Science supports need to subject gene-edited plants to strict safety assessments

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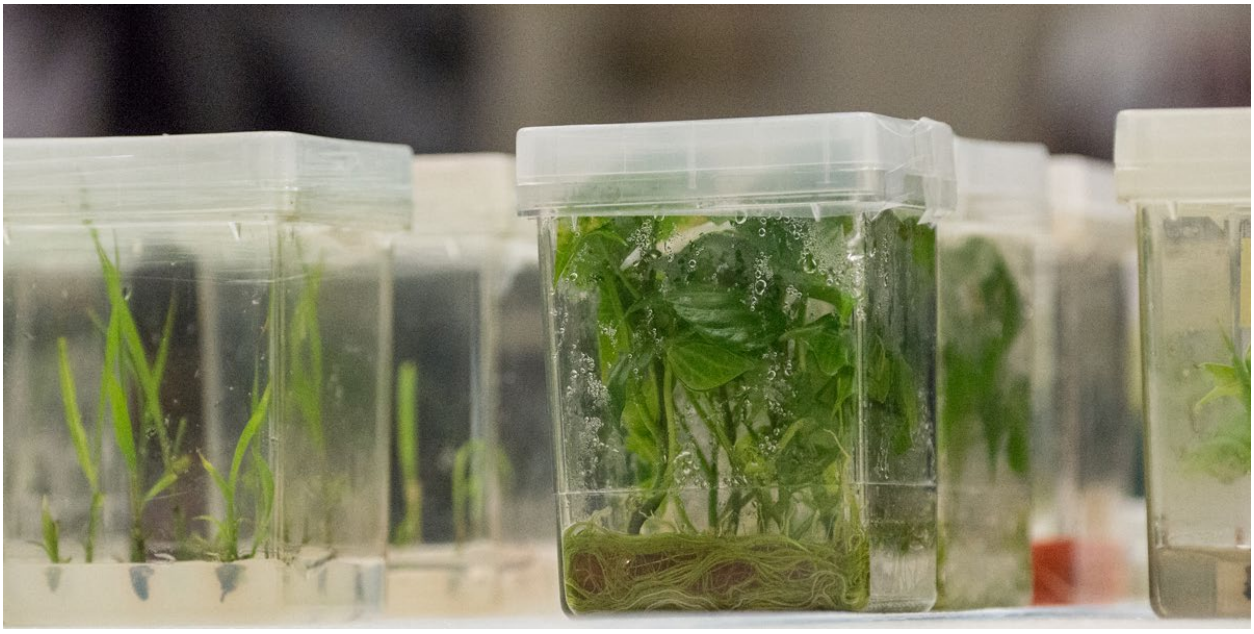

Compiled by Claire Robinson, editor, GMWatch.org. Technical advisor: Michael Antoniou, PhD, Head, Gene Expression and Therapy Group, King's College London, UK

We've been asked to produce an up-to-date list of scientific papers that support the need to subject gene-edited plants to stringent safety assessments – at least as stringent as those already applied to older-style transgenic GM crops. That list – which is not comprehensive but gives an idea of the current state of knowledge – is below. For each paper, we've given an explanation of the findings and their implications.

All these papers inform our understanding of unintended and potentially risky effects of gene editing. On this topic, there are currently far more papers being published in the medical research field in experiments using human and animal cells, compared with papers investigating such effects in plants.

However, the problems found thus far with human and animal cell gene editing

will also affect plant gene editing. To what extent is unknown, as not enough research has been done. Most plant research instead focuses on product development.

This important knowledge gap points to the necessity of strict oversight and regulation of gene editing in food crops, as well as farm animals.

Unintended effects in gene-edited plants carry the risk of changing the plant's biochemistry and thus producing unexpected toxins or allergens. Controlled laboratory animal feeding studies show that these problems have arisen with the first generation of GM crops.[1]

No animal feeding trials have been carried out with new gene-edited plants, so claims of food safety are based on assumptions and not on experimental evidence.

Peer-reviewed papers on regulatory and biosafety issues around gene editing

1) Gelinksky E and Hilbeck A (2018). 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(1):52. https://enveurope.springeropen.com/articles/10.1186/s12302-018-0182-9 (open access)
The main points of this commentary are:
* The current wave of enthusiasm for the new genetic engineering methods, with its claim to make good on the failed promises of the previous wave, seems to point more to an admission of failure of the last generation of genetic engineering than to a true change of paradigm.
* Regulation is being portrayed as a ban on research and use, which is factually incorrect, and the judges of the European Court of Justice are being defamed as espousing "pseudoscience".
* This highly polarised position dominates the media reporting of the new techniques and the court's ruling.
* Advocates of the new genetic engineering techniques appear to believe that their benefits are so clear that furnishing reliable scientific evidence is unnecessary.
* One-sided and biased reporting in the media often has the appearance of spin and lacks journalistic ethics that require journalists to report on different positions in a balanced and factual manner instead of taking positions and becoming undeclared advocates themselves.
Further reading: GMWatch, "European Court of Justice ruling on new GM methods is scientifically justified". 15 January 2019.
2) Eckerstorfer MF et al (2019). An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification

techniques (nGMs). Front. Bioeng. Biotechnol. https://doi.org/10.3389/fbioe.2019.00031

This analysis argues that the products of new GMO techniques cannot be assumed to be safe but must be subjected to a pre-market risk assessment tailored to the specific GMO in question. Key messages are:

- The characteristics of some genome editing tools, such as the small extent of the DNA sequence change or how precisely the editing tool can be targeted to a specific site, cannot be considered an indication of safety of new GMOs.
- All new GM techniques can result in unintended changes of different types and frequencies.
- New GM plants do not have a history of safe use and should not be exempted from biosafety assessments.

Further reading: GMWatch, "Safety checks needed for new GM products – new scientific analysis". 26 April 2019.

3) Kawall K (2019). New possibilities on the horizon: Genome editing makes the whole genome accessible for changes. Frontiers in Plant Science, 10:525. doi: 10.3389/fpls.2019.00525. https://www.frontiersin.org/articles/10.3389/fpls.2019.00525/full

This publication reviews applications of CRISPR/Cas in plants and shows some differences from conventional mutagenesis used in plant breeding and from spontaneous mutations. In conventional breeding and spontaneous mutations, some regions in the genome undergo changes less frequently than others because these regions are especially protected by repair mechanisms in the cell. CRISPR/Cas applications can bypass these naturally occurring processes.

Further reading: GMWatch, "New scientific publication shows differences between genome editing and conventional breeding". 4 May 2019.

Peer-reviewed papers on unintended effects of gene editing on human cells

4) Kosicki M et al (2018). Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nature Biotechnology 36:765–771. https://www.nature.com/articles/nbt.4192

This research from the medical gene-editing field, carried out in human cells, shows that CRISPR/Cas9 gene editing can cause greater genetic damage than was previously thought, arising from the cell's innate repair machinery that goes into action after the initial targeted CRISPR-induced DNA cut. Many CRISPR-edited cells had large genetic rearrangements such as DNA deletions and insertions. Potential consequences in gene therapy include triggering cancer.

GMWatch comment: The CRISPR/Cas9 technique as used in plants is the same, as are the mechanisms of DNA repair. These repair mechanisms are beyond the control of the genetic engineer, however "precise" the initial intended CRISPR-induced DNA cut is. Such off-target effects in food plants could affect food safety, including producing unexpected toxicity or allergenicity.

Further reading: GMWatch, "CRISPR causes greater genetic damage than previously thought". 17 July 2018.

5) Mou H et al. (2017). CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. Genome Biology 18:108. DOI: 10.1186/s13059-017-1237-8.

This study in human cells unexpectedly found large deletions resulting from single CRISPR-induced cuts, in some cases in excess of 500 base units of DNA. In some cases, subregions of genes ("exons") that carry information for the protein(s) for which they encode were deleted. This resulted in the formation of novel gene structures encoding truncated forms of proteins.

GMWatch comment: On the level of a whole living organism, such novel proteins could either be benign or harmful.

6) Tuladhar R et al (2019). CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nature Communications vol 10, Article number: 4056, 6 Sept. https://www.nature.com/articles/s41467-019-12028-5

This research investigated outcomes in human cells when CRISPR was used to knock-out a gene function by disrupting its normal base unit sequence. This disruption takes the form of DNA base unit insertions and deletions ("indels"). Indels are produced by the DNA repair process known as non-homologous end joining (NHEJ), which gets activated by the cell in order to repair the cut ends of the DNA molecule once the CRISPR has made its double-strand DNA break. The study found that instead of the intended outcome of destroying the function of a CRISPR-targeted gene, in 50% of cell lines investigated, the indels resulted in an alteration of the gene's DNA base unit sequence, so that it now produced new types of mRNAs (messenger RNA molecules) or proteins.

GMWatch comment: There is every reason to believe that unexpected outcomes of the type described in this study will take place in CRISPR'd plants where the aim is a gene knock-out by NHEJ-indel formation, since these processes are similar in plants and animals. The result in food crops could be unexpected toxicity or allergenicity.

Further reading: GMWatch, "CRISPR causes unexpected outcomes even at the intended site of genetic modification". 16 April 2019.
7) [UPDATE 4 Feb 2020] Smits AH et al (2019). Biological plasticity rescues target activity in CRISPR knock outs. Nat Methods 16, 1087–1093. https://www.ncbi.nlm.nih.gov/pubmed/31659326

This study in human cells revealed a major unintended effect from the CRISPR-Cas9 gene-editing tool. CRISPR edits intended to knock out the function of a gene failed to do so. Instead, proteins were still produced from the damaged genes. Many of those proteins were still functional, but they were also mutant,[28] which means they could gain a novel function, with unknown consequences.

GMWatch comment: The study has major implications for the food safety of gene-edited plants, as they could turn out to be unexpectedly toxic or allergenic. CRISPR-edited plants with gene knockouts should be subjected to stringent safety checks, as they could contain new proteins or compounds that pose a food safety risk. These include the non-browning mushroom that has been de-regulated in the US.

The developer of the mushroom stated that it did not need to be regulated since it was free from transgenes (genes inserted from another organism) and only contained "small deletions in a specific gene".

However, these findings, as well as those of Tuladhar and colleagues (above) suggest that the developer and the US regulators should revisit their assessment. The "small deletion" in a single gene in the CRISPR-edited mushroom may have led to the production of new proteins and altered biochemistry that put consumer health at risk.
Further reading: GMWatch, Researchers assumed CRISPR-mediated disruption of genes was turning them off – but they were wrong. 11 Jan 2020.
8) [UPDATE 4 Feb 2020] Sansbury BM et al (2019). Understanding the diversity of genetic outcomes from CRISPR-Cas generated homology-directed repair. Commun Biol 2, 1–10. https://www.nature.com/articles/s42003-019-0705-y
This study reported that a new tool for rapidly analyzing CRISPR edits has revealed the frequent production of unintended edits around the site of the intended cut in the DNA. Eric Kmiec, the lead author, commented that this is different from the risk of CRISPR causing "off-target" mutations by drifting from the intended site and making random cuts across the genome.

GMWatch comment: The conclusion that must be drawn is that improving the precision of the initial CRISPR edit cannot solve this problem.

Further reading: GMWatch, New tool for rapidly analyzing CRISPR edits reveals frequent unintended edits. 6 Jan 2020.

Peer-reviewed papers on unintended effects of gene editing in plant cells

9) Wolt JD et al (2016). Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047. https://www.ncbi.nlm.nih.gov/pubmed/27902801

This paper has a table of CRISPR-induced off-target effects (genetic damage) in gene-edited plants. The authors correctly note, "development of plant genome editing has not yet fully considered potential off-target mismatches that may lead to unintended changes within the genome" and suggest ways to reduce these effects.

GMWatch comment: The authors make clear that reducing unintended effects in plant genome editing is a work in progress, which has not yet succeeded in its aims.

10) Zhu C et al (2017). Characteristics of genome editing mutations in cereal crops. Trends in Plant Science 22:38-52. https://www.ncbi.nlm.nih.gov/pubmed/27645899

This review examines the types of mutations (DNA damage) caused in plants by different gene-editing techniques, including CRISPR and Zinc Finger Nuclease (ZFN) tools. The authors found that the outcomes varied in efficiency, accuracy, and mutation structure, depending on the characteristics of the editing tool used and the genome targeted. They noted that of the editing tools examined, "CRISPR… is more susceptible to off-target effects and great care is required during target selection to minimize the likelihood of unwanted mutations".

11) Tang X et al (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biology 19:84. https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1458-5

This study analysed gene-edited rice plants engineered with two different CRISPR editing tools for off-target and on-target effects. While the study found that the CRISPR tools in themselves did not introduce many off-target mutations in the plants, many off-target mutations resulted from other aspects of the CRISPR genetic manipulation process – namely tissue culture and Agrobacterium infection. When making CRISPR'd plants, tissue culture is always used and Agrobacterium infection is commonly used to deliver the editing tool – the latter element adding to the mutations introduced by the tissue culture. Thus the study found that the

CRISPR process, taken as a whole, causes large numbers of off-target mutations.

GMWatch comment: The use of the words "highly specific" in the title of the paper could be considered misleading, given the findings. This paper shows that process-based regulation, which takes account of the inherent uncertainties of specific techniques used in developing GM plants, is necessary.

Further reading: GMWatch, "New study claimed to show safety of CRISPR shows the opposite". 29 January 2019.

Peer-reviewed paper on gene-edited cattle

12) Norris AL et al (2020). Template plasmid integration in germline genome-edited cattle. Nat Biotech 38(2): 163-164. https://www.nature.com/articles/s41587-019-0394-6

This research by experts at the US Food and Drug Administration (FDA), which at the time of writing was published on a pre-peer-review website, found gene-editing errors in the genome of cattle engineered using the TALEN tool to not grow horns. The developer, Recombinetics, had declared that there were no off-target changes in the genome. Yet the FDA scientists found that genes conferring resistance to three different antibiotics had unexpectedly been incorporated into the animals' genome, at the targeted editing site.

GMWatch comment: The risk with antibiotic resistance genes is that they will transfer to disease-causing bacteria that will then become resistant to antibiotics, threatening human and animal health. This episode shows that GMO developers cannot be trusted to "regulate" themselves and that stringent and independent process-based regulation of gene-edited products and animals is necessary. If purely product-based (trait-based) regulation had been in place, as promoted by supporters of gene-editing technology in agriculture, this important change would have been missed, as these cattle would have been treated only as hornless cattle, just like any naturally hornless cattle.

Further reading: GMWatch, "Gene-edited hornless cattle: Flaws in the genome overlooked". 9 August 2019; Jonathan Latham, PhD, "Gene-editing unintentionally adds bovine DNA, goat DNA, and bacterial DNA, mouse researchers find". Independent Science News, 23 September 2019.

Peer-reviewed papers on gene-edited mice

13) Ono R et al (2019). Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. Communications Biology 2: 57 https://www.nature.com/articles/s42003-019-0300-2.pdf?origin=ppub

This study found that DNA from the E. coli genome can integrate into the target

organism's genome, as well as the delivery plasmid. Acquisition of E. coli DNA was found to be quite frequent. Insertion of long unintended DNA sequences occurred at 4% of the total number of edited sites and 21% of these were of DNA from the E. coli genome. The source of the E. coli DNA was traced back to the E. coli cells that were used to produce the vector plasmid.
The study also found that plasmid and tissue culture DNA contaminants can be inserted by one of the cell's DNA repair mechanisms (Non-Homologous End Joining or NHEJ) into the genome targeted for editing, following a CRISPR cut. In this case, edited mouse genomes were found to acquire bovine DNA or goat DNA. This was traced to the use, in standard culture medium for mouse cells, of foetal calf serum and goat serum; that is, body fluids extracted from cows or goats. This serum contains DNA from whichever animal species it happened to have been extracted from, hence the insertion in some experiments of goat DNA (which occurred when goat serum was used instead of calf serum). Even more worrisome, amongst the DNA sequences inserted into the mouse genome were bovine and goat retrotransposons (jumping genes) and mouse retrovirus DNA (HIV is a retrovirus). Thus gene-editing is a potential mechanism for horizontal gene transfer of unwanted pathogens, including, but not limited to, viruses.[2]

Jonathan Latham, PhD commented on Independent Science News, "These findings… imply, at the very least, the need for strong measures to prevent contamination by stray DNA, along with thorough scrutiny of gene-edited cells and gene-edited organisms. And, as the Recombinetics case [of the gene-edited hornless cattle, see above] suggests, these are needs that developers themselves may not meet."[2]

Further reading: Jonathan Latham, PhD, "Gene-editing unintentionally adds bovine DNA, goat DNA, and bacterial DNA, mouse researchers find". Independent Science News, 23 September 2019.

14) Shin HY et al. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nature Communications 8, Article number: 15464.
doi:10.1038/ncomms15464. https://www.ncbi.nlm.nih.gov/pubmed/28561021

This study looked at the molecular consequences of 17 CRISPR gene-editing events in four different gene regions of the mouse genome. The researchers found that CRISPR editing resulted in unexpected types of indels at all 17 sites in the mouse genome.

This was a surprise, as the DNA repair mechanism following the cutting of the DNA by CRISPR (known as non-homologous end-joining) is generally believed to result in approximately nine base unit deletions or insertions of a few base units. However, the authors found that depending on the site being targeted, the size of the

deletion was unexpectedly large – up to 600 base units of DNA. This was particularly the case where the site targeted harboured DNA repeats – that is, stretches of DNA with repetition in the DNA sequence.

Furthermore, the authors demonstrated for the first time that the deletion resulting from the DNA repair was asymmetrically located compared with the actual CRISPR cut site. This means it was almost invariably located either upstream or downstream from the cut site, rather than symmetrically spanning the cut site, as previously assumed.

Such large deletions from a single CRISPR cut had not been clearly defined in previous studies. The authors mention that this was because the technology that is generally used to determine the extent of the deletion (known as polymerase chain reaction or PCR) had not been appropriately applied; that is, the PCR analysis was used in such a way that it would only detect small indels.

This analytical failure has two consequences:
1. The intended alteration in a given genome region could have been far more extensive than intended and thus unexpectedly damage elements in the genome – for example, sections of targeted genes, or neighbouring genes to the target site and/or their regulatory elements
2. Large deletions would have been missed altogether.

The authors recommended that whole genome sequencing is necessary to look for such unintended effects, rather than the standard PCR screening method, which could miss them.
15) [UPDATE 22 Feb 2020] Skryabin BV et al. (2020). Pervasive head-to-tail insertions of DNA templates mask desired CRISPR-Cas9–mediated genome editing events. Science Advances 12 Feb 2020: Vol. 6, no. 7, eaax2941. DOI: 10.1126/sciadv.aax2941. https://advances.sciencemag.org/content/6/7/eaax2941
This study shows that when the CRISPR/Cas system was used in an SDN-2 ("gene modification") gene editing procedure aimed at engineering insertion of genetic material in mice, a high frequency was found of insertions of multiple copies of the DNA molecules used as a template for bringing about the desired gene modifications. The researchers were concerned by the fact that the insertions could not be detected using standard PCR analysis. This in turn led to what they called "a high rate of falsely claimed precisely edited alleles" (gene variants).

In other words, scientists have been unduly claiming precision for CRISPR when in reality it is not precise.

The researchers used an extended PCR analytical method and found that in most cases, there were multiple head-to-tail insertions of the template repair DNA molecule.

The lead authors of the study, Boris Skryabin and Timofey Rozhdestvensky, told The Scientist magazine that their findings could have relevance for gene editing across all kingdoms of life, from plants to human cells. They warned that duplications could lead to dangerous frameshift mutations, resulting in misshapen proteins.

GMWatch comment: Misshapen proteins could have unpredictable consequences for the food safety of gene-edited crops and foods – for example, producing unexpected toxicity or allergenicity.
Further reading: GMWatch, "Yet more problems with CRISPR – with consequences for food safety". 22 Feb 2020.
Katarina Zimmer, "CRISPR can create unwanted duplications during knock-ins". The Scientist, 19 Feb 2020.
Jonathan Latham, "Researchers are substantially undercounting gene-editing errors, concludes a new paper". Independent Science News, 25 Feb 2020.

Notes

1. See Krimsky S (2015). An illusory consensus behind GMO health assessment. Science, Technology & Human Values. & August. http://sth.sagepub.com/content/early/2015/08/05/0162243915598381; Hilbeck A et al (2015). No scientific consensus on GMO safety. Env Sci Europe 27(1):4. http://www.enveurope.com/content/27/1/4/abstract; references collected in Robinson C, Antoniou M, and Fagan J (2018), GMO Myths and Truths, 4th edition. Chelsea Green Publishing. https://www.amazon.com/GMO-Myths-Truths-Citizens-Genetically/dp/0993436722
2. Latham J, Gene-editing unintentionally adds bovine DNA, goat DNA, and bacterial DNA, mouse researchers find. Independent Science News, 23 September 2019.

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