Advice Differences in EU positions regarding Annex I of the NGT-legislation

COGEM-advies CGM/250516-01

1. Introduction

COGEM was asked by the Ministry of Infrastructure and Water Management to provide scientific advice on the differences between the European Parliament's (EP) position and the Council of the European Union's position regarding Annex I of the new legislation on plants produced using "new genomic techniques" (NGTs). Furthermore, COGEM was asked to assess whether the EP's position aligns with the European Commission's (EC) proposal, which is based on the premise that NGT1 plants - defined according to the criteria in Annex I - are plants that could occur naturally or are developed through conventional breeding techniques. Lastly, the COGEM was asked to what extent it considers the criteria unambiguously verifiable and widely applicable in implementation practice.

Since 2023, the EC published a proposal, and the EP and the Council both adopted a position on the legislation that allows for the deliberate release into the environment or placement on the market of NGT plants, within the European Union (EU). The techniques falling under the scope of NGTs include targeted mutagenesis and cisgenesis. The proposed legislation distinguishes between two categories of NGT plants. First, NGT1 refers to plants whose modifications are comparable to those achievable by conventional breeding methods, and these plants would therefore be exempt from the obligations of the GMO legislation. Second, NGT2 plants pertain to plants with genetic modifications that go beyond what is possible using conventional techniques. These plants would require a case-by-case risk assessment.

In this advice, COGEM examines the positions of the EP and the Council with respect to Annex I, evaluating their differences, scientific implications, and determining if they allow for modifications comparable to conventional breeding methods. Further assessment includes the verifiability and clarity of the criteria they propose.

2. Previous COGEM advice

In recent years, COGEM has repeatedly highlighted that current GMO regulations regarding GM plants are outdated and fail to align with advances in scientific and technological developments, necessitating updates.[1] COGEM has recommended exempting plants produced through targeted mutagenesis and cisgenesis, but not intragenesis, from the GMO regulation, as their safety profiles are comparable to conventionally bred crops.[1,2,3,4] Additionally, COGEM noted that enforcing the GMO regulation will become increasingly challenging with these new techniques, as detecting certain modifications is only possible if the specific changes are known in advance.[5,6]

COGEM was broadly positive about the European Commission's proposal and the European Food Safety Authority's (EFSA) justification for the criteria in Annex I.[7,8,9] COGEM did advise limiting the scope of the proposal to land plants only and further clarifying the text in Annexes I, II, and III.[7] At a

later point, COGEM offered specific recommendations for improving Annex I, including setting a limit on the number of modifications permissible in a gene, taking polyploidy of plants into account, and removing the term "targeted" in cisgenesis or inversions.[10]

3. The European Parliament's (EP) position and the Council of the European Union's position regarding Annex I

Several modifications are allowed in Annex I of the EC proposal for a plant to be considered equivalent to 'plants that could also occur naturally or be produced by conventional breeding techniques' (i.e., NGT1 plants). These modifications are (1) the insertion or substitution of up to 20 nucleotides; (2) the deletion of any number of nucleotides; (3) the insertion or substitution of a continuous DNA sequence existing in the breeder's gene pool and (4) the inversion of sequences. When this advice refers to modifications, it specifically refers to these four types. The annexes in the positions of the EP and the Council can be found below.

Annex I – European Parliament position
Criteria of equivalence of NGT plants to conventional plants

A NGT plant is considered equivalent to conventional plants if the following conditions referred to in points 1 and 1a are met:

(1) The number of the following genetic modifications, which can be combined with each other, does not exceed 3 per any protein-coding sequence taking into account that mutations in introns and regulatory sequences are excluded from this limit:
- (a) substitution or insertion of no more than 20 nucleotides;
- (b) deletion of any number of nucleotides;

(1a) The following genetic modifications, which can be combined with each other, do not create a chimeric protein that is not present in species from the gene pool for breeding purposes or does not interrupt an endogenous gene;
- (a) insertion of continuous DNA sequences existing in the gene pool for breeding purposes;
- (b) substitution of endogenous DNA sequences with continuous DNA sequences existing in the gene pool for breeding purposes;
- (c) inversion or translocation of continuous endogenous DNA sequences existing in the gene pool for breeding purposes.

Annex I – Council of the European Union position
Criteria of equivalence of NGT plants to conventional plants

A NGT plant is considered equivalent to conventional plants when it differs from the recipient/parental plant by no more than 20 genetic modifications per monoploid genome of the types referred to in points 1 to 4, in any DNA sequence sharing sequence similarity with the targeted site that can be predicted by bioinformatic tools.

Criteria specific to the use of targeted mutagenesis:

(1) substitution or insertion of no more than 20 nucleotides;
(2) deletion of any number of nucleotides;

Criteria specific to the use of cisgenesis:

(3) On the condition that the genetic modification does not interrupt an endogenous gene or that the resulting combination of DNA sequences in the recipient plant already occurs in a species from the breeders' gene pool
    (a) insertion of a continuous DNA sequence existing in the breeders's gene pool;
    (b) substitution of an endogenous DNA sequence with a continuous DNA sequence existing in the breeders's gene pool;
(4) Targeted inversion of a sequence of any number of nucleotides

4. Differences between the positions of the European Parliament and the Council of the European Union and equivalence of the described criteria to conventional breeding techniques

The annexes of the European Parliament (EP) and Council align on several criteria. However, there are differences on the exact wording of the criteria and the conditions of their use. In the following sections COGEM examines the annexes of the EP and the Council, assessing their differences, evaluating their scientific implications and determining if they allow for modifications comparable to conventional breeding methods. The differences in criteria and their implications will be addressed top-to-bottom of the Annex I in the subsequent sections.

4.1 Number of modifications allowed

COGEM notes that the exact scope of the number of modifications is up for interpretation, and further elaboration on the lack of clarity of the criteria can be found in paragraph 5.2. The interpretation of the positions given below is what the COGEM deems to be most likely reading of the text.

The EP's position appears to permit unlimited modifications across the genome, provided there are no more than three deletions or insertions/substitutions of 20 nucleotides within a single protein-coding sequence. This approach allows the possibility of large-scale changes, for example, a stacked combination of substitutions or insertions of up to 20 nucleotides in any non-protein-coding sequence. This includes any regulatory sequence or any sequence coding for a Non-Coding RNA.[11]

During random mutagenesis, dozens of mutations in genes are expected to occur, as mentioned in EFSA's technical paper.[9] However, the more modifications are clustered in a single region of the genome the more unlikely this is to occur through mutagenesis. Although in theory, this would be

possible with unlimited repeats of mutagenesis steps, it stretches the definition of equivalence to conventional breeding. Therefore, COGEM is of the opinion that unlimited or clustered modifications are not equivalent to what is realistically possible to achieve by conventional breeding.

The Council position sets a limit of 20 modifications across the entire plant genome, without restrictions on their locations. As mentioned earlier, modifications spread across the genome align with the EC's premise of equivalence, and the number of 20 modifications is in line with the conclusions of EFSA's technical paper.[9]

However, introducing these 20 modifications within a single protein coding sequence could result in alterations that are more in line with transgenesis than conventional breeding. Thus, the Council's position exceeds what is possible with conventional breeding in this regard.

As stated in COGEM's prior advice, it is possible to expect up to 20 nucleotides to be altered within a single gene, including both regulatory and coding regions, with conventional breeding techniques.[7,10] Both positions, with the EP's allowing for 3 modifications of 20 nucleotides to be changed in any protein coding sequences and the Council's allowing for 20 modifications of 20 nucleotides, exceed the limit of 20 nucleotides per gene as set out previously by COGEM. The EP's position which sets a limit on the number of modifications in a protein coding sequence more closely reflects COGEM's opinion on the matter.

4.2 Insertion of continuous DNA sequences

The Annex I in the EP position does not explicitly mention cisgenesis, but instead refers to the "insertion or substitution of continuous DNA sequences existing in the gene pool for breeding purposes". This is in line with how the Council defines cisgenesis and therefore does not result in a meaningful difference between the two.

The EP Annex I excludes the creation of "a chimeric protein that is not present in species from the breeding gene pool" when inserting or substituting continuous DNA sequences, or the disruption of endogenous genes. This position would allow for new combinations of DNA sequences as long as they do not result in a chimeric protein. This could theoretically result in new combinations that are not found in the breeders' gene pool, for example by combining regulatory sequences with coding sequences of other genes. Furthermore, chimeric proteins are defined in the EP position as joining two sequences that originally code for separate proteins.[a] This allows for the combination of a protein coding sequence with an initially non-coding region. By placing a protein-coding sequence upstream of a DNA region that typically doesn't produce a protein, the previously non-coding region can now be translated into a protein and fused with the newly added coding sequence.

The COGEM notes that creation of chimeric proteins also occurs naturally[12] and it would therefore not be needed to exclude the formation of such chimeric proteins to fulfil the criteria of equivalence. If

[a] Position of the European Parliament on adoption of the Regulation on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 and Directive 98/44/EC. Page 41, section 15b - "Chimeric protein" means proteins created through the joining of two or more genes or parts of genes that originally coded for separate proteins. [Am. 29]

there are additional reasons for prohibiting the creation of fused proteins, specifying these reasons in the text would provide further clarification.

The Council's position also similarly restricts insertion or substitution of continuous DNA sequences, but instead of excluding the formation of chimeric proteins, it states that the resulting combination may not interrupt an endogenous gene or result in a DNA sequence that is not already present in the breeders' gene pool.

By excluding any formation of new DNA sequences not existing in the breeder's gene pool, the Council does not allow for intragenesis. The Council's position even goes further by explicitly mentioning intragenesis as not being allowed in the main body of the text.

Previously the COGEM has stated that complex forms of intragenesis using and combining multiple genetic elements should not be considered equivalent to conventional breeding techniques.[13] As the EP's position can be interpreted as allowing intragenesis, it is not in line with the COGEM's opinion on equivalence to conventional breeding techniques.

4.3 Inversions

In the EP's position, inversions or translocations are only allowed for continuous endogenous DNA sequences and are not allowed to disrupt endogenous genes.

In contrast, the Council's position is that an inversion of any number of nucleotides is allowed with no further caveats. The Council's position thus allows for the disruption of endogenous genes and the creation of DNA sequences not already present in the breeders' gene pool via inversions. This strategy may also be applied to silence endogenous genes.

The Council's position makes no explicit mention of translocations, but as translocations can be considered as a form of cisgenesis COGEM sees no functional difference between the positions in this regard. Furthermore, the Council's position allows for the disruption of endogenous genes, whereas the EP's position does not. In nature, and when induced by conventional techniques, inversions may occur at any position in the genome. Thus, by not allowing the possible disruption of endogenous genes the EP's position does not align with the premise of equivalence to conventional techniques outlined in the EC proposal, whereas the Council's position does.

5. Verifiability, and clarity of Annex I

In the previous paragraphs COGEM assessed the differences in the EP's and Councils positions regarding Annex I, evaluating their scientific implications and determining if the allowed modifications are comparable to what can be obtained by conventional breeding methods. Additionally, the COGEM was asked to what extend it considers the criteria unambiguously verifiable and widely applicable in implementation practice. Significant challenges remain in ensuring both verifiability and traceability throughout the breeding and production pipeline and in the clarity of Annex I, which will be discussed below.

5.1 *Verifiability*

It is possible to confirm whether a NGT1 plant contains specific intentional modifications as reported by the applicant. While testing for the presence of these changes is feasible, it can be costly, especially if a plant contains a large number of modifications, and the volume of plants needing testing increases as NGTs become widely adopted. In contrast, ensuring that no unintended or additional modifications are present is significantly more challenging. Proving the absence of unintentional changes would require whole-genome sequencing combined with extensive bioinformatics analysis, which introduces significant logistical and financial hurdles. Without such comprehensive testing, it would be a significant hurdle to confirm that a plant does not contain modifications falling under the classification of NGT2 or even be transgenic.

In the current positions, it is clearly stated that when any seed is sold or made available in any way, it should be made clear whether the plant's reproductive material belongs to a NGT category. Furthermore, it is stated that the production chains that wish to remain free of NGTs can do so to safeguard consumer trust. However, traceability becomes increasingly complex if adoption grows, despite documentation providing a mechanism for tracking these plants. For instance, if NGT1 crops are grown alongside conventional varieties, the risks of unintentional mixing or outcrossing increase significantly. This challenge becomes even more pronounced when farmers save and reuse seeds. In such scenarios, untracked NGT1 plants - derived from saved or crossed seeds - could proliferate unchecked. Such practices could undermine labeling and traceability throughout the pipeline, reducing the practicality of their use.

COGEM notes that the risks associated with NGT1 plants, if they fulfil the criteria for equivalence, are comparable to those of conventionally bred plants, and that there are no additional environmental risks associated with their use, even when not tracked.

5.2 *Clarity of the annexes*

While the current positions outline important criteria for plant breeding, the annexes could benefit from greater clarity to ensure consistent understanding and application. Some elements of the text leave room for different interpretations, particularly regarding the scale and scope of permissible modifications. Enhancing clarity and explicitly defining the intent of specific criteria would make the Annex I more accessible and practical.

For example, in the Council's position, Criterion 3 of Annex I refers to the disruption of endogenous genes, but does not define what constitutes a gene, leaving room for different interpretations. In

contrast, the Council's position - unlike the EP's position - explicitly mentions intragenesis as undesirable in the text. By identifying intragenesis[b,14] as undesirable, it indicates that "endogenous genes" in this context include both regulatory and coding sequences. Otherwise, insertions combining regulatory elements (e.g., promoters) with coding sequences could potentially lead to intragenesis. Providing a similar level of specificity in other key areas would help stakeholders to better understand which types of modifications align with the NGT1 classification.

Clarity is especially important for cases where the result of a modification might be possible in one criterion but is specifically excluded in another one. For example, criterion 3 of the Council's position states that no new combinations of DNA sequences can be created and criterion 1a of the EP's positions states that no chimeric proteins can be created. However, in both cases such results can be achieved through the deletion of intervening sequences between two protein-coding sequences, a natural process that is also found in conventional breeding. This inconsistency may cause confusion, which could be avoided by clearly specifying which outcomes are considered undesirable in the text.

Additionally, the wording concerning the size and number of permissible modifications needs to be further refined. For example, in the EP's position, it is unclear whether three separate insertions of up to 20 nucleotides each are allowed within a single coding sequence, or whether the total insertions and substitutions must not exceed 20 nucleotides in total, spread across a maximum of three locations. Similarly, the Council's proposal leaves room for different interpretations regarding whether "20 nucleotide modifications in total" are allowed, or whether it permits 20 individual modifications, each composed of up to 20 nucleotides. Clear use of language could support uniform interpretation and implementation of the criteria.

In summary, COGEM concludes that both the Annex of the EP and the EC I require clarity on some aspects to avoid ambiguity in interpretations. Specifying which outcomes are considered undesirable in the text and using more precise wording in the definition of the criteria would ensure the legislation is clearly defined and easier to apply.

6. Conclusion

In summary, COGEM identifies key differences between Annex I of the European Parliament (EP) and that of the Council of the European Union.

Firstly, the EP's position permits far more modifications than that of the Council. COGEM is of the opinion that setting no limit on the number of modifications, as well as allowing for the occurrence of

[b] Position of the European Parliament on adoption of the Regulation on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 and Directive 98/44/EC. Page 7, section 2
- Intragenic plants result from the use of intragenesis techniques, but can be also obtained by through cisgenesis techniques in the strict sense. In the latter case, new developments of site-directed modification also offer the possibility to target the insertion of continuous DNA sequences other than complete genes (for example promoters or regulatory sequences), from the breeders' gene pool at specific loci in the genome. When the insertion of such fragments occurs within an endogenous gene, interrupting it, this leads to the formation of a rearranged gene in the recipient plant and, as such, the plant should also be considered intragenic, except in those particular cases in which the resulting DNA sequences in the recipient plant already occur in species from the breeder´s gene pool.

multiple clustered modifications, does not align with the initial premise of equivalence to conventional breeding techniques set out by the European Commission.

Secondly, both positions differ in their wording concerning combinations of new sequences. For example, the EP specifies only that the formation of chimeric proteins is prohibited, allowing for intragenesis by combining regulatory and protein-coding sequences, whereas the Council's position does not allow for such results. COGEM is of the opinion that plants produced by intragenesis should not be deemed equivalent to plants derived by conventional breeding techniques.

Lastly, the EP's position on inversions is more stringent than that of the Council, specifying that no genes may be interrupted. The EP's position does not align with the premise of equivalence, as inversions can naturally occur at any location within the genome.

To maintain the possibility of NGT1 free production chains would require tracing of NGT1 plants. This tracing may present challenges if these plants become widely adopted, particularly if testing is intended to verify for instance that they do not mix with organic food products.

For both Annexes, certain terms used throughout the text would benefit from further clarification, either within the Annex itself or in other relevant sections of the text. Specifically, the text needs better definitions of the types of intended modifications that are permitted and also explicitly state those that are not permitted to avoid ambiguity and misinterpretation.

Table 1. Summary of the comparison between Annex 1 in the EP and the Council position and their equivalence to conventional breeding techniques according to COGEM.

Subject	EP position	Council position	Difference	Equivalence to conventional techniques
Number of modifications allowed	Unlimited, except for 3 deletions, insertions, or substitutions in protein-coding sequences.	Limited to 20 modifications, with no restriction on location.	The EP position allows far more modifications but limits changes in protein-coding sequences.	The EP's unlimited modification allowance is not equivalent to conventional techniques. Both allow closely stacked modifications, which is not equivalent to conventional techniques.
Insertion of continuous DNA sequences	Allowed if it does not interrupt an endogenous gene or creates chimeric proteins.	Allowed if it does not interrupt an endogenous gene or create DNA sequences not present in the breeder's pool.	The EP position does not allow chimeric protein formation but permits intragenesis by combining regulatory and coding sequences.	Extensive recombination of regulatory and coding sequences, as allowed by the EP, is not equivalent to conventional techniques.
Inversions	Allowed if they target continuous endogenous sequences and do not disrupt endogenous genes.	Allowed if they target endogenous sequences.	The Council position allows disruption of endogenous genes, while the EP position does not.	Disruption of endogenous genes occurs naturally and with conventional techniques. Thus, the Council's position, not the EP's, is equivalent to conventional techniques.

Referenties

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