidy modification and the synthesis of allopolyploid species) (Gallais, 2019).

Many of these conventional techniques do not use molecular knowledge of the genetic variation mobilised, or else only in the form of statistical associations with markers. The genetic variation resulting from conventional methods can therefore only be described *a posteriori* (e.g. with genome sequencing).

Regarding quantitative traits, conventional breeding can lead to large-scale phenotypic modifications in the long term, probably mobilising numerous genes as well as spontaneous mutations.

Regarding mono- and oligogenic traits, large-scale phenotypic modifications can be introduced instantly by cross-breeding, although this must be followed by backcrossing. The larger the number of genes underlying a trait, the more difficult it becomes to combine favourable alleles in the same genotype, and the greater the risk of introducing unintended genetic material through the “hitchhiking” of genetically linked mutations. Some specific techniques produce major modifications in genomes, ranging from ploidy modification to the synthesis of new genomes.

Pan-genome studies describe the types and number of genetic variations in cultivated plants. There are around one million substitutions and small indels, and around 10,000 to 100,000 structural variants. Most indels are around 1 kb in length. Very large structural variants (inversions, translocations, duplications) are also observed, but much less frequently. Gene presence-absence variation is significant in all species, with around 30% to 60% of core genes conserved in all varieties.

The heterogeneity of genetic variation along genomes is due to heterogeneous selective pressures on genes. The regions surrounding highly selected genes are low in variation, particularly for a recent selection event with a low rate of recombination (this is known as a selective sweep). In allopolyploids, the different genomes can have different levels of genetic variation. Core genes are essential genes that are probably conserved by purifying selection, while facultative genes play an important role in phenotypic variation between varieties for adaptive traits such as disease resistance.

Concerning random mutagenesis, the types of mutations induced differ depending on the technique used. Mutagenesis using ethyl methanesulphonate (EMS) mainly induces G/C and A/T transitions (Szurman-Zubrycka *et al.*, 2023). In rice, Li *et al.* (2016) noted that mutagenesis induced by irradiation induced more transversions, in particular C/T transversions, than observed between unmutagenised varieties. Compared with the EMS method, irradiation induces more structural variants, with fast-neutron irradiation producing a high proportion of deletions (Wang *et al.*, 2012).

TILLING[9] populations have been described in various cultivated plants, enabling the number of mutations obtained by EMS treatment to be characterised (Jacob *et al*., 2018; Szurman-Zubrycka *et al*., 2023). Typically, random mutagenesis using EMS results in one mutation every 100 to 1,000 kb, provided that large populations (1,000 to 10,000 individuals) are used. It is therefore theoretically possible to achieve at least one mutation in each gene. However, these figures vary depending on the species and ploidy level. One study in particular showed that considerably higher numbers of mutations can be achieved by EMS-induced mutagenesis in tetraploid wheat (2,705 mutations per line on average and 35 mutations per kb) and hexaploid wheat (5,351 mutations per line on average and 39 mutations per kb). For this study, mutations were only sought in the exome (Krasileva *et al*., 2017). The distribution of mutations appears to be uniform along the genome in rice (Li *et al*., 2016) and uniform between gene and non-gene regions in wheat (Krasileva *et al*., 2017).

3.2. Analysis of the equivalence criteria proposed in Annex I

3.2.1. Statement of equivalence criteria between NGT plants and conventional plants

Annex I to the Proposal for a Regulation of 5 July 2023 on NGTs sets out *Criteria of equivalence of NGT plants to conventional plants* as follows:

> *A NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than 20 genetic modifications of the types referred to in points 1 to 5, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.*
>
> *1) substitution or insertion of no more than 20 nucleotides;*
>
> *2) deletion of any number of nucleotides;*
>
> *3) on the condition that the genetic modification does not interrupt an endogenous gene:*
>
> *a) targeted insertion of a contiguous DNA sequence existing in the breeder's gene pool;*
>
> *b) targeted substitution of an endogenous DNA sequence with a contiguous DNA sequence existing in the breeder's gene pool;*

[9] Targeted Induced Local Lesions in Genomes (TILLING): a conventional breeding technique used to select mutants carrying mutations of interest by carrying out a high-throughput molecular analysis of a library of DNA from the offspring of a genotype treated with a mutagenic agent.

4)	*targeted inversion of a sequence of any number of nucleotides;*
5)	*any other targeted modification of any size, on the condition that the resulting DNA sequences already occur (possibly with modifications as accepted under points (1) and/or (2)) in a species from the breeders' gene pool.*

3.2.2. Analysis by criterion

As a preamble to this analysis by criterion, the WG points out that the qualifiers "acceptable" and "tolerable", used to describe certain parameters, thresholds and conditions associated with the criteria, relate to the robustness of the comparison, i.e. its ability to correctly classify plants in either of the two categories of NGTs based on their definitions, and not to the acceptability or tolerability of the NGT plants thus categorised.

3.2.2.1. Criterion 0 – General conditions

A NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than 20 genetic modifications of the types referred to in points 1 to 5, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.

- **Clarification**

This sentence sets out the general conditions applicable to category 1 NGT plants. Based on its understanding of this proposal, the WG on "Biotechnology" notes the following key points:

- the conditions relate exclusively to a targeted site and to similar DNA sequences that can be predicted using bioinformatic tools;
- a maximum number of 20 genetic modifications is accepted in the above sites;
- each type of genetic modification must correspond to one of the types described in the points developed subsequently.

Based on its understanding of the proposed threshold, the WG suggests clarifying the text by replacing "when it differs (…) by no more than" with "that does not differ by more than". This would give the following, which is easier to understand: "Any NGT plant that does not differ by more than 20 genetic modifications (...) from the recipient/parental plant is considered equivalent to conventional plants".

- **Techniques concerned**

These general conditions apply *a priori* to all possible techniques for obtaining a NGT plant, i.e. techniques capable of targeted mutagenesis and cisgenesis (including intragenesis). The focus on sequences sharing similarity with a targeted site suggests that non-targeted techniques are not covered by these conditions. This point will be discussed in the comments.

- **Comments**

- Definition of "targeted site":

No details are given in the Proposal for a Regulation with regard to the term "targeted site". The WG on "Biotechnology" considers that the definition of this site is decisive in the application of each of the proposed criteria.

Two aspects need to be considered: (1) the region that will be used to identify similar sequences that could be unintentionally modified due to the lack of specificity of the technique, and (2) the region in which genetic modifications of the various types listed in the following points will be searched for. These two aspects could be separated in a composite definition: (1) a sequence restricted around the targeted site for the search for sequence similarities, and (2) a broader sequence, suitable for the search for associated genetic modifications.

Concerning the site sequence used for the similarity search: the WG considers that, given the heterogeneity of the techniques potentially implemented, it would seem inappropriate to set a standard size for the sequences considered in each application. The WG recommends that the size and location of the sequence used for the similarity search be defined based on the specific technique implemented to target the modification.

Similarly, the WG on "Biotechnology" considers that the region in which modifications will be searched for should be established based both on the type of intentional modification carried out at the site concerned and on the type of modifications searched for.

For example, for a technique using CRISPR-Cas9, the site used for the similarity search could only correspond to the recognition region of the guide RNA (which is around 20 nucleotides long) or a small window including this region. The entire targeted gene could be considered for the search for unintended genetic modifications. This would provide a way to detect deletions liable to occur in the targeted gene but outside the site targeted by the modification, as reported in the review by Sturme *et al*. (2022).

For cisgenesis, the WG considers that the site used for the similarity search could be a given window around the insertion site depending on the targeting technique used, and the sequence used to search for genetic modifications could cover a wider window, including the inserted cisgenic sequence; for large deletions via DNA breaks at sites flanking the window to be removed, these two sites should be considered together for the similarity search, while wider adjacent regions should be used to search for associated modifications.

The WG therefore recommends that the definition of "targeted site" be clarified so that the resulting criteria remain appropriate. In the absence of such a definition, the WG notes that there is a risk of distortions between dossiers, related to the interpretation of each requester.

- <u>Identification of similar sequences:</u>

The determination of "any DNA sequence sharing sequence similarity with the targeted site" will depend not only on the sequence considered by reference to the "targeted site", but also on a similarity threshold (or a given percent identity), the genomic data available for the species in question, and the software and parameters used.

- <u>Maximum threshold of 20 genetic modifications:</u>

The technical paper of the European Commission services (EC, 2023) justifies this threshold by citing the objective of excluding NGT plants with complex modifications unlikely to be obtainable by conventional breeding techniques. In this paper, the example of obtaining a low-gluten wheat line illustrates the ability of CRISPR-Cas9 to simultaneously modify up to 35 of the 45 genes in wheat encoding the alpha-gliadin protein (Sánchez-León *et al*., 2018). As this

result cannot be achieved using conventional techniques, a NGT plant modified in this way cannot be considered equivalent to conventional plants.

While the objective of such a threshold is clear, the WG considers there is no justification for choosing 20 as the maximum number of genetic modifications accepted in order for a NGT plant to be considered equivalent to conventional plants. The technical paper's rationale is based on the identification of 30 to 100 mutations per plant induced by random mutagenesis. The WG considers that this comparison seems irrelevant given that the number of these mutations varies depending on the intensity of the mutagenic treatment and that breeders then remove unintended mutations by genetic segregation, which they would not necessarily do following targeted mutagenesis.

Furthermore, on such a basis of numerical equivalence, it would be expected that the definition of a maximum number of genetic modifications accepted per genome would depend on the size of the genome and would therefore not be set in absolute terms. If a parallel is drawn with the number of mutations induced by random mutagenesis, it is expected that for an equivalent density of mutations in a genome, a greater number will be induced in a larger genome.

Regarding the consideration of ploidy, the WG notes that in polyploids, a greater number of modifications will need to be made to achieve a given phenotype due to the functional redundancy of the homeologous genes. For example, in hexaploid wheat, targeted modifications had to be made to the three homeologous alleles of the *MLO* gene to confer resistance to cereal powdery mildew (Wang *et al*., 2014). Therefore, for a given threshold of genetic modifications, low-ploidy plants will be favoured in that they will have the ability to accumulate more single genetic modifications.

Moreover, as the likelihood of obtaining similar mutations in different homoeologs using conventional breeding techniques is very small, it seems logical that these plants are less likely to achieve category 1 status due to the complexity of the modifications made with NGTs.

On the other hand, if it is established that these mutations exist in the breeders' gene pool, obtaining such a line using conventional techniques would not be impossible but would be extremely laborious due to the large number of crosses it would require. In this specific case, where targeted mutagenesis would make it easier and faster to obtain a combination of modifications that could also be achieved using conventional techniques, equivalence would be justified.

This discussion shows that a numerical threshold is not always the most appropriate criterion for deciding whether a NGT plant is equivalent to a conventional plant.

The WG on "Biotechnology" underlines that this comment is also valid for the opposite situation where certain modifications themselves have a very low likelihood of being produced using conventional breeding techniques. Therefore, when the desired phenotype can only be obtained through a single modification to the sequence of a gene and this modification is not present in the breeder's gene pool, the likelihood of obtaining it by conventional breeding or identifying it in natural populations becomes very low. For example, the WG notes that obtaining a tomato variety with increased GABA content required the introduction of a stop codon at a specific point in the sequence of the *SlGAD3* gene to suppress the expression of the C-terminal regulatory region (Nonaka *et al.*, 2017). The authors report that in a previous study using 4,588 mutagenised lines, the two mutations identified via TILLING in the *SlGAD3* gene were not located at a position that could have affected the expression of the C-terminal region. In the WG's opinion, this example shows that the low likelihood of obtaining a plant

using conventional breeding techniques is not necessarily related to the combination of a large number of modifications.

Lastly, the WG on “Biotechnology” notes that a numerical approach to genetic modifications is likely to lead to practical difficulties in counting, both for the requester and for the authorities responsible for examining the request, as some modifications may or may not be considered as being the sum of different types of modifications set out in Annex I.

- “Off-target” genetic modifications in sequences similar to the target:

The WG underlines that these general conditions mean that unintended genetic modifications induced due to a lack of specificity, in sites with a certain degree of homology to the target, will be counted among the 20 accepted genetic modifications, without being considered in terms of potential negative effects. The technical paper, whose equivalence rationale is based on a simple molecular analysis of the occurrence of certain types of genetic modifications, does not address the issue of risk.

The WG notes that the number of these unintended genetic modifications decreases as the specificity of NGT targeting techniques increases, and that it is well below the number of modifications induced by certain techniques such as random mutagenesis that are considered conventional in the Proposal for a Regulation (Sturme *et al.*, 2022). It should be noted, however, that the latter modifications are largely removed via gene segregation in the selection steps following random mutagenesis, which is not necessarily the case with NGTs.

The WG on “Biotechnology” recommends removing these unintended modifications whenever possible from NGT plants, and when this is impossible or difficult (e.g. for plants with vegetative propagation or a long life cycle), assessing the potential risks and only tolerating them if they are considered negligible.

- Genetic modifications in the rest of the genome:

The WG on “Biotechnology” underlines that these general conditions, which focus on a maximum number of acceptable genetic modifications at the targeted site and in similar sequences, seem to ignore, rather than exclude, possible modifications occurring in the rest of the genome. Is any modification outside the target and similar sequences (apart from the presence of transgenic elements, excluded from the definition of NGT plants) therefore tolerated?

For example, with non-targeted cisgenesis/intragenesis, none of the proposed criteria would apply due to the absence of a target, but the current wording of this criterion would cause the modification to be tolerated without even being counted. As Annex I has been drafted, while it does not relate to modifications outside of targets and similar sequences, it does not explicitly exclude them.

This is also true for any unintended modifications occurring outside the site and similar sites, such as insertions/deletions, frameshift mutations, and any type of structural variant, which could occur at the temporary insertion site of the NGT effectors: apart from transgenic elements, these would be tolerated without conditions or counting.

The WG on “Biotechnology” requests an explicit clarification of the genetic modifications, excluding transgenic elements, that could be induced in the rest of the genome.

3.2.2.2. Criterion 1

1) substitution or insertion of no more than 20 nucleotides;

- **Clarification**

This point indicates that substitutions and insertions are accepted up to a maximum insert size of 20 nucleotides (at the targeted site and in similar sequences, according to the general conditions).

The technical paper specifies that a substitution can be considered as a combination of a deletion and an insertion. In accordance with criterion 2 applicable to deletions, no conditions apply to the substituted sequence.

- **Techniques concerned**

A priori, criterion 1 relates to modifications induced intentionally through targeted mutagenesis techniques. The technical paper states that “very large insertions as part of structural rearrangements should be considered insertions of a cisgene, which are covered by criterion 3”.

Furthermore, it is specified that cisgenic sequences obtained by any other targeted modification of any size can tolerate Type 1 modifications (see criterion 5).

Lastly, this criterion could potentially be applied to account for any unintended modifications consisting of small insertions outside the breeders’ gene pool, whether induced through targeted mutagenesis or through cisgenesis by insertion/substitution covered by criterion 3.

- **Comments**

- <u>Maximum threshold of 20 nucleotides:</u>

While the proposed threshold of 20 nucleotides does not exceed the observations reported in the scientific literature for the size of genetic modifications resulting from the application of conventional breeding techniques (more frequently observed below 10 nucleotides but reaching up to 50 nucleotides for small insertions, according to the technical paper), the WG on “Biotechnology” stresses that the size of a modification alone does not provide any information as to its functional consequences. This maximum substitution/insertion limit of 20 nucleotides has no biological significance or basis.

In an exon, if the number of bases modified is a multiple of 3, this can modify the amino acid sequence of the encoded protein, while other sizes of modifications (any size that is not a multiple of 3) will cause a frame shift and a stop codon, which can result either in the gene not being expressed or in a truncated protein. In a promoter region, the modification can suppress or modify the expression of a gene. In a regulatory gene, or transcription factor, the expression of a large number of genes in the same regulatory pathway can be modified, etc.

The WG on “Biotechnology” underlines that the genetic modifications observed in varieties obtained using conventional techniques are the result of further breeding, subsequent to the

application of a possible mutagenic treatment. Any potential undesirable modifications are removed by genetic segregation in favour of the desired modifications, usually through phenotypic selection and not based on the molecular size of the modification. There is nothing to suggest that the undesirable modifications removed differ in size from those conserved.

Furthermore, the technical paper points out that the size of 20 nucleotides is the limit below which it becomes very unlikely that a random sequence will be unique in a genome (Broothaerts *et al.*, 2021). The WG on "Biotechnology" considers that this limit should vary depending on the size of the genome and that it should be higher for large and/or polyploid genomes.

It follows from this statistical estimate that a sequence that is less than 20 nucleotides long will have a very high likelihood of already being naturally present in the genome. The WG on "Biotechnology" notes, however, that the functional nature of these pre-existing sequences in the genome can, depending on their genomic location, be different from that of an identical sequence intentionally introduced at a different location for a specific functional purpose.

Coding sequences only account for a small proportion of a genome. For example, in maize, annotated genes represent around 8% of the genome (Hufford *et al.*, 2021). It is therefore possible that identical sequences pre-existing in a genome have no biological/functional role, unlike a sequence that is substituted or inserted intentionally into a given target or unintentionally into a similar sequence. It should also be noted that some transcription factor binding motifs and transcription initiation motifs, which can activate or repress gene expression, are less than 20 nucleotides long.

The WG on "Biotechnology" concludes that there is no scientific basis for accepting (in the sense of equivalence) substitutions or insertions based on their size. Furthermore, the maximum threshold of 20 nucleotides for an insertion or substitution has not been shown to be particularly appropriate for defining equivalence to conventional plants.

3.2.2.3. Criterion 2

2) deletion of any number of nucleotides;

- **Clarification**

This point indicates that any deletion is accepted unconditionally (at the targeted site and in similar sequences, according to the general conditions).

- **Techniques concerned**

A priori, criterion 2 relates to modifications induced through targeted mutagenesis techniques. Furthermore, it is specified that cisgenic sequences obtained by any other targeted modification of any size can tolerate Type 2 modifications (see criterion 5). Lastly, this criterion could potentially be applied to account for any unintended modifications induced through targeted mutagenesis or cisgenesis.

- **Comments**

The WG on "Biotechnology" considers that this unconditional deletion criterion does not seem justified in view of the literature on pan-genome analyses, which show that the size distribution of structural variants is strongly biased in favour of sizes of around one kilobase or less. Large variants exist but are much less common and tend to be translocations, inversions, or duplications.

The WG considers that, in terms of functional consequences, the deletion of an entire gene would be identical to gene presence-absence variation, which is widely observed in conventionally bred varieties.

However, the WG underlines that even for these limited sizes, the deletions maintained in these varieties have gone through the breeder's selection steps. Presence-absence variation in non-essential genes outside pan-genomes is the result of natural selection. Size alone would therefore not necessarily guarantee equivalence between newly induced deletions in NGT plants and deletions in plants obtained using conventional techniques.

Lastly, large deletions that would result in the simultaneous suppression of a large number of linked genes are seldom observed in varieties obtained using conventional techniques.

The WG on "Biotechnology" concludes that structural variant-type deletions observed in conventional plants are usually limited to around one kilobase in size. The functional consequences of these deletions should be characterised regardless of their size.

3.2.2.4. Criterion 3

3) on the condition that the genetic modification does not interrupt an endogenous gene:

a) targeted insertion of a contiguous DNA sequence existing in the breeder's gene pool;

b) targeted substitution of an endogenous DNA sequence with a contiguous DNA sequence existing in the breeder's gene pool;

- **Clarification**

The WG on "Biotechnology" questioned the meaning of "contiguous DNA sequence". Two objects, such as two nucleotides, can be contiguous, but one object cannot be contiguous on its own. In light of the technical paper and the latest EFSA opinion on cisgenesis (EFSA GMO Panel, 2022), it would seem that the correct phrase should be "continuous DNA sequence" (EFSA uses "a single intact and continuous sequence" in its opinion), which distinguishes between a cisgenic sequence in the strict sense of the term and a composite and rearranged sequence, characteristic of intragenesis.

The condition specifying that the modification must not interrupt an endogenous gene should be accompanied by a statement clarifying what is meant by "gene". This could refer to the coding sequence or all the introns and exons, the 5' and 3' untranslated regions, the promoter and terminator, or even the regulatory elements for expression potentially located several kilobases upstream or downstream of the coding sequence.

According to the technical paper, this criterion should exclude any intragenesis, which would mean that the insertion of the DNA sequence in question outside an endogenous gene should

be sufficiently far from any genetic element belonging to a gene to prevent the resulting sequence from being considered a sequence rearrangement.

Similarly, according to the same argument aimed at avoiding an intragenic result, the "targeted substitution of an endogenous DNA sequence with a contiguous DNA sequence existing in the breeder's gene pool", "on the condition that the genetic modification does not interrupt an endogenous gene" could be interpreted as having to be sufficiently broad to encompass an entire endogenous sequence. This sequence could potentially consist of a gene with all its components that, as with insertion, should be sufficiently far from any genetic element belonging to neighbouring genes.

- **Techniques concerned**

The techniques concerned are those capable of targeted cisgenesis by insertion or substitution.

- **Comments**

- What is the target?

The insertion of the continuous DNA sequence is supposed to be targeted outside an endogenous gene, with no additional conditions required of the target. This raises the following question of consistency in treatment: what is the difference between a targeted insertion in a site that does not interrupt an endogenous gene, with no additional conditions required of the targeted site, and an insertion using non-targeted transformation techniques that has been verified afterwards as not interrupting an endogenous gene? According to the proposed criterion, only the first case could be exempted from the requirements of GMO legislation under category 1, whereas the two products could be considered equivalent in that they do not interrupt an endogenous gene.

Some NGT techniques may enable the target of cisgenesis to be defined so that the cisgenic sequence is inserted at the site of the orthologous gene or sequence, possibly substituting the new allele for the original allele where appropriate, resulting in a clearer equivalence to a conventional plant into which the same allele has been introduced by introgression.

No details are given concerning a potential orthologous relationship between the original sequence and the new sequence.

The WG on "Biotechnology" considers that targeting the cisgenic sequence at the site of the orthologous sequence would avoid any positional effects, characteristic of standard genetic transformation techniques, associated with a new insertion site.

- Exclusion of intragenesis:

The WG on "Biotechnology" notes that this criterion only applies to cisgenesis in the strict sense of the word (it does not apply to intragenesis). The technical paper specifies that this criterion therefore excludes intragenesis from all the criteria; this should be made more explicit in Annex I.

The WG on "Biotechnology" considers that excluding plants obtained through intragenesis from category 1 NGT plants is justified. This is because the rearrangements between different sequences from the breeder's gene pool that are

characteristic of intragenic sequences could not occur naturally or be obtained using conventional techniques.

3.2.2.5. Criterion 4

4) targeted inversion of a sequence of any number of nucleotides;

- **Clarification**

This point indicates that any inversion is accepted, regardless of its size (at the targeted site and in similar sequences, according to the general conditions).

The use of the word “targeted” should be explained in this criterion, considering the general conditions applicable to each of the types of modifications listed.

- **Techniques concerned**

A priori, this criterion relates to unintended modifications induced at the targeted site and in similar sequences through targeted mutagenesis or targeted cisgenesis techniques.

- **Comments**

As with the unconditional deletion criterion, this inversion criterion without size conditions does not seem justified in light of the literature on pan-genome analyses (see criterion 2).

Moreover, suitable analytical techniques would be required to detect this type of event.

3.2.2.6. Criterion 5

5) any other targeted modification of any size, on the condition that the resulting DNA sequences already occur (possibly with modifications as accepted under points (1) and/or (2)) in a species from the breeders’ gene pool.

- **Clarification**

According to the technical paper, the aim of this criterion is to “consider possible outcomes (DNA sequences) that might be shown to occur in a species from the breeders’ gene pool but that might not be covered by the previous criteria”. It is also specified that “this criterion provides a derogation only from criterion 3 and from the condition that the genetic modification does not interrupt an endogenous gene”.

The WG on “Biotechnology” considers that this criterion is unclear. It could refer to another form of targeted cisgenesis carried out, in addition to criterion 3, possibly within an endogenous gene, through any targeted modification of a type other than those mentioned above, which could therefore include targeted mutagenesis without size conditions, provided that the resulting sequence exists in the breeders’ gene pool, where this sequence may be modified by deletions or by substitutions or insertions of sequences less than 20 nucleotides long. This criterion should therefore be clarified and examples should be given to illustrate it.

- **Techniques concerned**

These are likely to be targeted mutagenesis techniques (e.g. SDN-2, ODM, base editing, or prime editing) or targeted insertion/substitution techniques (e.g. prime editing or SDN-3) resulting in a cisgenic sequence.

- **Comments**

The objective of being exhaustive, as stated in the technical paper, is not surprising given the criterion's lack of specificity. The limiting conditions set out in the previous criteria are no longer valid: any modification seems to be permitted as long as the resulting sequence is a cisgenic sequence at the targeted site; this sequence is allowed to vary within the limits defined in criteria 1 and 2 (any deletion and possible substitutions or insertions of sequences less than 20 nucleotides long).

It seems inconsistent to allow variations in this cisgenic sequence when they did not appear to be tolerated under criterion 3.

On the other hand, allowing any type of modification as long as the resulting sequence is a cisgenic sequence is consistent with the equivalence approach focused on the molecular nature of the product obtained. However, the techniques used will remain limited to the targeted mutagenesis and cisgenesis techniques defined for NGT plants.

The fact that the plural is used in the wording of the criterion ("on the condition that the resulting DNA sequences already occur (...) in a species from the breeders' gene pool") casts doubt on the possibility that this may be referring to fragments of rearranged sequences collectively resulting in an intragenic sequence.

The WG on "Biotechnology" considers that a criterion causing intragenic plants to be exempted from the requirements of GMO legislation would not be justifiable.

3.2.3. Other general comments

3.2.3.1. Points requiring clarification

The WG on "Biotechnology" considers that on the whole, Annex I is unclear.

The WG emphasises that:

- certain terms are not defined in the Proposal for a Regulation and need to be clarified ("conventional plants", "targeted site", "similarity", "gene", "contiguous DNA sequence");
- conversely, certain terms defined in the Proposal for a Regulation are not used in Annex I, even though they would be enlightening, such as "mutagenesis", "cisgenesis", and "intragenesis";
- the terms that are both defined in the Proposal for a Regulation and used in Annex I are confusing; these include "NGT plants", which refers to a subset of plants obtained through NGTs.

The WG on "Biotechnology" considers that certain clarifications provided in the technical paper should be included in Annex I, such as – if this is indeed the case – the exclusion of intragenesis and non-targeted cisgenesis from acceptable techniques for category I plants.

3.2.3.2. The scientific basis of the criteria

The Commission's technical paper states that "similar genetic modifications obtained by different techniques are not expected to present different risks" and that "if certain type and number of mutations can be introduced by both conventional breeding techniques and NGTs, also the type of traits associated to these mutations would not be different between the techniques". It concludes that it is sufficient to only consider the type and number of mutations to assess equivalence and that the related effects do not need to be taken into account.

The WG on "Biotechnology" considers that there is no scientific basis for trait or risk level equivalence between two categories of plants with equivalent genetic variations or modifications that would be defined solely by their type, size, and number.

The WG reiterates that genetic variability and genetic variations observed in nature are the product of thousands of years of evolution, drift, and natural selection. Genetic variations and modifications observed in varieties produced through conventional breeding techniques have been selected by breeders. In both cases, genetic variations and modifications associated with negative effects are removed; these may relate to the plant's fitness or its selective value in nature, or to the agronomic and qualitative characteristics sought by humans in conventional breeding programmes. These variations and modifications are removed or selected based not on their type, size, or number, but on their potential biological impact.

The WG on "Biotechnology" underlines that the functional or biological consequences of a given genetic variation or modification are not determined by its type or size.

Nevertheless, based on an analysis of the proposed equivalence approach, which focuses on the types, sizes, and number of genetic modifications, **the WG on "Biotechnology" considers that the thresholds not to be exceeded, set at 20 genetic modifications per plant, at the targeted site and in similar sequences, and at 20 nucleotides for insertions and substitutions, are not justified. This is also true for the acceptance of any deletion or inversion without conditions and, to a lesser extent, for targeted cisgenesis without any orthology conditions applied to the target.**

The lack of consideration for potential modifications outside targeted sites and similar sequences (with the exception of transgenic elements, as per the definition of NGT plants) is not justified either.

3.3. Conclusions of the Working Group on "Biotechnology"

The WG on "Biotechnology" reiterates the context of this internal request:

The Proposal for a Regulation on NGTs adopted by the European Commission on 5 July 2023 intends to exempt certain plants genetically modified through NGTs from GMO legislation on the grounds that they are equivalent to conventional plants.

To this end, it proposes equivalence criteria that would ensure that NGT plants that meet these criteria (category 1 NGT plants) could have been produced using conventional breeding techniques.

The WG on “Biotechnology” reiterates the key question in the internal request: to what extent can plants defined in this way actually be considered equivalent to plants obtained through conventional techniques?

To answer this question, the WG on “Biotechnology” first set out to clarify the proposed equivalence criteria, in particular the terms and concepts needed to understand them; it also defined the various sets of techniques concerned, capable of inducing the genetic modifications in question. It then went on to analyse the scientific aspects in greater detail, criterion by criterion, and raised a number of questions, highlighting the associated limitations and examining the scientific basis of the criteria more broadly.

Following its work, the WG on “Biotechnology” notes the following points:

1) Lack of clarity:
   - The equivalence criteria are anything but clear, in particular because ambiguous terms are used (“targeted site”, “similarity”, “gene”, “breeders’ gene pool”, “contiguous DNA sequence”);
   - The criteria focus solely on genetic modifications at a “targeted site” and in similar sequences. The “targeted site” and similar sequences need to be defined; their definition will determine the genetic modifications in question;
   - The exclusion of intragenic plants from category 1 NGT plants is not explicitly stated in the criteria and should be clarified;
   - The exclusion of plants obtained through non-targeted cisgenesis from category 1 NGT plants is not explicitly stated in the criteria and should be clarified.
2) Insufficient scientific rationale for the claimed equivalence between NGT plants meeting the proposed criteria and conventional plants:
   - The proposed maximum number of acceptable genetic modifications is insufficiently justified;
   - The possibility or likelihood of a given modification or combination of modifications being achieved through conventional techniques should be considered;
   - Based on pan-genome analyses, the acceptance of deletions and inversions regardless of their size is not scientifically justified;
   - The lack of consideration for unintended genetic modifications potentially located outside the targeted sites and similar sequences (apart from transgenic elements) is not justified.
3) Failure to take account of the relationship between the proposed equivalence criteria and the associated risk:
   - The technical paper states that categories of plants that are equivalent in terms of the type, size, and number of genetic variations and modifications would have equivalent traits and risk levels. This assumption has no scientific basis;
   - The proposal for a maximum number of acceptable genetic modifications has no scientific basis in terms of risk: the associated risk is not directly proportional to any number of modifications;
   - The proposal for a maximum accepted size of insertions and substitutions makes no biological sense; the functional consequences and risks potentially associated with an insertion are not proportional to the length of its nucleotide sequence;

- The acceptance of any deletion or inversion without considering the functional consequences and the potential associated risks is not justified;
- The fact that unintended genetic modifications, induced due to a lack of specificity in sequences similar to the target, are included in the proposed number of genetic modifications without their possible negative effects being considered, is not justified.

Therefore, the analysis of the proposed criteria for equivalence between NGT plants and conventional plants led the WG on “Biotechnology” to consider the issue more broadly. The WG recommends that these equivalence criteria, which are based solely on molecular aspects and which, moreover, are insufficiently justified, should take account of the traits of the plants and the possible associated risks.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) highlights that in response to a formal request from the French Ministries of the Environment and Agriculture, and prior to the publication of the Proposal for a Regulation, it initiated an expert appraisal on methods for assessing the risks associated with NGT plants and their socio-economic impact. This expert appraisal is currently in progress and should be available in the first quarter of 2024. It is focusing in particular on identifying the adaptations that need to be made to the methodology for assessing the health and environmental risks associated with transgenic plants when the assessment concerns plants obtained through site-directed mutagenesis using CRISPR-Cas9 and related techniques.

Following the publication of the Proposal for a Regulation, ANSES decided to issue an internal request to analyse the criteria defining category 1 NGT plants, considered equivalent to conventional plants, set out in Annex I of this Proposal for a Regulation and justified in a technical paper published by the European Commission in October. This analysis is addressed in this Opinion.

The conclusions of the WG on “Biotechnologies” are threefold and pertain to the need to clarify the definitions and scope, the scientific basis for the criteria, and the need for the equivalence criteria to take account of potential risks.

Firstly, the Agency agrees with the WG experts that functional consequences and potential risks are not taken into account in the equivalence criteria. However, it underlines that this is already the case in the legislative framework for GMOs resulting from Directive 2001/18/EC. Indeed, the fact that only plants derived from transgenesis require a dossier demonstrating the control or absence of risks, and that plants obtained through conventional techniques including random mutagenesis are exempted from this requirement, was part of the debate held and the choices made when the GMO framework was being established, with part of the argument residing in the conventional nature of the techniques and their long history of use. ANSES notes that for NGTs, the equivalence criteria approach extends the dividing line between plants subject to assessment and those not subject to assessment, according to the logic of the current texts.

A corollary of this regulatory construction is that the terms and definitions used need to be particularly clear and unambiguous. ANSES therefore endorses and supports the experts’

requests for clarification. While some of the lack of precision may result from the diversity of the texts on which the Proposal for a Regulation is based (JRC report, EFSA opinion, etc.), it is important that the final text be self-supporting. The first major shortcoming is the absence of a definition of the conventional plants with which the decision-making rule has to establish a comparison. Some other important clarifications are also recommended, including: making explicit the exclusion of non-targeted cisgenesis from the NGT techniques covered, making a clearer distinction between intragenesis and cisgenesis, specifying the materials included in insertions (the wording of the JRC and EFSA texts on which the Proposal for a Regulation is based is more precise than the term “genetic material” used in this proposal), defining the sites targeted by NGT operations, applying criterion 3 only to cisgenesis in the strict sense (excluding intragenesis), and finally, clarifying the “breeders’ gene pool”.

Lastly, ANSES endorses the conclusions of the WG on “Biotechnology” regarding the various limitations of the scientific rationale for the equivalence criteria. In particular, the Agency underlines that the possibility of replacing certain absolute thresholds with thresholds based on the length of plant genomes should be considered.

Prof Benoît VALLET

KEYWORDS

OGM, nouvelles techniques génomiques, NTG, techniques d'obtention conventionnelles, mutagenèse ciblée, mutagenèse dirigée, cisgenèse, législation, réglementation, Union européenne

GMO, new genomic techniques, NGT, conventional breeding techniques, targeted mutagenesis, cisgenesis, legislation, regulation, European Union

REFERENCES

Anzalone, Andrew V., Xin D. Gao, Christopher J. Podracky, Andrew T. Nelson, Luke W. Koblan, Aditya Raguram, Jonathan M. Levy, Jaron A. M. Mercer, et David R. Liu. « Programmable Deletion, Replacement, Integration and Inversion of Large DNA Sequences with Twin Prime Editing ». *Nature Biotechnology* 40, nº 5 (mai 2022): 731-40. https://doi.org/10.1038/s41587-021-01133-w.

Broothaerts W, Jacchia S, Angers A, Petrillo M, Querci M, Savini C, Van Den Eede G, et Emons H. « New Genomic Techniques: State-of-the-Art Review », nº KJ-NA-30430-EN-N (online) (2021). https://doi.org/10.2760/710056 (online).

Charlesworth B and Charlesworth D. « Elements of evolutionary genetics ». Roberts and Company Publishers, Greenwood Village, Colorado USA. ISBN 978-0-9815194-2-5 (2010).

Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration JO L 106 du 17 avril 2001, pp. 1-39.

European Commission services, Regulation on new genomic techniques (NGT) – Technical paper on the rationale for the equivalence criteria in Annex I, 16 Oct. 2023.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2012. Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA Journal 2012;10(2):2561. [33 pp.] doi:10.2903/j.efsa.2012.2561. Available online: www.efsa.europa.eu/efsajournal.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins E, Bresson J-L, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Naegeli H, Nogu_e F, S_anchez Serrano JJ, Savoini G, Veromann E, Veronesi F, Casacuberta J, Fernandez Dumont A, Gennaro A, Lenzi, P, Lewandowska A, Munoz Guajardo IP, Papadopoulou N and Rostoks N, 2022. Updated scientific opinion on plants developed through cisgenesis and intragenesis. EFSA Journal 2022;20(10):7621, 33 pp. https://doi.org/10.2903/j.efsa.2022.7621.

Gallais. « De la domestication à la transgénèse - Évolution des outils pour l'amélioration des plantes » - André Gallais (EAN13 : 9782759220243) | Librairie Quae : des livres au coeur des sciences ». 2019. https://www.quae.com/produit/1167/9782759220243/de-la-domestication-a-la-transgenese.

Gill, Rafaqat A., Federico Scossa, Graham J. King, Agnieszka A. Golicz, Chaobo Tong, Rod J. Snowdon, Alisdair R. Fernie, et Shengyi Liu. « On the Role of Transposable Elements in the Regulation of Gene Expression and Subgenomic Interactions in Crop Genomes ». *Critical Reviews in Plant Sciences* 40, nº 2 (4 mars 2021): 157-89. https://doi.org/10.1080/07352689.2021.1920731.

HCB (High Council for Biotechnology). « Scientific Opinion of the Scientific Committee in response to the referral of 2 July 2020 concerning the draft decree amending Article D.531-2 of the French Environment » (Ref. HCB-2020.07.07-1). (2020) (Paris, HCB), 44 pp. Available online: http://www.hautconseildesbiotechnologies.fr.

He, Yubing, Michael Mudgett, et Yunde Zhao. « Advances in Gene Editing without Residual Transgenes in Plants ». *Plant Physiology* 188, nº 4 (28 mars 2022): 1757-68. https://doi.org/10.1093/plphys/kiab574.

Hufford, Matthew B., Arun S. Seetharam, Margaret R. Woodhouse, Kapeel M. Chougule, Shujun Ou, Jianing Liu, William A. Ricci, et al. « De Novo Assembly, Annotation, and Comparative Analysis of 26 Diverse Maize Genomes ». *Science (New York, N.Y.)* 373, nº 6555 (6 août 2021): 655-62. https://doi.org/10.1126/science.abg5289.

Jacob, Pierre, Adi Avni, et Abdelhafid Bendahmane. « Translational Research: Exploring and Creating Genetic Diversity ». *Trends in Plant Science* 23, nº 1 (1 janvier 2018): 42-52. https://doi.org/10.1016/j.tplants.2017.10.002.

Krasileva, Ksenia V., Hans A. Vasquez-Gross, Tyson Howell, Paul Bailey, Francine Paraiso, Leah Clissold, James Simmonds, et al. « Uncovering Hidden Variation in Polyploid Wheat ». *Proceedings of the National Academy of Sciences of the United States of America* 114, n(07 February 2017): E913-21. https://doi.org/10.1073/pnas.1619268114.

Li, Guotian, Mawsheng Chern, Rashmi Jain, Joel A. Martin, Wendy S. Schackwitz, Liangrong Jiang, Miguel E. Vega-Sánchez, et al. « Genome-Wide Sequencing of 41 Rice (Oryza Sativa L.) Mutated Lines Reveals Diverse Mutations Induced by Fast-Neutron Irradiation ». *Molecular Plant* 9, nº 7 (6 juillet 2016): 1078-81. https://doi.org/10.1016/j.molp.2016.03.009.

Nonaka, Satoko, Chikako Arai, Mariko Takayama, Chiaki Matsukura, et Hiroshi Ezura. « Efficient Increase of Ɣ-Aminobutyric Acid (GABA) Content in Tomato Fruits by Targeted Mutagenesis ». *Scientific Reports* 7, nº 1 (1 août 2017): 7057. https://doi.org/10.1038/s41598-017-06400-y.

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.

Quiroz, Daniela, Mariele Lensink, Daniel J. Kliebenstein, et J. Grey Monroe. « Causes of Mutation Rate Variability in Plant Genomes ». *Annual Review of Plant Biology* 74 (22 mai 2023): 751-75. https://doi.org/10.1146/annurev-arplant-070522-054109.

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed JO L 268 du 18.10.2003, pp. 1-23.

Sánchez-León, Susana, Javier Gil-Humanes, Carmen V. Ozuna, María J. Giménez, Carolina Sousa, Daniel F. Voytas, et Francisco Barro. « Low-Gluten, Nontransgenic Wheat Engineered with CRISPR/Cas9 ». *Plant Biotechnology Journal* 16, nº 4 (avril 2018): 902-10. https://doi.org/10.1111/pbi.12837.

Sturme, Mark H. J., Jan Pieter van der Berg, Lianne M. S. Bouwman, Adinda De Schrijver, Ruud A. de Maagd, Gijs A. Kleter, et Evy Battaglia-de Wilde. 2022. « Occurrence and Nature of Off-Target Modifications by CRISPR-Cas Genome Editing in Plants ». *ACS Agricultural Science & Technology* 2 (2): 192-201. https://doi.org/10.1021/acsagscitech.1c00270.

Szurman-Zubrzycka, Miriam, Marzena Kurowska, Bradley J. Till, et Iwona Szarejko. « Is it the end of TILLING era in plant science? » *Frontiers in Plant Science* 14 (2023). https://www.frontiersin.org/articles/10.3389/fpls.2023.1160695.

Wang TL, Uauy C, Robson F, Till B (2012) Tilling in extremis. Plant Biotechnology journal 10, 761-772. https://onlinelibrary.wiley.com/doi/10.1111/j.1467-7652.2012.00708.x

Wang, Yanpeng, Xi Cheng, Qiwei Shan, Yi Zhang, Jinxing Liu, Caixia Gao, et Jin-Long Qiu. « Simultaneous Editing of Three Homoeoalleles in Hexaploid Bread Wheat Confers Heritable Resistance to Powdery Mildew ». *Nature Biotechnology* 32, nº 9 (septembre 2014): 947-51. https://doi.org/10.1038/nbt.2969.

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ANNEX 1

RÉPUBLIQUE FRANÇAISE
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Decision No 2023-182

INTERNAL REQUEST

The Director General of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES),

Having regard to the Public Health Code, and in particular its Article L. 1313-3 giving ANSES the prerogative to issue an internal request on any question with a view to accomplishing its missions,

Has decided the following:

Article 1: The French Agency for Food, Environmental and Occupational Health & Safety is issuing an internal request to conduct an expert appraisal whose characteristics are listed below.

1.1 Themes and objectives of the expert appraisal

Scientific analysis of Annex I of the European Commission's Proposal for a Regulation of 5 July 2023 on new genomic techniques (NGTs) – Review of the proposed equivalence criteria for defining Category 1 NGT plants.

1.2 Background of the internal request

Since the adoption of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs), scientific and technical progress has led to the development of new genetic modification techniques that have raised questions as to whether their products fall within the definition of GMOs and the scope of this Directive.

Following the judgment of the Court of Justice of the European Union in Case C-528/16 (Judgment of the Court of Justice of 25 July 2018), which concluded that new mutagenesis techniques fell within the scope of this Directive while raising practical issues of applicability, the European Council invited the European Commission to carry out a study regarding the status of "new genomic techniques" (NGTs) under Union law (Council Decision (EU) 2019/1904 of 8 November 2019).

In its study published on 29 April 2021, the Commission concluded that current European Union (EU) legislation on GMOs was not appropriate for some of these NGTs, which were formally defined for the first time as being "techniques that are capable of altering the genetic material of an organism and that have emerged or have been developed since 2001". In particular, the risk assessment provided for in the current legislation was deemed sometimes inadequate or disproportionate, and the detection requirements were considered often inapplicable for plants and plant products obtained through targeted mutagenesis or cisgenesis techniques.

The Proposal for a Regulation on NGTs adopted by the European Commission on 5 July 2023, which intends to provide an appropriate and measured response to the questions raised by the emergence of these new techniques, uses the principle of *lex specialis*. This means that the rules set out in the Proposal for a Regulation are meant to take precedence over existing legislation, in particular Directive 2001/18/EC and Regulation (EC) No 1829/2003 as regards the placing on the market of plants and products or parts of products derived from such plants for use in food and/or feed.

Among the plants modified by NGTs (in the pre-existing sense of the term), the Proposal for a Regulation defines a NGT plant as 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". Two categories of NGT plants are distinguished. Category 1 plants, which could also occur naturally or be produced by conventional breeding, are defined by criteria for equivalence to conventional plants set out in Annex I to the Proposal for a Regulation. A technical paper by the European Commission published on 16 October 2023 provides the rationale for these equivalence criteria. Once their status is recognised, these plants will no longer be subject to EU legislation on GMOs. On the other hand, NGT plants that are not category 1 are category 2 and will remain covered by GMO legislation, subject to specific provisions and derogations.

This legislative proposal will pose various challenges for ANSES at different stages, from the adoption procedure to the implementation of the Regulation. In the short term, the Regulation is due to be adopted under the ordinary legislative procedure, involving debates and voting in the European Parliament and Council. At this stage of the negotiations, ANSES could contribute to:

- informing public decision-making, by providing an analysis of the regulatory proposal to the French competent authorities to accompany the forthcoming debates and voting in the Council;
- informing and enlightening public and legislative debate, by publishing an analysis for society, including ordinary citizens and stakeholders, including members of the European Parliament who will take part in the forthcoming debates and voting in this body.

In light of time constraints, the analysis carried out will focus on reviewing the proposed equivalence criteria for defining Category 1 NGT plants. This is considered to be one of the most sensitive points in the legislative proposal, since it creates a category of genetically modified plants that would be exempted from the requirements of EU GMO legislation.

This internal request supplements the formal request relating to groundwork on methods for assessing the risks associated with the use of GMOs in food and feed, which includes a methodological discussion on possible adaptations to the requirements for assessing the health and environmental risks associated with plants derived from NGTs, in particular CRISPR-Cas9 and related techniques (2023 work programme of the Risk Assessment Department, Sheet 1.1.4 METHEVALOGM).

1.3 Questions on which the expert appraisal work will focus

The Proposal for a Regulation on NGTs adopted by the European Commission on 5 July 2023 intends to exempt certain plants genetically modified through NGTs from GMO legislation on the grounds that they are equivalent to conventional plants. This internal request focused on the criteria used in the Proposal for a Regulation to conclude whether certain NGT plants are equivalent to conventional plants.

The plants concerned by this equivalence, i.e. Category 1 NGT plants, are jointly defined by:

- the scope of the plants considered in the Proposal for a Regulation, referred to as "NGT plants" in this Regulation,
- the application of the criteria for "equivalence to conventional plants" set out in Annex I to the Proposal for a Regulation.

The joint analysis of the criteria in Annex I and of the associated rationale in the European Commission's technical paper, and the definition of NGT plants considered in the Regulation, should be carried out with the aim of answering the following key question: to what extent can plants defined in this way actually be considered equivalent to conventional plants?

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For the dual purpose of clarification and critical analysis, this work will aim to:

- clarify the equivalence criteria for defining Category 1 NGT plants as set out in Annex I to the Proposal for a Regulation and underpinned by the technical paper, to improve the readability of the legislative proposal,
- highlight potential questions and limitations in the definition of these criteria, by reviewing their scientific basis and considering the usefulness of additional criteria.

1.4 Estimated duration of the expert appraisal

The expert appraisal is expected to run until the end of November.

Article 2: An opinion will be issued and published by the Agency following completion of the work.

Signed in Maisons-Alfort on 6 November 2023

Prof Benoit VALLET
Director General