

PSGR

Physicians & Scientists for Global Responsibility

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Submission

Proposal P1055. Definitions for gene technology and new breeding techniques

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PSGR would welcome an opportunity to speak to this submission.

Physicians and Scientists for Global Responsibility Charitable Trust (PSGR) work to educate the public on issues of science, medicine, technology (SMT). PSGR work to encourage scientists and physicians to engage in debate on issues of SMT, particularly involving genetics and public and environmental health.

SUMMARY

Gene technology is currently evolving in its capacity to potentially benefit and harm society. To date, more papers have been published on unintended outcomes and risks of gene editing in medical research on human and animal cells and laboratory animals, as compared with plants. These results have implications for the gene editing of animals that are farmed. The problems found with human and animal gene editing are increasingly being confirmed in plant gene editing.

Unintended mutational (DNA-damaging) outcomes summarized below occur after the gene-editing tool has completed its task of creating a double-strand DNA break. The mutations occur as a consequence of the cell's DNA repair machinery, over which the genetic engineer has no control. Even if scientists eventually succeed in avoiding off-target mutations, most of the unintended mutations described can still occur at the intended gene-editing site.

These effects can combine to alter the biochemical function of the plant in unexpected ways, resulting in the production of novel proteins. The consequences are an alteration in the plant's protein and biochemical function, which could lead to poor crop performance and/or the production of novel toxins and allergens or higher levels of existing toxins and allergens

The lack of full control of the gene-editing procedure, as well as gaps in our knowledge of outcomes, emphasise the need for strict regulation of gene editing in food crops and farm animals. Regulation must start from consideration of the genetic engineering process used to create the gene-edited organism - *process-based regulation* - so that regulators know where things can go wrong and what to look for.

High yielding super-crops with good agronomic and qualitative characteristics have already been produced and will continue to be produced as a result of conventional plant breeding programmes worldwide. Given that the current body of published research knowledge, as briefly outlined in this submission, points to serious concerns around food safety:

- a) PSGR **disagrees** with the proposal to adopt the United States Department of Agriculture (USDA) revised definition for 'genetic engineering' and submits that the definition should not be limited to nucleic acids.
- b) PSGR **agrees** that it is important to regulate gene-edited foods in a manner that recognises their risk. Risk arises from biological and chemical characteristics and via the rapid take-up and application of NBT foods throughout the global food chain.
- c) PSGR **proposes** that ALL gene-edited food and refined ingredients should remain designated as GM food for Code purposes.
- d) PSGR **proposes** that the new definition includes all technology that can alter a pathway or molecule of an organism, that then changes/has potential to change chemical, biological traits of organisms, viruses or related replicating elements.

1. THE NEED FOR REGULATION

New GM plants do not have a history of safe use and should not be exempted from biosafety assessments.

In 2018, the European Court of Justice (EU) confirmed that plants developed by novel genomic techniques for directed mutagenesis should be regulated as GMOs. Thus, they should have to undergo an environmental risk assessment (ERA) prior to deliberate release, or being placed on the market. The authors of a review on this topic recommended that further specific guidance for the ERA and monitoring should be developed to facilitate a focused assessment approach for genome-edited (GE) plants.¹

In another review, the author highlights the case-specific risk assessment of crop plants derived from site-directed nucleases (SDN). It was found that nearly half of plants with so-called market-oriented traits contain complex genomic alterations induced by SDN-1 applications, which may pose new types of risks.²

All plants altered by new genetic modification techniques (nGM) considered in further literature reviews of published studies, can result in unintended changes of different types and frequencies. The rapid development of nGM plants can compromise the detection and elimination of unintended effects. In addition to case-specific pre-market risk assessments, appropriate molecular characterizations to identify unintended changes and/or confirm the absence of unwanted transgenic sequences has been recommended.^{3 4 5}

Technological advances and commercial incentives have exponentially increased the potential for rapid deployment into the environment of organisms modified using nGM techniques. This acceleration of pace of probable exposures to humans and environments amplifies the potential for harm.⁶ The FSANZs responsibility to protect human and environmental health, implies an obligation to consider the implications of scalar effect. In order to achieve this, ongoing regulation - and transparency in regulation is required. nGM alterations to minor food ingredients cannot ethically be downplayed as of no consequence.

2. CHANGES INDUCED BY GENE EDITING ARE NOT THE SAME AS HAPPENS IN NATURE

¹ Eckerstorfer MF et al (2021). Biosafety of genome editing applications in plant breeding: Considerations for a focused case-specific risk assessment in the EU. *BioTech* 2021, 10(3), 10; <https://doi.org/10.3390/biotech10030010>

² Kawall K (2021). The generic risks and the potential of SDN-1 applications in crop plants. *Plants* 10(11). 10.3390/plants10112259 <https://www.mdpi.com/2223-7747/10/11/2259/htm>

³ Eckerstorfer MF et al (2019). *Front Bioeng Biotechnol* 7:31. <https://www.frontiersin.org/articles/10.3389/fbioe.2019.00031/full>

⁴ Gelinksky E and Hilbeck A (2018). *Environ Sci Europe* 30(1):52. <https://enveurope.springeropen.com/articles/10.1186/s12302-018-0182-9>

⁵ Kawall K et al (2020). *Environmental Sciences Europe* volume 32, Article number: 106 (2020) <https://enveurope.springeropen.com/articles/10.1186/s12302-020-00361-2>

⁶ Heinemann et al (2021) Differentiated impacts of human interventions on nature: Scaling the conversation on regulation of gene technologies. *Elementa: Science of the Anthropocene* 9 (1): 00086. DOI 10.1525/elementa.2021.00086

Gene editing makes the whole genome accessible for changes - unlike naturally occurring genetic changes.

The major aim in plant breeding is to develop and improve plants by altering specific traits to increase their yield and quality. The basis for the development of new cultivars in conventional breeding is genetic variation, which occurs naturally due to spontaneous mutation and meiotic recombination, or can be induced by mutagenesis. DNA damage can occur randomly at any part of the genome, subsequently leading to mutations. Cellular mechanisms stop the progression of the cell cycle where DNA damage is sensed and also activate repair mechanisms to protect distinct areas of the genome and sustain genomic integrity.⁷

New GE technologies, e.g. CRISPR/Cas9, are now making the entire genome accessible for any desired change. These new techniques circumvent mechanisms that protect certain areas of the genome by targeting nucleases to specific genomic regions, thereby increasing the probability of the induction of genomic alterations.⁶

3. UNINTENDED MUTATIONS

Below is a selection of studies showing different types of unintended mutations resulting from gene editing that can affect the functioning of multiple gene systems. The consequences are an alteration in the plant's protein and biochemical function, which could lead to poor crop performance and/or the production of novel toxins and allergens or higher levels of existing toxins and allergens.

(a) Off-target mutations

Gene-editing tools, especially CRISPR, are prone to causing mutations (damage) to the organism's DNA at locations other than the intended edit site ("off-target mutations"). This can alter the function of other genes, with unknown consequences to biochemical composition and function.

Assessing the specificity of CRISPR-Cas9 for increasing editing efficiency as well as the potential for unanticipated downstream effects from off-target mutations is an important regulatory consideration for agricultural applications.⁸ With respect to designer nuclease technologies, such as zinc-finger nucleases and meganucleases, much remains to be learned about their detailed behavioural characteristics in different plant species.⁹

(b) Large deletions and rearrangements of DNA at both off-target and on-target gene editing sites

⁷ Kawall K (2019). *Frontiers in Plant Science* 10:525. <https://www.frontiersin.org/articles/10.3389/fpls.2019.00525/full>

⁸ Wolt JD et al (2016). *The Plant Genome* 9(3):10.3835/plantgenome2016.05.0047.

<https://access.onlinelibrary.wiley.com/doi/full/10.3835/plantgenome2016.05.0047>

⁹ Zhu C et al (2017). *Trends in Plant Science* 22(1):38–52. <https://www.ncbi.nlm.nih.gov/pubmed/27645899>

Large deletions and rearrangements of the plant's genome, which can involve thousands of base units of DNA, have been observed following CRISPR gene editing. These mutations can affect the functioning of many genes, leading to alterations in the plant's protein and biochemical composition.^{10 11 12 13 14}

(c) Genomic rearrangement resulting from shattering of chromosomes (chromothripsis) at on-target gene editing sites

CRISPR gene editing for gene therapy applications can lead to massive damage to chromosomes. The phenomenon is known as chromothripsis. The fact that the damage occurs "on-target" - at the intended edit site - means that any attempts to target the CRISPR gene editing more precisely will not solve this problem.¹⁵

(d) Creation of new gene sequences leads to new RNA and protein products

Alteration of the genetic code of the targeted gene can produce mutant forms of the protein it encodes for, new RNA, and new protein products. These outcomes can lead to changes in the plant's biochemistry.^{16 17 18}

(e) Gene-editing process-induced mutations

The gene editing process, taken as a whole (including plant tissue culture and GM transformation procedure), induces hundreds of unintended mutations throughout the genome of the plant. This can affect multiple gene functions with unknown consequences to protein biochemistry and metabolic activity.¹⁹

(f) Insertion of foreign and contaminating DNA into genome at editing sites

Following creation of a double-strand DNA break by the CRISPR gene-editing tool, the repair can unexpectedly include the insertion and rejoining of the broken DNA ends of the recombination template DNA used in SDN-2 and -3, or the insertion of contaminating DNA present in materials used in the plant tissue culture. This insertion of extraneous DNA in the genome of the plant, which can take place at off-target sites as well as the intended on-target editing site, has the effect of introducing new gene functions, as well as disrupting the function of host genes.

¹⁰ Biswas S et al (2020). *Journal of Genetics and Genomics*. May 21. <https://www.sciencedirect.com/science/article/pii/S1673852720300916>

¹¹ Höijer I et al (2021). CRISPR-Cas9 induces large structural variants at on-target and off-target sites in vivo that segregate across generations. *bioRxiv*. doi: <https://doi.org/10.1101/2021.10.05.463186>. <https://www.biorxiv.org/content/10.1101/2021.10.05.463186v1>

¹² Kosicki M et al (2018). *Nature Biotechnology* 36:765–771. <https://www.nature.com/articles/nbt.4192>

¹³ Mou H et al. (2017). *Genome Biology* 18:108. <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-017-1237-8>

¹⁴ Shin HY et al. (2017). *Nature Communications* 8, 15464 (2017). <https://www.ncbi.nlm.nih.gov/pubmed/28561021>

¹⁵ Leibowitz ML et al (2021). Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. *Nat Genet*. 2021 Jun;53(6):895-905. doi: 10.1038/s41588-021-00838-7. Epub 2021 Apr 12. <https://pubmed.ncbi.nlm.nih.gov/33846636/>

¹⁶ Mou H et al. (2017). *Genome Biology* 18:108. <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-017-1237-8>

¹⁷ Tuladhar R et al (2019). *Nat Commun* 10, 4056 (2019). <https://www.nature.com/articles/s41467-019-12028-5>

¹⁸ Smits AH et al (2019). *Nat Methods* 16, 1087–1093. <https://www.nature.com/articles/s41592-019-0614-5>

¹⁹ Tang X et al (2018). *Genome Biology* 19:84. <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1458-5>

These effects can combine to alter the biochemical function of the plant in unexpected ways.

Reports^{20 21 22 23} describe insertion of the whole plasmid DNA molecules that acted as the recombination template for the SDN-2 or SDN-3 procedure. The insertion of these plasmid DNA templates will invariably result in at least one antibiotic resistance gene being incorporated in the genome, as these are a component of plasmids. This risks the transfer of antibiotic resistance genes to disease-causing bacteria in the environment and more worryingly, in the gut of the consumer, which would compromise medical use of antibiotics.

4. CONVENTIONAL PLANT BREEDING (NON GENE-EDITING, NON GM) SOLUTIONS AND SUCCESSES ALREADY IN USE

There are currently numerous super-crops grown worldwide that are high-yielding, pest-resistant, disease-resistant, drought-tolerant, flood-tolerant or low in gluten. They are the result of successful conventional plant breeding programmes. Plant breeding is often aided by genetic marker-assisted selection techniques. Examples of such crops include flood-tolerant rice, low gluten wheat and blight resistant potatoes.

Using crop wild relatives (CWR) in crop improvement is also producing crops with higher nutritional value. Whilst more complicated than breeding with domesticated relatives, the successful new crops have emerged and are emerging, particularly in developing nations, where food security can be limited.

Pre-breeding aims to isolate desired genetic traits (e.g. disease resistance) from un-adapted material like CWR and introduce them into breeding lines that are more crossable with modern, elite varieties. Pre-breeding broadens the elite gene-pool by recapturing lost beneficial genetic diversity.

As an example, chickpea is an excellent source of dietary fibre, calcium and magnesium. Recent research using CWR shows that even more can be done with the popular legume to combat malnutrition.²⁴

Another study, published in *Crop Science* also looks at boosting the nutrient content of chickpea, by crossing it with its hardy, weeding wild relatives.²⁵

²⁰ Norris AL et al (2020). *Nat Biotech* 38(2):163-164. <https://www.nature.com/articles/s41587-019-0394-6>

²¹ MEDIA ARTICLE: Molteni M (2020). *WIRED*, 24 July. <https://www.wired.com/story/a-crispr-calf-is-born-its-definitely-a-boy/>

²² Skryabin BV et al. (2020). *Science Advances* 6(7), eaax2941. <https://advances.sciencemag.org/content/6/7/eaax2941>

²³ Ono R et al (2019). *Communications Biology* 2: 57. <https://www.nature.com/articles/s42003-019-0300-2.pdf?origin=ppub>

²⁴ Sharma, S. Lavale, S.A., Nimje, C. and Singh, S. (2020). Characterization and identification of annual wild Cicer species for seed protein and mineral concentration for chickpea improvement. <https://doi.org/10.1002/csc2.20413>

²⁵ Kilian, B., Dempewolf, H., Guarino, L., Werner, P., Coyne, C. and Warburton, M.L (2020). *Crop Science* special issue: Adapting agriculture to climate change. A walk on the wild side. <https://doi.org/10.1002/csc2.20418>