

GENE EDITING: UNEXPECTED OUTCOMES AND RISKS^{1 2}

GM Watch, Technical advisor: Dr Michael Antoniou

More papers have been published on unintended outcomes and risks of gene editing in medical research on human and animal cells and laboratory animals, compared with plants.

The results have implications for the gene editing of farm animals. The problems found with human and animal gene editing are increasingly being confirmed in plant gene editing.

The 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. So 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.

This lack of full control of the gene-editing procedure, as well as gaps in our knowledge of outcomes, point to 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.

NEED FOR REGULATION

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

Eckerstorfer MF et al (2019). *Front Bioeng Biotechnol* 7:31. ³

Gelinsky E and Hilbeck A (2018). *Environ Sci Europe* 30(1):52. ⁴

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

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

Kawall K (2019). *Frontiers in Plant Science* 10:525. ⁵

¹ "Gene Editing: Unexpected Outcomes and Risks." *GM Watch*. Last modified August 30, 2020. <https://gmwatch.org/en/67-uncategorised/19499-gene-editing-unexpected-outcomes-and-risks>

² For more details on individual studies, see Robinson, Claire, and Michael Antoniou (Technical Advisor). "Science Supports Need to Subject Gene-Edited Plants to Strict Safety Assessments." Last modified November 20, 2019. <https://gmwatch.org/en/news/archive/2019/19223-peer-reviewed-papers-with-results-that-support-the-need-to-subject-gene-edited-plants-to-strict-safety-assessments>

³ Eckerstorfer, Michael F., Marion Dolezel, Andreas Heissenberger, Marianne Miklau, Wolfram Reichenbecher, Ricarda A. Steinbrecher, and Friedrich Waßmann. "An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (NGMs)." *Frontiers in Bioengineering and Biotechnology* 7 (2019). <https://www.frontiersin.org/articles/10.3389/fbioe.2019.00031/full>

⁴ Gelinsky, Eva, and Angelika Hilbeck. "European Court of Justice Ruling Regarding New Genetic Engineering Methods Scientifically Justified: A Commentary on the Biased Reporting about the Recent Ruling." *Environmental Sciences Europe* 30, no. 1 (December 20, 2018): 52. <https://enveurope.springeropen.com/articles/10.1186/s12302-018-0182-9>

⁵ Kawall, Katharina. "New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes." *Frontiers in Plant Science* 10 (2019). <https://www.frontiersin.org/articles/10.3389/fpls.2019.00525/full>

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.

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.

Wolt JD et al (2016). *The Plant Genome* 9(3):10.3835/plantgenome2016.05.0047. ⁶

Zhu C et al (2017). *Trends in Plant Science* 22(1):38–52. ⁷

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

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.

Biswas S et al (2020). *Journal of Genetics and Genomics*. May 21.⁸

Kosicki M et al (2018). *Nature Biotechnology* 36:765–771.⁹

Mou H et al. (2017). *Genome Biology* 18:108.¹⁰

Shin HY et al. (2017). *Nature Communications* 8, 15464 (2017).¹¹

⁶ Wolt, Jeffrey D., Kan Wang, Dipali Sashital, and Carolyn J. Lawrence-Dill. "Achieving Plant CRISPR Targeting That Limits Off-Target Effects." *The Plant Genome* 9, no. 3 (2016): plantgenome2016.05.0047.

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

⁷ Zhu, Changfu, Luisa Bortesi, Can Baysal, Richard M. Twyman, Rainer Fischer, Teresa Capell, Stefan Schillberg, and Paul Christou. "Characteristics of Genome Editing Mutations in Cereal Crops."

Trends in Plant Science 22, no. 1 (2017): 38–52. <https://www.ncbi.nlm.nih.gov/pubmed/27645899>

⁸ Biswas, Sukumar & Tian, Jiaqi & Rong, Li & Chen, Xiaofei & Luo, Zhijing & Chen, Mingjiao & Zhao, Xiangxiang & Zhang, Dabing & Persson, Staffan & Yuan, Zheng & Shi, Jianxin. (2020). Journal Pre-proof Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. *Journal of Genetics and Genomics*.

10.1016/j.jgg.2020.04.004. <https://www.sciencedirect.com/science/article/pii/S1673852720300916>

⁹ Kosicki, Michael, Kärt Tomberg, and Allan Bradley. "Repair of Double-Strand Breaks Induced by CRISPR–Cas9 Leads to Large Deletions and Complex Rearrangements." *Nature Biotechnology* 36, no. 8 (September 2018): 765–771. <https://www.nature.com/articles/nbt.4192>

¹⁰ Mou, Haiwei, Jordan L. Smith, Lingtao Peng, Hao Yin, Jill Moore, Xiao-Ou Zhang, Chun-Qing Song, et al. "CRISPR/Cas9-Mediated Genome Editing Induces Exon Skipping by Alternative Splicing or Exon Deletion." *Genome Biology* 18, no. 1 (June 14, 2017): 108.

<https://genomebiology.biomedcentral.com/articles/10.1186/s13059-017-1237-8>

¹¹ Shin, Ha Youn, Chaochen Wang, Hye Kyung Lee, Kyung Hyun Yoo, Xianke Zeng, Tyler Kuhns, Chul Min Yang, Teresa Mohr, Chengyu Liu, and Lothar Hennighausen. "CRISPR/Cas9 Targeting Events Cause Complex Deletions and Insertions at 17 Sites in the Mouse Genome." *Nature Communications* 8 (31 2017): 15464. <https://www.ncbi.nlm.nih.gov/pubmed/28561021>

Creation of new gene sequences leads to new RNA and protein products

Iteration 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.

Mou H et al. (2017). *Genome Biology* 18:108.

Tuladhar R et al (2019). *Nat Commun* 10, 4056 (2019).¹²

Smits AH et al (2019). *Nat Methods* 16, 1087–1093.¹³

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.

Tang X et al (2018). *Genome Biology* 19:84.¹⁴

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. These effects can combine to alter the biochemical function of the plant in unexpected ways. Reports (Norris et al., 2020; Skryabin et al., 2020; Molteni 2020) 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.

Norris AL et al (2020). *Nat Biotech* 38(2):163-164.¹⁵

MEDIA ARTICLE: Molteni M (2020). *WIRED*, 24 July.¹⁶

Skryabin BV et al. (2020). *Science Advances* 6(7), eaax2941.¹⁷

Ono R et al (2019). *Communications Biology* 2: 57.¹⁸

¹² Tuladhar, Rubina, Yunku Yeu, John Tyler Piazza, Zhen Tan, Jean Rene Clemenceau, Xiaofeng Wu, Quinn Barrett, et al. "CRISPR-Cas9-Based Mutagenesis Frequently Provokes on-Target mRNA Misregulation." *Nature Communications* 10, no. 1 (September 6, 2019): 1–10. <https://www.nature.com/articles/s41467-019-12028-5>

¹³ Smits, Arne H., Frederik Ziebell, Gerard Joberty, Nico Zinn, William F. Mueller, Sandra Clauder-Münster, Dirk Eberhard, et al. "Biological Plasticity Rescues Target Activity in CRISPR Knock Outs." *Nature Methods* 16, no. 11 (November 2019): 1087–1093. <https://www.nature.com/articles/s41592-019-0614-5>

¹⁴ Tang, Xu, Guanqing Liu, Jianping Zhou, Qirong Ren, Qi You, Li Tian, Xuhui Xin, et al. "A Large-Scale Whole-Genome Sequencing Analysis Reveals Highly Specific Genome Editing by Both Cas9 and Cpf1 (Cas12a) Nucleases in Rice." *Genome Biology* 19, no. 1 (July 4, 2018): 84. <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1458-5>

¹⁵ Norris, Alexis L., Stella S. Lee, Kevin J. Greenlees, Daniel A. Tadesse, Mayumi F. Miller, and Heather A. Lombardi. "Template Plasmid Integration in Germline Genome-Edited Cattle." *Nature Biotechnology* 38, no. 2 (February 2020): 163–164. <https://www.nature.com/articles/s41587-019-0394-6>

¹⁶ "A Crispr Cow Is Born. It's Definitely a Boy." *Wired*, July 24, 2020. <https://www.wired.com/story/a-crispr-calf-is-born-its-definitely-a-boy/>

¹⁷ Skryabin, Boris V., Delf-Magnus Kummerfeld, Leonid Gubar, Birte Seeger, Helena Kaiser, Anja Stegemann, Johannes Roth, et al. "Pervasive Head-to-Tail Insertions of DNA Templates Mask Desired CRISPR-Cas9-Mediated Genome Editing Events." *Science Advances* 6, no. 7 (February 1, 2020): eaax2941. <https://advances.sciencemag.org/content/6/7/eaax2941>

¹⁸ Ono, Ryuichi, Y. Yasuhiko, K. Aisaki, S. Kitajima, J. Kanno, and Y. Hirabayashi. "Exosome-Mediated Horizontal Gene Transfer Occurs in Double-Strand Break Repair during Genome Editing." *Communications Biology* (2019). <https://www.nature.com/articles/s42003-019-0300-2.pdf>