

Analysis statement by ENSER (European Network of Scientists for Social and Environmental Responsibility) on the EU Commission's new GM proposal.¹

Here for Annex 1 on NGT "equivalence criteria"²

7 July 2023

The EU Commission's proposal is scientifically unacceptable, removes the provisions of the precautionary principle and puts the public and environment at risk. Critical scientific expertise and its supporting scientific evidence was completely ignored. The proposal follows exclusively the guidance and assertions of the public and private biotechnology sector - and is therefore to be classified as one-sided. In the following, we briefly explain why this is so - with scientific reasoning and evidence. We focus on the Annex I only, for now.

ANNEX I

The Commission's proposal text **is given in italics and in blue**. It is quoted verbatim.

"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 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."

Text interpretation:

This provision allows a new GM plant to have up to 20 (distinct) genetic modifications and – without any further scientific analysis and risk assessment - to be regarded as equivalent to conventional plants and to be marketed without labelling or traceability. The number "20" has long been under political discussion and does not portray a scientific basis regarding risks and safety impacts.

These 20 modifications can be any combination of the categories 1-5 listed below. There is wide room for interpretation: a) there are 20 different interventions allowed, meaning 20 different targets within the genome; or: b) all occurring modifications at target sites are added up and must not exceed 20. If, for example, a plant is diploid, that is it has 2 sets of chromosomes and thus every

¹ https://food.ec.europa.eu/system/files/2023-07/gmo_biotech_ngt_proposal.pdf

² https://food.ec.europa.eu/system/files/2023-07/gmo_biotech_ngt_proposal_annex.pdf

gene occurs twice, then the sum of 20 modifications would allow to target 10 different genes or DNA sequences. Whether a) or b), either could indeed result in a very complex and deeply altered organism, for example with altered metabolic pathways and modulated by their respective genomic and environmental context.

According to the provision, genetic modifications of narrowly defined *off-target* sites would be included in this counting, as long as they take place in sequences similar to the intended target site and that can be “predicted by bioinformatic tools”.

Such sequence-specific -but not gene-specific- “off-target” modifications would simply be added to the total without any further need of assessment. Furthermore, there are no requirements to identify or assess off-target modifications that are non-sequence-specific and that are known to arise due to the processes used.

This paragraph of the annex 1 thus explicitly *only includes* changes at *intended* target sites or at highly similar off-target sites. It fully ignores and excludes any other widely known changes that will take place due to the processes of genetic modification, such as:

- Large scale genome-wide mutations, due to the processes involved in inserting a DNA sequence (randomly) into the plant genome via GM transformation techniques³ and commonly in combination with tissue culture. In this case it would be the transformation of the plant with the gene for a ‘genome editing tool’ (such as for the CRISPR/Cas DNA cutters) . As the insertion of such genes is currently a necessity for producing ‘genome edited plants’, the definition of NGT plants is explicitly allowing for the “temporary” insertion of foreign DNA “during the development of the NGT plant”⁴ – and where this transgene is later removed in the next plant generations by selection, but not so the genome-wide mutations. For example, when transforming grapevine with such a CRISPR/Cas transgene, (Wang et al. 2021)⁵ found in the resulting GM plants 9,325-12,959 new point mutations, with 230-377 of them occurring within the coding sequence of genes. Such mutations can impact regulatory processes or, when within coding sequences, can alter gene function or resulting protein functions. Consequently they constitute risks.
- Near target site mutations, including larger deletions, translocations and inversions which have been found following CRISPR/Cas actions (see ENSSER statement January 2023)⁶.

Comments & conclusion:

Notably, it does not say the plants described under this section *are* equivalent, but *are considered* equivalent to conventional plants. This of course is a *legal* definition illustrating the political negotiation process, *not a scientific* definition. It is a return to the concept of 'substantial equivalence' used in the first EU GMO legislation of the 1990s and discarded in the later, i.e. current, legislation as an unscientific and unreliable concept. This lack of scientific grounding and evidence is most notably depicted in the arbitrariness of numbers of modifications or base-pair

³ Common GM transformation techniques include *Agrobacterium* mediated gene transfer, bio-ballistics or shotgun technique, and electroporation.

⁴ *‘NGT plant’ means a genetically modified plant obtained by targeted mutagenesis or cisgenesis, or a combination thereof, on the condition that it does not contain any genetic material originating from outside the breeders’ gene pool that temporarily may have been inserted during the development of the NGT plant;* (COM proposal 5 July 2023, p. 27, Article 3, definition 2)

⁵ Wang X, Tu M, Wang Y, Yin W, ... Wang Z. (2021). Whole-genome sequencing reveals rare off-target mutations in CRISPR/Cas9-edited grapevine. *Horticulture Research* 8(1):114. doi:10.1038/s41438-021-00549-4

⁶ ENSSER position statement on CRISPR/Cas gene editing, 7 February 2023. <https://ensser.org/publications/2023/ensser-position-statement-on-crispr-cas-gene-editing/>

insertions/substitutions allowed to count as ‘equivalent’, with equivalence clearly not being equitable with safety.

It is not clear whether the 20 modifications stipulated are the maximum for a “final” plant (the one being marketed), or just for the one that is currently worked upon or worked with. A question thus is if “20” includes the addition of modifications derived from serial modifications and from ‘stackings’ of different modified traits via breeding with different NGT lines, or whether ‘stacking’ via breeding with two newGM (NGT) plants would in fact allow for 40 or potentially unlimited numbers of modifications in total.

What is to follow in Annex 1 is a catalogue of points that allows to go way beyond what conventional breeding could possibly achieve, and goes even further beyond what would be possible without the “use of modern advanced techniques” that are being explicitly allowed to push the definition of “breeders’ gene pool” (see footnote⁷) for any species open into the extreme, and where it will be legally difficult to exclude any genetic sequence sourced from the database of almost any higher plant (see point 3 below).

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

Text interpretation of this ‘equivalence’ criterion:

- a) “20 nucleotides”: This is an arbitrary and (almost) random choice of a number of what would constitute “equivalence” without any evidence and data showing why 19 (or 1 for that matter) would be fine and safe, but 21 would not.
- b) This criterion point allows for two different types of repair actions at the target site (i.e. the DNA breakage or cutting site)
 - i. The insertion of or substitution with **random nucleotides** during NHEJ repair (non-homologous end joining – SDN1).
 - ii. The insertion of or substitution with a **specific predetermined sequence of nucleotides that is added** either as a template in a SDN2 application or as an insertable sequence via SDN3 (both require homologous repair pathways).

Comments and conclusions:

It is well known that it is not the size of a mutation (modification/injury) that matters, but the change itself that matters and that gives rise to risks through altered functional processes in the GM organisms. There is no evidence-based reason to believe why a change of 19 nucleotides (or any other number in fact) would constitute equivalence, or safety for that matter, or why a change of 22 would not. There are some possible reasons why 20 might have been chosen but they are entirely of a technical nature (i.e. what biochemical procedures are possible) and do not justify safety conclusions. It has been suggested that a sequence of 18-25 nucleotides is needed for ease of detection (e.g. utilising primers⁸). Furthermore, it has also been suggested that 18-21 nucleotides are sufficient for RNAi capability, which for example could be utilised as an inbuilt insecticidal toxin or pesticidal compound (especially when combined with other modifications allowed under points 1-5).

Whilst on one hand this provision allows for the accidental, that is, unintentional insertion or substitution of up to 20 nucleotides -as it is common that these occur in the process-, it also allows for the intentional 20 nucleotide modifications. And whilst both versions hold serious risks to the

⁷ ***‘breeders’ gene pool’ means 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;*** (COM proposal 5 July 2023, p. 27, Article 3, definition 6)

⁸ <https://the-dna-universe.com/2022/09/05/primer-design-guide-the-top-5-factors-to-consider-for-optimum-performance/>

environment, such as to pollinators, herbivores and food chains or potentially decomposer or soil organisms (for example via RNAi activity, see below), no requirements are made for easy identification, risk and safety assessments or labelling. **The notion that “small” DNA changes come with small or no risks is at odds with scientific evidence and reasoning.** It is exactly because small changes can easily achieve the disruption of whole genes and impact whole functional pathways that this genome editing technology is being put forward as a tool in the first place.

“(2) deletion of any number of nucleotides;”

The removal (at target sites) of whole genes, any parts of genes, such as stretches of coding DNA sequences as well as the removal of regulatory DNA sequences is allowed and ‘equivalent’. This could for example mean the targeted removal of repressors or activators of specific genes, or it could mean the removal of a whole gene except for the regulatory sequences and to replace the removed sequence with a 20 base-pair sequence aimed to produce pesticidal components (esp. via RNAi), without any risk assessment requirements despite severe risks to health and the environment, including beneficial insects such as pollinators and pest predators.

Whilst this could be seen as 2 sets of genetic modification (first deletion, then insertion), it may in fact only count as one according to point (5) below. Again, there is no solid data to warrant this criterium, as targeted deletions can substantially alter functions and timings of processes within the plant, most likely with multiple knock-on effects and unpredictable risks. Thus, exclusion from risk assessment is not warranted, certainly not based on scientific reasoning and with precaution in mind.

“(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;”

This mostly refers to cisgenesis, but goes beyond as it refers to the “breeders’ gene pool” as defined in the proposal itself (p. 23, Article 3, point 11). This has been defined extremely wide and allows to resort to genetic material from very different and - as currently defined - even fully unrelated species (see footnote 5).

Anything that can be transferred via modern advanced techniques, including embryo rescue and sequential ‘bridge crosses’ (moving a gene from outside the ‘crossable’ gene pool into the gene pool via (sequential) crosses with intermediates).

It appears that once a new DNA sequence or gene has been constructed, irrespective of the steps (under 1-5) involved, this sequence now becomes part of the dubiously defined “breeders’ gene pool” and the insertion of such a sequence into a new recipient would count as only one of the 20 allowed or alternatively as two in a diploid (4 in tetraploid) plant if counting is for each individual allele.

“Breeders’ gene pool”: The argument within the debate and presentation in the whole proposal is wrongly construed to suggest that it is solely ‘foreign’ DNA that constitutes a safety problem and that gives rise to risks. The insertion of any DNA sequence, irrespective of its origin, has the power to alter regulatory and functional aspects of the plant. By excluding unintended effects on one hand and by artificially expanding and maximising the actual and conceptual ‘breeders’ gene pool’ and legally minimising what is to be considered ‘foreign’ on the other hand, it leaves the scientific basis and the precautionary principle and the regulator’s obligation of care gone with the wind.

Furthermore, the regulatory definition of what constitutes “conventional” breeding and can be undertaken without risk assessment and notification is becoming increasingly dubious and questionable and will require close attention in the near future.

In order to claim that a gene or DNA sequence is part of the breeders’ gene pool it appears that it is sufficient to be able to show that, in theory, a gene or gene sequence can be moved via bridge crosses, irrespective if this would be via 1, 2, 10 or more intermediates.

“(4) targeted inversion of a sequence of any number of nucleotides;”

This provision allows for any section of DNA, be it 20 nucleotides or 2000 nucleotides, to be turned around (inversion). This may have been included because such inversions are found to unintentionally (accidentally) occur near or at DNA double strand breaks (CRISPR/Cas cutting sites). This would allow to *ignore unintentional disruptions and modifications* near the target site (such as deletions, insertions/translocations, inversions etc.) and ignore the risks that arise from such modifications, here inversions.

It also allows for *intentional* inversions -should it be possible to perform this- and to do so without requirements for detailed analysis and risk assessment. Once it exists, it will become part of the ominous ‘breeder’s gene pool’ and can be included at the discretion of anybody with no responsibility for its consequences.

There is no scientific basis to declare or document with peer-reviewed factual evidence that this does not bear serious risks, as inversions within a gene can, for example, suddenly disrupt or change the run of the sequence, giving rise to new RNA and potentially new proteins and again with nobody holding any responsibility and liability in case this change turns out detrimental.

“(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.”

This provision ensures that whatever else might occur in future or has not been explicitly mentioned under 1-4, can be claimed as ‘equivalent’ and as not requiring any further analysis or risk assessment either. This point includes the whole spectrum of possible accidental and unintentional mutations/modifications that occur near or at target sites, from deletions to duplications to scrambling and inversions.

This point also ensures that once genes or gene sequences have been genetically modified either via an up to 20 nucleotide insertion or substitution (point 1) or any size of deletion at any place of target (point 2), such modified genes can now be considered as part of the ‘breeder’s gene pool’ and can be combined with any number of other such altered traits. This enables a continuous development of new DNA sequences with unproven and undocumented claims of both benefit and safety, allowing for the building up of promoters, signal sequences, short genes etc. ad libitum.

This paragraph is ultimate the *carte blanche* to cover for any as of yet not-invented and/or unintentional changes that do now or may still occur in the future and hence will escape notification, documentation, risk assessments, traceability and labelling under any circumstances. It might also be that there are techniques in the pipeline that will enable such modifications in an intentional manner with yet completely unknown consequences and side effects.

IN CONCLUSION:

The intended exemptions from the GM regulations are exemptions from risk assessment, traceability, detectability and labelling and provision of reference material. They are far reaching and go way beyond not only what current conventional breeding could possibly achieve, but **open the door widely to new risks with nobody holding responsibility and liabilities**. We cannot think of a more lax regime that would not simply amount to abandoning regulations altogether. It is one step away from nothing, regarding liability and responsibility and from almost fully concentrated profitability through patentability in the hands of a few. The exemptions are not based on science but merely based on legal definitions with arbitrary choice of numbers of modifications or numbers of nucleotides based on unsupported claims or apparent needs from the biotechnology sector.

Unintended modifications that are all well documented in the scientific literature and, thus, widely known to occur throughout the genome due to the various processes of genetic modifications (such as transformation processes involved in the insertion of the transgene for CRISPR/Cas DNA cutters) are explicitly excluded from any requirements for detection and analysis. They have become invisible to the regulatory process, despite their known high risk potential.⁹

Unintentional modifications such as deletions, inversions, translocations, duplications, scramblings etc. at or next to the target site are being legalised as part of the exempted modification method, and, again, irrespective of their known risks and consequences.

In conclusion, the Annex (with related definitions) is a serious departure from evidence-based science and risk assessment as well as from the precautionary principle. To exempt technologies and their applications from any risk assessments on the basis that they are new (including those that have not even been developed yet) is anti-scientific and reckless and **does in no way comply with the obligations of governments and regulators to ensure safety for the people and the environment**.

New genetic modification technologies (and their applications) that have not been trialled and tested for years, including under real field conditions over years and systematically assessed for their impacts on health and environment should by definition not be exempted but rather strictly regulated and monitored.

Serious abuse and unintentional misuse of CRISPR-Cas, which is easy to use and cheap for any lay person, will likely follow from this deregulation.

Finally, it is important to note, that the organisms that are being covered by these regulations are the whole spectrum of plants. Whilst major annual crops are in the foreground of the discussions, the regulation will equally cover (and be exempted re Annex 1) all other plants genetically modified with the new techniques, including wild plants, forest trees, rare, indigenous crops etc., with potentially severe impacts on environments, sustainability and health.

⁹ Products of new genetic modification techniques should be strictly regulated as GMOs, ENSSER, 2017, <https://ensser.org/publications/ngmt-statement/>