On-target effects of genome editing techniques:

(Un)repaired DNA damage, a hinderance to safety and development?

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Introduction

Genome editing encompasses new forms of genetic engineering techniques being increasingly applied to the development of agricultural and biomedical technologies, by 'editing' the DNA of living organisms (i.e. their 'genome'), including plants, animals and human cells. The genome editing process involves the use of biological molecules (enzymes) which cut the DNA and various mechanisms which then repair it. Arguments over the safety and efficacy of genome editing techniques at the molecular level often hinge on aspects of their 'precision' in targeting DNA sequences of interest, and the 'indistinguishability' of engineered traits from mutations that may arise naturally.

Claims of genome editing precision or specificity have been repeatedly challenged by the accumulation of studies showing unintended effects, such as 'off-target' modification of additional regions of the genome to the 'target site' chosen by the developers. Evidence to date also reveals the issue of unintended 'on-target' effects that include various forms of genetic damage that scar edited genomes. This genetic damage is a common side-effect of the process of genome editing, resulting from error-prone mechanisms of DNA repair following genome editing induced DNA breaks, and the deployment of additional, less understood DNA repair pathways by the cell. However, unintended effects at the target site have received much less attention, and efforts to characterise and detect them are not routinely performed. Not only do such effects fundamentally challenge the notion of indistinguishability and safety, but they raise important questions regarding whether such side-effects are contributing to a bottleneck of gene edited crops reaching commercialisation.

What do genome editing tools actually do to DNA?

Common genome-editing tools, such as CRISPR-based systems, use chemicals (enzymes) called endonucleases which cut DNA. In most cases, they cut both strands of DNA, resulting in 'double-strand breaks'. These genome editing tools are described as targeted and precise, because they can be engineered to cut a specific DNA sequence of choice. For example, they can be directed to cut a gene involved in the susceptibility of a plant to a herbicide, and thus aim to modify the gene to exert tolerance to a given herbicide. In the case of CRISPR systems, the targeted nature is conferred by guide RNA (gRNA) sequences complimentary to the target DNA sequence to be modified, which then direct the CRISPR enzyme (usually CRISPR/Cas9 endonuclease) (sometimes known as 'DNA scissors') to the target site. These guide RNAs are synthesised in the laboratory to target a sequence of interest and introduced to cells along with the rest of CRISPR machinery. This is usually

delivered in the form of transgenic DNA constructs (i.e. constructs that include 'transgenic' or foreign DNA, from different organisms) that encode for CRISPR enzyme and the guide RNA. Other genome editing techniques such as TALENs, meganucleases (MNs) and zinc-finger nucleases (ZFNs), do not deploy gRNAs to target specific DNA sequences, but are instead protein-based enzymes that have DNA recognition sites to bind and cleave particular DNA sequences of interest. The initiation of DNA damage in the form of double-stranded breaks is the first step of the genome editing process. However, after this stage, the outcomes that result are not determined by the engineer but instead by the cell, which activates its own DNA repair machinery to repair the broken DNA. How the cell decides to repair the DNA will result in divergent outcomes, either unintended or intended.

As detailed below, while developers routinely make claims that this process is predictable, understood and well-defined, the complex outcomes of DNA repair processes are not completely understood, nor are they entirely controllable. Such uncertainties add further justifications to widespread calls to regulate genome edited technologies under legislation covering genetically modified organisms (GMOs), warranting careful scrutiny of the biosafety implications of genetic damage being documented in edited organisms and cells (Agapito-Tenfen et al., 2018; Eckerstorfer et al., 2019; ENSSER, 2021; Testbiotech, 2021).

An introduction to DNA repair pathways

The repair of DNA damage is critical for the maintenance of genome integrity in all living organisms. Human cells for example, may develop 10 - 50 lesions in their DNA a day with double strand breaks induced by environmental agents or also occurring spontaneously (Vilenchik & Knudson, 2003). If left unrepaired, double strand breaks can lead to carcinogenesis (cancer) or cell death due to resulting DNA and chromosomal damage. Chromosomes are long DNA molecules with part or all of the genetic material of an organism and a gene is a section of DNA which contains instructions to make a protein, which may be essential for the life of the organism. Damage may include gross chromosomal rearrangements and large-scale translocations, and/or large or small insertions and deletions of parts of the DNA. Mis-repair of double-strand breaks in DNA is a major source of diseases such as cancers in people (Bunting & Nussenzweig, 2013), which may have similar consequences in animals. Such genetic damage in plants also raises safety and socio-economic concerns, with potential changes in gene function and genome integrity potentially altering the safety profile of food crops, or their agronomic performance.

Organisms have developed sophisticated response pathways to safeguard genomic integrity, with organisms harbouring various mechanisms to repair DNA damage. In mammals and plants there are numerous conserved DNA repair pathways for double strand break repair. Understanding is still incomplete and evolving, with evidence continuing to emerge on the various pathways involved, and the underlying mechanisms (Chapman et al., 2013; Symington & Gautier, 2011). Most attention to date has focused on two major pathways for repairing double-stranded breaks in DNA, non-homologous end-joining (NHEJ) and, homology-directed repair (HDR).

Non-homologous end-joining (NHEJ) repair pathway

NHEJ is thought to be the predominant repair pathway in mammals and plants (Beucher et al., 2009; Karanam et al., 2012; Weinthal et al., 2013). NHEJ involves the fusion of two broken ends of DNA, which can be either error free (Schimmel et al., 2019), or be error prone depending on various known and yet to be understood factors (Ceccaldi et al., 2016; Schimmel et al., 2019). An error-free repair re-constructs the original DNA sequence before the double strand break (DSB). The specific architecture of the DNA break is thought to influence choice of repair mechanisms, with some studies suggesting that different outcomes may result depending on how the DNA ends have been cut, for example, if both strands are cut to the same length ('blunt' cut), or if one DNA strand is longer and thus left with a strand 'overhang', which may introduce errors as processing of these ends to allow for re-joining may require deleting these incompatible DNA 'overhangs'. If errors are introduced, it is understood to generally result in small insertions and deletions, called 'indels', of less than 20 DNA bases, commonly seen in plants (Charbonnel et al., 2011; Gorbunova & Levy, 1997; Lloyd et al., 2012).

Homology-directed repair (HDR) pathway

HDR is another pathway for repairing double-stranded breaks, which requires a DNA template with the correct sequence, to guide the DNA repair process, for example, another copy of the same genetic region (located on a sister chromatid, i.e. the other strand of a pair of chromosomes). This pathway is considered to be the most accurate. It can be further broken down in to at least two sub-pathways depending on the mechanism of action and definitions used, (these are known as homologous recombination and single strand annealing) (Haber, 1995). Of note, how such DNA pathways are defined, distinguished and categorised is still debated, reflecting the evolving state of knowledge around DNA repair pathways, with single-strand annealing sometimes falling outside of HDR categorisations.

Alternative end-joining (Alt-EJ) repair pathway

A third pathway for repairing double-stranded breaks that is generally omitted from discussions on repair of genome editing-induced DNA damage, has numerous names including the umbrella term, alternative end-joining (Alt-EJ) that includes microhomology-mediated end-joining (MMEJ), and theta-mediated end joining (TMEJ). This Alt-EJ pathway is intrinsically error-prone, and as a result, a common driver of human genetic disease, as well as genetic variation. It is thought to function by using short homologous sequences (microhomology) between the broken ends to align them back together for repair. Moreover, it has a tendency to re-join double -strand ends from different chromosomes, promoting chromosomal translocations and rearrangements (Simsek & Jasin, 2010; Y. Zhang & Jasin, 2011). Until recently it was thought that this pathway was only deployed in the absence of classical non-homologous end joining pathways in a cell, it is now understood to be a highly regulated and independent pathway (Hanscom & McVey, 2020). While some describe this as another 'alternative' or back-up form of NHEJ, others describe it as a separate pathway in its own right, now understood to be the only DNA repair mechanism for double strand breaks in certain biological contexts (Schimmel et al., 2019).

RNA role in DNA repair processes

More recent research is also revealing an important role for RNA in DNA repair processes, with various RNA species thought to be involved in different aspects of multiple repair pathways (Bader et al., 2020). This is particularly notable for studies on mammalian organisms where much focus in biomedical fields such as cancers are a research priority, though some studies also point to the roles of RNA in plant DNA repair mechanisms (Schalk et al., 2017; Xu et al., 2007). Numerous studies have now documented RNA-templated DNA repair in various organisms including plants and animals (Brambati et al., 2020; Keskin et al., 2014; Zong et al., 2020). Whereas RNA-binding proteins have been found to facilitate DNA repair, persistent RNA:DNA hybrids have shown to promote inaccurate repair and thus genome instability, if not removed in a timely way (Brambati et al., 2020). They do this by inhibiting HDR pathways, instead promoting error prone repair pathways, leading to subsequent outcomes such as increases in DNA translocations (Cohen et al., 2018). RNA-DNA hybrids are thus thought to exert both physiological and pathological roles, playing important functions in DNA repair, but with a propensity to lead to errors under certain conditions.

On the other hand, stand-alone RNA molecules, just like DNA, can serve as templates for HDR repair pathways, either directly, or when copied to 'complimentary' DNA (cDNA) sequences (Keskin et al., 2014). Insertions of RNA-derived cDNA copies into double strand break sites is well documented, and though incompletely understood, may be mediated by NHEJ or Alt-EJ pathways. Retrotransposons, types of mobile genetic elements present in mammalian genomes that actively replicate and re-insert themselves into the genome, are also found at DNA break sites (Teng et al., 1996). The process by which such genetic elements integrate under normal conditions remains incompletely understood, as do the mechanisms underlying their involvement in DNA repair pathways in mammalian cells and in other organisms.

Reductive (mis)perception of repair pathway deployment during the genome editing process

The common narrative often promoted by GMO developers is that genome editing can be used to perform different applications depending on which DNA repair pathway is deployed by the cell. Such narratives focus solely on cells deploying two of the known repair pathways, homologous recombination (HR) or non-homologous end-joining (NHEJ) to achieve different engineered outcomes. Whether it may be altering a few bases of DNA e.g. to inactivate a gene to confer a new trait, or insertion of foreign genetic material to confer a new trait, these different intended outcomes are commonly described as site-directed nuclease (SDN) -1, -2 and -3 applications. These categories were invented for an EU Commission report (Lusser et al., 2011), though the definitions have no legal basis and are simplistic and roughly defined with no clear distinction between categories SDN-2 and SDN-3. Nonetheless, they have been incorporated into mainstream descriptions of genome editing applications, with undue influence over regulatory discussions.

The intended outcome of Site-Directed Nucleases-1 (SDN-1) is a site-specific small random mutation in the form of an insertion or deletion (also called 'indels'). For Site-Directed

Nuclease-2 (SDN-2), the intended outcome is a site-specific pre-determined point mutation (specific nucleotide substitutions of a single or a few nucleotides or small insertions or deletions, i.e. changing one, or a few, chemical letters in the DNA sequence). For SDN-2, an exogenous (i.e. external) DNA template is delivered to the cells simultaneously with the genome editing machinery for achieving desired nucleotide sequence. For Site-Directed Nuclease-3 (SDN-3), the intended outcome is the insertion of a longer DNA sequence (e.g. a gene from a different species, known as a transgene) at a specific target site. Exogenous DNA fragments or gene cassettes up to several kilo base pairs (kbp) in length can be inserted to a desired site in the genome or into a specific gene . As such, for SDN-2 and -3, the engineer delivers a DNA template with the desired changes in it, along with the genome editing machinery. This DNA template is then used by DNA repair machinery to guide the correct repair of the DNA break to compliment that of the DNA template which includes the desired changes in it (Puchta & Fauser, 2013).

Genome editing proponents make concerted efforts to promote the notion of precision and predictability of DNA repair following genome editing-induced double-strand breaks. The idea is that SDN-1 can be achieved by relying on the cell to deploy NHEJ-mediated repair, while insertions can be achieved for SDN-2 and -3 outcomes if the cell choses to use HR to repair the break based on the introduced DNA template. However, studies are revealing a wide range of more complex, unintended DNA repair by-products including large deletions, large insertions (including of transgenic 'foreign' DNA), rearrangements and chromosomal translocations that do not easily conform to this reductive understanding of genome editing-induced DNA repair (see below).

Studies are now suggesting that additional factors, such as the deployment of the third alternative end-joining pathway, among other complexities, may play a role in mediating the range of unintended modifications being observed. Therefore, how each repair pathway relatively contributes to genome-edited mediated DNA breaks has been under characterised (Bothmer et al., 2017; van Overbeek et al., 2016). Indeed, knowledge of DNA repair pathways is still an evolving field, leaving knowledge gaps and uncertainties around the extent and type of genetic damage being caused, and any notion of predictability and thus subsequent safety of genome editing applications.

Genome editing results in complex, erroneous DNA repair

Current evidence suggests that repair of genome editing-induced DNA damage results in complex outcomes that go beyond the intended changes and mechanisms described by genome editing promoters. A recent paper reports that slow and erroneous DNA repair appears to predominate following CRISPR-Cas9 induced DNA breaks (Brinkman et al., 2018), with an estimated error rate between 20-100% depending on the target site. Such findings suggest that repair of CRISPR-induced cuts may not be representative for the repair of naturally occurring double-strand breaks (Brinkman et al., 2018; Kawall, 2019), which is thought to be highly precise. For example, human cells have been estimated to suffer 10-50 double-strand breaks a day, yet assessments of the levels of erroneous repair in adults, in the absence of the use of genome editing techniques, suggest very low rates of errors (see (Brinkman et al., 2018). While the mechanism of increased errors during the genome editing process was not fully investigated, the authors suggest that errors may result from

interference in DNA repair by the CRISPR machinery's prolonged interaction with the DNA break site that may inhibit the repair process. Another study that detected the majority of outcomes from SDN-1 applications, found that the most frequent outcomes were deletions of 20 base pairs, as opposed to smaller deletions supposedly expected for SDN-1 (Sant'Ana et al., 2020). Consistently, unintended gross chromosomal damage such as duplications, rearrangements, translocations, chromosomal fusions, insertions and deletions have been documented in edited cells or organisms (Adikusuma et al., 2018; Biswas et al., 2020; Brunner et al., 2019; Burgio & Teboul, 2020; Cullot et al., 2019; Kosicki et al., 2018; Ledford, 2020; Nelson et al., 2019; Q. Zhang et al., 2018; Zuccaro et al., 2020).

Vector backbone insertions (both on and off-target) have been documented in numerous studies, including mammals and plants (Biswas et al., 2020; Liu et al., 2021; Norris et al., 2020; Ono et al., 2019; Roberts et al., 2017; Q. Zhang et al., 2018). Studies assessing DNA repair mechanisms underlying *de novo* (i.e. new) integration events point towards the deployment of both NHEJ and Alt-EJ mediated repair, being deployed instead of the homologous recombination pathway desired by the engineers (Canaj et al., 2019; Schimmel et al., 2019; Wyatt et al., 2016; Zelensky et al., 2017). Canaj et al., (2019) suggest that the ligation (joining up) of donor DNA templates to double-stranded breaks may underlie these outcomes, which may be influenced by certain chemical modifications of the template DNA ends. Studies have also reported accidental insertions of foreign DNA not introduced directly by the genetic engineering process, including integration of serum-derived goat and bovine genes in edited mouse cells, deriving from the culture medium used to grow the cells (Ono et al., 2019). Insertion of DNA templates has also been associated with single-stranded DNA templates used to promote single-strand annealing pathways in zebrafish (Boel et al., 2018).

Equally intriguing, are the observations of RNA-derived DNA insertions, and thus the involvement of RNA in mediating genome editing-induced DNA repair. RNA-derived DNA integrations have been documented in mammalian cells, including those derived from mRNA molecules, the guide RNA of the CRISPR-cas9 machinery, as well as retrotransposons (Jeon et al., 2019; Ono et al., 2015; Onozawa et al., 2014). The insertion of retrotransposons was postulated by Jeon et al., (2019) to increase the complexity of modification outcomes, including exacerbating the issue of mosaicism - the generation of distinct genome mutations in different cells of an organism. It is perhaps a matter of debate whether such organisms harbouring *de novo* insertions of RNA-derived sequences would be strictly defined as 'transgenic', but nonetheless, such observations point towards clear biosafety risks associated with such genomic instability. Large insertions can potentially disrupt genes at the site of integration, as well as activity of nearby genes (a frequent outcome seen in classic/commercial GMOs). Of note, the association of CRISPR guide RNAs with DNA break sites in the form of DNA-RNA hybrids has also been suggested to promote error-prone repair by interfering with the repair process (Bothmer et al., 2017), pointing towards intrinsic factors to bear in mind when using guide RNA-based genome-editing tools.

It is worth considering that particular DNA elements may exert specific types of effects that may have implications for safety, further underlying the need for thorough characterisation of edited organisms. Uncertainties arise from potential effects associated with particular RNA elements. For example, retrotransposons are thought to destabilise the genome in various ways (Zong et al., 2020). Moreover, new data suggests that certain retroelements are favoured at certain genome editing sites, increasing the unpredictability of potential

outcomes (Jeon et al., 2019). Most fundamentally, such observations challenge the notion that techniques that do not include the introduction of foreign DNA as part of the engineering process (either as vectors encoding for genome editing machinery, or DNA templates for SDN-2 and SDN-3 applications), can be assumed to create gene edited organisms which do not carry exogenous DNA sequences.

Other on-target effects include large-scale deletions, translocations, duplications and rearrangements. A recent example was the use of SDN-1 'genome editing' to disrupt a gene in rice to make semi-dwarf varieties (Biswas et al., 2020). The authors reported a variety of mutations, insertions, deletions and rearrangements of DNA, which varied with different rice varieties. Unintended insertion of plasmid DNA (small circular DNA molecules used in the genetic engineering process) was also detected. The rice also displayed reduced yield, which was dependent on the genetic background of the variety, though the mechanism for this remains unclear. Separate findings of large-scale deletions and translocations (as well as high frequency insertions of vector DNA) in CRISPR edited cells (Liu et al., 2021) led researchers to conclude that high fidelity CRISPR systems that aim to increase specificity by reducing off-target effects, cannot overcome on-target effects resulting from deleterious repair by-products. Analysis of the repair outcomes suggested that Alt-EJ repair pathways were responsible for the complex repair outcomes. Consistently, blocking the NHEJ pathway leads to an increase in large deletions and translocations (100pb-300kb in size) (Liu et al., 2021; van Overbeek et al., 2016). The high frequency of translocations prompted the authors to warn that experiments aiming to perform editing of multiple genes at once (multiplexing), "would induce tremendous translocations between any two target sites". A recent genome edited wheat that used CRISPR systems to modify 35 genes to reduce gluten content exemplifies that use of multiplexing (Sánchez-León et al., 2018). However, analysis of unintended effects was restricted merely to looking for off-target activity at 6 sites. Such multiplexing capacities are promoted as one of the benefits of genome editing that promotes efficient modifications of multiple genes simultaneously.

Added complexity in predicting how genome editing-induced DNA damage may be repaired, is based on how different DNA break architectures can impact the choice of DNA repair mechanisms deployed by the cell, though current understanding is still lacking. Early evidence suggests that DNA breaks that are staggered, i.e. one strand is longer than the other, may promote either HDR or Alt-EJ outcomes, with blunt ends promoting NHEJ. It has been observed for example, that when CRISPR-based 'nickases' (modified enzymes designed to create single-strand rather than double-strand breaks) are used to cause staggered DNA breaks, Alt-EJ is deployed at higher rates than NHEJ, with increased errors reported (Bothmer et al., 2017). This is worth bearing in mind when developers attempt to generate staggered ends with the intention of promoting HDR mediated repair, and reduce off-target effects.

Moreover, it is often assumed that CRISPR/Cas 9 systems generate blunt ends, while TALENs, CRISPR/Cas12a and ZFNs cause staggered DNA breaks. However, recent studies suggest that CRISPR/Cas9 can also generate staggered ends (Gisler et al., 2019; Zuo & Liu, 2016). Whether this results directly from the cutting itself, or whether the DNA ends are subsequently processed to generate staggered ends is not clear. Gizler et al., (2020) further observed that individual guide RNAs for each CRISPR target may mediate the generation of blunt or staggered ends. Knowledge gaps surrounding the exact architecture

of DNA breaks caused by genome editing machinery make predicting any potential unintended effects from erroneous repair very challenging.

Genome edited crops must be regulated and assessed for unintended effects

In the field of medical research, unintended effects of genome editing are largely undisputed, as highlighted by recent reports on the state of play by prestigious medical organisations and researchers who warn against potential unintended effects that may result in diseases such as cancers (National Academy of Medicine (U.S.) et al., 2020). However, in other fields of genome editing research such as agriculture, the rates and implications of unintended effects are constantly challenged and dismissed by proponents, based on reductive understanding of DNA repair processes, and thus notions of precision and predictability of genome editing tools (EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) et al., 2020).

An illustrative example of on-target effects that support the case for strict characterisation and regulation of genome editing technologies, is the detection of foreign DNA unintentionally inserted into the genome of the recently developed genome edited 'hornless' cow. Instead of 'editing' the cow, an unintentional transgenic organism was generated by the capturing of unintentional DNA sequences to patch up the break site. The cow harbours DNA originating from the vector DNA, the vehicle used to deliver the DNA encoding for the editing machinery into the cells (including antibiotic resistance genes) (Norris et al., 2020). The developers missed these unintended outcomes based on the incorrect assertion that integration events were not possible. Such a finding highlights the critical need for comprehensive molecular characterisation and analytical methods that allow for the detection of large-scale alterations that would otherwise be missed. Even in cases where some checks are done, the methods used are usually not sufficient to detect most unintended changes.

Unintended effects resulting in erroneous repair of DNA breaks are not routinely studied, and standard analytical protocols will miss large-scale on-target alterations. As recently observed in edited human cells, approximately 16 % of sampled cells had large unintended changes that would usually be missed with conventional detection protocols (Alanis-Lobato et al., 2020). It is thus worth referring to Agapito-Tenfen et al., (2018), with regard to the concept of indistinguishability being defined by someone's choice of what to measure. What is chosen for knowing, also means choosing what remains unknown. The authors thus rightly highlight that new analytical methods are indeed undermining claims of indistinguishability. These methods should be deployed routinely for assessing edited organisms that may be released into the environment or food systems. Filling in these knowledge gaps also goes beyond biosafety assessment, but may also assist in enabling the traceability of commercialised food products that is required to ensure consumer choice and uphold regulatory decisions on unapproved gene edited organisms. Proper characterisation of on-target alterations can thus serve to assist in regulatory mechanisms to preserve traceability and consumer choice at the individual and national level.

Moreover, further understanding of these complex DNA repair outcomes is needed, as they also pertain to efficacy of this technology. While proponents regularly claim that genome

editing is needed to generate useful traits for addressing serious societal problems of food insecurity and climate change, to date only two genome edited crops have been commercialised, suggesting potential bottlenecks in development of successful lines. The efficacy implications of unintended effects, including off-target changes and erroneous DNA repair outcomes, are yet to be fully understood. Further research is thus warranted before unsubstantiated claims about the benefits of genome editing technologies are used to rush through changes to GMO legislation that could remove requirements for important health and safety assessments.

Associating indistinguishability with safety also fails to acknowledge that such genome editing technologies, along with others (e.g. external RNA-based products, gene drive technologies and others that are moving engineering tools directly into the field), are increasing the magnitude and scale of human intervention. As highlighted by Heinemann et al. (2021), mutations introduced by genome editing or other genetic technologies, are not reliant on the processes of evolution, but instead can be driven by human activity, to ensure such mutations establish and spread in the environment (Heinemann et al., 2021). Genome editing is flexible and cheap, promoting widespread use, increasing the extent of genetic changes being pursued, and thus the likelihood of large-scale environmental introduction, with unknown consequences. Technologies that perform genetic engineering in the field, such as pollen-mediated, or viral-mediated delivery of genome editing machinery in the open environment, further confirm the need for thorough risk assessments and regulations (Sirinathsinghji, 2019).

Conclusions

Many questions remain regarding the mechanistic underpinnings of DNA repair pathways in the genome editing process, and how this may impact safety and efficacy of genome edited organisms:

- New and alternative DSB repair pathways have shown to play crucial role in DNA repair outcomes in a variety of species, with current understanding still evolving
- Evidence to date clearly indicates that genome edited DNA repair outcomes are highly complex and variable, with a multitude of unintended effects that demonstrate a lack of ‘precision’, ‘efficiency’ and ‘indistinguishability’ from naturally arising mutations
- Such unintended DNA repair outcomes will determine the efficiency and safety of gene-editing technologies applied to organisms
- Complexity of unintended effects resulting from repair of genome-editing induced DNA damage remains an active field of research that is under acknowledged by those advocating for deregulation of genome editing technologies for food and environmental applications, yet remain uncontested in the medical field
- The current categorization of gene-editing techniques into SDN1, SDN2 and SDN3 does not reflect the variety of pathways leading to genome editing outcomes and thus cannot be used to determine regulations of genome edited organisms, such as suggestions to exclude SDN-1 and SDN-2 applications from GMO legislation

- Underestimation of on-target DNA changes leads to the under evaluation and analysis of unintended effects that need to be systematically characterised to ensure safety prior to the release of genome edited organisms into the environment
- Genome edited organisms must be strictly regulated to allow for thorough characterisation of the full spectrum of unintended effects associated with the technology in the form of risk assessment and management, ensuring safety and efficacy of edited organisms. Further, regulations are vital to ensure labelling and traceability of products in order to operationalise citizen and farmer rights to decide what to grow and consume, and to facilitate any recall/removal from the food chain and the environment following any unanticipated risk events.

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