4. Gene editing is risky and its products can be unsafe

MYTH

The precision and control of gene editing mean that it is safe-by-design.

REALITY

The unintended outcomes of gene editing lead to risks, which are poorly understood.

Claims that gene editing is "breeding", that it is "precise", and that outcomes are "nature-identical" are often made to imply that gene-edited organisms will be safe-by-design.

Some GMO developers have gone further, explicitly claiming that gene-edited plants are just as safe as conventionally bred ones.

Some GMO developers claim that gene-edited plants are just as safe as conventionally bred ones

Bayer claims that compared with conventional breeding, CRISPR/Cas gene editing is "simpler, faster and more precise, with no impact on the safety of the final crop compared to traditional plant breeding".[1] And Corteva says that CRISPR-edited plants are "as safe as plants found in nature or produced through conventional breeding".[2]

The agbiotech industry argues that it would therefore be "disproportionate" to subject these products to GMO regulatory requirements aimed at ensuring their safety.[3] Corteva sees no need to conduct safety testin on its gene-edited crops

and says it tests CRISPR-produced plants in "the same way" as it tests conventionally bred plants.[4]

However, as we have seen in previous chapters, gene editing is not precise, nor are the outcomes identical to those of conventional breeding. While the initial cut in the DNA can be targeted to a specific region of the genome, the subsequent DNA repair process causes unwanted mutations both at on-target and off-target sites in the genome.[5,6,7]

> Unintended genetic changes will alter the pattern of gene function within the organism

Techniques common to both gene editing and older transgenic GM methods, such as tissue culture and GM transformation, will lead to additional mutations (see chapter 2).

These unintended genetic changes will alter the pattern of gene function within the organism. In plants, this can alter biochemical pathways and lead to compositional changes, which, scientists warn, could include the production of novel toxins and allergens or altered levels of existing toxins and allergens.[8,9,10]

GENE EDITING CAN UNINTENTIONALLY ADD FOREIGN DNA IN THE GENOME

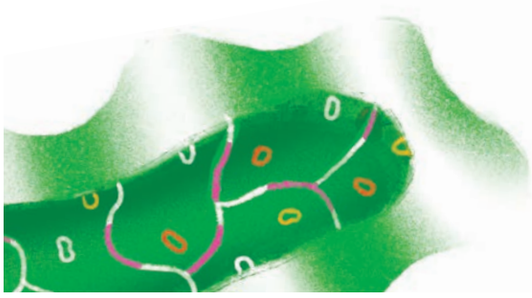
The presence of unintended mutations has been well documented in human and animal cells and has begun to gain more attention in plants.[11] However, another unwanted outcome of gene editing has received little attention and it is unclear to what extent it occurs in animal and plant cells and what the effects might be.

> Edited mouse genomes unintentionally acquired bovine or goat DNA

This outcome was highlighted in a study by Japanese researchers. The study found that even SDN-2 (gene alteration) applications of CRISPR/Cas gene editing, which aim not to introduce foreign DNA, resulted in the unintended incorporation of foreign and contaminating DNA into the genome of gene-edited organisms.[12] This unwanted result is not restricted to CRISPR but has been found with other types of gene editing, too.[13]

Specifically, the researchers looked at the effects of CRISPR/Cas gene editing in mouse cells and embryos and found that edited mouse genomes unintentionally acquired bovine or goat DNA. This was traced to the use, in standard culture medium for mouse cells, of foetal calf serum and goat serum extracted from cows or goats.[12]

Even more worrisome, amongst the DNA sequences inserted into the mouse genome were bovine and goat retrotransposons (jumping genes) and mouse retrovirus DNA12



(retroviruses include cancer-causing "onco-retroviruses" and human immunodeficiency virus, HIV, which can lead to AIDS). Thus gene editing is a potential mechanism for horizontal gene transfer (the transfer of genetic material by any method other than "vertical" transmission of DNA from parent to offspring) of disease-causing organisms, including, but not limited to, viruses.[14]

The study also found that DNA from the genome of E. coli bacteria can inadvertently integrate into the target organism's genome. The source of the E. coli DNA was traced to the E. coli bacterial cells used to produce the vector plasmid. The plasmid is a small circular DNA molecule that carries the genes giving instructions for the manufacture of the CRISPR/Cas components (and in SDN-2 applications, the DNA repair template) into the cells. Importantly, the researchers used standard methods of vector plasmid preparation, so this type of contamination could happen routinely.[12]

These findings are clearly relevant to gene-edited animals, but how do they relate to plant gene editing? Tissue culture medium containing components from animals is not used in making gene-edited plants, so the presence of animal DNA is not a concern.

However, in cases where genetic engineers deliver the gene-editing tool into plant cells encoded by a plasmid, there are two ways in which foreign DNA can become inadvertently integrated into the genome of the plant being edited. First, the plasmid encoding the gene-editing tool, either as a whole, or fragments thereof, can become integrated. Second, DNA from the genome of the E. coli bacteria used to propagate the plasmid can often contaminate the final plasmid preparation used in the gene-editing process, and thus could end up being integrated into the gene-edited plant's genome.

Gene editing is a potential mechanism for horizontal gene transfer of disease-causing organisms, including, but not limited to, viruses

Foreign plasmid or bacterial genomic DNA could be inadvertently incorporated during plant gene editing. Therefore regulators must legally oblige developers to conduct appropriate in-depth molecular genetic characterisation of their products to ascertain if such an outcome has taken place or not.

SDN DISTINCTIONS NOT USEFUL FOR JUDGING RISK

The distinction between SDN-1, -2, and -3 is not useful for differentiating levels of risk for each type of gene-edited organism. This is because SDN-1, -2, and -3 refer to the intention of the gene editing and not the actual outcome, whereas the outcome of a gene-editing event can be very different from the intention.

Also, even small changes in the genome can cause large effects.[15,16] The London-based molecular geneticist Dr Michael Antoniou said, "The size of genetic changes does not determine risk, since small genetic changes may result in dramatic and novel effects. For example, a small deletion or insertion following a gene-editing event could result in creating a new gene sequence, which can give rise to a novel mutant protein with unknown functional consequences. This is why all of the mutations caused by gene editing must be assessed on the basis of what they do, as well as what type and how numerous they are."

The size of genetic changes does not determine risk, since small genetic changes may result in dramatic and novel effects

SDN-1 and -2 applications are often assumed to be less disruptive than SDN-3 because there is no intention to permanently integrate foreign DNA into the genome. However, there is no evidence that the mutations caused are fewer, smaller, or less risky in type. In fact, major mutations, including large deletions, insertions, and rearrangements of DNA, have been found to be generated even by SDN-1 procedures.[17,18]

Indeed, all types of gene editing – SDN-1, -2, and -3 – can be carried out at multiple locations of the genome using multiplex approaches, which target several genes at once, or in repeated, sequential applications.[19,20,21] Thus claims that the changes made are "small" and "similar to what might happen in nature" are misleading, as several individually small changes can combine to produce an organism that is very different from the parent organism. While even small changes can produce large effects, a number of small changes made via gene editing can result in even greater changes, which increases the possibility of unintended alterations in the edited plant's biochemistry and overall composition, with unknown consequences for both crop performance and the health of the consumer.

Thus the risks of both small and large changes must be carefully assessed. Although unwanted genetic changes have been studied in gene-edited organisms to some extent, no safety studies have been carried out with gene-edited products. Such studies are compulsory under EU laws before a GMO product can be placed on the market.

GENE-EDITED CATTLE CONTAINED ANTIBIOTIC RESISTANCE GENES

Claims of nature-identical or safe-by-design gene-edited products should be viewed with scepticism, as demonstrated by the case of the gene-edited hornless cattle.

In 2019 researchers at the US Food and Drug Administration (FDA) analysed the genomes of two calves13 that had been gene edited by the biotech company Recombinetics using the TALEN tool in an SDN-3 (gene insertion) procedure. The aim of the genetic manipulation was to prevent the animals from growing horns by inserting into their genome the POLLED gene, taken from conventionally bred hornless cattle.

Recombinetics scientists had claimed that the gene editing used in the cattle was so precise that "our animals are free of off-target events".[22] The company's executives had told Bloomberg in 2017, "We know exactly where the gene should go, and we put it in its exact location," and "We have all the scientific data that proves that there are no off-target effects."[23]

A commentary by academic researchers, some of whom were associated with Recombinetics, claimed that the gene editing used in the cattle was precise, that the changes brought about are largely identical to what could have arisen naturally, and that any animals with unwanted traits would be excluded from breeding programmes.[24]

> These claims were proven false by what the FDA scientists found

However, all these claims were proven false by what the FDA scientists found.

At one of the target sites of the gene-editing procedure within the calves' genome, the POLLED gene had inserted as planned. However, at the other intended gene editing site, two copies of the entire circular plasmid DNA construction that carried the

POLLED sequence, which acted as the repair template DNA in the SDN-3 procedure, had been unintentionally integrated. These unintentionally integrated plasmids contained complete gene sequences that confer resistance to three antibiotics (neomycin, kanamycin, and ampicillin).[13]

It is not known if the presence of these antibiotic resistance genes could affect the health of the animal or of people who consume its products. However, one risk that merits investigation is that these genes could transfer to disease-causing bacteria, which would then become resistant to antibiotics, threatening human and animal health.[25]

The Recombinetics scientists had missed these unintended effects because they used inadequate analytical methods.[22] Tad Sontesgard, CEO of Acceligen, a subsidiary of Recombinetics that owned the animals, said, "It was not something expected, and we didn't look for it". He admitted that a more complete check "should have been done".[23]

As a result of the FDA scientists' discovery, Brazil cancelled its plans to create a herd of the gene-edited hornless cattle.[26]

> Developers cannot be trusted to self-regulate and determine for themselves whether the changes induced by gene editing are safe

Developers cannot be trusted to self-regulate and determine for themselves whether the changes induced by gene editing are safe or the same as could happen in nature. Strict regulation must be in place to ensure thorough screening for unintended effects. As commonly used screening methods will miss many mutations, a combination of long-range PCR and long-read DNA sequencing must be used, as noted in chapter 2. In addition, safety studies must be conducted to better understand the risks to public health and the environment posed by the gene-edited organism.

WHY GENE EDITING RATHER THAN BREEDING?

The failure of the gene-edited hornless cattle venture raises an obvious question: Why didn't the developers simply cross the gene into the elite Holstein breed through breeding, instead of gene editing the Holstein?

The team of academic scientists cited above, some of whom were associated with Recombinetics, wrote that in principle, conventional breeding could achieve this end, but in practice the cost would be prohibitive: "No breeder can afford to undertake this approach."[24] In a separate paper, Recombinetics scientists cited a shortage of breeding sires producing commercially available POLLED semen and the poor "genetic merit" of polled Holstein sires – they said breeding for the POLLED trait brings along other undesirable traits such as poor milk yield.[22]

The supposedly slow speed of conventional breeding programmes relative to gene editing was cited by both sets of authors.[22,24]

However, this does not seem to be true for Europe.[27] According to a breeder of polled Holsteins in Pennsylvania, USA, Europeans "aggressively selected for the trait, and now they are years ahead of us as far as polled genetics. Animal welfare legislation in Europe based on consumer pressure will drive even further use of polled."[27]

Hendrik Albada, co-owner of the Hul-Stein Holstein herd in the Netherlands, said polled sires are popular in Europe based on genetic merit alone – almost 10% of the cows in Germany in 2015 were bred to a polled bull.[27]

It seems that conventional breeding has already achieved what GMO advocates claimed could only be done quickly through gene-editing technology. The cost and time involved are not prohibitive; polled cattle are produced with high genetic merit; and good progress has been made in availability of polled sires.

This example shows that society needs to critically evaluate claims that gene editing is the only or best solution to a given problem.

ORGANISMS WITH UNWANTED MUTATIONS MAY NOT BE REMOVED FROM BREEDING PROGRAMMES

GMO developers often claim that gene-edited organisms with genetic errors and unwanted traits will be eliminated from breeding programmes,[24] or that the errors can be removed by subsequent backcrossing; thus they are nothing to worry about.

> Experience with first-generation GM crops shows that backcrossing as conducted by GMO developers does not reliably remove unwanted traits

However, the case of the gene-edited cattle that turned out to unexpectedly contain antibiotic resistance genes (see above) shows that GMO developers cannot be relied upon to identify genetic errors and unwanted traits13 and that strict regulation must be in place to enforce thorough screening.[28]

Experience with first-generation GM crops shows that backcrossing as conducted by GMO developers does not reliably remove unwanted traits and that crops with such traits have reached the market.

For example, in the case of glyphosate-tolerant NK603 maize, an increase in certain compounds was found in the GM crop compared with the non-GM parent, which could prove either protective or toxic, depending on context. In addition, metabolic imbalances were found in the GM maize, which could affect nutritional quality.[29] These unwanted changes may explain adverse health impacts observed from consumption of the maize.[30] In the case of GM MON810 Bt insecticidal maize, it contained an allergen, zein, that was not present in the parent crop.[31] It is possible that the developer did not notice these changes, or if they did, deemed them unimportant.

With GM vegetatively propagated crops, such as potatoes, bananas, and fruit trees, the presence of large numbers of unwanted mutations is inevitable. This is because propagation takes place not by seeds produced by sexual reproduction (pollination), but by various asexual methods, including growing from tubers (e.g. potatoes), cuttings (e.g. bananas), and grafting (e.g. fruit trees such as apples) – generating a new plant from a part of the parent plant. This means that mutations caused by genetic engineering processes (including gene editing) cannot be bred out by backcrossing and will persist into the final marketed product.

GENE-EDITED ORGANISMS NOT SAFER THAN OLDER-STYLE GMOS

It is a common misconception that gene-edited organisms are safer than older-style GMOs. But there is no scientific basis to this notion, as confirmed by Bayer scientist Dr Larry Gilbertson, who said that the risks of new techniques like gene editing and older techniques of genetic modification are the same: "I don't think there's a fundamental difference in the risk between these two technologies since they're both fundamentally just changes in DNA."[32]

> "The risks linked to the use of those new techniques/ methods of mutagenesis might prove to be similar to those which result from the production and release of a GMO through transgenesis"
> - European Court of Justice

In 2018 this scientific reality was reflected in the European Court of Justice ruling that gene-edited organisms (called in the case "new techniques/ methods of mutagenesis") must be regulated in the same way as older-style GMOs.
The court explained:

"The risks linked to the use of those new techniques/ methods of mutagenesis might prove to be similar to those which result from the production and release of a GMO through transgenesis,
since the direct modification of the genetic material of an organism through mutagenesis

makes it possible to obtain the same effects as the introduction of a foreign gene into the organism (transgenesis) and those new techniques make it possible to produce genetically modified varieties at a rate out of all proportion to those resulting from the application of conventional methods of mutagenesis."[33]

Gene-editing techniques pose new and different risks compared with older-style transgenic GM techniques. Some scientists therefore argue that the EU's risk assessment guidelines should be expanded to take these risks into account.[8,15,16] Interestingly, neither the Bayer scientist, nor the European Court of Justice, nor the scientists who warn of the special risks of gene editing support the notion that gene-edited organisms are safer than older-style transgenic GMOs. These claims are based on marketing concerns, not science.

COMPARING GENE EDITING WITH MUTATION BREEDING IS MISLEADING

Advocates of gene editing claim that it is more precise and thus safer than mutation breeding.[34] But this claim is misleading because it is the wrong comparison. Although mutation breeding is used alongside conventional breeding, it is a minority method that cannot be equated to conventional breeding. The standard method of conventional breeding is cross-breeding and selection of desired traits. The process can be made quicker and more efficient by using the biotechnologies known as marker assisted selection and genomic selection[35,36] (use of these technologies does not in itself result in a GMO). Standard conventional breeding has an undeniable history of safe use and is the technique that should be used as the comparator to gene-edited crops.

As we have seen in chapter 3, gene editing is different from mutation breeding and would lead to different risks. Just how risky mutation breeding is for health and environment remains unknown because controlled studies have not been done, though there is suggestive evidence that it may be less risky than gene editing.[8]

Just how risky mutation breeding is for health and environment remains unknown because controlled studies have not been done

Nevertheless, for the plant itself, mutation breeding is widely recognized as risky, unpredictable, and inefficient at producing beneficial mutations. Plant cells can be killed by exposure to the chemical or radiation, while many of the resulting plants are deformed, non-viable, and/or infertile.[37,38,39]

Mutation breeding is recognised under EU law as genetic modification. It is exempted from the requirements of the regulations because (despite the absence of research on risk) it is deemed to have a history of safe use.[40] But this clearly does not apply to gene editing, which has no history of use, let alone safe use.[8]

REGULATORY OVERSIGHT CRUCIAL

Gene editing technology produces unintended outcomes, which can pose risks to human and animal health and the environment. Even if developers are optimistic that unwanted outcomes can be eliminated, they do not:

- properly screen for them – arguably because that would defeat the purpose of using gene editing to gain time
- reliably remove them
- always have the ability to remove them (with vegetatively propagated crops).

For these reasons, stringent regulatory oversight is crucial, as FDA scientist Steven M. Solomon recommended for gene-edited animals in the US,[28] and as the European Court of Justice has ruled with regard to all gene-edited organisms in the EU.[33]

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