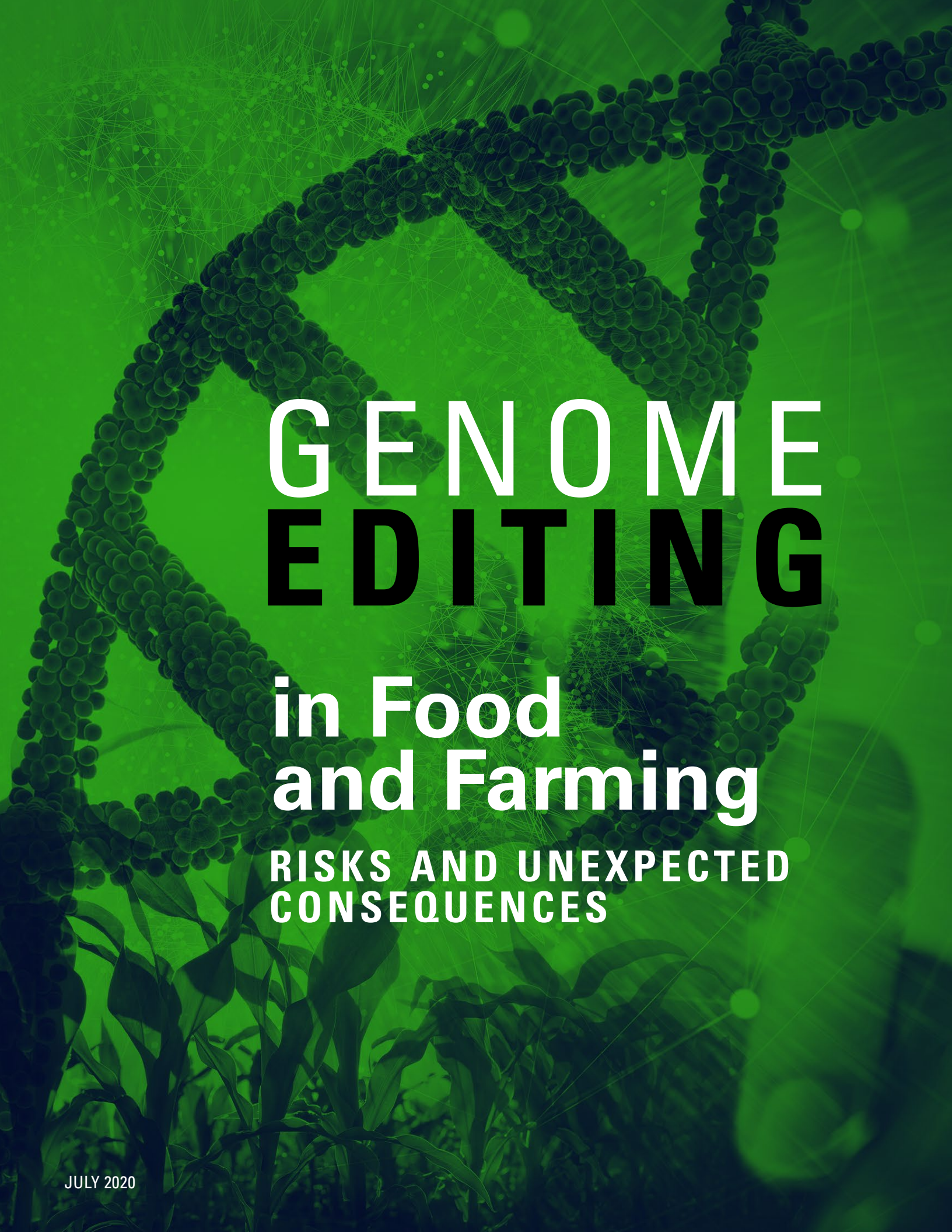


GENOME EDITING

in Food and Farming

RISKS AND UNEXPECTED CONSEQUENCES

JULY 2020

Acknowledgements

This report was updated by Dr. Janet Cotter and adapted by the Canadian Biotechnology Action Network (CBAN) from the 2018 report “Gene-edited organisms in agriculture: Risks and unexpected consequences” written by Dr. Janet Cotter, Logos Environmental, UK, and Dana Perls, Friends of the Earth U.S., with review assistance from Dr. Jonathan Latham of the Bioscience Resource Project.

Logos Environmental is a scientific consultancy for environmental non-governmental organizations set up in 2015. Contact: jcotter11@gmail.com.

The Canadian Biotechnology Action Network (CBAN) brings together 16 groups to research, monitor and raise awareness about issues relating to genetic engineering in food and farming. CBAN members include farmer associations, environmental and social justice organizations, and regional coalitions of grassroots groups. CBAN is a project on the shared platform of the MakeWay Charitable Society. **www.cban.ca**

Friends of the Earth U.S. fights to protect our environment and create a healthy and just world, with more than one million members and activists across all 50 states working to make this vision a reality. Friends of the Earth U.S. is part of the Friends of the Earth International Federation, a network in 74 countries working for social and environmental justice. **www.foe.org**

Any errors or omissions in this report are the responsibility of the Canadian Biotechnology Action Network.

Logos Environmental

Friends of the Earth United States

cban Canadian Biotechnology Action Network

Table of Contents

GLOSSARY & ACRONYMS OF GENOME EDITING TECHNIQUES iv

EXECUTIVE SUMMARY v

INTRODUCTION 1

WHAT IS GENOME EDITING? 2

Box 1: Genome editing techniques 4

Box 2: Classification of genome editing 4

GENOME EDITING APPLICATIONS 6

GENETIC ERRORS CAUSED BY GENOME EDITING 8

Off-target effects 8

Unintended on-target effects 9

Interference with gene regulation 10

Intended and unintended insertion of DNA 11

Box 3: Case Study
Unintended Foreign DNA in Genome-Edited Hornless Cows 12

UNEXPECTED AND UNPREDICTABLE EFFECTS
IN GENOME-EDITED ORGANISMS 14

GENE DRIVES 16

REGULATION 18

CONCLUSION 21

FURTHER READING 22

REFERENCES 23

Glossary

DNA: Deoxyribonucleic acid. In plants and animals, a molecule inside a cell's nucleus that carries genetic information.

Gene: A section (or sections) of DNA in a genome that codes for protein production via an intermediary, mRNA.

Genetic engineering: Also commonly called genetic modification, leading to the creation of genetically modified organisms (GMOs). Genetic engineering makes changes directly to the genetic material of an organism, without mating, by introducing genetic material or using techniques that induce change to an organism's genome. The material introduced into the cell is produced, or at least handled, in the laboratory by humans.

Genetic errors: In the context of genome editing, genetic errors are either unintended changes to the DNA (such as rearrangements or deletions), or changes in RNA and protein composition (e.g. by misreading of DNA). Genetic errors resulting from genome editing can lead to unexpected and unpredictable effects in the resulting genetically modified organism (GMO).

Genome: The entire set of genetic material in an organism, including DNA.

Genome editing or gene editing: A collection of genetic engineering techniques that alter the genetic material of organisms. The aim is to insert, delete or otherwise change a DNA sequence at a specific, targeted site or sites in the genome. Also called "genome engineering."

Host: The organism that is undergoing genome editing.

Nuclease: An enzyme that can cut DNA. Different nucleases can break either one or both strands of DNA.

Off-target effects: Unintended changes to other (non-target) genes.

Plasmid: Small, circular, double-stranded DNA molecules found in bacteria. They can be genetically engineered to contain genes of interest and introduced into the cell of a host organism, where the DNA may be taken up into the genome of the host, as in first-generation genetic engineering. In genome editing, the DNA coding for the genome editing components and the plasmid may or may not be assimilated into the host organism's genome, intentionally or unintentionally.

RNA: Ribonucleic acid. In a cell, there are different types of RNA, including messenger RNA (mRNA), which carries genetic information from DNA and directs the production of proteins.

Site-directed nuclease (SDN): The technology that guides DNA cutters to a target location to make a cut to DNA, as part of genome editing systems such as CRISPR. The SDN consists of a guide (generally constituted of RNA) and a DNA cutter (a nuclease). Often called "molecular scissors."

Unintended on-target effects: Errors that occur when the intended change is achieved at the target location but also triggers genetic errors.

Acronyms of genome editing techniques

(See Box 1: Genome editing techniques & Box 2: Classification of genome editing)

CRISPR: Clustered regularly interspaced short palindromic repeat

TALENs: Transcription activator-like effector nucleases

ZFN: Zinc-finger nuclease

ODM: Oligonucleotide-directed mutagenesis

Executive Summary

Genome editing (also called gene editing) is a collection of new genetic engineering techniques that alter the genetic material of plants, animals and microbes, creating genetically modified organisms (GMOs). These techniques aim to change DNA in the cell of an organism. This allows a novel trait to be induced without necessarily inserting genes from another organism or producing a novel protein, as is the case with almost all of the currently commercialized products of genetic engineering.

Many studies have now shown that genome editing can create genetic errors, such as "off-target" and "on-target" effects, leading to unexpected and unpredictable outcomes in the resulting GMO.

Genome editing can be imprecise. For example, genome editing techniques can make unintended "edits" to genes that were not the target of the editing system, giving rise to off-target effects. The genome editing technique called CRISPR-Cas9 appears to be particularly prone to creating off-target effects.

Genome editing can also result in unintended on-target effects, when a technique is successfully used to make the intended change at the target location, but also triggers genetic errors. For example, unintended on-target effects can change the way that a gene is read and processed into proteins, with potential implications for food and environmental safety. Additionally, genome editing can inadvertently cause extensive deletions and complex re-arrangements of the host's DNA.

New scientific publications also indicate that the integration of unwanted DNA during the genome editing process is more common than previously thought. For example, foreign DNA was unexpectedly found in genome-edited hornless cows that were claimed to be free of foreign DNA, demonstrating the need for systematic risk assessment.

There are several types of possible genetic errors that need to be investigated in each GMO resulting from genome editing, including unintended DNA integration. However, as yet, there are no standard protocols for the detection of off-target or on-target effects of genome editing.

Some types of intended changes to genetic material induced by genome editing techniques are sometimes described as "mutations" because only very small parts of DNA are altered and no novel genes have been intentionally introduced. However, **even small changes in a DNA sequence can have big effects**. The orchestration of gene function in an organism is part of a complex regulatory network that is still poorly understood. This means it is not possible to predict the nature and consequences of all the interactions between altered genetic material and other genes within an organism. Making one genetic change could, for example, impact an organism's ability to express or suppress other genes.

As the evidence in this report shows, even when a genome-edited organism does not contain foreign genes or express a novel protein, it cannot be considered safe for environmental release or human consumption on this basis alone. **Although widely promoted on the basis of specificity and precision, genome editing – like all other types of genetic engineering – can cause unexpected and unpredictable effects**.

In addition to opening up possibilities to genetically engineer a wider range of species, (more animal species, for example) for many different traits, genome editing techniques have facilitated experimentation with powerful "gene drives," where genome-edited organisms are being designed to speed up the inheritance process and push new genes through entire populations of a species, with potentially serious and irreversible impacts.

In this report, we provide an overview of genome editing techniques being explored in agriculture, and the range of potential unexpected effects that can arise from them. The report draws on recent scientific publications, in a rapidly evolving field of research. The purpose of providing this information is to encourage broad public discussion about the potential implications of using genetic engineering, and specifically the new techniques of genome editing, in food and farming, and the ways in which decisions about the use of the technology should be made.

Introduction

> Gone are the days when life was shaped exclusively by the plodding forces of evolution. We are standing on the cusp of a new area, one in which we will have primary authority over life's makeup and all its vibrant and varied outputs. Indeed, we are already supplanting the deaf, dumb, and blind system that has shaped genetic material on our planet for eons and replacing it with a conscious, intentional system of human-directed evolution."
>
> – Jennifer A. Doudna and Samuel H. Sternberg, 2017[1]

Widespread excitement over new genetic engineering techniques called "genome editing" or "gene editing", and the wide-ranging promises for their applications, mirrors those that accompanied the first-generation of genetic engineering. As with earlier genetic engineering, genome editing techniques are moving towards commercial application even while our knowledge of the mechanisms that underlie how genomes work remains incomplete.

Scientist Jennifer Doudna, discussing the genome editing technique CRISPR that she co-developed, called it "a transformative genome engineering technology"[2] and believes the technique gives us the ability to "rewrite the code of life" and control evolution.[3] At the same time, she says that the main mechanism that is triggered to accomplish these changes (cell repair) is "a process we don't fully understand, sort of magic happens, this is where the editing actually happens."[4]

In the laboratory, genome editing is a new set of research tools that are being used to increase our understanding of the functions of genes and the regulation of the genome. At the same time, these new techniques are being used to genetically engineer more crop plants and also farm animals.

Genome editing techniques are often described as being precise.[5] In fact, some argue that genome editing is so precise that the resulting agricultural products can automatically be considered safe and can therefore be exempt from government regulation and safety assessment.[6] However, as detailed in this report, research is showing that these techniques can cause genetic errors, and even precise edits do not necessarily result in precise outcomes. **Like all genetic engineering, genome editing can result in unexpected effects in the resulting organism**.

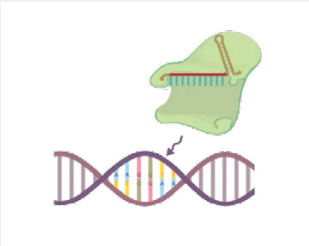
Genetic engineering in agriculture was first introduced to the public over twenty-five years ago with a description of precision, speed, and tremendous promise. However, the technology failed to deliver the products it promised,[7] and was ultimately described as "ham-fisted."[8] Now, as then, advances in genetic engineering such as genome editing are being widely celebrated as the future of agriculture. However, the technologies have limitations and risks with respect to food and environmental safety.

What is genome editing?

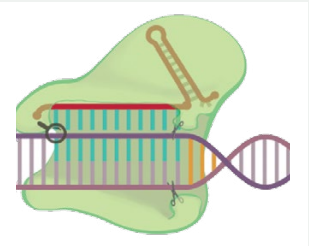
Genome editing, also called gene editing, is a term used to describe a collection of new techniques that alter the genetic material (usually DNA) of plants, animals and microbes. In general, these techniques consist of different types of DNA "editing" systems that aim to insert, delete or otherwise change a DNA sequence at specific, targeted sites in the genome. The organism's genetic material is changed, not through the breeding process, but directly and artificially by humans, making these techniques a type of genetic engineering, resulting in the creation of genetically modified organisms (GMOs).

Genome editing techniques are a type of genetic engineering, resulting in the creation of genetically modified organisms (GMOs).

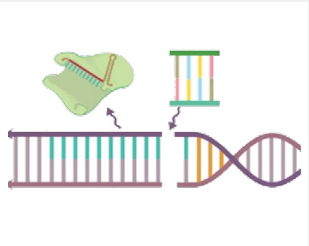
How Genome Editing Works



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



The DNA cutter docks onto the target site and cuts through the DNA.



The repair of DNA is then initiated and occurs either with (SDN-2) or without (SDN-1) a synthetic repair template. Alternatively, genes can be inserted (SDN-3).

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

Genome editing is a set of new genetic engineering techniques that alter the genetic material of plants, animals and microbes, most often using DNA cutters that are guided to a location within an organism's DNA and used to cut the DNA. This cut DNA is then repaired by the cell's own repair mechanism, which creates "edits" or changes to the organism.

Genome editing systems are comprised of molecular components that are programmed to make changes (perform "edits") at a target location in the genome. The most frequently used genome editing technique is CRISPR-Cas9 or CRISPR, but other techniques follow similar principles (see Box 1: Genome editing techniques).

The CRISPR editing system uses the technology of site-directed nucleases (SDNs), which are guided DNA cutters, often referred to as "molecular scissors." The editing system consists primarily of a guide (RNA) and a DNA cutter (nuclease). In plants, commonly a package ("cassette") of DNA encoding the CRISPR components is inserted into the organism's genome at random, using first-generation genetic engineering techniques. **The CRISPR system then guides the DNA cutter to a location on the organism's DNA, to cut specific, targeted DNA.** When a cut is made to DNA, the cell responds by repairing the damage, and it is through this repair mechanism that the system makes "edits", which can be insertions, deletions, or other changes to DNA. Afterwards, the inserted CRISPR DNA is bred out of the genome-edited plant via conventional breeding, so that the resulting genetically engineered organism (GMO) may no longer be transgenic (i.e. no longer contain genes from another species). Some genome editing approaches, known as "DNA-free" genome editing,[9] do not require a DNA cassette to be inserted. Instead, only the guide and cutter are introduced. Alternatively, DNA in the form of a plasmid is introduced into the cell,[10] but not expected to join with the host's DNA. These two latter methods of introducing the CRISPR components into the genome are common for genome editing animals.

During genome editing, the cut made to DNA triggers the cell's own repair mechanism, and the type of repair is key to how genome-edited organisms are classified. There are three types of genome editing, dependent on how the repair is achieved: one that does not use a synthetic repair template (SDN-1), one that uses a synthetic repair template (SDN-2) and one where a gene (or genes) is inserted (SDN-3) (see Box 2: Classification of genome editing).[11]

Genome editing is a form of genetic engineering, resulting in the creation of genetically engineered organisms (commonly called genetically modified organisms or GMOs).[1] The principal difference between earlier genetic engineering and this new generation of techniques is that, while both change genetic material directly, with genome editing, genes do not necessarily have to be permanently incorporated into the organism to produce the new trait.

Currently, most commercial genetically engineered crop plants, which are predominantly herbicide tolerant and insect resistant,[12] were made using first-generation genetic engineering techniques. This earlier genetic engineering – devised in the 1970s and first commercialized in the mid-1990s – inserts genes at a random location in an organism's genome.[13] If those genes are from a different, unrelated species (often called "foreign" genes), then the resulting genetically engineered organism is transgenic. Almost all of the genetically engineered crops currently on the market are transgenic, with the inserted gene(s) producing a protein that, for example, makes the plant tolerant to a particular herbicide (e.g., Roundup Ready soy) or toxic to certain plant pests (e.g., Bt corn).

1 The terms genetic engineered (GE) and genetically modified (GM) are used interchangeably. The term GM is widely used in Europe, as well as in the North American marketplace. Many national regulatory regimes and international agreements use the term genetic modification. The U.S. and Canadian governments both refer to genetic engineering. Canadian regulation defines GM broadly to include conventional breeding as well as genetic engineering (including genome editing). In international convention, the use of the term GM refers to genetic engineering and excludes conventional breeding.

BOX 1:

GENOME EDITING TECHNIQUES

Genome editing techniques such as **CRISPR, TALENs, ZFN and meganucleases*** use the technology of site-directed nucleases (SDNs), which are guided DNA cutters, often referred to as "molecular scissors." The SDN consists of a guide and a DNA cutter, and makes a cut at the location on the genome where the DNA change is intended to take place. The cutter cuts the DNA, which then undergoes repair carried out by the cell's own repair mechanism.

CRISPR, the most frequently used technique, commonly uses a type of DNA cutter called "Cas9," and it is therefore often referred to as the **CRISPR-Cas9** genome editing system. Other types of cutters, such as Cpf1 (also called Cas12a) and Cas13a (which edits mRNA), can also be used.[17] In addition, new CRISPR strategies are under development, including those that silence genes,[18] as well as prime and base editing.[19]

The genome editing technique known as **oligonucleotide-directed mutagenesis (ODM)** does not use a guided DNA cutter, 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.[20]

*See Glossary, page iv

There are different classes of genome editing, depending on how the repair to the cut DNA is achieved.

BOX 2:

CLASSIFICATION OF GENOME EDITING

There are different classes of genome editing, depending on how the repair to the cut DNA is achieved, although the distinction between categories can be blurred. Different components can accompany the **site-directed nuclease (SDN)**. For example, a synthetic DNA template is often used to direct the cell's repair and achieve a particular change in the DNA.[21] The use of these different components give rise to three different types of genome editing:

1) **SDN-1:** No repair template is used;
2) **SDN-2:** A repair template is used;
3) **SDN-3:** Genes are inserted during the genome editing process and are left in the organism in order to confer the novel trait. If the genes are from other species, this type of genome editing results in a transgenic organism.

In some countries, these different classes of genome editing are regulated differently (see Regulation).

Though they are often referred to as being among a range of "new breeding techniques", genome editing techniques are wholly different from conventional breeding. Conventional breeding has been used by farmers and breeders for thousands of years[14] to develop plant and animal varieties with desired traits, such as crops with resistance to pests and diseases. Conventional breeding relies on male and female mating to produce offspring with desired traits that are then selected for further breeding. In contrast, **genome editing, like other techniques of genetic engineering, directly alters the genetic material of an organism using laboratory techniques**.

There is controversy over the terms used to describe genome editing techniques. Genome editing is often called "gene editing," but this terminology does not include the alteration of multiple genes or gene regulators, which is possible with these techniques. The term "editing" has also been criticized,[15] because it likens genome editing to a precise and predictable text editor, when, in fact, the techniques of genome editing can give rise to several different types of genetic errors, as described in this report. The term "editing" also invites the perception of DNA as linear text, while genetic processes are complex and multidimensional. Genome editing has also been termed "genome engineering", implying greater intervention than "editing."[16] In this report, we use the term genome editing.

The term "editing" has been criticized, because it likens genome editing to a precise and predictable text editor, when, in fact, the techniques of genome editing can give rise to several different types of genetic errors.

Genome editing applications

Genome editing techniques are enabling experiments with new traits, and with a wide range of plant and animal species. In particular, genome editing has facilitated the creation of genetically engineered animals, which was more technologically difficult with earlier genetic engineering techniques, resulting in numerous scientific publications on genome-edited farm animals.[22]

Studies have demonstrated initial feasibility for a number of genome-edited plants and animals. However, the majority of these "proof of concept" studies, although they demonstrate that the desired DNA change can be achieved, do not examine (or do not examine thoroughly) the resulting GMO for possible genetic errors, such as off- and on-target effects (see Genetic errors caused by genome editing).[23]

With farm animals, feasibility studies include the development of pigs with resistance to certain diseases,[24] "super-muscly" pigs,[25] and hornless cows.[26] There is also research on genome editing insects and rodents for use in gene drives (see Gene Drives). Genome-edited traits being researched in plants include drought tolerance in corn, virus resistance in cucumbers, altered flowering time in tomatoes, and altered composition in soybeans.[27]

However, one of the most common plant traits in development is herbicide tolerance,[28] the trait that overwhelmingly dominates current genetically engineered crop acres globally.[29] In fact, **the first genome-edited organism commercialized in North America is a herbicide-tolerant canola**, developed by the company Cibus to be tolerant to the herbicide sulfonylurea.[30] It is on the market in Canada and the U.S. The only other genome-edited crop plant currently on the market is a soybean with higher oleic acid content, developed by the company Calyxt, and sold in the U.S. only.[31]

Genome-edited crops that could be commercialized soon include high-fiber wheat (from Calyxt, expected to be marketed "as early as 2022")[32] and a waxy corn by DuPont (now Corteva; expected to be launched in 2020).[33] However, although many other experiments are reported in the scientific literature and media, **there is no reliable way to anticipate or identify what GMOs are in the product pipeline**. The processes to develop, regulate and commercialize new products are not transparent to the public, and are affected by many variables that shift over time, including technical limitations, commercial viability and interest. Ultimately, it is not possible to determine which products are most likely to be approved or commercialized.

CIBUS' NON-TRANSGENIC CANOLA

The first genome-edited organism commercialized in North America is a herbicide tolerant canola, developed through the use of oligonucleotide directed mutagenesis (ODM) (see Box 1: Genome editing techniques). It was developed by the U.S. company Cibus, to be tolerant to the herbicide sulfonylurea, and is now sold under the seed brand Falco™. It was introduced in the U.S. in 2016, and in Canada in 2018.

Cibus initially advertised the canola as "non-GMO"[34] but is now more commonly advertising it as "non-transgenic,"[35] with some reference to it also being "non-GMO."[36] However, as of the 2018 court decision in the European Union, the canola would be regulated as a GMO in Europe (see Regulation).

In 2019, the company claimed that "products derived using Cibus' patented technology have been certified as non-GMO in countries including the United States, Canada, Argentina and Chile and the process for review is underway in the European Union and Japan."[37] However, North America's largest non-GMO product certifier, the Non-GMO Project, defines genome editing as GM and will not certify the Cibus canola as Non-GMO Project Verified.[38]

Genetic errors caused by genome editing

Genome editing can cause genetic errors, including "off-target" effects in the genome, unintended "on-target" effects, interference with gene regulation, and intended and unintended insertion of DNA. These genetic errors are important because they can lead to unexpected and unpredictable effects in the resultant genome-edited organisms, that could be important for food and environmental safety (see Unexpected and unpredictable effects in genome-edited organisms).

OFF-TARGET **EFFECTS**

Genome editing can be imprecise, creating genetic errors such as "off-target" effects, which are changes to other genes that were not intended.

In genome editing, an editing system (such as CRISPR-Cas9) is introduced into the genome, but this editor can make mistakes. **The editor can make unintended edits to the host's DNA at additional sites to the target location**, if the DNA sequences are similar. These unintended alterations cause genetic errors known as off-target effects.[39] Such unintended changes may be close to the target gene or can be at distant locations within the genome.

The frequency of off-target effects depends on the genome editing technique (i.e. ZFN, TALENs, etc.) and the protocol (e.g. dosage) used,[40] but **the CRISPR-Cas9 system appears to be particularly prone to creating off-target effects**.[41] In addition, many crops, such as corn, wheat and sugar beet, have multiple sets of genomes (i.e. are polyploid). This means there are similar and/or repeated gene sequences in these organisms, which increases the likelihood that they will be unintentionally changed during the genome editing process.[42]

Genome editing can be imprecise, creating genetic errors such as off-target effects, which are changes to other genes that were not intended.

Off-target effects have been observed in studies on genome-edited plants, such as rice, soybean[43] and wheat.[44] Off-target effects are also a concern in genome-edited farm animals, such as pigs and cattle,[45] and have been detected in genome-edited pigs,[46] mice[47] and human cells.[48]

Off-target effects can cause changes in biochemistry or protein production, both of which are important for food and environmental safety (see Unexpected and unpredictable effects in genome-edited organisms). Most studies on the potential uses of genome editing techniques in agriculture consider off-target effects to be both a major challenge and a major concern.[49] Despite this, **few studies search thoroughly for off-target effects**.[50]

As yet, there are no standard protocols for detecting off-target effects. They can either be examined by predicting potential off-target sites in the genome and then examining the DNA in those sites to see if they have been unintentionally changed, or by sequencing the whole genome. Whole genome sequencing enables a search for off-target effects genome-wide instead of being limited to only computationally predicted sites, but it is costly.[51] At the moment, most studies that examine for off-target effects use computation of predicted off-target sites.[52]

Studies examining only those sites predicted by computation may miss off-target effects. Even with whole genome sequencing, some studies report a lack of detectable off-target effects in genome-edited organisms.[53] This could be either because off-target effects are not present, or they are present but it is difficult to distinguish between off-target effects and natural genetic variation.[54]

UNINTENDED ON-TARGET EFFECTS

Genome editing can also cause unintended "on-target" effects, where the intended change occurs at the target location but also triggers genetic errors. Unintended on-target effects can occur even in the absence of off-target effects. On-target effects refer to an assortment of possible genetic errors, including errors in the way genes are processed or "read," as well as rearrangements and deletions of parts of the host's DNA. Even though they are called on-target effects, they may occur at some distance from the intended edit.

During normal cell function, parts of genes within DNA are "read" to produce an intermediary product, called messenger RNA (mRNA). This mRNA is then used as a template to produce proteins. **Unintended on-target effects can be caused by both large and small changes to DNA.** Even a small intended edit of the gene (e.g. an insertion or deletion of just one DNA base pair), although on-target, can disrupt the way a gene is read and processed into proteins. The mRNA may form in a different way (i.e. the alternative splicing mechanism is disrupted) or important parts of the gene (those coding for protein production) may be missed or "skipped" completely.[55] In addition, **genome editing can inadvertently cause duplications, extensive deletions and complex rearrangements of DNA sections.**[56] These deletions and rearrangements can also cause genes to be misread.

Genome editing can also cause unintended on-target effects, where the intended change occurs at the target location but also triggers genetic errors.

The misreading of DNA has the potential to produce unintentionally altered (or malformed) proteins. For example, one study using a laboratory culture of CRISPR genome-edited human cells found a malformed protein produced in error when DNA was misread as a result of the genome editing process.[57] Another study found unexpected changes in protein expression or mRNA in approximately 50% of commercially available CRISPR-edited human cell lines.[58]

Food allergens are mostly proteins, so altered proteins could have significant implications for food safety.[59] Concerns with the allergenicity of proteins have long been an important preoccupation with GMOs created by standard genetic engineering techniques. For example, "Starlink" corn was approved in the U.S. in 1998 for animal, but not human, consumption, due to concerns over the potential allergenicity of the protein (Cry9C) produced by the inserted Bt toxin that conferred insect resistance.[60] After it was discovered contaminating human food supplies in the U.S. and Canada in 2000,

products with Starlink corn were recalled from shelves at great cost to food companies, and the corn was withdrawn from the market.[61]

The misreading of DNA in a genome-edited plant or animal could, like first-generation GMOs, also impact biodiversity. For example, if the chemistry of a genome-edited plant or animal is changed by the misreading of DNA, it could potentially produce a compound that is toxic to the wildlife that feeds on it. Despite these possible impacts, **unintended on-target genetic errors created by the genome editing process may be missed because detailed, genome-wide examinations of DNA are not routinely performed, and there are no standard protocols for such examinations**.[62] Similarly, there is rarely any examination of gene products, such as mRNA and proteins,[63] which could be important to food safety.

INTERFERENCE WITH GENE REGULATION

In addition to altering an organism's DNA, **genetic engineering 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 (silenced) 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. The precise way this regulatory network operates is still poorly understood, as exemplified by recent advances in our

Even precise edits do not necessarily result in precise outcomes.

knowledge of how gene expression is regulated.[64] For example, for several decades, a dominant theory in molecular biology, known as the central dogma, 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 one another.[65] Also, DNA that does not produce proteins was previously thought to be "junk" DNA with no identified purpose, but recent scientific research now considers much of this DNA important for controlling gene expression in plants, animals[66] and in human genomes.[67]

There have already been reports of an unexpected response from the cell regulatory network during genome editing. In experiments with human cells, the cuts in DNA created by CRISPR were unexpectedly found to kill cells or stop them from growing.[68] **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 edits to DNA may inadvertently affect the operation of the organism's regulatory network. This could result in the organism's own (unedited) genes not responding as they should, by being produced incorrectly, in the wrong amount, or at the wrong time. **Any unexpected and unpredictable effects in the genome-edited organism could result in alterations to biochemical pathways or protein composition, which could have implications for food and environmental safety**.

INTENDED **AND UNINTENDED** INSERTION OF DNA

Several genome editing techniques are in development (see Box 1: Different genome editing techniques). Although, in the future, genes may not need to be inserted to perform genome editing, currently **genes encoding the editing components are commonly inserted into a plant's genome in order to perform the genome editing**. Most market-oriented genome-edited plants in development have genes encoding for gene-editing components inserted into them at random locations.[69] However, instead of these genes conferring a novel trait, they code for the CRISPR components that perform the genome editing process. This applies to plants developed by SDN-1 and SDN-2 types of genome editing (see Box 2: Classification of genome editing), which aim to have no transgenes in them.

In the case of CRISPR, a package of DNA (DNA "cassette") containing the CRISPR components is commonly inserted into the plant's genome at a random location, in exactly the same way as earlier genetic engineering techniques. This DNA codes for the CRISPR components. This complex targets a specific location in the host genome to perform the genetic change. Afterwards, the inserted CRISPR DNA is bred out via conventional breeding so that the GMO may no longer be transgenic (i.e. no longer contains genes from another species). The genome-edited high-fiber wheat produced by Calyxt was developed in this way.[70] However, it is inevitable that, **in some cases, not all the inserted DNA will be removed and some of the DNA encoding for genome editing components will inadvertently remain in the genome-edited organism**.[71]

When DNA is inserted in this manner into a plant's genome, the insertion may not be precise. Just as earlier genetic engineering could not direct the location for the insertion of transgenes and control their copy number, **multiple copies and additional fragments of the DNA cassette can be unintentionally introduced into the plant's genome**.[72] The insertion of DNA coding for genome editing components can also cause sections of the host's organism's DNA to become rearranged, as has often happened with first-generation genetically engineered crops.[73] This is in addition to any deletions and rearrangements caused by the genome editing process itself (see Unintended on-target effects).

In some genome-edited plants and animals, DNA coding for CRISPR components is introduced as a plasmid into the organism's cell and performs the genome editing without becoming integrated with the organism's own genome, as is claimed with DuPont's genome-edited waxy corn.[74] However, **any introduced DNA could unintentionally become integrated, at random, into the organism's genome**.[75] Unintended integration of genome editing components (including any additional copies and fragments) has been found in both plants and animals, not only from CRISPR, but also from TALENs.[76] In a recent case, cows that had been genome-edited via TALENs to be hornless[77] were found to have DNA from the genome editing process, including antibiotic resistance genes, unintentionally incorporated into their genomes (see Box 3: Case Study – Unintended Foreign DNA in Genome-Edited Hornless Cows).[78] **Integration of unwanted DNA from the genome editing process appears to be more common than previously thought but a failure to identify integration events has led to a high rate of falsely claimed precision**.[79]

Both unintended integration of DNA coding for CRISPR components, and additional fragments or rearrangements of intended DNA insertions, can give rise to unexpected effects in genome-edited organisms, in a similar manner to first-generation genetic engineering. It is, therefore, important that checks for unintended DNA and unintended effects from DNA insertion (intended or unintended) are carried out.

BOX 3: CASE STUDY

Unintended Foreign DNA in Genome-Edited Hornless Cows

"We can't know if we don't look."

– Steven M. Solomon, Director Center for Veterinary Medicine, US Food and Drug Administration, 2020[80]

"It was not something expected, and we didn't look for it."

– Tad Sonstegard, CEO, Acceligen, agricultural subsidiary of Recombinetics, 2019[81]

The development of genome-edited hornless cows[82] (via TALENs, see Box 1: Genome editing techniques) was celebrated as an illustration of the power and ease of genome editing, and discussed as a demonstration of why genome-edited animals did not need to be regulated. As key university researchers involved in the project argued, "The effects of genome editing are largely identical to those of the natural processes that continually create variation in the genomes of food animals. From this point of view, it is hard to see why the process of genome editing to introduce defined genetic changes should be regulated."[83] However, **the case of the hornless cows shows the potential for errors in the genome editing process and the need for independent safety assessment**.

The dairy cows were genome-edited to be hornless (polled), to eliminate the practice of manually dehorning cows. Two cows were developed by university researchers in collaboration with the U.S. company Recombinetics. The developers reported that they were created without foreign genes[84] and "our animals are free of off-target effects".[85] However, in 2019, researchers at the U.S. Food and Drug Administration (FDA) found unexpected foreign DNA in the cows (on-target effects).[86]

FDA researchers found two antibiotic resistance genes and various other gene sequences from bacteria in both cows. This genetic material was from the DNA carrier (a plasmid; see What is genome editing) that had been used to introduce the repair template into the cows' cells. The FDA also found an extra copy of the DNA repair template, at the target site.

The genome-edited cows were checked for off-target effects but not for unintended on-target effects. In their 2016 study, the developers reported that the polled gene had been successfully changed and there were no unintended insertions or deletions from the cutting and repair process.[87] In 2017, the executive chair of Recombinetics said, "We know exactly where the gene should go, and we put it in its exact location," and concluded, "we have all the scientific data that proves that there are no off-target effects." He also said that the cows were "100% bovine [cow]".[88] However, upon discovery of the extra DNA, Recombinetics said, **"The company did not directly screen for the presence of the plasmid, it should have."**[89]

The extraneous DNA found in the cattle does not necessarily present a safety threat. This was stressed by the FDA in a related commentary that argued for government

regulation,[90] as well as in an editorial in the journal *Nature Biotechnology* that argued against mandatory government safety assessments.[91] The editorial authors argued against regulation while acknowledging that the extra DNA was "unexpected and initially missed" and that **"gene editing is not the neat precise process that proponents tout."** [emphasis added].

The FDA scientists suggested a number of reasons why the template plasmid may not have been detected in the cows. They said that their discovery "highlights a potential blind spot in standard genome editing screening methods" and that, across the scientific literature, **"we suspect that integration errors are either under-reported or overlooked."**[92] [emphasis added].

In this case, the discovery of unexpected DNA was not made by the developers, and it was only incidentally found by the FDA. The FDA researchers were not screening the genome-edited cows as part of any government regulatory process but because they were using the cows' genome sequences to test a new bioinformatics method.[93] This is significant because, from the outset, the developers argued that regulating genome-edited animals was not necessary to ensure safety. In fact, the publication of the key scientific papers on the hornless cows was accompanied by arguments for and against regulation.[94]

The director of the FDA's Center for Veterinary Medicine wrote that the FDA's finding emphasized the agency's "critical role in risk-based evaluation of intentional genome alterations."[95] However, this conclusion was disputed in a 2020 editorial in *Nature Biotechnology*, which argued that **the FDA's proposal to regulate all GM animals "canonizes a precautionary stance to genome-edited animals" and, as such, "makes no economic sense."**[96]

Once the cows were found to be carrying extra DNA, the company stressed that their cows were only research animals[97] ("testing regulatory systems with our experimental gene-edited animal(s) is of interest"[98]) and there "never was a commercial intent for these animals or their offspring."[99] However, executives had, in 2017, mentioned that there were "multimillion-dollar deals in the final steps of negotiations."[100] In 2019, the genome-edited dairy cows were being incorporated into a breeding program in Brazil that was then cancelled because the cows no longer qualified for Brazil's regulatory exclusion of non-transgenic organisms, because they contained foreign DNA.[101]

> "Our analysis demonstrated that genome editing in animals can have unintended consequences, and in this case, it caused foreign DNA to be integrated into the animals' genomes. While the existence of an unintended alteration does not necessarily mean that the genome edit is unsafe to animals or consumers, it does show that both scientists and regulators need to be alert to the potential for such unintended alterations to take place."
>
> – Steven M. Solomon, director of the FDA's Center for Veterinary Medicine, February 2020[102]

Unexpected and unpredictable effects in genome-edited organisms

As with all genetically engineered organisms, genetic errors introduced by genome editing (e.g. off-target or unintended on-target effects) can result in unexpected effects in the genome-edited organism. Unexpected effects are unpredictable because they are caused by genetic errors and the lack of a full understanding of how genomes work means that effects cannot be reliably predicted. They can include changes in the chemistry, biochemical pathways or protein composition. Such effects could have implications for food and environmental safety if they alter toxicity (via chemical changes) or allergenicity (via changes in protein composition). Importantly, unexpectedly malformed proteins (i.e. with changed composition) have already been observed as an effect of genome editing (see Unintended "on-target" effects).

The intended changes to genetic material induced by some types of genome editing techniques (types SDN-1, SDN-2 and ODM) are sometimes described by developers as "mutations"[103] because only very small parts of DNA (e.g. one or a few base pairs) are altered, and no novel (or foreign) genes are intentionally introduced. While mutations do occur naturally, and indeed are an important source of genetic variability in breeding plants and animals, the process is very different from the direct changing of genetic material with genome editing. Importantly, **it does not follow that a genome-edited organism with only a small change to its DNA is always "safe."** To evaluate environmental and food safety, the changes to DNA (both intended and unintended) and other genetic material (e.g. mRNA) need to be carefully assessed.

As with all genetically engineered organisms, genetic errors introduced by genome editing can result in unexpected effects in the genome-edited organism.

Many genes are multifunctional, particularly in animals.[104] This means that a gene that is rendered dysfunctional, even by a single base change, could have an essential, unrelated function elsewhere in the organism. For example, in the case of genome-edited pigs reported to be resistant to porcine reproductive and respiratory syndrome virus (PRRS), the gene that has been knocked out (CD163) is known to be important in defending against other infections and to regulate blood composition.[105] Therefore, **intentionally disabling just one single gene (often referred to as a "genetic tweak")[106] could have important consequences for other traits in the animal or plant**.

Genetic errors caused by genome editing can result in unexpected effects in the resulting GMO, irrespective of whether or not genes for a novel trait have been introduced. For example, super-muscly genome-edited pigs were found to have an extra vertebra compared to unaltered pigs, even though no genes had been inserted.[107] Although pigs can have slightly different numbers of vertebrae, the underlying mechanism of this change is not known, but is thought likely to be associated with the gene knocked out to make them super-muscly.[108]

While there are many "proof of concept" publications about what genome editing might achieve, there are, as yet, no studies examining their potential environmental impacts. There are large gaps in the scientific literature on how new traits may impact the environment, particularly if they introduce novel compounds. For example, first-generation genetically engineered plants altered to produce omega-3 fatty acids unexpectedly induced toxic effects on caterpillar larvae, deforming wings in the adult butterfly and raising concerns about how this crop might affect the food web.[109] Ultimately, the full impacts of genetically engineered organisms on the environment cannot be predicted. Biological, ecological and social systems are interrelated and interdependent.

Gene Drives

Genome editing techniques, particularly CRISPR (type SDN-3) systems, have enabled the possibility of "genes drives." Gene drives are a technology through which a few genetically engineered individuals are designed to intentionally push new genes through an entire population of a species.[110] The gene drive mechanism ensures that the new genes will be inherited by all offspring (as opposed to an expected half of the offspring in normal inheritance) in subsequent generations.[111] Thus, **gene drives are designed to alter the genetic make-up of an entire wild population, or to eradicate a population or species**.

Gene drives are being proposed for pest and disease control in agriculture.[112] However, the most advanced research so far is in insects, specifically in gene drive systems that would alter genes to prevent mosquitoes from reproducing effectively, thus reducing the size of particular mosquito populations.[113] The research group Target Malaria, funded by the Bill and Melinda Gates Foundation among others,[114] aims to use gene drive mosquitoes to reduce the population of *Anopheles gambiae* mosquitoes, which transmit the parasite that causes malaria.

In agriculture, applications are being discussed to alter genes so that agricultural pests such as a type of fruit fly (spotted wing drosophila)[115] and pigweed (*Palmer amaranth*)[116] do not reproduce effectively, in order to make them ultimately go extinct. Researchers are also devising a type of gene-drive mechanism for mammals,[117] and have outlined a hypothetical gene drive system intended to drive a desired trait though a herd or population of a farm animal.[118]

Unlike GMOs that have thus far been engineered for confined use in agricultural production, gene drive organisms are expressly designed for intentional release into the wild. Releasing gene drive organisms can therefore be understood as a form of ecosystem engineering.[119] **Once released, gene drive organisms cannot be recalled, and the changes they create in the genetic make-up of the population are most likely irreversible**. Acknowledgement of this challenge has led to theorizing various means to control or reverse gene drives, however these exist only as mathematical models and are accompanied by their own complex risks.[120]

Scientists and others are already warning that the consequences of gene drives could be severe if there are any unexpected effects from the genome editing process[121] (e.g. from off-target effects[122]), or if other (e.g. ecological) unintended consequences arise.[123] The overarching concern is that genetic change to, or elimination of, an organism in the wild could disrupt ecosystems in unpredictable ways.

In 2018, governments at the United Nations Convention on Biological Diversity (CBD) agreed to apply the precautionary approach[2] in relation to the regulation of gene drives, including to conduct risk assessments and establish safety measures to prevent potential adverse effects.[124] Governments also agreed on the need to seek or obtain the approval of potentially affected indigenous peoples and local communities prior to considering any release. The CBD is further considering experiences, challenges and needs that may arise in relation to the risk assessment of gene drives.[125] Ultimately, however, the risks of releasing gene drives cannot be identified before they are deployed, suggesting that a precautionary approach would lead to a zero deployment of gene drives.

There is widespread agreement that research into gene drive systems requires some form of international regulation.[126] **However, some scientists and civil society organisations argue that gene drives cannot be regulated at all, and are calling for a global moratorium on gene drive research**.[127]

Ultimately, the risks of releasing gene drives cannot be identified before they are deployed, suggesting that a precautionary approach would lead to a zero deployment of gene drives.

2 The precautionary approach prioritizes the protection of human health and the environment when faced with scientific uncertainty and gaps in our knowledge. The approach is based on Precautionary Principle, as defined in the UN 1992 Rio Declaration on Environment and Development, which states that, "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."

Regulation

> The advent of genome editing offers an opportunity to rethink the regulatory approach to the products of biotechnology."
>
> –Young et al., 2020[128]

There is international debate about how genome-edited plants and animals for use in agriculture should be regulated, if at all.[129] The emergence of genome editing techniques has led to renewed arguments for reducing regulation.[130] However, as discussed in this report, the use of new genome editing techniques will challenge regulators with new traits and new processes, with increasing complexity and ongoing uncertainty.

There are divergent regulatory approaches to genome editing around the world. In some countries, certain genome-edited classes are excluded or included under existing GMO regulations (see Box 2: Classification of Genome editing). Other countries are changing regulation and definitions of genetic engineering in order to account for the new techniques. While some jurisdictions regulate based on the process used to genetically engineer organisms (e.g. the European Union), others regulate the end ("novel") products on a case-by-case basis (e.g. Canada).

All regulatory authorities around the world regulate transgenic genome-edited plants (type SDN-3) as GMOs.[131] However, some countries (Argentina, Brazil, Australia, Japan and the U.S.) have decided that non-transgenic genome-edited organisms should not be regulated in the same way as products of earlier genetic engineering:

- **Argentina** (2015) passed a resolution to not regulate products that do not have transgenic material (no "novel combination of genetic material"), unless their features lead to a significant risk hypothesis.[132]
- **Brazil** (2018) has classified non-transgenic genome-edited organisms as non-GMO.[133]
- **Australia** (2019) amended its regulations to exclude techniques that do not use a DNA repair template (SDN-1), or do not introduce other genetic material.[134]
- **Japan** (2019) will also not regulate or require labelling of genome-edited foods unless they contain transgenes.[135]
- In the **United States** most genome-edited crops would have already fallen outside existing environmental regulation which focused on GM crop plants that pose a plant pest risk".[136] However, new rules published in June 2020 also explicitly exclude plants produced through a range of genome editing classes, including those that do not use a repair template, make one small change (a single base pair substitution), and introduce a gene known to be in the plant's gene pool.[137] These same crops may or may not undergo a food safety assessment because the Food and Drug Administration's "premarket food safety consultation" is, technically, voluntary.[138]

The European Union and Canada are relying on existing regulations rather than creating new rules for the new techniques:

- The **European** Court of Justice (2018) ruled that all genome-edited organisms, including those developed by ODM, SDN-1 and SDN-2, meet the definition of GMOs in current European Union regulations.[139]
- In **Canada**, existing regulation means that all organisms with "novel traits" are regulated regardless of the technology used to produce them.[140] This means that most, or all, genome-edited products will be subject to government safety evaluation.

Many countries are thereby also excluding some products of genome editing from existing GMO labelling requirements. The new U.S. *National Bioengineered Food Disclosure Standard*, which will come into full effect in 2022, will mandate labelling for "bioengineered" foods but excludes those that do not contain detectable genetic material and "for which the modification could not otherwise be obtained through conventional breeding or found in nature."[141] Canada has no mandatory labelling for any genetically engineered foods. In addition to providing transparency in the marketplace, labelling and traceability can allow for post-market monitoring of safety and assist efficient food recalls if necessary.[142]

As discussed in the sections above, all genome editing techniques can create genetic errors that can lead to unexpected and unpredictable effects in the resulting genome-edited organism. Such effects can go unnoticed if they are not adequately screened for. **CRISPR and other new genome editing techniques can lead to unintended consequences and therefore require robust regulation**.

One of the primary concerns for food safety is the question of unintended alteration to protein composition as a result of genetic engineering. This is because allergens are proteins, so any inadvertent change in protein composition could cause the genome-edited plant or animal to trigger allergies in humans.[143] Importantly, altered proteins have already been identified as an unintended outcome of genome editing.[144] Protein composition needs to be examined carefully in genome-edited foods. Evaluations of GMOs need to identify **all potential hazards, no matter how theoretical they may initially appear**.

Such oversight is needed in order to avoid potentially damaging impacts that could otherwise be overlooked. For example, it is important that regulations include comprehensive examination for off-target effects, and that any found are fully evaluated to determine if they have caused any changes to chemistry or protein production. As discussed, however, there are, as yet, no standardized protocols to screen for off-target and on-target effects. The case of the genome-edited hornless cows illustrates that, even when developers state that they are certain their products show no unintended effects, third-party oversight is necessary (see Box 3: Case Study – Unintended Foreign DNA in Genome-Edited Hornless Cows).[145]

Arguments to exclude some or all genome-edited products from government safety evaluation are being made. A 2020 editorial in the journal *Nature Biotechnology* argues that "mandatory oversight could be phased out" for genome-edited animals in the U.S. and replaced with a system whereby the U.S. FDA "exercises discretion over which genome-edited animals are regulated according to the hazard represented by the introduced trait."[146] A similar argument is being made by the biotechnology industry in Canada, where the lobby group CropLife Canada recommends a tiered regulatory system for genome-edited products, one that predetermines the risk potential of products based on "complexity and familiarity" and could thereby exclude some products from a full scientific evaluation.[147]

There are already long-standing critiques from many scientists and civil society organizations of GMO regulatory regimes around the world, largely focused on the need for increased independence and transparency.[148] For example, governments continue to largely rely on information and data generated and submitted by the same companies or institutions that are requesting product approval. In Canada, for example, all information submitted for safety evaluation is classified as confidential business information and is therefore not made available for review by independent scientists.[149] This also means that much of the science behind regulatory decisions is not peer-reviewed. This is significant because peer-review and independent corroboration are defining features of the scientific method.[150]

This report focuses on describing the science of genome editing and the associated risks that could have implications for food and environmental safety. However, **the regulation of GMOs also affects our economies and societies, and there is increased discussion about the limitations of relying on solely "science-based" regulation for genome editing**.[151] In particular, the use of genome editing to genetically engineer humans has prompted calls for mechanisms to enable debate over ethical questions.[152] These include, for example, a proposal to establish an interdisciplinary network to function as a global observatory, "as a crucial step to determining how the potential of science can be better steered by the values and priorities of society."[153]

Excluding non-scientific considerations, such as possible economic and social impacts, can narrow the scope of scientific evaluation itself. For example, without consulting farmers, any regulatory process can miss or underestimate potential long-term environmental and agronomic impacts of the products being evaluated.[154] The Royal Society of Canada's Expert Panel on the Future of Food Biotechnology argued that health and environmental safety issues "though largely scientific in nature, often cannot be addressed fully without reference to broader ethical, political and social issues and assumptions."[155] This is in part because defining the scope of risk and levels of acceptable risk inherently involves value judgements.

A strictly scientific assessment could lead to regulatory agencies reviewing products for approval that may have little or no social worth. In Canada, for example, some farmer associations have requested an economic risk assessment of all GMOs before market release, in a process that would include consultations with farmers.[156] In the absence of such participatory processes, and in the context of a high level of corporate consolidation in the global seed and agrochemical markets,[157] companies are developing and commercializing products (e.g. GM glyphosate-tolerant alfalfa) that have little utility and, on the contrary, may pose serious risks to farming systems and the environment.[158]

Conclusion

Genome editing techniques are leading to the commercialization of new genetically engineered plants and animals for food, and yet the orchestration of gene function in organisms is part of a complex regulatory network that is still poorly understood.

There is an increasing amount of evidence showing that genome editing techniques now being explored are not as precise as originally claimed. Describing these new genetic engineering techniques as enabling "edits" to the genome suggests a level of precision that has not yet been achieved, and may not be possible.

It is clear that genome editing can give rise to genetic errors. These include off-target effects, unintended on-target effects, interference with gene regulation, and effects from intended and unintended insertion of DNA. Genetic errors can result in unexpected and unpredictable effects in the resultant GMO. Unexpected effects such as altered protein composition could impact the food and environmental safety of genome-edited plants and animals. Ultimately, the precision of genome editing techniques and the food and environmental safety of genome-edited products cannot be assumed. Rather, robust regulation with thorough risk assessment is required to ensure food and environmental safety.

Genome editing, with such powerful applications and profound implications, requires precautionary regulation as well as mechanisms to consider societal values.

Genome editing could facilitate many new GMOs coming to market, enhancing the need to grapple with ongoing social and economic considerations. Additionally, genome editing is enabling the particularly powerful technology of gene drives, which pose profound environmental and social risks. Given the complexity of the ecosystems into which they would be introduced, assessing the full risks of gene drive organisms prior to their release is not possible. Any release of gene drive organisms is highly likely to be impossible to recall, with unpredictable and irreversible consequences.

Genome editing, with such powerful applications and profound implications, requires precautionary regulation as well as mechanisms to consider societal values.

Further Reading

Summary of research into the unexpected exon skipping as a consequence of CRISPR genome editing: Sharpe, J.J. & Cooper, T.A. (2017) Unexpected consequences: exon skipping caused by CRISPR-generated mutations. Genome Biology 18: 109.

Review of off-target and unintended on-target effects in plants and their detection detection methods: Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C. & Wilhelm R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environmental Evidence 8: 27.

Commentary from the US Food and Drug Administration (FDA) on why regulation of genome-edited animals is necessary: Solomon, S.M. (2020) Genome editing in animals: why FDA regulation matters. Nature Biotechnology 38: 142-142.

Framework for a European Union risk assessment of genome-edited crops, including a description of unintended effects. Eckerstorfer, M.F., Heissenberger, A., Reichenbecher, W., Steinbrecher, R.A. & Waßmann, F. (2019) An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification techniques (nGMs). Frontiers in Bioengineering and Biotechnology. 7:31.

Detailed overview of gene drives technology and associated issues: Critical Scientists Switzerland (CSS), European Network of Scientists for Social and Environmental Responsibility (ENSSER) and Federation of German Scientists (FGS/VDW) (2019) Gene drives: a report on their science, applications, social aspects, ethics and regulation.

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.

Involvement of society and reflection of societal values in the regulation of genome-edited organisms: Hartley, S., Gillund, F., van Hove, L. & Wickson, F. (2016) Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453.

References

1 Doudna, J. & Sternberg, S. (2017) A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution. Houghton Mifflin Harcourt. Boston, New York. pp. 243-244.

2 Doudna, J. (2018) The Science and Ethics of Genome Editing, Presentation to Convergence Science Network, February 13. Retrieved from https://www.youtube.com/watch?v=gC_x2XKJjQo.

3 Doudna, J. & Sternberg S. (2017) A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution. Houghton Mifflin Harcourt. Boston, New York.

4 Doudna, J. (2018) CRISPR Biology and Biotechnology: The Future of Genome Editing. Lecture at the Science History Institute, Ullyot Public Affairs Lecture, November 16. Retrieved from https://www.youtube.com/watch?v=mO0xFBQox-Q

5 E.g. Sauer, N.J., Mozoruk, J., Miller, R.B., Warburg, Z.J., Walker, K.A., Beetham, P.R., Schopke, C.R. & Gocal, G.F. (2016) Oligonucleotide-directed mutagenesis for precision gene editing. Plant Biotechnology Journal 14: 496-502; Voytas, D.F. & Gao C (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biology 12: e1001877.; Hartung, F. & Schiemann, J. (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. Plant Journal 78: 742-752.

6 Bratlie, S., Halvorsen, K., Myskja, et al. (2019) A novel governance framework for GMO. EMBO Reports 20: e47812.; Custers, R. (2017) The regulatory status of gene-edited agricultural products in the EU and beyond. Emerging Topics in Life Sciences 1 : 221–229. Conko, G., Kershen, D.L., Miller H. & Parrott, W.A. (2016) A risk-based approach to the regulation of genetically engineered organisms. Nature Biotechnology 34: 493-503.

7 Canadian Biotechnology Action Network (2015) Where in the world are GM foods and crops? Retrieved from www.gmoinquiry.ca/where

8 Ainsworth, C. (2015) A new breed of edits. Nature (outlook) 528: S15-S16.

9 Malnoy, M., Viola, R., Jung, M.H. Koo, O. J., Kim, S., Kim, J. S., Velasco, R., & Nagamangala Kanchiswamy, C . (2016) DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Frontiers in Plant Science 7: 1904.

10 Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F, & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38:163-164.

11 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.

12 International Service for the Acquisition of Agri-biotech Applications (2018) Global status of commercialized biotech/GM crops: 2018. Retreived from http://www.isaaa.org/resources/publications/briefs/54/default.asp

13 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.

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

15 Gelinsky, E. & Hilbeck, A. (2018) European Court of Justice ruling regarding new genetic engineering methods scientifically justified: a commentary on the biased reporting about the recent ruling. Environmental Sciences Europe 30: 52; O'Keefe, M., Perrault, S., Halpern, J., Ikemoto, L., Yarborough, M. & UC North Bioethics Collaboratory for Life & Health Sciences (2015) "Editing" genes: a case study about how language matters in bioethics. The American Journal of Bioethics 15: 3-10.

16 Cong, L., Ran, F.A., Cox, D. et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.

17 Abudayyeh, O.O., Gootenberg, J.S., Essletzbichler, P. et al. (2017) RNA targeting with CRISPR-Cas13. Nature 550: 280-284; Tang, X., Lowder, L.G., Zhang, T. et al. (2017) A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. Nature plants 3: 17103; Kim, H., Kim, S.T., Ryu, J., Kang, B.C., Kim, J.S. & Kim, S.G. (2017) CRISPR/Cpf1-mediated DNA-free plant genome editing. Nature Communications 8: 14406; 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.

18 McDonald, J.I., Celik, H., Rois, L.E., Fishberger, G., Fowler, T., Rees, R., Kramer, A., Martens, A., Edwards, J.R. & Challen, G.A. (2016) Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. Biology Open 5: 866-874; Huang, Y.H., Su, J., Lei, Y., Brunetti, L., Gundry, M.C., Zhang, X., Jeong, M., Li, W. & Goodell, M.A. (2017) DNA epigenome editing using CRISPR-Cas SunTag-directed DNMT3A. Genome Biology 18: 176; Larson, M.H., Gilbert, L.A., Wang, X., Lim, W.A., Weissman, J.S. & Qi, L.S. (2013) CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nature Protocols 8: 2180-2196.

19 Anzalone, A.V., Randolph, P.B., Davis, J.R. et al. (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576: 149-157; Mishra, R., Joshi, R.K. & Zhao, K. (2019) Base editing in crops: current advances, limitations and future implications. Plant Biotechnology Journal 18: 20-31; Qin, L., Li, J., Wang, Q. et al. (2020) High-efficient and precise base editing of C•G to T•A in the allotetraploid cotton (*Gossypium hirsutum*) genome using a modified CRISPR/Cas9 system. Plant Biotechnology Journal 18: 45-56; 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.

20 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.

21 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.

22 Eriksson, S., Jonas, E., Rydhmer, L., & Röcklinsberg, H. (2018) Invited review: breeding and ethical perspectives on genetically modified and genome edited cattle. Journal of Dairy Science 101: 1–17; West, J., & Gill, W. W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

23 Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C. & Wilhelm, R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environmental Evidence 8: 27; Hahn, F. & Nekrasov, V. (2019) CRISPR/Cas precision: do we need to worry about off-targeting in plants? Plant Cell Reports 38: 437-441.

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

25 Cyranoski, D. (2015) Super-muscly pigs created by small genetic tweak. Nature (news) 523: 13-14.

26 Young, A.E., Mansour, T.A., McNabb, B.R. Owen, J.R., Trott, J.F., Brown, C.T. & Van Eenennaamet, A.L. (2019) Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. Nature Biotechnology 38, 225–232. Carlson, D.F., Lancto, C.A., Zang, B., Kim, E-S., Walton, M. Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. (2016) Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34: 479-481.

27 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.

28 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

29 International Service for the Acquisition of Agri-biotech Applications (ISAAA) (2018) Global status of commercialized biotech/GM crops: 2018. Retreived from http://www.isaaa.org/resources/publications/briefs/54/default.asp

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

31 Calyxt (2020) Our products. Retrieved from https://calyxt.com/our-products/

32 Calyxt (2020) What's next? Retrieved from https://calyxt.com/innovation-pipeline/

33 Bomgardner, Melody M. (2017) CRISPR: A new toolbox for better crops. Chemical and Engineering News, June 12. Retrieved from https://cen.acs.org/articles/95/i24/CRISPR-new-toolbox-better-crops.html

34 Arnason, R. (2018) Non-GM canola causes stir among farmers. The Western Producer, February 2. Retrieved from https://www.producer.com/2018/02/non-gm-canola-causes-stir-among-farmers/

35 Cibus (2020) Innovating traditional plant breeding. Retrieved from https://www.cibus.com/our-technology.php; Falco (2020) Falco website. Retrieved from https://www.falcoseed.com/ca/

36 Cibus (2019) Cibus achieves critical milestones for three non-GMO traits to increase canola yield. Press Release, November 6. Retrieved from https://www.cibus.com/press-release.php?date=100619

37 Ibid.

38 Roseboro, K. (2018) New GMO technologies present big challenges to non-GMO supply chain, certification. The Organic & Non-GMO Report, October 30. Retrieved from https://non-gmoreport.com/articles/new-gmo-technologies-represent-major-challenges-to-non-gmo-supply-chain/

39 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: 3; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107.

40 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: 3.

41 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.

42 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.

43 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: 3; Yin, K., Gao, C. & Qiu, J-L. (2017) Progress and prospects in plant genome editing. Nature Plants 3: 17107.

44 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.

45 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; Carey, K., Ryu, J., Uh, K., Lengi, A.J., Clark-Deener, S., Corl, B.A. & Lee, K. (2019) Frequency of off-targeting in genome edited pigs produced via direct injection of the CRISPR/Cas9 system into developing embryos. BMC Biotechnology 19: 25.

46 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.

47 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.

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

49 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: 3; 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.

50 Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C. & Wilhelm, R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environmental Evidence 8 : 27; Proudfoot, C., Carlson, D.F., Huddart, R. et al. (2015) Genome edited sheep and cattle. Transgenic research 24: 147-153; Li, W.R., Liu, C.X., Zhang, X.M. et al. (2017) CRISPR/Cas9-mediated loss of FGF5 function increases wool staple length in sheep. The FEBS Journal 284: 2764-2773.

51 Carey, K., Ryu, J., Uh, K., Lengi, A.J., Clark-Deener, S., Corl, B.A. & Lee, K. (2019) Frequency of off-targeting in genome edited pigs produced via direct injection of the CRISPR/Cas9 system into developing embryos. BMC Biotechnology 19: 25.

52 Modrzejewski D, Hartung, F., Sprink, T., Krause, D., Kohl, C. & Wilhelm R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environmental Evidence 8: 27.

53 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; Li, J., Manghwar, H., Sun, L. et al. (2018) Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnology Journal 17: 858-868.

54 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; Li, J., Manghwar, H., Sun, L. et al. (2018) Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnology Journal 17: 858-868.

55 Sharpe, J.J. & Cooper, T.A. (2017) Unexpected consequences: exon skipping caused by CRISPR-generated mutations. Genome Biology 18: 109; 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: 108; 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; Tuladhar, R., Yeu, Y., Tyler Piazza, J., et al. (2019) CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nature Communications 10: 4056.

56 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; Owens, D.D.G., Caulder, A., Frontera, V. et al. (2019) Microhomologies are prevalent at Cas9-induced larger deletions. Nucleic Acids Research 47: 7402-7417; Simeonov, D.R., Brandt, A.J., Chan, A.Y.A. et al. (2019) A large CRISPR-induced bystander mutation causes immune dysregulation. Communications Biology 2: 70.

57 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.

58 Tuladhar, R., Yeu, Y., Tyler Piazza, J., et al. (2019) CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nature communications 10: 4056.

59 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.

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

61 Canadian Biotechnology Action Network (2019) GM Contamination in Canada: the failure to contain living modified organisms – incidents and impacts. Retrieved from www.cban.ca/ContaminationReport2019

62 Hahn, F. & Nekrasov, V. (2019) CRISPR/Cas precision: do we need to worry about off-targeting in plants? Plant Cell Reports 38: 437-441; 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: 108; Simeonov, D.R., Brandt, A.J., Chan, A.Y.A. et al. (2019) A large CRISPR-induced bystander mutation causes immune dysregulation. Communications Biology 2: 70; Wang, L., Shao, Y., Guan, Y. (2015) Large genomic fragment deletion and functional gene cassette knock-in via Cas9 protein mediated genome editing in one-cell rodent embryos. Scientific Reports 5: 17517.

63 Tuladhar, R., Yeu, Y., Tyler Piazza, J., et al. (2019) CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nature Communications 10: 4056.

64 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.

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

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

67 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.

68 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.

69 USDA-APHIS (2018) Am I regulated under 7 CFR part 340. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated

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

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

72 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.

73 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.

74 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

75 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

76 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; Ono, R., Ishii, M., Fujihara, Y., Kitazawa, M., Usami, T., Kaneko-Ishino, T., Kanno, J., Ikawa, M. & Ishino, F. (2015) Double strand break repair by capture of retrotransposon sequences and reverse-transcribed spliced mRNA sequences in mouse zygotes. Scientific Reports 5: 12281; Ono, R., Yasuhiko, Y., Aisaki, K., Kitajima, S., Kanno, J.& Hirabayashi, Y. (2019) Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. Communications Biology 2: 57; Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F. & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164; Young, A.E., Mansour, T.A., McNabb, B.R. Owen, J.R., Trott, J.F., Brown, C.T. & van Eenennaam, A.L. (2020) Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. Nature Biotechnology 38: 225–232.

77 Young, A.E., Mansour, T.A., McNabb, B.R. Owen, J.R., Trott, J.F., Brown, C.T. & Van Eenennaamet, A.L. (2019) Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. Nature Biotechnology 38, 225–232. Carlson, D.F., Lancto, C.A., Zang, B., Kim, E-S., Walton, M. Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. (2016) Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34: 479-481.

78 Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F. & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164.

79 Skryabin, B.V., Kummerfeld, D.M., Gubar, L. et al. (2020) Pervasive head-to-tail insertions of DNA templates mask desired CRISPR-Cas9-mediated genome editing events. Science Advances 6: eaax2941.

80 Solomon, S.M. (2020) Genome editing in animals: why FDA regulation matters., Nature Biotechnology (correspondence) 38: 142–143.

81 Regalado, A. (2019) Gene-edited cattle have a major screwup in their DNA. MIT Technical Review, August 19. Retrieved from https://www.technologyreview.com/2019/08/29/65364/recombinetics-gene-edited-hornless-cattle-major-dna-screwup/

82 Young, A.E., Mansour, T.A., McNabb, B.R. Owen, J.R., Trott, J.F., Brown, C.T. & Van Eenennaamet, A.L. (2019) Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. Nature Biotechnology 38: 225–232; Carlson, D.F., Lancto, C.A., Zang, B., Kim, E-S., Walton, M. Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. (2016) Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34: 479-481.

83 Carroll, D., Van Eenennaam, A., Taylor, J. et al. (2016) Regulate genome-edited products, not genome editing itself. Nature Biotechnology 34: 477–479.

84 Maxmen, A. (2017) Gene-edited animals face US regulatory crackdown. Nature (news) January 19. doi:10.1038/nature.2017.21331

85 Carlson, D.F., Lancto, C.A., Zang, B., Kim, E-S., Walton, M. Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. (2016) Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34: 479-481.

86 Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F. & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164.

87 Carlson, D.F., Lancto, C.A., Zang, B., Kim, ES., Walton, M. Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. (2016) Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology 34: 479-481.

88 Piore, A. (2017) This genetics company is editing horns off milk cows. Bloomberg, October 12. Retrieved from https://www.bloomberg.com/news/articles/2017-10-12/this-genetics-company-is-editing-horns-off-milk-cows

89 Recombinetics Inc. (2019) Company Statement & FAQs on plasmid insertion found in first gene-edited bull, October 1. Retrieved from http://recombinetics.com/2019/10/01/company-statement-faqs-plasmid-remnant-found-first-gene-edited-bull/

90 Solomon, S.M. (2020) Genome editing in animals: why FDA regulation matters., Nature Biotechnology (correspondence) 38: 142–143.

91 Anon. (2020) Course correction. Nature Biotechnology (editorial) 38: 113.

92 Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F. & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164.

93 Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F. & Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164; Latham, J. & Wilson, A. (2019) FDA Finds unexpected antibiotic resistance genes in 'gene-edited' dehorned cattle. Independent Science News, August 12. Retrieved from: https://www.independentsciencenews.org/news/fda-finds-unexpected-antibiotic-resistance-genes-in-gene-edited-dehorned-cattle/

94 Carroll, D., Van Eenennaam, A., Taylor, J. et al. (2016) Regulate genome-edited products, not genome editing itself. Nature Biotechnology 34: 477–479; Anon (2020) Course correction. Nature Biotechnology (editorial) 38: 113; Solomon, S.M. (2020) Genome editing in animals: why FDA regulation matters. Nature Biotechnology 38: 142–143.

95 FDA (2020) FDA Expertise advancing the understanding of intentional genomic alterations in animals. Press Statement, February 07. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-expertise-advancing-understanding-intentional-genomic-alterations-animals

96 Anon (2020) Course correction. Nature Biotechnology (editorial) 38: 113.

97 Van Eenennaam, A. L. (2019) Responsible science takes time. Nature Research, October 7. Retrieved from https://bioengineeringcommunity.nature.com/users/310649-alison-l-van-eenennaam/posts/54229-responsible-science-takes-time

98 Recombinetics Inc. (2019) Company statement & FAQs on plasmid insertion found in first gene-edited bull, October 1. Retrieved from http://recombinetics.com/2019/10/01/company-statement-faqs-plasmid-remnant-found-first-gene-edited-bull/

99 Ibid.

100 Piore, A. (2017) This genetics company is editing horns off milk cows. Bloomberg, October 12. Retrieved from https://www.bloomberg.com/news/articles/2017-10-12/this-genetics-company-is-editing-horns-off-milk-cows

101 Molteni, M. (2019) Brazil's plans for gene-edited cows got scrapped – here's why. Wired, 26 Aug. Retrieved from https://www.wired.com/story/brazils-plans-for-gene-edited-cows-got-scrappedheres-why/

102 FDA (2020) Expertise advancing the understanding of intentional genomic alterations in animals. Press Statement, February 07. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-expertise-advancing-understanding-intentional-genomic-alterations-animals

103 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.

104 Ramírez-Sánchez, O., Pérez-Rodríguez, P., Delaye, L. & Tiessen, A. (2016) Plant proteins are smaller because they are encoded by fewer exons than animal proteins. Genomics, Proteomics & Bioinformatics 14: 357-370.

105 Reiner, G. (2016) Genetic resistance - an alternative for controlling PRRS? Porcine Health Management 2: 27.

106 See, e.g. Cyranoski, D. (2015) Super-muscly pigs created by small genetic tweak. Nature (news) 523: 13-14; Cohen, J. (2018) Scientists tweak DNA in viable human embryos. Science (news) August 20. Retrieved from https://www.sciencemag.org/news/2018/08/scientists-tweak-dna-viable-human-embryos

107 Qian, L., Tang, M., Yang, J. et al. (2015) Targeted mutations in myostatin by zinc-finger nucleases result in double-muscled phenotype in Meishan pigs. Scientific Reports 5: 14435.

108 Ibid.

109 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; Colombo, S.M., Campbell, L.G., Murphy, E.J., Martin, S.L. & Arts, M.T. (2018) Potential for novel production of omega-3 long-chain fatty acids by genetically engineered oilseed plants to alter terrestrial ecosystem dynamics. Agricultural Systems 164: 31-37.

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 Ibid.

112 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.

113 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

114 Target Malaria (2020) Who we are. Retrieved from https://targetmalaria.org/who-we-are/

115 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.

116 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

117 Grunwald, H.A., Gantz, V.M., Poplawski, G., Xu, X-R.S., Bier, E. & Cooper, K.L. (2019) Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline. Nature 566: 105–109.

118 Gonen, S., Jenko, J., Gorjanc, G., Mileham, A.J. Whitelaw, C.B.A. & Hickey, J.M. (2017) Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs. Genetics Selection Evolution 49: 3; 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.

119 Critical Scientists Switzerland (CSS), European Network of Scientists for Social and Environmental Responsibility (ENSSER) and Federation of German Scientists (FGS/VDW) (2019) Gene drives: a report on their science, applications, social aspects, ethics and regulations. Retrieved from https://ensser.org/publications/2019-publications/gene-drives-a-report-on-their-science-applications-social-aspects-ethics-and-regulations/

120 Ibid.

121 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; ETC Group (2018) Forcing the farm: how gene drive organisms could entrench industrial agriculture and threaten food sovereignty. Retrieved from http://www.etcgroup.org/content/forcing-farm.

122 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.

123 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

124 Friends of the Earth International and ETC Group (2018) United Nations hits the brakes on gene drives. Press release November 29. Retrieved from http://www.etcgroup.org/content/united-nations-hits-brakes-gene-drives; United Nations Convention on Biological Diversity (CBD) (2018) Decision adopted by the Conference of the Parties to the Convention on Biological Diversity 14. Synthetic biology. CBD/COP/DEC/14/19. Retrieved from https://www.cbd.int/doc/decisions/cop-14/cop-14-dec-19-en.pdf

125 United Nations 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; United Nations Convention on Biological Diversity, Twenty-fourth meeting of the Subsidiary Body on Scientific, Technical and Technological Advice, 18-13 May 2020. Meeting Documents. Retrieved from https://www.cbd.int/meetings/SBSTTA-24

126 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. Retrieved from https://www.independentsciencenews.org/environment/gene-drives-a-scientific-case-for-a-complete-and-perpetual-ban/

127 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; Latham, J. (2017) Gene drives: a scientific case for a complete and perpetual ban. Independent Science News February 13. Retrieved from https://www.independentsciencenews.org/environment/gene-drives-a-scientific-case-for-a-complete-and-perpetual-ban/

128 Young, A.E., Mansour, T.A., McNabb, B.R., Owen, J.R., Trott, J.F., Brown, C.T. & Van Eenennaam, A.L. (2020) Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. Nature Biotechnology 38: 225–232.

129 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.

130 Jones, H.D. (2015) Future of breeding by genome editing is in the hands of regulators. GM Crops & Food 6: 223-232; Smyth, S.J. (2017) Canadian regulatory perspectives on genome engineered crops. GM Crops & Food 8: 35-43; Lassoued, R., Phillips, P.W.B., Smyth, S.J. & Hesseln, H. (2019) Estimating the cost of regulating genome edited crops: expert judgment and overconfidence. GM Crops & Food, 10: 44-62.

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

132 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.

133 National Biosafety Technical Commission (CTNBio) (2018) Normative Resolution No. 16, January 15. Retrieved from https://agrobiobrasil.org.br/wp-content/uploads/2018/05/Normative-Resolution-16-of-January-15-2018.pdf

134 Australian Government. (2019) Gene Technology Amendment (2019 Measures No. 1) Regulations. Retrieved from https://www.legislation.gov.au/Details/F2019L00573

135 Farid,M., Cao, J., Lim,Y., Arato,T., & Kodama, K. (2020) Exploring factors affecting the acceptance of genetically edited food among youth in Japan. International Journal of Environmental Research and Public Health 17: 2935.

136 USDA (2020) USDA SECURE Rule paves way for agricultural innovation. Press release. May 14. https://www.usda.gov/media/press-releases/2020/05/14/usda-secure-rule-paves-way-agricultural-innovation; 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

137 USDA (2020) Movement of Certain Genetically Engineered Organism. May 18. https://www.aphis.usda.gov/brs/fedregister/BRS_2020518.pdf

138 USDA (2020) USDA SECURE Rule paves way for agricultural innovation. Press release. May 14; 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

139 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

140 Ellens, K.W., Levac, D., Pearson, C., Savoie, A., Strand, N., Louter, J. & Tibelius, C. (2019) Canadian regulatory aspects of gene editing technologies. Transgenic Research 28: 165-168.

141 USDA (2018) National Bioengineered Food Disclosure Standard, Doc. No. AMS-TM-17-0050. https://www.federalregister.gov/documents/2018/12/21/2018-27283/national-bioengineered-food-disclosure-standard

142 Canadian Biotechnology Action Network (2019) GM Contamination in Canada: the failure of living modified organisms – incidents and impacts. Retrieved from www.cban.ca/ContaminationReport2019

143 Health Canada (2006) Guidelines for the safety assessment of novel foods. Section 4.1.1.5: Allergenicity considerations. Retrieved from https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidelines-safety-assessment-novel-foods-derived-plants-microorganisms/guidelines-safety-assessment-novel-foods-2006.html

144 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.

145 FDA (2020) FDA Expertise Advancing the Understanding of Intentional Genomic Alterations in Animals, Press Statement, February 07. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-expertise-advancing-understanding-intentional-genomic-alterations-animals

146 Anon (2020) Course correction. Nature Biotechnology (editorial) 38: 113.

147 CropLife Canada. (2020) Gene editing in agriculture is here – will Canada be a leader or watch from the sidelines? March 15. https://croplife.ca/gene-editing-agriculture-will-canada-leader-watch-sidelines/

148 Royal Society of Canada, Expert Panel on the Future of Food Biotechnology (2001) Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada. Retrieved from https://rsc-src.ca/en/elements-precaution-recommendations-for-regulation-food-biotechnology-in-canada

149 Canadian Biotechnology Action Network (2015) Are GM Foods and Crops Well Regulated? Retrieved from https://gmoinquiry.ca/regulation/

150 Royal Society of Canada, Expert Panel on the Future of Food Biotechnology (2001) Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada. Retrieved from https://rsc-src.ca/en/elements-precaution-recommendations-for-regulation-food-biotechnology-in-canada

151 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.

152 Ibid.

153 Jasanoff, S. & Hurlbut, B.J. (2018) A global observatory for gene editing. Nature 555: 435-437.

154 Dowling, D. & Lewington, D. (2013) Request for environmental assessment of genetically engineered roundup ready alfalfa under the Environmental Bill of Rights, Ontario. Submitted to the Environmental Commissioner of Ontario. Retrieved from http://www.cban.ca/FarmersAlfalfaRequestON

155 Royal Society of Canada, Expert Panel on the Future of Food Biotechnology (2001) Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, p.3. Retrieved from https://rsc-src.ca/en/elements-precaution-recommendations-for-regulation-food-biotechnology-in-canada

156 ACORN et al. (2017) Request for urgent action to prevent economic harm due to GM alfalfa. Open letter to Minister of Agriculture and Agri-Food, Canada, July 19. Retrieved from https://www.nfu.ca/wp-content/uploads/2018/01/2017-06-16-joint-alfalfa-letter-MacAulay-EN.pdf Manitoba Forage Seed Association. Testimony to the Standing Committee on Agriculture and Agri-Food, 7 June 2010. Retrieved from https://www.ourcommons.ca/DocumentViewer/en/40-3/AGRI/meeting-26/evidence.

157 Clapp, J. (2018) Mega-mergers on the menu: corporate concentration and the politics of sustainability in the global food system. Global Environmental Politics 18: 12-33.

158 Dowling, D. & Lewington, D. (2013) Request for Environmental Assessment of Genetically Engineered Roundup Ready Alfalfa Under the Environmental Bill of Rights, Ontario. Submitted to the Environmental Commissioner of Ontario. Retrieved from http://www.cban.ca/FarmersAlfalfaRequestON