Gene-edited organisms in agriculture:

Risks and unexpected consequences

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Friends of the Earth

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About Logos Environmental

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Gene editing creates GMOs; it entails humans directly altering genetic material.

Executive Summary

Gene-edited crops that have bypassed USDA oversight include: white button mushrooms, wheat, soybeans and waxy corn.

In the U.S., companies are racing to incorporate genetically modified organisms (GMOs) produced using new genetic engineering technologies such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and other "gene editing" (or "genome-editing") techniques into our food system with little to no oversight and public disclosure, despite scientific research that is demonstrating the potential for significant unintended consequences.

For example, in a recent study published in Nature Biotechnology, scientists from the Wellcome Sanger Institute in the UK found that new genetic engineering techniques like CRISPR may cause "genetic havoc"[1]. Specifically, researchers found large deletions and rearrangements of DNA[2] near the target site that were not intended by researchers.[3] Prior to that study, two recent independent studies published in Nature Medicine, one by the biotech company, Novartis and the other by the Karolinska Institute, found that cells genetically engineered with CRISPR "have the potential to seed tumors",[4] or may initiate tumorigenic mutations.[5]

Earlier studies found that gene-edited plants such as soybeans had off-target effects, in which gene editing occurred at unintended locations with DNA sequences similar to the targeted location.[6] These studies are a small sample of the growing research demonstrating the unintended consequences and surprise impacts that may result from genetically engineering organisms.

The new genetic engineering techniques are being proposed for a wide range of applications from pharmaceuticals to genetic therapy in humans to agriculture.[7] Within agricultural proposals, the most common trait for gene-edited plants is herbicide tolerance.[8] This prevalence implies that, like current genetically engineered crops, the application of techniques like CRISPR will further entrench a chemical-intensive approach to agriculture. In fact, the first product to go to market was Cibus' SU Canola™, which is resistant to the herbicide sulfonylurea.

The unexpected and unintended effects of all genetically engineered organisms, regardless of whether 'traditional' or gene-edited genetic engineering techniques have been used, have the potential to cause environmental and human health problems.[9] While some studies describe gene editing such as CRISPR as "precise,"[10] most studies have been "proof of concept" studies that look at specific intended changes that might be achieved. But these studies haven't looked at collateral effects of gene editing, such as unintended changes to DNA in other genes. As the current research shows, precise edits do not necessarily result in precise outcomes. Additional concerns about gene editing applications in agriculture include increased agrochemical use, effects on pollinators, impacts from stacking genetically engineered traits and genetic contamination of crops' wild relatives.

While recent studies raise concerns about unintended effects, more research is needed to understand the implications of CRISPR and other engineering techniques on non-target genes and surrounding ecosystems. Yet food products such as the CRISPR mushroom[11] are being allowed into fields and onto the market in the U.S., with insufficient evidence to demonstrate their safety,[12] without regulatory oversight and without being labeled as GMO products.

In this report, we highlight the unintended effects and potential risks related to gene editing applications in agriculture as reported in peer-reviewed scientific studies. We emphasize significant research and data gaps in the analysis of how the unintended genetic mutations resulting from gene editing may impact human health and ecosystems. The report provides recommendations for further research and points to the lack of regulatory oversight in the U.S. We also address the question of whether gene editing in agriculture is necessary, as modern conventional breeding offers an alternative, and possibly better, option in the development of new varieties of plants and animals.

What is gene editing?

Gene editing is a set of new genetic engineering techniques for altering the genetic material of plants, animals and microbes, such as bacteria, using "molecular scissors" that are aimed at a location on the organism's DNA and used to cut the DNA. This cut DNA is then repaired by the cell's own repair mechanism.

These techniques result in GMOs. Any artificial manipulation that invades living cells for the purpose of altering its genome[13] in a direct way, including gene editing, constitutes genetic engineering.

CRISPR

One of the most popular and recent types of gene editing technologies is CRISPR. CRISPR cuts DNA at a specific location using molecular scissors known as site-directed nuclease (SDN). It then inserts, deletes or otherwise alters a specific gene. Although CRISPR has been touted for its potential to be a precise genetic engineering tool, recent studies caution that using CRISPR can have unintended effects on DNA and gene regulation and could create serious problems, like potentially interacting with a cancer prevention gene in human cells[14].

Gene drives

Gene drives, using CRISPR, are proposed to engineer the genetics of entire populations[15,16] by forcing a specific trait through generations of a species and bypassing the process of natural selection. Once released, gene drive organisms cannot be recalled, and any changes to the genetic makeup of the population they induce are most likely irreversible. Hence, the genetic changes to a population are likely to persist for a very long time, possibly permanently. This may result in far-reaching and unpredictable consequences for society and the environment.

Proposed uses of gene drives are still in the "proof of concept" stage. They include genetically engineering mosquitoes to prevent effective reproduction, thus reducing the mosquito population as a vector of diseases,[17] or altering the genes of agricultural pests to suppress their populations[18]. While such applications appear to promise societal benefits, concerns surrounding gene drives are severe. Given the magnitude of risk, 170 civil society organizations from around the globe are urging

Gene-edited traits could be stacked with other GMO traits, potentially affecting toxicity to wildlife.

a moratorium on gene drive development.[19] Scientists have likewise cautioned that gene drives could foster far-reaching, harmful impacts if any unintended effects were to occur.[20]

The need for regulatory oversight of gene-edited plants and animals in agriculture

Initial scientific assessments of CRISPR and other new genetic engineering techniques and the high potential for unintended consequences demonstrate the importance of a robust governance structure and a precautionary approach to gene editing.[21] Yet, the current regulatory structure in the U.S. is a patchwork of weak oversights split between the U.S. Environmental Protection Agency (EPA), U.S. Department of Agriculture (USDA) and U.S. Food and Drug Administration (FDA). As a result, some of the most common types of gene editing technologies, such as CRISPR, can avoid essential regulation and assessment in the U.S. The EPA requires virtually no assessment of the environmental impact of gene-edited organisms, while the USDA only regulates gene-edited plants if they involve plant pests or are themselves plant pests.[22] The FDA has no mandatory requirement for food safety assessment and technically has authority to assess gene-edited animals, but the standards for doing so are unclear.[23] Once they are on the market in the U.S., gene-edited products may not be identifiable to consumers or retailers, as the current proposed GMO labeling regulation under debate in the U.S. may not cover gene-edited organisms.[24]

Given the prevalence of unintended consequences from genetic engineering applications, all genetic engineering techniques should fall within the scope of government regulatory oversight of genetic engineering and GMOs. In July 2018, the European Court of Justice set an important precedent by ruling that second wave genetic engineering techniques, like ODM (oligonucleotide-directed mutagenesis) and CRISPR, will be included within the European regulations developed for first-wave genetic engineering technologies.[25]

The United Nations Convention on Biological Diversity (CBD) is also leading important international dialogue about the governance of second-wave genetic engineering. The CBD is currently deliberating global recommendations for precautionary guidelines to govern genetic engineering with particular attention to gene drives.

Conclusion

Research and regulations are not keeping pace with developments in genetic engineering. New genetic engineering techniques, like CRISPR, require further analysis in the context of agricultural ecosystems and the food system as a whole in order to properly assess their potential risks and hypothetical benefits. Along with science-based assessments of health and environmental risks to address significant gaps in scientific knowledge, the scope of analysis should be expanded to include social, cultural and ethical considerations as well as extensive public discussion to determine the future of gene editing in agriculture. More robust research and regulations on gene editing are needed across the international community, with special attention given to potential impacts on human and environmental health alongside inclusive public discourse on the topic.

Alternatives to gene editing are proving to be less risky and highly effective.[26] Assisted by a growing understanding of DNA and genomes, techniques like genomic selection[27] and marker-assisted selection can now speed up the selection of desirable traits in conventional breeding. Such approaches have already achieved success in producing disease-resistant crops[28] and improving cattle, pig and chicken breeding.[29] Innovative conventional breeding options such as these should be explored further as a viable solution to developing a precautionary, safe, equitable, sustainable and just food system.

Key Findings

- Gene-edited organisms are prone to unintended and unexpected effects at the molecular level that may pose a threat to human health and the environment if commercialized without comprehensive mandatory safety assessment and oversight.
- Gene drives, designed to drive a particular trait through the entire population of a species, could have far-reaching and unpredictable negative consequences for organisms and the environment.
- The prevalence of herbicide-tolerant gene-edited plant proposals[30] implies that gene editing applications will further entrench a chemical-intensive approach to agriculture.
- In the U.S., current regulations may allow gene-edited organisms into the environment and onto the market without assessments or labeling.
- There are gaps in research about how unintended consequences at the genetic level may impact the whole organism or interact with complex environmental factors. More robust research is needed, particularly about potential impacts on human and environmental health.

Recommendations for international and national regulators

- Any deliberate, artificial manipulation that invades living cells for the purpose of altering its genome in a directed way, including gene editing, constitutes genetic engineering. All genetic engineering techniques should fall within the scope of government regulatory oversight of genetic engineering and GMOs.
- The products of all techniques of genetic engineering, including gene editing, should be regulated using the Precautionary Principle to protect human health and the environment.
- Oversight and regulations should include independent assessment for safety and other long-term impacts before entering the market or environment, and products of all genetic engineering should be labeled and traceable.

Gene editing can produce large deletions and complex rearrangements of the organism's own DNA.

Overview

"These CRISPR-modified crops don't count as GMOs."[31] "This gene editing tech might be too dangerous to unleash."[32] Headlines about new genetic engineering techniques have spread across the globe. The genetic engineering techniques may be new, but GMOs and their associated environmental impacts, such as the increased use of glyphosate-based Roundup herbicide,[33] have been of concern for the past 20 years. Now, genetic engineering is being trumpeted under a different guise, using names such as gene editing or genome editing. Despite the publicity of its potential applications in agriculture,[34] gene editing is raising concerns.

Gene editing is genetic engineering, as it involves using laboratory techniques to alter DNA.[35] Although promoted on the basis of largely unsubstantiated claims of specificity and precision, gene editing techniques — like all types of genetic engineering — can cause unexpected and unpredictable effects. Despite this, gene-edited plants are bypassing USDA oversight,[36] meaning there is no environmental safety assessment of these genetically engineered crops, even though they could have far-reaching consequences.

This report discusses the concerns with gene editing as documented by published scientific studies, highlights the gaps in scientific assessments and points to the need for regulatory oversight requiring health and environmental safety assessments of gene-edited organisms. Many scientific studies have now highlighted specific genetic errors that can be created by gene editing — including so called "off-target" effects. However, studies have not yet been conducted on what the implications of these errors might be for food and environmental safety. The scope of published studies also shows a gap in broad public dialogue about the use of gene-edited plants and animals in agriculture and about how they are assessed, labeled and employed (if at all) in agriculture. For any gene-edited organism, it is vital that detailed studies are performed prior to any outdoor growing or entry into the food chain. These studies should evaluate any potential negative impacts on human and animal health, the environment and biodiversity and should all be taken into consideration by regulators in accordance with the Precautionary Principle.[37]

Issues with gene-edited organisms

Technical

- Gene-edited organisms are a new type of genetic engineering, leading to the creation of GMOs.
- Gene editing is prone to generating genetic errors, leading to unexpected effects in the resulting GMO.
- Food and environmental safety could be affected by the unexpected effects caused by gene editing.

Societal values

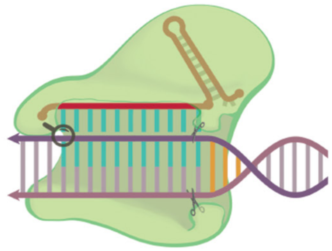
- The regulatory system lacks consideration of societal values.
- Gene-edited organisms in food may not be labeled as GMOs.
- Gene-edited organisms are not essential for agriculture – advanced conventional breeding is delivering new varieties.

Regulatory

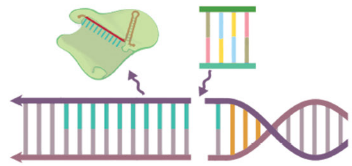
- Gene-edited organisms require careful assessment of any genetic errors and unexpected effects.
- Loopholes in the U.S. regulatory system allow GMOs to evade risk assessment.
- Gene-edited organisms undergo risk assessment in many other countries and regions of the world, e.g. EU.

How gene editing works

"Molecular scissors" (nucleases) are guided to a location (the target site) on an organism's DNA.



The molecular scissor complex docks onto the target site and cuts through the DNA.



The repair of DNA is then initiated and occurs either with (SDN2) or without (SDN1) a synthetic repair template. Alternatively, genes can be inserted (SDN3).

The DNA is now "edited." However, in reality, gene editing is prone to creating unintended changes and errors that can lead to unexpected effects in the gene-edited organism.

Defining genetic engineering

Current genetically engineered crops, e.g., Roundup Ready soy and Bt corn, have been made using "standard" genetic engineering. Standard genetic engineering — as devised in the 1970s — inserts genes (made up of DNA) at a random location into an organism's own DNA, or genome.[38] If those genes are from a different organism (often called "foreign" genes), then the resulting GMO is transgenic. Almost all current commercial genetically engineered crops are transgenic, with the inserted gene(s) normally producing a protein that makes the plant tolerant to a particular herbicide (e.g., Roundup Ready soy), or toxic to certain plant pests (e.g., Bt corn).

Genetic engineering is very different from conventional breeding. Conventional breeding has been used by farmers and breeders for thousands of years[39] to develop plant and animal varieties with desired traits, such as grain or milk with superior qualities or resistance to pests and diseases. In plants and animals, conventional breeding relies on normal male and female mating to produce offspring with desired traits that are then selected for further breeding. In contrast, genetic engineering does not rely on mating to obtain desired traits. Instead, researchers directly alter the genetic material of an organism using laboratory techniques. It is this direct alteration of genetic material by humans that defines genetic engineering in the U.S.[40] and underpins the definition of a GMO in the United Nations[41] and the European Union[42].

Genetic engineering does not rely on mating to obtain desired traits. Instead, humans directly alter the genetic material of an organism by using laboratory techniques.

What is gene editing?

Gene editing (also called genome editing) is a set of new genetic engineering techniques for altering the genetic material of plants, animals and microbes, such as bacteria. All such techniques use a synthetic molecular guide with the goal of changing DNA while it is present in the organism, i.e., in situ. The change in the organism's genetic material is achieved not through the breeding process (as in conventional breeding), but directly and artificially by humans using the same, or similar, laboratory techniques as genetic engineering. This means that gene editing, like genetic engineering, produces GMOs.

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The most talked-about gene editing technique is CRISPR[43], but there are several gene editing techniques that all follow the same basic principles. Molecular scissors are aimed at a location on the organism's DNA and cut the DNA. This cut DNA is then repaired by the cell's own repair mechanism. The type of repair is the key to how gene-edited organisms are classified. Depending on how the repair is achieved (see Box, right), there are three different types of gene editing: one that uses a synthetic repair template, one that doesn't use a synthetic repair template and one where a gene (or genes) are inserted.[44]

The principal difference between "standard" genetic engineering and gene editing is that genes do not necessarily have to be inserted into the organism to produce a new trait,[45] although the molecular scissors must be introduced into the organism. The resulting gene-edited organism may or may not produce a novel protein as part of the novel trait, as most current commercialized GMOs do. However, even if a gene-edited organism does not contain foreign genes or express a novel protein it cannot be considered "safe" for the environment or food on this basis alone. In addition, the developer may, or may not, have introduced foreign DNA and may or may not know if it is still there (see Intended and unintended insertion of DNA). As explained in Unexpected Effects below, the process of gene editing, like standard genetic engineering, can give rise to unexpected and unpredictable effects in the resulting GMO.

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Different types of gene editing

Gene editing techniques such as CRISPR, TALEN, ZFN and meganucleases[46] guide molecular scissors (known as site-directed nucleases, SDNs) to the location on the genome where the DNA change is intended to take place. Depending on the technique, these guided molecular scissors are in the form of synthetic proteins, or synthetic RNA-protein combinations. The molecular scissors cut the DNA, which then undergoes repair using the cell's own repair mechanism. Often, a synthetic DNA template is used to direct the repair in such a way that a particular change in the DNA is achieved.[47] This gives rise to different types of gene editing:

1) No repair template is used (SDN type 1 or SDN1)
2) A repair template is used (SDN type 2 or SDN2)
3) Genes are inserted during the gene editing process, usually giving the resulting organism a particular trait. This gene editing results in a transgenic organism if the genes are from other species. (SDN type 3 or SDN3).

The most common type of molecular scissors used with CRISPR is called "Cas9," so people often refer to the CRISPR-Cas9 system, but other types of molecular scissors are also possible, e.g., Cpf1.[48] In addition, a new CRISPR strategy is under development called base editing. Base editing uses molecular scissors that don't cut all the way through the DNA, but unravel the DNA, allowing a small (single base pair) change to the DNA to take place.[49] However, the concerns regarding genetic errors created during the gene editing process still apply to base editing.

The gene editing technique known as oligonucleotide-directed mutagenesis (ODM) does not use guided molecular scissors, but instead introduces a short strand of DNA that attaches itself to the organism's DNA at a particular location and causes a change to that DNA.[50]

Although often described as "precise," genetic engineering, including gene editing, is prone to creating genetic errors.

Gene editing applications under development

Gene editing is being proposed for a wide range of potential traits. Pilot or "proof of concept" studies demonstrating feasibility for both plants and animals have been published in scientific journals. On animals, they include the development of pigs with resistance to certain diseases[51] and "double-muscled" beef cattle, which raise ethical concerns because the over-developed muscles can cause breathing problems[52] and other health issues. There is also research on gene-edited insects for use in gene drives (see Gene Drives). For plants, gene-edited traits under development include drought tolerance in corn, virus resistance in cucumbers, altered flowering time in tomatoes and altered composition in soybean.[53] However, the most common trait for plants is herbicide tolerance.[54] This suggests that, like current genetically engineered crops, the primary interest in gene editing is developing herbicide-tolerant crops. In fact, one of the first gene-edited products to be commercialized in North America was an herbicide-tolerant canola.[55]

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Unexpected effects with gene editing

Although gene editing techniques are often described as "precise"[56] compared to standard genetic engineering, these techniques can cause unintended alterations to genetic material (as described below), just like standard genetic engineering. Indeed, the same claim of accuracy and specificity was the basis of standard genetic engineering techniques now known to induce errors (see Intended and unintended insertion of DNA).[57] Such unintended alterations or genetic errors can give rise to unexpected effects. Furthermore, even when the intended alteration occurs, unexpected effects can occur because gene-edited organisms may behave differently in the natural environment than expected from laboratory experiments. Tissue culture may also be involved in the gene-edited process, as it often is with standard genetic engineering techniques, which can also result in unexpected changes (mutations to DNA) effects.[58] While there are many "proof of concept" studies demonstrating what intended changes gene editing might achieve, there is a complete lack of studies on what the implications of any unexpected effects arising from the gene editing process and/or the engineered trait could be for food and environmental safety.

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Most studies on the potential uses of gene editing techniques in agriculture consider off-target effects to be both a major challenge and a major concern.

Unexpected "off-target" effects

One of the main ways gene editing can be imprecise and create genetic errors is by causing "off-target" effects — changes to other genes that were not intended. Off-target effects could unintentionally alter important genes, causing changes in chemistry or protein production — both of which are important for food and environmental safety. Most studies on the potential uses of gene editing techniques in agriculture consider off-target effects to be both a major challenge and a major concern,[59] and as detailed below, many studies have now detected off-target effects in gene-edited plants and animals.

Off-target effects are caused by the gene editing process occurring at additional unintended location(s) with similar DNA sequences to the intended (target) location. This gene editing causes an unintended change to the DNA at an unintended (off-target) location.[60] The frequency of off-target effects depends on the gene editing technique and the exact protocol used,[61] but the CRISPR-Cas9 system appears to be particularly prone to off-target effects.[62] Such off-target effects have been detected in many studies on gene-edited plants, e.g., rice, soybean[63] and wheat[64].

Off-target effects are also a concern in gene-edited farm animals, such as pigs and cattle,[65] and have been detected in gene-edited mice[66] and human cells[67]. However, some studies report a lack of detectable off-target effects in gene-edited animals.[68] This could be because, although the off-target effects may be present, it is difficult to distinguish between off-target effects and natural genetic variation.[69] In order to evaluate any potential risks posed by any gene-edited plant or animal that is grown in the environment and/or is intended for food, it is important that any off-target effects are fully evaluated to determine if they have caused any changes to chemistry or protein production.

Unexpected "on-target" effects

In addition to off-target effects, gene editing can also cause "on-target" effects, where the intended change occurs at the intended location, but has a different outcome than expected. A small insertion or deletion of DNA within a gene, even if on-target, could change the way a gene is read and processed into proteins in problematic ways. Essentially, genes in DNA are "read" to produce an intermediary product (RNA[70]), which is then processed into proteins. Studies have found that CRISPR can inadvertently cause extensive deletions and complex re-arrangements of DNA.[71] These deletions and re-arrangements of DNA by CRISPR may cause important parts of the gene (those coding for protein production) to be "missed" when the DNA is read.[72] This misreading of DNA has the potential to produce altered proteins. Food allergens are mostly proteins, so altered proteins could have significant implications for food safety.[73] Concerns with the allergenicity of proteins have long been an important concern with GMOs created by standard genetic engineering techniques. For example, genetically engineered Starlink corn was only approved for animal, not human, consumption in the U.S. because of concerns over the potential allergenicity of the inserted insect resistant gene (Bt Cry9C). After it was found contaminating human food supplies, it was withdrawn from the market.[74]

The misreading of DNA in a gene-edited plant or animal could impact biodiversity. For example, if the chemistry of a gene-edited plant or animal were changed by the misreading of DNA, it could produce a compound that is toxic to the wildlife that feeds on it. These types of concerns regarding human and ecological safety mean that gene-edited organisms need to be analyzed for any on-target effects, and the implications of on-target effects need to be carefully evaluated.

Gene edits to DNA may unintentionally affect the operation of the organism's regulatory network. This could result in the organism's own (unedited) genes not being expressed as they should be, perhaps expressed in the wrong amount, the wrong composition or at the wrong time.

Interference with gene regulation

In addition to altering an organism's DNA, gene editing may have unintended impacts on an organism's ability to express or suppress other genes. Within an organism, genes are switched on (expressed) and off in different parts of the organism at different times as the organism grows, reproduces or responds to environmental factors such as light, heat or drought. In addition, genes interact with each other, either suppressing or reinforcing their expression. The orchestration of gene function in an organism is part of a complex regulatory network. However, the precise way that this regulatory network operates is complex and still poorly understood, as exemplified by recent advances in our knowledge of how gene expression is regulated.[75] For example, for several decades, a dominant theory in molecular biology was that each gene had a single function (i.e., produces one protein), but it is now known that genes can have several functions and interact with each other.[76] Similarly, DNA that did not produce proteins was thought to be "junk" DNA, but it is now thought that much of this junk DNA is important for controlling gene expression in plants, animals[77] and human genomes[78].

There have already been reports of an unexpected response from the cell regulatory network during gene editing. In experiments with human cells, the cuts in DNA created by CRISPR were unexpectedly found to kill cells or stop them from growing.[79] The lack of understanding about how genomes are regulated means it is not possible to predict the nature and consequences of all the interactions between altered genetic material (whether intentionally or unintentionally altered) and other (unedited) genes within the organism. This means that gene edits to DNA may unintentionally affect the operation of the organism's regulatory network. This could result in the organism's own (unedited) genes not being expressed as they should be, perhaps by being expressed in the wrong amount, the wrong composition or at the wrong time.

As these examples show, scientists' understanding of genetics and how genes are regulated is still highly provisional. Gene editing may even be "precise," but the outcomes are not always precise. Just like all genetically engineered organisms, gene-edited organisms may exhibit unexpected and unpredictable effects as a result of unforeseen interactions between the altered genetic material, the organism's own (unedited) genes and its regulatory network. Any unexpected and unpredictable effects could result in alterations to biochemical pathways or protein composition that could have implications for food and environmental safety.

Gene editing may even be "precise," but the outcomes are not always precise.

Intended and unintended insertion of DNA

Many variations of gene editing are in development. However, most of the gene-edited plants developed so far have used a similar process to conventional GMOs. During a typical CRISPR gene editing process, a DNA "cassette" (a suite of genes) containing the CRISPR components is inserted into the organism's genome at a random location — in the exact same way that standard GMOs are created. The inserted cassette produces the CRISPR-Cas9 complex of protein and RNA that performs the genetic change. Afterwards, the inserted CRISPR DNA cassette may then be bred out via conventional breeding so the organism is no longer transgenic (i.e., it no longer contains genes from another species). The gene-edited high fiber wheat produced by Calyxt, a biotech startup, was developed in this way.[80] However, it is inevitable that not all the inserted DNA will

The most common trait for gene-edited plants is herbicide tolerance.

always be removed. Despite this, no procedures, safety-related or otherwise, are in place to evaluate with this eventuality.[81]

In some gene-edited plants, the CRISPR DNA cassette is introduced into the organism's cell and performs the gene editing without becoming integrated with the organism's own genome, as is claimed with DuPont's gene-edited waxy corn.[82] However, the introduced DNA could unintentionally become integrated, at random, into the organism's genome.[83]

When DNA is inserted into an organism's genome, the insertion may not be precise. Whether the DNA is intentionally or unintentionally inserted, multiple copies and additional fragments of the DNA cassette can be introduced into the organism's genome.[84] The insertion of DNA can also cause sections of the organism's own DNA to become rearranged, as has often happened with standard genetically engineered crops.[85] Even though the inserted DNA may be subsequently removed through conventional breeding, it is especially possible that fragments could remain undetected and rearrangements of the organism's own DNA could persist. Additional fragments and rearrangements of DNA could give rise to unexpected effects in gene-edited organisms, creating the same concerns as current GMOs. For example, the gene editing could have implications for food and environmental safety if it alters the chemistry (and therefore the toxicity) or the protein composition (and therefore allergenicity) of the organism.

Potential effects of gene-edited organisms on biodiversity and the environment

There are many "proof of concept" publications about what gene editing might achieve, but none of the potential products from gene editing have been examined for what their engineered trait (or any unexpected effects) might mean for the environment and biodiversity. There are large gaps in the scientific knowledge. As with standard genetically engineered crops, there are many concerns regarding negative impacts on biodiversity, including the effects of genetically engineered crops on butterflies[86] and increased use of herbicides such as glyphosate.[87] Unfortunately, many warnings about standard GMOs' negative impacts on the environment and biodiversity have been ignored. For example, warnings from scientists about the inevitable rise of glyphosate (the active ingredient in Roundup) tolerant weeds and diminishing biodiversity in agricultural fields[88] associated with the use of Roundup Ready genetically engineered crops[89] went unheeded by regulators. The warnings that genetically engineered crops could not be controlled have played out with ongoing contamination of food from genetically engineered crops worldwide.[90] Such warnings must not go unheeded with gene-edited organisms.

It's important that any potential impacts on biodiversity from the engineered trait(s) in gene-edited crops are evaluated prior to being grown outdoors. For example, how might changes such as flowering time impact pollinators that rely on flower nectar for food? Such effects are important as many crop species are essential for agricultural biodiversity and pollinators are important for agriculture.[91] In addition, many crops have wild relatives, and these traits could contaminate those wild relatives.[92] If the trait was to produce a toxic compound, e.g., as an insecticide, this could impact biodiversity if the trait became prevalent in the wild. What might be the impacts of using gene editing in combination with standard genetic engineering in plant crops? Scientists do not yet fully understand the effects of stacking several genetically engineered traits (e.g. tolerance to multiple herbicides and/or multiple types of insect resistance) into a single variety, including how the combinations affect toxicity to wildlife and how all the new genes resulting from genetic engineering interact with one another.[93]

Small changes — big effects?

The changes to genetic material induced by some types of gene editing techniques (types SDN1 and SDN2) are sometimes described as "mutations"[94] because only very small parts of DNA (one or a few base pairs) are altered. However, small changes can have big effects. For example, in humans, the disorder known as sickle cell anemia is caused by a single change (a point mutation to a single base pair) in the person's DNA.[95] Although mutations do occur naturally, and indeed are an important source of genetic variability in breeding plants and animals, it doesn't follow that a gene-edited organism with only a small change to the organism's DNA is always "safe". To evaluate environmental and food safety, the changes to DNA (both intended and unintended) would have to be carefully evaluated.

Although mutations do occur naturally, and indeed are an important source of genetic variability in breeding plants and animals, it doesn't follow that a gene-edited organism with only a small change to the organism's DNA is always "safe".

Gene editing (types SDN1 and SDN2, with no foreign genes inserted) could result in greater changes to the genome than just one or a few base pairs if it were to be applied repeatedly, targeted at several genes at once or if the various techniques were used in combination.[96] For gene editing type SDN3, with genes inserted, it is conceivable that not only could several functional genes be inserted at once, but the changes could result in extensive changes to the genome, so it becomes almost unrecognizable compared to the original organism. Such extensive changes would fall within the scope of "synthetic biology"[97] and have, so far, been achieved for simple organisms, such as bacteria and yeast.[98] Therefore, even small edits produced by gene editing techniques can be significant and could potentially result in big changes.

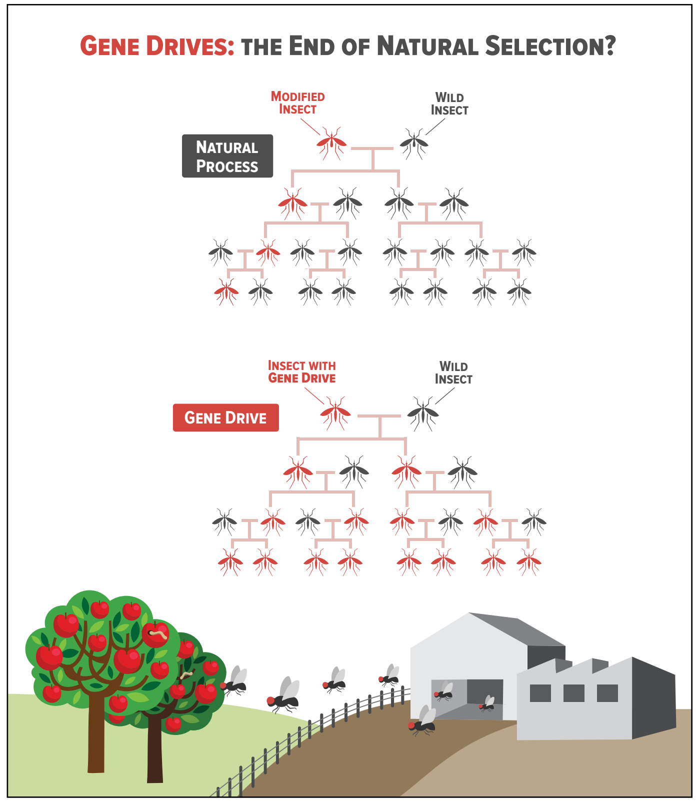
Gene drives

Gene editing techniques, particularly CRISPR (SDN3) systems, have facilitated the possibility of "genes drives." With gene drive systems, a few gene-edited individuals are used to spread new genes through the entire population of a species.[99] The gene drive mechanism ensures that the specified new genes will be inherited by every single offspring (as opposed to an expected half of the offspring in normal inheritance) in each subsequent generation.[100] Examples of proposed gene drive systems include altering genes to prevent mosquitoes from reproducing effectively, reducing the size of mosquito populations,[101] and a reduction in the susceptibility of mosquitoes to becoming infected with the malarial parasite[102]. In agriculture, potential gene drive applications include altering genes so that agricultural pests such as a type of fruit fly (spotted wing drosophila)[103] and pigweed (Palmer amaranth)[104] don't reproduce effectively, suppressing their population numbers. Such potential gene drives are currently at the "proof of concept" stage, but there is a concerted research effort into this field.[105]

Once released, gene drive organisms cannot be recalled and any changes to the genetic make-up of the population they induce are most likely irreversible. Hence, the genetic changes to a population are likely to persist for a very long time, possibly permanently. Alongside concerns about who defines what is and is not an agricultural "pest,"[106] scientists are already warning[107] that the consequences of gene drives could be severe should any unexpected effects (for example, those arising from the off-target effects of gene editing[108]) or unintended consequences arise.[109]

The National Academy of Sciences[110] warns that: *"Gene drives developed for agricultural purposes could also have adverse effects on human wellbeing. Transfer of a suppression drive to a non-target wild species could have both adverse environmental outcomes and harmful effects on vegetable crops ... for example, Palmer amaranth is a damaging weed in the United States, but a related Amaranthus species, with which Palmer amaranth can interbreed, is cultivated for food in Mexico, South America, India, China, and Africa. The escape of a suppression drive in Palmer amaranth could affect non-targeted species and negatively impact valued Amaranthus vegetable crops. There are currently no national regulatory mechanisms worldwide that adequately address field testing and environmental releases of gene-drive modified organisms."*

Gene drives have raised significant concerns among scientists and there have been calls for an international moratorium on gene drive research.[111] Although only currently at the "proof of concept" stage, concerns are so high among scientists, there is widespread agreement that even research into gene drive systems requires some form of international regulation.[112] The United Nations Convention on Biodiversity is considering how to address the issue of gene drives and their possible adverse environmental impacts.[113] However, it is not yet clear whether gene drives for either insects (e.g., mosquitoes) or agricultural plant pests will be regulated at all within the U.S.[114]



Gene drive organisms cannot be recalled as they are designed to irreversibly change the make-up of a population, giving scientists cause for alarm.

The need for regulatory oversight of gene-edited plants and animals in agriculture

Currently, there is international debate about how gene-edited plants and animals in agriculture should be regulated.[115] Gene editing in agriculture is new. It has only recently become commercially feasible, with the CRISPR technique dating from approximately 2012[116] and only a few commercialized products, currently limited to plants. Many other countries and regions are in the process of revising GMO regulations to account for gene-edited organisms. Gene-edited plants with genes from another species inserted (type SDN3 gene-editing) into them are generally regarded as GMOs by regulatory authorities around the world, as they are very similar to transgenic organisms.[117] However, there is deliberation in several countries about whether gene-edited organisms which are not transgenic but which have "edited" DNA (type SDN1 and SDN2 gene editing) should be regulated in the same way as GMOs produced from standard genetic engineering techniques. For example, in the EU, the European Court of Justice ruled that all gene-edited organisms (including those developed by ODM, SDN1 and SDN2) will be included within the European GMO regulations.[118] Australia undertook a public consultation during late 2017/early 2018[119] and appears likely to regulate gene-edited organisms developed using SDN2 as GMOs, but the situation regarding SDN1 is unclear[120]. In Argentina, regulators are employing a case-by-case regulation dependent on whether there is a novel combination of genetic material.[121] For Canada, all organisms with "novel traits" are regulated under novel food guidelines which require assessment of all novel food products, whether produced by conventional breeding or genetic engineering, including gene editing.[122]

In the U.S., the USDA has decided that for genetically engineered plants, it *"does not regulate or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques as long as they are not plant pests or developed using plant pests."*[123] Gene-edited crops may not be classified as plant pests, nor developed from plant pests, and it is usually unclear whether they could have been developed by standard (conventional) breeding. In this way, gene-edited crops are evading regulatory oversight in the U.S.

Normally in the U.S., the developer of a genetically engineered organism would be required to submit a detailed application for the organism to become deregulated (i.e., cultivated without any further notification). However, since 2011, it is estimated that more than 30 transgenic organisms, mostly plants,[124] and at least six gene-edited plants (see below) have been able to bypass USDA oversight. The USDA arbitrarily regards these organisms as not requiring regulation because they do not involve genes from known plant pests or are not themselves known problematic plants.[125] Gene-edited crops that have bypassed USDA oversight[126] include: soybean with drought and salt tolerance, camelina (false flax, similar to canola) with increased oil content, green bristlegrass with delayed flowering time, waxy corn with altered starch composition, white button mushroom with anti-browning properties[127] and high fiber wheat.[128] Although these crops may or may not undergo a voluntary food safety assessment by the FDA,[129] their status of being non-regulated means that they can be grown outdoors in the open environment without any environmental safety assessment.

The lack of an environmental safety assessment for gene-edited plants raises the possibility that any unexpected and unintended effects present could cause adverse effects on the environment and biodiversity. Such unexpected or unintended effects might go unnoticed by the developers of the product. For exampzle, researchers in the UK using standard genetic engineering techniques to produce genetically engineered plants with omega-3 oils suddenly became concerned after it was found the omega-3 oils unexpectedly produced toxic effects on caterpillar larvae, deforming wings in the adult butterfly.[130] Such an unexpected but potentially significant effect would almost certainly go unnoticed if there was no requirement to perform any kind of environmental safety assessment on gene-edited crops. The exemption of gene editing techniques in environmental safety assessments could have far-reaching negative consequences. For example, growing gene-edited plants outdoors allows them to cross-pollinate with neighboring crops or their wild relatives, facilitating the spread of their altered genes.

Many scientists are alarmed that gene-edited plants are unregulated in the U.S. As one scientific journal discussed: *"The approach to oversight of GM crops at the US Department of Agriculture shows how a regulatory system can stray from science. GM crop regulations at that agency depend on its authority to control*

The exemption of gene editing techniques in environmental safety assessments could have far-reaching negative consequences.

Gene-edited organisms that do not contain foreign genes or express a novel protein cannot be considered "safe" for the environment or food; unintended effects arising from the gene editing process and any new trait must be carefully considered.

plant pests and noxious weeds. It is a system that had some relevance to the first generation of such crops, many of which were designed using genetic elements from plant pathogens. It is rapidly losing relevance in the face of NBTs [New Breeding Technologies]. In more than two dozen cases, the agency has determined that a particular NBT plant variety does not fall under its purview for regulation because it does not entail the use of a plant pest and is unlikely to yield a noxious weed. These might have been scientifically sound decisions, but they were not made for scientifically sound reasons."[131]

It is not clear whether gene-edited animals would be examined for any potential environmental impacts in the U.S. because there are no specific references to genetically engineered animals in the EPA regulations. However, they would be assessed for food safety under the FDA.[132]

Since its inception, U.S. regulatory oversight for GMOs has been repeatedly criticized for failing to robustly assess the risks of GMOs.[133] Robust regulatory oversight is essential to ensure that the risks of gene-edited organisms are considered prior to them entering the environment or the food chain. Detailed analyses of any unintended effects, inserted genes and new trait(s) in gene-edited organisms are required. Gene-edited organisms that do not contain foreign genes or express a novel protein cannot be considered "safe" for the environment or food; unintended effects arising from the gene editing process and any new trait must be carefully considered.

One of the primary concerns for the food safety of GMOs, including gene-edited organisms, is whether there is any unintended alteration to protein composition. This is because allergens are proteins, so any inadvertent changes in protein composition could cause the gene-edited plant or animal to trigger allergies in humans (or animals) when eaten.[134] In the assessment of gene-edited organisms, all hazards need to be identified, no matter how theoretical they might initially appear. Otherwise, there is a danger that potentially damaging impacts could be overlooked. For example, concerns regarding the negative impacts of genetically engineered Bt crops on biodiversity were only articulated a few years after Bt corn had been commercialized[135] and it was later observed that unexpected changes in the chemistry of genetically engineered Bt corn increased the attractiveness of the Bt corn to aphid pests.[136]

Regulatory oversight is essential to ensure that potential risks of gene-edited organisms are considered prior to them entering the environment or food chain.

Gene-edited organisms require careful examination for unintended effects if they are to be used in agriculture.

In order to avoid any negative impacts on food and environmental safety from gene-edited organisms, there is a need for comprehensive scientific examination of all the potential risks prior to environmental release or entry into the food chain.

Proposals for the regulation of gene-edited organisms in agriculture range from ensuring that they are regulated as genetically engineered organisms[137] to, at the very least, examining products from gene editing to see if they contain any DNA sequences from the gene editing process[138]. In order to avoid any negative impacts on food and environmental safety from gene-edited organisms, there is a need for comprehensive scientific examination of all the potential risks prior to environmental release or entry into the food chain. These scientific findings should inform regulatory and oversight requirements. In particular, the Precautionary Principle needs to be applied, meaning that full scientific certainty of a possible harm is not needed before preventative action is taken.

In the U.S., it is still unclear whether gene-edited food will be labeled. In the proposed U.S. labeling regulations[139] for food made from genetically engineered ingredients, the term "bioengineering" refers to genetic material with inserted genes and could exclude a gene-edited food ingredient if the genetic material was "edited" without genes being inserted. Although the proposal is seeking feedback on whether or not to include food produced by gene editing, there is a risk that gene-edited food will go unlabeled as bioengineered, genetically engineered or GMO, disempowering the consumers seeking non-genetically engineered foods. The issue of labeling is also important because the international body for organic agriculture excludes genetically engineered (including gene-edited) organisms from organic systems.[140] Without labeling, it will be impossible to know whether food is gene-edited or conventionally bred.

Regulation and governance are more than science

This report largely focuses on the scientific aspects of gene editing in agriculture, such as the risks and potential consequences to food safety and biodiversity. However, the use (or non-use) of gene-edited organisms in agriculture also depends on societal values. There is a growing awareness that a science-based risk assessment for gene-edited organisms is limited in scope.[141] Proposals to expand the scope of the regulations and governance of GMOs beyond scientific concerns include: recognition of the underlying values and assumptions shaping science and innovation, respect for ethical, societal and cultural values, ensuring the sustainability of agricultural systems and the consideration of a range of alternatives to genetically engineered food. [142] One study offers that expanding the scope of governance necessitates the involvement of a broad range of people from different societal sectors to manage the complexity of issues, forming what has been termed a "cooperative governance network."[143]

There are alternatives to gene editing in the development of new varieties of plants and animals. Conventional breeding has progressed greatly in the past decade,[144] aided by knowledge of DNA and genomes but using this knowledge to assist, rather than replace, conventional breeding. Techniques such as genomic selection and marker-assisted selection allow the selection of desirable traits in plant or animal offspring from conventional breeding based on DNA analysis, greatly speeding up the development of new varieties. [145] These approaches have already produced disease-resistant crops,[146] flood tolerant rice[147] and crops with increased yield[148]. These same approaches are also used in cattle, pig and chicken breeding[149]. It is key to address the question of whether gene editing in agriculture is necessary to begin with.

Conclusion and recommendations

Gene editing encompasses a suite of new genetic engineering techniques that are being applied to living organisms intended to be used in agriculture. Robust scientific studies document that gene-edited organisms, like standard GMOs, are prone to unexpected and unpredictable effects arising from genetic errors including: off-target effects, on-target effects, interference with genetic regulations and both the intended and unintended insertion of DNA. The engineered trait may also show unexpected interactions with the environment. Applications like gene drives pose particular risks as there can be no recall of such gene-edited organisms and the genetic changes they drive through a population are intended to be permanent.

Despite the significant body of scientific literature demonstrating unintended consequences from gene editing, gene-edited plants are evading regulatory oversight in the U.S. and may already be cultivated in the environment without any safety assurances. There is a risk that food derived from gene-edited crops and animals may go unlabeled, disempowering consumers seeking non-genetically engineered foods and subjecting them to risks such as allergens.

Given the scientific findings of potential unintended consequences from gene editing and the lack of studies concerning health and ecological impacts, government regulatory oversight of all genetic engineering techniques should follow the Precautionary Principle. The products of all genetic engineering techniques (both standard and gene-edited) should be independently assessed for food and environmental safety and other impacts prior to being released into the environment or marketed. They should also be traceable and labeled as GMOs.

While it is critical for more scientific studies to be conducted about the specific impacts of the unintended consequences of gene editing on agricultural systems, ecological systems, human and animal health, the discussion regarding the use of gene editing in agriculture also needs to go further than a science-based risk assessment to encompass wide public discussion about the future of agriculture.

Suggestions for further reading

- Overview of gene editing techniques, their regulation and consumer concerns: Trans Atlantic Consumer Dialogue (2016) Resolution on consumer concerns about new genetic engineering techniques. http://tacd.org/new-policy-resolution-on-consumer-concerns-about-new-genetic-engineering-techniques/
- Off-target effects generated by CRISPR in plants: Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835. https://dl.sciencesocieties.org/publications/tpg/articles/9/3/plantgenome2016.05.0047
- Potential environmental and social impacts of agricultural pest control using gene drives: Courtier-Orgogozo, V., Morizot, B. & Boëte, C. (2017) Agricultural pest control with CRISPR based gene drive: time for public debate. EMBO Reports 18: 878-880. http://embor.embopress.org/content/18/6/878
- Involvement of society and reflection of societal values in the regulation of gene-edited organisms: Hartley, S., Gillund, F., van Hove, L. & Wickson, F. (2016) Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453. http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002453
  Sarewitz, D. (2015) Science can't solve it. Nature. 522: 412–413. https://www.nature.com/news/crispr-science-can-t-solve-it-1.17806

References

1 Begley, S. (2018) Potential DNA damage from CRISPR "seriously underestimated," study finds. July 16 2018. Retrieved from https://www.scientificamerican.com/article/potential-dna-damage-from-crispr-seriously-underestimated-study-finds/.

2 DNA stands for deoxyribonucleic acid. DNA contains the genetic information and is present in every cell in every living organism and many viruses. The basic building block of DNA is a pair of amino acids, known as a base-pair.

3 Kosicki, M., Tomberg, K. & Bradley, A. (2018) Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nature Biotechnology 36: 765-771.

4 Begley, S. (2018) A serious new hurdle for CRISPR: edited cells might cause cancer, two studies find. June 11 2018. Retrieved from https:/www.statnews.com/2018/06/11/crispr-hurdle-edited-cells-might-cause-cancer/

5 Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., & Taipale, J. (2018) CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nature Medicine 24: 927–930; Ihry, R.J. Worringer, K.A., Salick, M.R. et al. (2018) p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nature Medicine 24: 939-946.

6 Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107.

7 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9.

8 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Kaskey, J. (2018) BASF to crank up R&D `two gears' with Bayer seeds, next CEO says. Bloomberg Technology April 12 2018. Retrieved from https://www.bloomberg.com/news/articles/2018-04-12/basf-to-crank-up-r-d-two-gears-with-bayer-seeds-next-ceo-says

9 Hixson, S.M., Shukla, K., Campbell, L.G., Hallett, R.H., Smith, S.M., Packer, L. & Arts, M.T. (2016) Long-chain omega-3 polyunsaturated fatty acids have developmental effects on the crop pest, the cabbage white butterfly *Pieris rapae.* PLoS ONE 11: e0152264.

10 Sauer, N.J., Narváez-Vásquez, J., Mozoruk, J. et al. (2016) Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiology 170: 1917–1928; Hartung, F. & Schiemann, J. (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. The Plant Journal 78: 742–752; Voytas, D.F. & Gao, C. (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biology 12: e1001877.

11 Waltz, E. (2016) Gene-edited CRISPR mushroom escapes US regulation. Nature (news) 532: 293.

12 Cibus (2014) Cibus announces approval of first commercial product SU Canola™ in Canada. Press Release 18 March 2014. Retrieved from https://www.cibus.com/press_release.php?date=031814

13 The genome is the complete set of DNA, including genes, in an organism.

14 Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., & Taipale, J. (2018) CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nature Medicine 24: 927–930

15 US National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. Retrieved from https://www.nap.edu/download/23405

16 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

17 Hammond, A.M. & Galizi, R. (2017) Gene drives to fight malaria: current state and future directions. Gene drives to fight malaria: current state and future directions. Pathogens and Global Health 111: 412-423; Hammond, A., Galizi, R., Kyrou, K. et al. (2016) A CRISPR–Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotechnology 34: 78–83; National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

18 Buchman, A., Marshall, J.M., Ostrovski, D., Yang, T. & Akbari. O.S. (2018) Synthetically engineered *Medea* gene drive system in the worldwide crop pest *Drosophila suzukii*. Proceedings of the National Academy of Sciences 115: 4725–4730.

19 Anon (2017) Drive safely. Nature (editorial) 552: 6.

20 Taning, C.N.T., Van Eynde, B., Yu, N., Ma. S. & Smagghe, G. (2017) CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. Journal of Insect Physiology 98: 245–257; Courtier-Orgogozo, V., Morizot, B. & Boëte, C. (2017) Agricultural pest control with CRISPR based gene drive: time for public debate. EMBO Reports 18: 878-880; US National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405; Esvelt, K.M., Gemmell, N.J. (2017) Conservation demands safe gene drive. PLoS Biology 15: e2003850. DeFrancesco, L. (2015) Gene drive overdrive. Nature Biotechnology 33: 1019-1021.

21 The Precautionary Principle underlies the Precautionary Approach, defined as "*In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.*" United Nations (1992). Rio Declaration on Environment and Development. Retrieved from https://www.cbd.int/doc/ref/rio-declaration.shtml

22 USDA Press (2018) Secretary Perdue Issues USDA Statement on Plant Breeding Innovation. USDA.gov. Retrieved from https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation

23 Center for Veterinary Medicine (2017) Animals with Intentionally Altered Genomic DNA. FDA.gov. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/default.htm

24 Agricultural Marketing Service (2018) National Bioengineered Food Disclosure Standard. Retrieved from https://www.regulations.gov/document?D=AMS_FRDOC_0001-1709

25 European Court of Justice (2018) Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive, Case C-528-16. ECLI:EU:C:2018:20. Retrieved on July 31, 2018 from https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf

26 McCouch, S. Gregory, G.J., Bradeen, J. et al. (2013) Agriculture: feeding the future, comments and opinion, Nature. https://doi.org/10.1038/499023a.

27 Crossa, J., Pérez-Rodríguez, P., Cuevas, J. et al. (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends in Plant Science 22: 961–975.

28 E.g., Jackson, L. (2011) Wheat cultivars for California. Retrieved from http://smallgrains.ucdavis.edu/cereal_files/WhtCV-DescLJ11.pdf

29 Meuwissen, T., Hayes, B & Goddard, M. (2016) Genomic selection: a paradigm shift in animal breeding. Animal Frontiers 6: 6–14.

30 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Kaskey, J. (2018) BASF to crank up R&D `two gears' with Bayer seeds, next CEO says. Bloomberg Technology April 12 2018. Retrieved from https://www.bloomberg.com/news/articles/2018-04-12/basf-to-crank-up-r-d-two-gears-with-bayer-seeds-next-ceo-says

31 Li, Y. (2018) These CRISPR-modified crops don't count as GMOs. UConn Today. Retrieved from https://today.uconn.edu/2018/05/crispr-modified-crops-dont-count-gmos-2/#

32 Molteni, M. (2017) This gene editing tech might be too dangerous to unleash. Wired. Retrieved from https://www.wired.com/story/this-gene-editing-tech-might-be-too-dangerous-to-unleash/

33 Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Science Europe 28: 30.

34 E.g., Future Farming (2017) Gene editing: what can it deliver for agriculture? Retrieved from https://www.futurefarming.com/Smart-farmers/Articles/2017/9/Gene-editing-what-can-it-deliver-for-agriculture-3079WP/

35 DNA stands for deoxyribonucleic acid. DNA contains the genetic information and is present in every cell in every living organism and many viruses. The basic building block of DNA is a pair of amino acids, known as a base-pair.

36 Waltz, E. (2018) With a free pass, CRISPR-edited plants reach market in record time. Nature Biotechnology 36: 6-7.

37 The Precautionary Principle underlies the Precautionary Approach, defined as "*In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.*" United Nations (1992) Rio Declaration on Environment and Development. Retrieved from https://www.cbd.int/doc/ref/rio-declaration.shtml

38 Shou, H., Frame, B.R., Whitham, S.A. & Wang, K. (2004) Assessment of transgenic maize events produced by particle bombardment or *Agrobacterium*-mediated transformation. Molecular Breeding 13: 201–208.

39 Conventional breeding is termed 'traditional breeding' by USDA. USDA (n.d.) Agricultural Biotechnology Glossary. Retrieved from https://www.usda.gov/topics/biotechnology/biotechnology-glossary

40 USDA (n.d.) Agricultural Biotechnology Glossary. Retrieved from https://www.usda.gov/topics/biotechnology/biotechnology-glossary; FDA (U.S.) (2015) Guidance for industry: voluntary labeling indicating whether foods have or have not been derived from genetically engineered plants. Retrieved from https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm059098.htm

41 United Nations Cartagena Protocol on Biosafety (2000) Article 3 Use of Terms. Retrieved from http://bch.cbd.int/protocol/text/; Codex Alimentarius Commission (2003) Principles for the risk analysis of foods derived from modern biotechnology CAC/GL 44-2003 (amended 2008 and 2011). Retrieved from http://www.codexalimentarius.net/download/standards/10007/CXG_044e.pdf

42 European Commission (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L106: 1-38.

43 CRISPR stands for 'clustered regularly interspaced short palindromic repeat'. Other genome-editing techniques include TALEN (transcription activator-like effector nucleases); ZFN (zinc-finger nucleases), meganucleases and ODM (oligonucleotide-directed mutagenesis).

44 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Sander, J.D. & Joung, J.K. (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotechnology 32: 347–355.

45 There is no insertion of genes with gene-editing types SD1 and SDN2.

46 CRISPR (clustered regularly interspaced short palindromic repeat); TALEN (transcription activator-like effector nucleases); ZFN (zinc-finger nucleases).

47 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Sander, J.D. & Joung, J.K. (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotechnology 32: 347–355.

48 Zetsche, B., Heidenreich, M., Mohanraju, P. et al. (2017) Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nature Biotechnology 35: 31–34; Gao, C. (2018) The future of CRISPR technologies in agriculture. Nature Reviews Molecular Cell Biology 19: 275–276.

49 Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I. & Liu, D.R. (2017) Programmable base editing of A-T to G-C in genomic DNA without DNA cleavage. Nature 551: 464–471; Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A. & Liu, D.R. (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533: 420–424; Gao, C. (2018) The future of CRISPR technologies in agriculture. Nature Reviews Molecular Cell Biology 19: 275–276.

50 Sauer, N.J., Narváez-Vásquez, J., Mozoruk, J. et al. (2016) Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiology 170: 1917–1928.

51 West, J. & Gill, W.W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

52 Ishii, T. (2017) Genome-edited livestock: Ethics and social acceptance. Animal Frontiers 7: 24–32.

53 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9.

54 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Kaskey, J. (2018) BASF to crank up R&D `two gears' with Bayer seeds, next CEO says. Bloomberg Technology April 12. Retrieved from https://www.bloomberg.com/news/articles/2018-04-12/basf-to-crank-up-r-d-two-gears-with-bayer-seeds-next-ceo-says

55 Cibus (2014) Cibus announces approval of first commercial product SU Canola™ in Canada. Press Release March 18 2014. Retrieved from https://www.cibus.com/press_release.php?date=031814.

56 Sauer, N.J., Narváez-Vásquez, J., Mozoruk, J. et al. (2016) Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiology 170: 1917–1928; Hartung, F. & Schiemann, J. (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. The Plant Journal 78: 742–752; Voytas, D.F. & Gao, C. (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biology 12: e1001877.

57 Wilson, A.K., Latham. J.R. & Steinbrecher, R.A. (2006) Transformation-induced mutations in transgenic plants: analysis and biosafety implications. Biotechnology and Genetic Engineering Reviews 23: 209-237.

58 Wilson, A.K., Latham. J.R. & Steinbrecher, R.A. (2006) Transformation-induced mutations in transgenic plants: analysis and biosafety implications. Biotechnology and Genetic Engineering Reviews 23: 209-237.

59 Jung, C., Capistrano-Gossmann, G., Braatz, J., Sashidhar, N. & Melzer, S. (2017) Recent developments in genome editing and applications in plant breeding. Plant Breeding 137: 1-9; Zhu, C., Bortesi, L., Baysal, C., Twyman, R.M., Fischer, R., Capell, T., Schillberg, S. & Christou, P. (2017) Characteristics of genome editing mutations in cereal crops. Trends in Plant Science 22: 38–52; Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107; West, J. & Gill, W.W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

60 Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107.

61 Wang, G., Du, M., Wang, J & Zhu, T.F. (2018) Genetic variation may confound analysis of CRISPR-Cas9 off-target mutations. Cell Discovery 4:18; Klein, M. Eslami-Mossallam, B., Arroyo, D.G. & Depken, M. (2018) Hybridization kinetics explains CRISPR-Cas off-targeting rules. Cell Reports 22: 1413–1423; Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047.

62 Zhu, C., Bortesi, L., Baysal, C., Twyman, R.M., Fischer, R., Capell, T., Schillberg, S. & Christou, P. (2017) Characteristics of genome editing mutations in cereal crops. Trends in Plant Science 22: 38–52.

63 Wolt, J.D., Wang, K., Sashital, D. & Lawrence-Dill, C.J. (2016) Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107.

64 Zhang, Y, Liang, Z., Zong, Y., Wang1, Y., Liu, J., Chen, K., Qiu, J-L. & Gao, C. (2016) Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nature Communications 7: 12617.

65 West, J. & Gill, W.W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6; Ryu, J., Prather, R.S. & Lee, K. (2018) Use of gene-editing technology to introduce targeted modifications in pigs. Journal of Animal Science and Biotechnology 9: 5.

66 Anderson, K.R., Haeussler, M., Watanabe, C. et al. (2018) CRISPR off-target analysis in genetically engineered rats and mice. Nature Methods 15: 512–514; Shin, H.Y., Wang, C., Lee, H.K., Yoo, K.H., Zeng, X., Kuhns, T., Yang, C.M., Mohr, T., Liu, C. & Hennighausen, L. (2017) CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nature Communications 8: 15464.

67 Carroll, D. (2013) Staying on target with CRISPR-Cas. Nature Biotechnology (News and Views) 31: 807-809.

68 E.g., Wang, X. & Chen, Y. (2016) P7003 Heritable multiplex gene editing via CRISPR/Cas9 exhibits no detectable genome-wide off-target effects in sheep. Journal of Animal Science 94: 177.

69 Wang, G., Du, M., Wang, J & Zhu, T.F. (2018) Genetic variation may confound analysis of CRISPR-Cas9 off-target mutations. Cell Discovery 4: 18.

70 RNA stands for ribonucleic acid. In a cell, there are different types of RNA including messenger RNA that carries genetic information from DNA that directs the production of proteins.

71 Kosicki, M., Tomberg, K., Bradley, A. (2018) Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nature Biotechnology 36: 765-771.

72 Sharpe, J.J. & Cooper, T.A. (2017) Unexpected consequences: exon skipping caused by CRISPR-generated mutations. Genome Biology 18: doi: 10.1186/s13059-017-1240-0; Mou, H., Smith, J.L., Peng, L. et al. (2017) CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. Genome Biology 18: doi: 10.1186/s13059-017-1237-8; Lalonde, S., Stone, O.A., Lessard, S., Lavertu, A., Desjardins, J., Beaudoin, M., Rivas, M., Stainier, D.Y.R. & Lettre, G. (2017) Frameshift indels introduced by genome editing can lead to in-frame exon skipping. PLoS ONE 12: e0178700; Kapahnke, M., Banning, A. & Tikkanen, R. (2016) Random splicing of several exons caused by a single base change in the target exon of CRISPR/Cas9 mediated gene knockout. Cells 5: 45.

73 McClain, S., Bowman, C., Fernández-Rivas, M., Ladics, G.S. & van Ree, R. (2014) Allergic sensitization: food- and protein-related factors. Clinical and Translational Allergy 4: 11.

74 Bucchini, L. & Goldman, L.R. (2002) Starlink corn: a risk analysis. Environmental Health Perspectives 110: 5–13.

75 See Waterhouse, P.M. & Hellens, R.P. (2015) Coding in non-coding RNAs. Nature 520: 41-42; Holoch, D. & Moazed, D. (2015) RNA-mediated epigenetic regulation of gene expression. Nature Reviews Genetics 16: 71-84.

76 Nilsen, T.W. & Graveley, B.R. (2010) Expansion of the eukaryotic proteome by alternative splicing. Nature 463: 457-463.

77 Biémont, C. & Vieira, C. (2006) Genetics: Junk DNA as an evolutionary force. Nature 443: 521-524.

78 Doolittle, W.F. (2012) Is junk DNA bunk? A critique of ENCODE. Proceedings of the National Academy of Sciences 110: 5294–5300; Kellis, M., Wold, B., Snyder, M.P. et al. (2014) Defining functional DNA elements in the human genome. Proceedings of the National Academy of Sciences 111: 6131–6138.

79 Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., & Taipale, J. (2018) CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nature Medicine 24: 927–930; Ihry, R.J. Worringer, K.A., Salick, M.R. et al. (2018) p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nature Medicine 24: 939-946.

80 USDA (2018) Request for regulatory status of "Nutritionally-enhanced wheat". Letter to Calyxt March 20 2018. Retrieved from https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/17-038-01_air_response_signed.pdf

81 Kim, J. & Kim, J-S. (2017) Bypassing GMO regulations with CRISPR gene editing. Nature Biotechnology (correspondence) 34: 1014-1015.

82 Dupont Pioneer (2015) Confirmation of regulatory status of waxy corn developed by CRISPR-Cas technology. Letter to USDA-APHIS. Retrieved from https://www.pioneer.com/CMRoot/Pioneer/About_Global/Non_Searchable/news_media/15-352-01_air_inquiry_cbidel.pdf

83 Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q., Liu, J., Zhang, H., Liu, C., Ran, Y. & Gao, C. (2017) Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nature Communications 8: 14261; Li, Z., Liu, Z.-B., Xing, A., Moon, B.P., Koellhoffer, J.P., Huang, L., Ward, R.T., Clifton, E., Falco, S.C., Cigan, A.M. (2015) Cas9-guide RNA directed genome editing in soybean. Plant Physiology 169 960–970; Dupont Pioneer (2015) Confirmation of regulatory status of waxy corn developed by CRISPR-Cas technology. Letter to USDA-APHIS. Retrieved from https://www.pioneer.com/CMRoot/Pioneer/About_Global/Non_Searchable/news_media/15-352-01_air_inquiry_cbidel.pdf

84 Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q., Liu, J., Zhang, H., Liu, C., Ran, Y. & Gao, C. (2017) Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nature Communications 8: 14261; Li, Z., Liu, Z.-B., Xing, A., Moon, B.P., Koellhoffer, J.P., Huang, L., Ward, R.T., Clifton, E., Falco, S.C. & Cigan, A.M. (2015) Cas9-guide RNA directed genome editing in soybean. Plant Physiology 169: 960–970.

85 Windels, P., Taverniers, I. Depicker, A. Van Bockstaele, E. & De Loose, M. (2001) Characterisation of the Roundup Ready soybean insert. European Food Research Technology 213: 107-112; Rang, A., Linke, B. & Jansen, B. (2005) Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. European Food Research Technology 220: 438-443; Hernández, M., Pla, M., Esteve, T., Prat, S., Puigdomènech, P. & Ferrando, A. (2003) A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3'-transgene integration sequence. Transgenic Research 12: 179–189; Wilson, A.K., Latham. J.R. & Steinbrecher, R.A. (2006) Transformation-induced mutations in transgenic plants: analysis and biosafety implications. Biotechnology and Genetic Engineering Reviews 23: 209-237.

86 Pleasants, J.M., Zalucki, M.P., Oberhauser, K.S., Brower, L.P., Taylor, O.R. & Thogmartin, W.E. (2017) Interpreting surveys to estimate the size of the monarch butterfly population: pitfalls and prospects. PLoS ONE 12: e0181245; Pleasants, J.M. & Oberhauser, K.S. (2013) Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conservation and Diversity 6: 135–144; Lang, A. & Otto, M. (2010) A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera. Entomologia Experimentalis et Applicata 135: 121–134.

87 Schütte, G. Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassalli, S., Ruohonen-Lehto, M., Saucy, A-G.W. & Mertens, M. (2017) Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. Environmental Science Europe 29: 5.; Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28: 3.

88 Heard, M.S., Hawes, C., Champion, G.T. et al. (2003) Weeds in fields with contrasting conventional and genetically modifies herbicide-tolerant crop – I. Effects on abundance and diversity. Philosophical Transactions of The Royal Society London B 358: 1819-1832; Heard, M.S., Hawes, C., Champion, G.T. et al. (2003) Weeds in fields with contrasting conventional and genetically modifies herbicide-tolerant crops. II. Effects on individual species. Philosophical Transactions of The Royal Society London B 358: 1833-1846; Roy, D.B., Bohan, D.A., Haughton, A.J. et al. (2003) Invertebrates and vegetation of the field margins adjacent to crops subject to contrasting herbicides regimes in the Farm Scale Evaluations of genetically modified herbicide –tolerant crops. Philosophical Translations of The Royal Society London B 358: 1879-1898;

89 Green, J.M. (2016) The rise and future of glyphosate and glyphosate-resistant crops. Pest Management Science 74: 1035-1039.

90 Price, B. & Cotter, J. (2014) The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. International Journal of Food Contamination 1: 5.

91 Calderone, N.W. (2012) Insect pollinated crops, insect pollinators and US Agriculture: trend analysis of aggregate data for the period 1992–2009. PLoS ONE 7: e37235.

92 Ellstrand, N.C. (2003) Dangerous Liaisons? When Cultivated Plants Mate with their Wild Relatives. Baltimore: Johns Hopkins University Press.

93 Bøhn, T. (2018) Criticism of EFSA's scientific opinion on combinatorial effects of 'stacked' GM plants. Food and Chemical Toxicology 111: 268–274; Hilbeck, A. & Otto. M. (2015) Specificity and combinatorial effects of *Bacillus Thuringiensis* cry toxins in the context of GMO environmental risk assessment. Frontiers in Environmental Science 3:71; Agapito-Tenfen, S-Z., Vilperte, V., Benevenuto, R.F., Rover, C.M., Traavik, T.I. & Nodari, R.O. (2014) Effect of stacking insecticidal *cry* and herbicide tolerance *epsps* transgenes on transgenic maize proteome. BMC Plant Biology 14: 346.

94 See Li, C., Unver, T. & Zhang, B. (2017) A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in cotton (*Gossypium hirsutum* L.). Nature Scientific Reports 7: 43902; Zhu, J., Song, N., Sun, S., Yang, W., Zhao, H., Song, W. & Lai, J. (2016) Efficiency and inheritance of targeted mutagenesis in maize using CRISPR-Cas9. Journal of Genetics and Genomics 43: 25-36.

95 Clancy, S. (2008) Genetic mutation. Nature Education 1: 187. https://www.nature.com/scitable/topicpage/genetic-mutation-441

96 Zetsche, B., Heidenreich, M., Mohanraju, P. et al. (2017) Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nature Biotechnology 35: 31–34; Wang, X. & Chen, Y. (2016) P7003 Heritable multiplex gene editing via CRISPR/Cas9 exhibits no detectable genome-wide off-target effects in sheep. Journal of Animal Science 94: 177, Raitskin, O. & Patron, N.J. Multi-gene engineering in plants with RNA-guided Cas9 nuclease. Current Opinion in Biotechnology 37: 69–75.

97 Friends of the Earth & ETC Group (2017) GMOs 2.0: Synthetic biology: a guide to protecting natural products. Retrieved from https://1bps6437gg8c169i0y1drtgz-wpengine.netdna-ssl.com/wp-content/uploads/2017/12/SynbioFreeCompanyGuide.pdf; ENSSER (2018) Products of new genetic modification techniques should be strictly regulated as GMOs. Retrieved from https://ensser.org/publications/ngmt-statement/; Secretariat of the Convention on Biological Diversity (2015) Synthetic Biology. CBD Technical Series no. 82, Montreal. Retrieved from https://www.cbd.int/ts/cbd-ts-82-en.pdf

98 Bao, Z., HamediRad, M., Xue, P., Xiao, H., Tasan, I., Chao, R., Liang, J. & Zhao, H. (2018) Genome-scale engineering of *Saccharomyces cerevisiae* with single-nucleotide precision. Nature Biotechnology 36: 505-508; Garst, A.D., Bassalo, M.C., Pines, G., Lynch, S.A., Halweg-Edwards, A.L., Liu, R., Liang, L., Wang, Z., Zeitoun, R., Alexander, W.G. & Gill, R.T. (2017) Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. Nature Biotechnology 35: 48-55.

99 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

100 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

101 Hammond, A.M. & Galizi, R. (2017) Gene drives to fight malaria: current state and future directions. Gene drives to fight malaria: current state and future directions. Pathogens and Global Health 111: 412-423; Hammond, A., Galizi, R., Kyrou, K. et al. (2016) A CRISPR–Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotechnology 34: 78–83; National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

102 Dong, Y., Simões, M.L., Marois, E. & Dimopoulos, G. (2018) CRISPR/Cas9 -mediated gene knockout of *Anopheles gambiae FREP1* suppresses malaria parasite infection. PLoS Pathogens 14: e1006898.

103 Buchman, A., Marshall, J.M., Ostrovski, D., Yang, T. & Akbari. O.S. (2018) Synthetically engineered *Medea* gene drive system in the worldwide crop pest *Drosophila suzukii*. Proceedings of the National Academy of Sciences 115: 4725–4730.

104 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

105 Courtier-Orgogozo, V., Morizot, B. & Boëte, C. (2017) Agricultural pest control with CRISPR based gene drive: time for public debate. EMBO Reports 18: 878-880.

106 Courtier-Orgogozo, V., Morizot, B. & Boëte, C. (2017) Agricultural pest control with CRISPR based gene drive: time for public debate. EMBO Reports 18: 878-880; National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

107 Taning, C.N.T., Van Eynde, B., Yu, N., Ma. S. & Smagghe, G. (2017) CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. Journal of Insect Physiology 98: 245–257; Courtier-Orgogozo, V., Morizot, B. & Boëte, C. (2017) Agricultural pest control with CRISPR based gene drive: time for public debate. EMBO Reports 18: 878-880; National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405; Esvelt, K.M. & Gemmell, N.J. (2017) Conservation demands safe gene drive. PLoS Biology 15: e2003850. DeFrancesco, L. 2015. Gene drive overdrive. Nature Biotechnology 33: 1019-1021; Latham, J. 2017. Gene drives: a scientific case for a complete and perpetual ban. GeneWatch 30-1. Retrieved from http://www.councilforresponsiblegenetics.org/GeneWatch/GeneWatchPage.aspx?pageId=583

108 Hammond, A.M. & Galizi, R. (2017) Gene drives to fight malaria: current state and future directions. Gene drives to fight malaria: current state and future directions. Pathogens and Global Health 111: 412-423.

109 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

110 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

111 Calloway, E. (2016) 'Gene drive' moratorium shot down at UN biodiversity meeting. Nature News. Retrieved from https://www.nature.com/news/gene-drive-moratorium-shot-down-at-un-biodiversity-meeting-1.21216

112 Anon (2017) Drive safely. Nature (editorial) 552: 6; Gemmell, N.J. (2017) Conservation demands safe gene drive. PLoS Biology 15: e2003850; Latham, J. (2017) Gene drives: a scientific case for a complete and perpetual ban. GeneWatch 30-1. Retrieved from http://www.councilforresponsiblegenetics.org/GeneWatch/GeneWatchPage.aspx?pageId=583

113 UN Convention on Biological Diversity (CBD) (2016) Decision adopted by the Conference of the Parties to the Convention on Biological Diversity XIII/17. Synthetic biology. CBD/COP/DEC/XIII/17. Retrieved from https://www.cbd.int/doc/decisions/cop-13/cop-13-dec-17-en.pdf

114 National Academies of Sciences, Engineering, and Medicine (2016) Gene Drives on the Horizon: advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. National Academies Press, Washington, D.C. Retrieved from https://www.nap.edu/download/23405

115 Hartley, S., Gillund, F., van Hove, L. & Wickson, F. (2016) Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453; Sarewitz, D. (2015) Science can't solve it. Nature 522: 412–413; Kuzma, J. & Kokotovich, A. (2011) Renegotiating GM crop regulation: targeted gene-modification technology raises new issues for the oversight of genetically modified crops. EMBO Reports 12: 883-888.

116 CRISPR Update (n.d.) CRISPR Timeline. Retrieved from http://www.crisprupdate.com/crispr-timeline/

117 Lusser, M. & Davies, H. (2013) Comparative regulatory approaches for groups of new plant breeding techniques. New Biotechnology 30: 437-46.

118 European Court of Justice (2018) Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive, Case C-528-16. ECLI:EU:C:2018:20. Retrieved on July 31, 2018. Retrieved from https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf

119 Office of the Gene Technology Regulator (Australia) (2018) Technical Review of the Gene Technology Regulations 2001. Retrieved from http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/reviewregulations-1; National Gene Technology Scheme (Australia) (2018) The third review of the national gene technology scheme. Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/Content/011C554B9847D6F0CA258169000FCBBE/$File/third-review-gene-technology-preliminary-report-2018.pdf

120 Office of the Gene Technology Regulator (Australia) (2018) Technical Review of the Gene Technology Regulations 2001. Retrieved from http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/reviewregulations-1; National Gene Technology Scheme (Australia) (2018) The third review of the national gene technology scheme. Retrieved from http://www.health.gov.au/internet/main/publishing.nsf/Content/011C554B9847D6F0CA258169000FCBBE/$File/third-review-gene-technology-preliminary-report-2018.pdf

121 Whelan, A.I. & Lema, M.A. (2015) Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. GM Crops & Food 6: 253–265.

122 Smyth, S.J. (2017) Canadian regulatory perspectives on genome engineered crops. GM Crops & Food 8: 35-43.

123 USDA (2018) Secretary Perdue issues USDA statement on plant breeding innovation. Press Release March 28 2018. Retrieved from https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation

124 Taning, C.N.T., Van Eynde, B., Yu, N., Ma. S. & Smagghe, G. (2017) CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. Journal of Insect Physiology 98: 245–257.

125 See APHIS (2016) response to "Request for APHIS confirmation that TRSOG101B Transgenic Sugarcane is not a regulated article." Retrieved from https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-035-01_air_response_signed.pdf

126 USDA (n.d.) Am I Regulated Under 7 CFR part 340? Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated

127 Waltz, E. (2018) With a free pass, CRISPR-edited plants reach market in record time. Nature Biotechnology 36: 6-7.

128 USDA (2018) Request for regulatory status of "Nutritionally-Enhanced Wheat". Letter to Calyxt 20th March 2018. Retrieved from https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/17-038-01_air_response_signed.pdf; Calyxt (2018) Calyxt's high fiber wheat deemed non-regulated by USDA. Press Release 21st March 2018. Retrieved from www.calyxt.com/calyxts-high-fiber-wheat-deemed-non-regulated-by-usda/.

129 FDA (U.S.) (2018) How FDA regulates food from genetically engineered plants. Retrieved from https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/ucm461831.htm

130 Hixson, S.M., Shukla, K., Campbell, L.G., Hallett, R.H., Smith, S.M., Packer, L. & Arts, M.T. (2016) Long-chain omega-3 polyunsaturated fatty acids have developmental effects on the crop pest, the cabbage white butterfly *Pieris rapae.* PLoS ONE 11: e0152264; Rothamsted Research (2016) Rothamsted Research comments on the recent study by Hixson et al. (2016) demonstrating that long-chain omega-3 polyunsaturated fatty acids have developmental effects on the crop pest, the cabbage white butterfly *Pieris rapae*. Retrieved from https://www.rothamsted.ac.uk/sites/default/files/project-files/Comment%20on%20Hixson%20et%20al.%202016.pdf

131 Anon (2016) Breeding Controls. Nature (editorial) 532: 147.

132 FDA (U.S.) (2018) Animals with intentionally altered genomic DNA. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/default.htm

133 See, e.g., Gurian-Sherman, D. (2017) A hole in the regulation of GMOs that Kudzu could fit through. Union of Concerned Scientists https://blog.ucsusa.org/doug-gurian-sherman/a-hole-in-the-regulation-of-gmos-that-kudzu-could-fit-through-402; Perls, D. (2017) Next-generation genetically modified foods need better regulation. STAT. Retrieved from https://www.statnews.com/2017/02/02/genetically-modified-foods-regulation/

134 FDA (U.S.) (2002) Guidance to industry for foods derived from new plant varieties. FDA Federal Register 57: 22984. Docket No. 92N-0139. Retrieved from https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096095.htm

135 Losey, J.E., Raynor, L.S. & Carter, M.E. (1999) Transgenic pollen harms monarch larvae. Nature 399: 214.

136 Faria, C.A., Wäckers, F.L., Pritchard, J., Barrett, D.A. & Turlings, T.C.J. (2007) High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. PLoS ONE 2: e600.

137 European Network for Social and Environmental Responsibility (2018) Products of new genetic modification techniques should be strictly regulated as GMOs. Retrieved from https://ensser.org/publications/ngmt-statement/

138 Kim, J. & Kim, J-S. (2017) Bypassing GMO regulations with CRISPR gene editing. Nature Biotechnology (correspondence) 34: 1014-1015.

139 Agricultural Marketing Service (2018) National Bioengineered Food Disclosure Standard. Retrieved from https://www.regulations.gov/document?D=AMS_FRDOC_0001-1709

140 International Federation of Organic Agriculture Movements (2016) Genetic engineering and genetically modified organisms. Position paper. Retrieved from https://www.ifoam.bio/sites/default/files/position_genetic_engineering_and_gmos.pdf; Wickson, F. Binimelis, R. & Herrero, A. (2016) Should organic agriculture maintain its opposition to GM? New techniques writing the same old story. Sustainability 8: 1105.

141 Jasanoff, S. & Hurlbut, B.J. (2018) A global observatory for gene editing. Nature 555: 435-437; Jordan, N.R., Dorn, K.M., Smith, T.M., Wolf, K.E., Ewing, P.M., Fernandez, A.L., Runck, B.C., Williams, A., Lu, Y. & Kuzma J. (2017) A cooperative governance network for crop genome editing. EMBO Reports 18: 1683-1687; Hartley, S., Gillund, F., van Hove, L., Wickson, F. (2016) Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453; Sarewitz, D. (2015) Science can't solve it. Nature 522: 412–413.

142 Jordan, N.R., Dorn, K.M., Smith, T.M., Wolf, K.E., Ewing, P.M., Fernandez, A.L., Runck, B.C., Williams, A., Lu, Y. & Kuzma J. (2017) A cooperative governance network for crop genome editing. EMBO Reports 18: 1683-1687; Hartley, S., Gillund, F., van Hove, L., Wickson, F. (2016) Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453.

143 Jordan, N.R., Dorn, K.M., Smith, T.M., Wolf, K.E., Ewing, P.M., Fernandez, A.L., Runck, B.C., Williams, A., Lu, Y. & Kuzma J. (2017) A cooperative governance network for crop genome editing. EMBO Reports 18: 1683-1687.

144 Gilbert, N. (2016) Frugal Framing. Nature (news feature) 533: 308-310. Gilbert, N. 2014. Cross-bred crops get fit faster. Nature (news in focus) Nature 513: 292.

145 Crossa, J., Pérez-Rodríguez, P., Cuevas, J. et al. (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends in Plant Science 22: 961–975.

146 E.g. Jackson, L. (2011) Wheat cultivars for California. Retrieved from http://smallgrains.ucdavis.edu/cereal_files/WhtCV-DescLJ11.pdf

147 E.g., Dar, M.H., de Janvry, A., Emerick, K., Raitzer D. & Sadoulet, E. (2013) Flood-tolerant rice reduces yield variability and raises expected yield, differentially benefitting socially disadvantaged groups. Nature Scientific Reports 3: 3315.

148 E.g., Fujita, D., Trijatmiko, K.R., Tagle, A.G. et al. (2013) *NAL1* allele from a rice landrace greatly increases yield in modern *indica* cultivars. Proceedings of the National Academy of Sciences 110: 20431 – 20436.

149 Meuwissen, T., Hayes, B & Goddard, M. (2016) Genomic selection: a paradigm shift in animal breeding. Animal Frontiers, 6: 6–14.