GENETICALLY ENGINEERED ANIMALS

From Lab to Factory Farm

Friends of the Earth

Acknowledgments

This report was written by Dr. Janet Cotter, Logos Environmental, UK and Dana Perls, M.C.P., Senior Food and Technology Campaigner, Friends of the Earth U.S.

Friends of the Earth would like to thank Anthony Jackson, GeneWatch UK; Tamara Lebrecht, GeneWatch UK; Louise Sales, Friends of the Earth Australia; and Katharina Kawall, Fachstelle Gentechnik und Umwelt, Germany for review of this report.

Friends of the Earth

About Friends of the Earth:

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

Visit www.foe.org to learn more.

Logos Environmental

About Logos Environmental:

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

Any errors or omissions in this report are the responsibility of Friends of the Earth U.S.

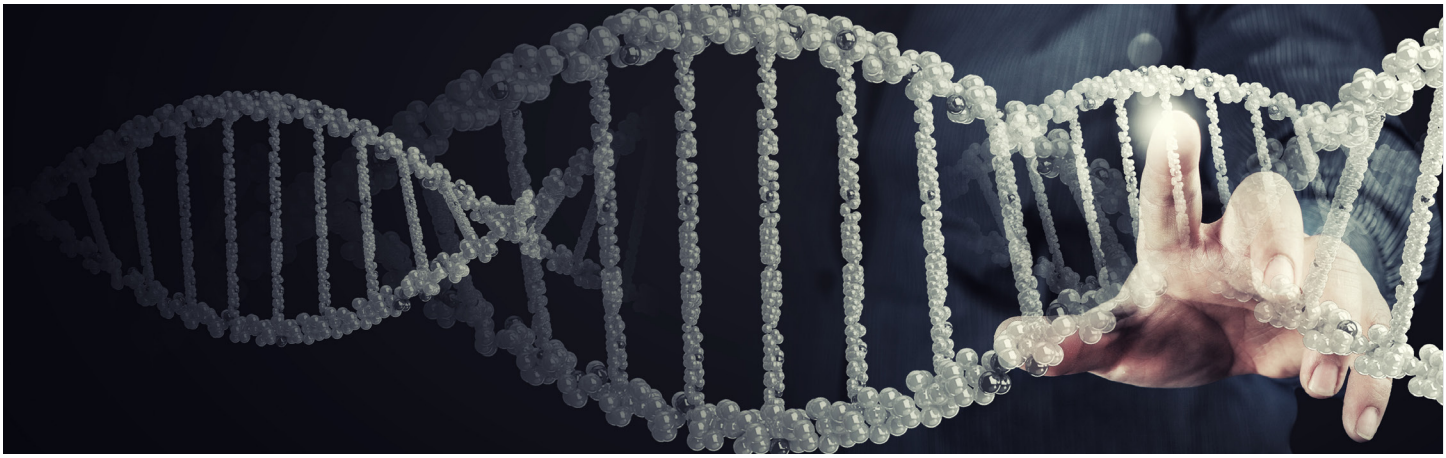


Table of Contents

Gene-edited farm animals, including cows, sheep, pigs and chicken are in the development pipeline.

Executive Summary

In the face of environmental degradation and biodiversity loss from industrial agriculture, it is critical to transition to sustainable and ecological farming systems.[1] But a new wave of research on genetically engineered animals is leading us in the opposite direction — by designing animals to better fit within industrial systems rather than addressing the underlying health, animal welfare and environmental problems associated with these systems.[2] A growing body of scientific evidence is finding that genetically engineered animals may present even more food safety, environmental and animal welfare issues for an already problematic industrial animal farming system.

The AquAdvantage salmon was the first genetically engineered animal approved for human consumption. Since its approval in 2015, concerns about engineering animals have only deepened. Emerging scientific literature reveals that genetic engineering techniques, including new gene editing techniques like clustered regularly interspaced short palindromic repeats, or CRISPR, are not as precise or predictable as initially thought, and can result in unintended physical and genetic mutations that may be inhumane, risky for the health of animals and consumers and environmentally unsustainable. Gene editing techniques may be subject to little to no regulatory oversight or safety assessment.

This report provides insight on health, environmental, ethical and consumer concerns raised by the proliferation of research on genetically engineered animals. We highlight potential risks related to gene editing applications in livestock agriculture as reported in peer-reviewed scientific studies. We emphasize gaps in research and data analysis about how unintended genetic errors resulting from gene editing may impact animal welfare, human health and the environment. We also raise questions about whether gene-edited livestock are necessary, and what a more sustainable, ethical and healthy path for our food system could look like.

The impetus of genetic engineering is to design animals that survive better in factory farms.

Engineering Animals for Factory Farms

The multitude of problems associated with factory farming are unlikely to be addressed and may be exacerbated by the use of genetically engineered farm animals in these systems.

In response to the problems created by concentrated animal feeding operations, or CAFOs, and instigated by the availability of new genetic engineering techniques such as CRISPR, researchers are developing a new generation of genetically engineered farm animals. The goals of these experiments generally fall into three categories: increased yield (e.g., "super-muscly" animals), increased cost-effectiveness in raising animals (e.g., disease resistance) and changes in the composition of the milk, meat or eggs (e.g., nutrition).

Examples of genetically engineered animals in development include "super-muscly" cows, sheep and pigs;[3] pigs resistant to the respiratory disease PRRSV;[4] and gene-edited chickens engineered to potentially produce non-allergenic eggs.[5] Some scientists argue that genetically engineered animals, such as pigs engineered to resist certain diseases, can improve animal welfare, however, the impetus is to design animals that will more easily survive in the cramped and filthy conditions common in CAFOs.

Other research explores the potential of gene drives for farm animals, a genetic engineering technology being developed to drive a desired trait though a herd or population. Although no gene drive system has yet been field tested or deployed,[6] studies suggest that — like previous impacts from genetically modified organisms, or GMOs — organisms might evolve to be resistant to gene drives,[7] and the technology could give rise to off-target effects, which may have severe health, welfare and ecological implications for animals or ecosystems.[8]

Feeding the nearly 10 billion animals raised annually in U.S. factory farms requires a staggering amount of land, genetically engineered seed and toxic pesticides, fertilizer, fuel and water.[9] Industrial animal agriculture is a leading cause of climate change, accounting for 16.5 percent of global greenhouse gas emissions.[10] Raising billions of animals in confinement also generates massive amounts of noxious manure that pollute our air and water — especially in nearby communities. Routine use of antibiotics in animal agriculture that allow animals to survive the unsanitary conditions common in factory farms contributes to the rise of antibiotic resistance, one of our most pressing public health problems.

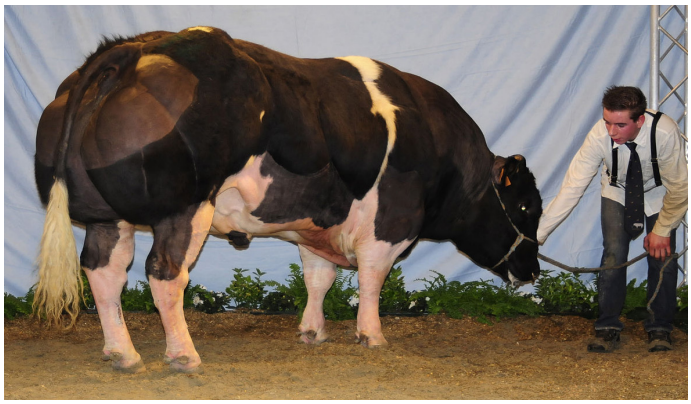


Image: lagriflanders/Flickr/cc

Gene-edited super-muscly animals will magnify welfare concerns currently associated with conventionally bred doubled-muscled animals.

Gene Editing and Unintended Consequences

Scientific studies have shown that the genetic engineering of animals via gene editing techniques like CRISPR and other new technologies can create unintended consequences and potentially harmful effects on animal health, from enlarged tongues to induced tumors. Yet development of genetically engineered animals is moving forward, funded by private companies or government grants, but with little public awareness.

Scientists from the Wellcome Sanger Institute in the UK published a study in *Nature*

Biotechnology that found new genetic engineering techniques like CRISPR may cause "genetic havoc" in cells. Researchers found large deletions and rearrangements of DNA near the target site that were not intended by researchers.[11] Chinese scientists at Nanjing Agricultural University found that gene editing resulted in rabbits having enlarged tongues. And Dr. Kui Li, a scientist from the Chinese Academy of Agricultural Sciences, found some gene-edited pigs had an extra spinal vertebra.[12]

These studies are just a few of the growing body of science demonstrating that gene editing techniques like CRISPR may not be as "precise" in their outcomes as researchers hope. For example, gene editing could cause genes not meant to be targeted to malfunction, and this could lead to health problems or other unintended outcomes in the genetically engineered animal.[13]

Food Safety Implications

Animal genomes are complex. Any genetic errors created by altering DNA could disrupt how genes function. This could potentially produce altered or novel proteins, which in turn could impact food safety. Indeed, one scientific study by Kapahnke and others, published in *Cell* in 2016, used a laboratory culture of human cells and found an altered protein produced in error from the gene editing process.[14] Because food allergens are mostly proteins, unintentionally altered proteins could have significant implications for food safety.

Animal Health and Welfare Implications

Genetic engineering of animals could magnify ethical and welfare concerns related to how animals are bred and the conditions in which they are raised.[15] As part of the genetic engineering process, animals are often cloned.[16] Cloning can lead to birth defects, spontaneous abortions and early postnatal death.[17] Even if cloning is not involved, the genetic engineering process raises welfare issues because the animals may suffer from genetic abnormalities that could cause genes to malfunction and create subsequent health problems in the animal.[18]

Health problems may arise in response to mutations at the cellular level as well. Two independent studies, one by the biotech company Novartis and the other by the Karolinska Institute, published in *Nature Medicine* in 2018 described that cells genetically engineered with CRISPR "have the potential to seed tumors," or may initiate tumorigenic mutations.[19] There is further concern that gene editing for certain traits can perpetuate problematic animal management practices. For example, a frequently-reported trait of gene-edited animals is resistance to various diseases, which could encourage keeping even larger numbers of animals in the close confinement and unsanitary, inhumane conditions that perpetuate disease in the first place.

Image: Rodrigo Gutierrez/Reuters

Genetically engineered animals could exacerbate the problems of factory farms.

Environmental Implications

Industrial animal agriculture contributes to significant levels of air, water and soil contamination. It is also a large contributor to greenhouse gas emissions. There is an urgent need to shift to models of animal farming that have inherently fewer environmental and health impacts.[20] However, instead of instigating this shift, the advent of genetically engineered farm animals will likely further entrench the paradigm of unsustainable, industrial agriculture and may exacerbate environmental problems associated with factory farms. In addition, genetically engineered animals may raise concerns about potential escape and crossbreeding with non-genetically engineered animals. Animals like pigs, goats, horses and rabbits may become feral when they escape from captivity,[21] leading to wild populations of genetically engineered animals.

Consumer Rejection

Societal concerns such as animal welfare suggest that many people are likely to have even more concerns about genetically engineered animals than genetically engineered crops. This suggests that they are likely to reject genetically engineered animals on ethical and welfare grounds, regardless of their trust in the regulatory system to address food safety and environmental concerns. A recent poll found that a majority of U.S. adults believe that engineering animals "to increase protein production" is "taking technology too far."[22] Partially in response to consumer concerns, more than 80 U.S. grocery store chains have committed to not selling genetically engineered salmon, the first genetically engineered animal to enter the U.S. market and approved for human consumption.[23]

Lack of Adequate Oversight and Assessment

Currently, the U.S. Food and Drug Administration (FDA) oversees the food safety aspects of genetically engineered animals,[24] but there are no specific regulations or guidance that cover related environmental impacts.[25] The U.S. has approved one genetically engineered animal for human consumption, the genetically engineered salmon, and regulates it as an "animal drug." It was approved despite many scientists and environmental groups raising serious concerns regarding the risks of escape of the genetically engineered salmon, potential negative impacts on wild salmon populations and concerns regarding food safety. One concern is that gene-edited animals could evade regulatory oversight in the U.S under enforcement discretion and follow the lead of Australia, which allows some gene editing techniques to be used in plants and animals and marketed as food without government regulation.[26]

Change the Farm, Not the Animal

A growing body of science is demonstrating that genetic engineering of animals may lead to unintended consequences for food safety, animal health and welfare and the environment. Many of the "solutions" offered by genetically engineered (including gene-edited) animals are in response to problems caused by current industrial livestock farming systems. However, genetically engineering animals will not address the root problems associated with factory farming, and in fact may entrench an unsustainable and inhumane model of livestock production.

While proponents claim there may be welfare and ecological benefits associated with some of the engineered traits, such as disease resistance or hornless cattle, these potential benefits are within the frame of intensive animal farming practices. However, small and mid-scale, high-welfare, diversified, ecologically regenerative and organic livestock production systems avoid many public health, animal welfare and environmental problems inherent in industrial animal agriculture. In addition, they have been shown to generate important ecological benefits, including carbon sequestration, soil fertility, water savings and reduced dependence on pesticides and fossil fuels.[27]

Recent reports by the United Nations warn that to avoid ecological catastrophe, we need to rapidly transition away from industrial agriculture and reduce consumption of factory farmed meat and dairy.[28] Based on the studies which exemplify the uncertainty and risks from gene editing, U.S. FDA regulations need to effectively regulate all gene-edited animals to ensure the safety of animals, consumers and the environment. Rather than creating genetically engineered animals to fit into factory farms, it is critical to develop sustainable and ecological animal agriculture systems that support animal welfare, preservation and restoration of biodiversity and public health.

The real solution to problems derived from factory farming is ecological agricultural systems.

Rather than addressing problems with CAFOs, animals are being genetically engineered to fit these systems.

Introduction

Intensively (or factory) farmed meat, egg and dairy production, in which large numbers of animals are kept in closely confined indoor conditions known as Concentrated Animal Feeding Operations (CAFOs), poses serious threats to the environment, public health and animal welfare.[29] Now there is potential for a new type of intensively farmed animal: the genetically engineered animal. New genetic engineering techniques, such as gene editing, have increased the technical feasibility of commercial production of genetically engineered animals. These genetically engineered animals may facilitate the redesign of animals to better fit within industrial systems rather than addressing the underlying health, animal welfare and environmental problems associated with CAFOs.[30] Genetically engineered animals may also exacerbate or add new food safety, environmental and animal welfare issues for an already problematic intensive animal farming system. Recent newspaper reports on gene-edited animals cite aborted pregnancies, "enlarged tongues" and extra vertebrae as unintended results of gene editing[31], but what other impacts might also occur? Like all types of genetic engineering, gene editing has unexpected and unpredictable outcomes. Would such genetically engineered animals be safe to eat? Would genetically engineered animals be acceptable to consumers?

To date, there are no commercially available genetically engineered farm animals in the U.S. and elsewhere, and only very few other commercial genetically engineered animals, e.g. the AquAdvantage salmon. As this report describes, many new genetically engineered animal traits are in the development pipeline. These threaten to bring many more genetically engineered animals to our farms and dinner plates.

This report gives an overview of the status of genetically engineered farm animals and current areas of research, such as gene drives for farm animals. Drawing from the published scientific literature, it details the concerns with genetically engineered farm animals and identifies gaps in current scientific knowledge. The report outlines the genetic errors that can be created by the gene editing processes — even by small changes, often called genetic "tweaks" to the DNA of an animal — and how these might affect the health and welfare of the animals, as well as consumer's health. It questions whether there is a need for genetically engineered animals in agriculture, especially given the ethical, health and welfare concerns. Finally, the report discusses the need for regulatory oversight requiring health, welfare and environmental safety assessments of genetically engineered, including gene-edited, animals and the lack of broad public dialogue about the use of gene-edited animals in agriculture.

Gene editing is likely to increase concerns for animal welfare.

Current problems with the intensive farming of animals

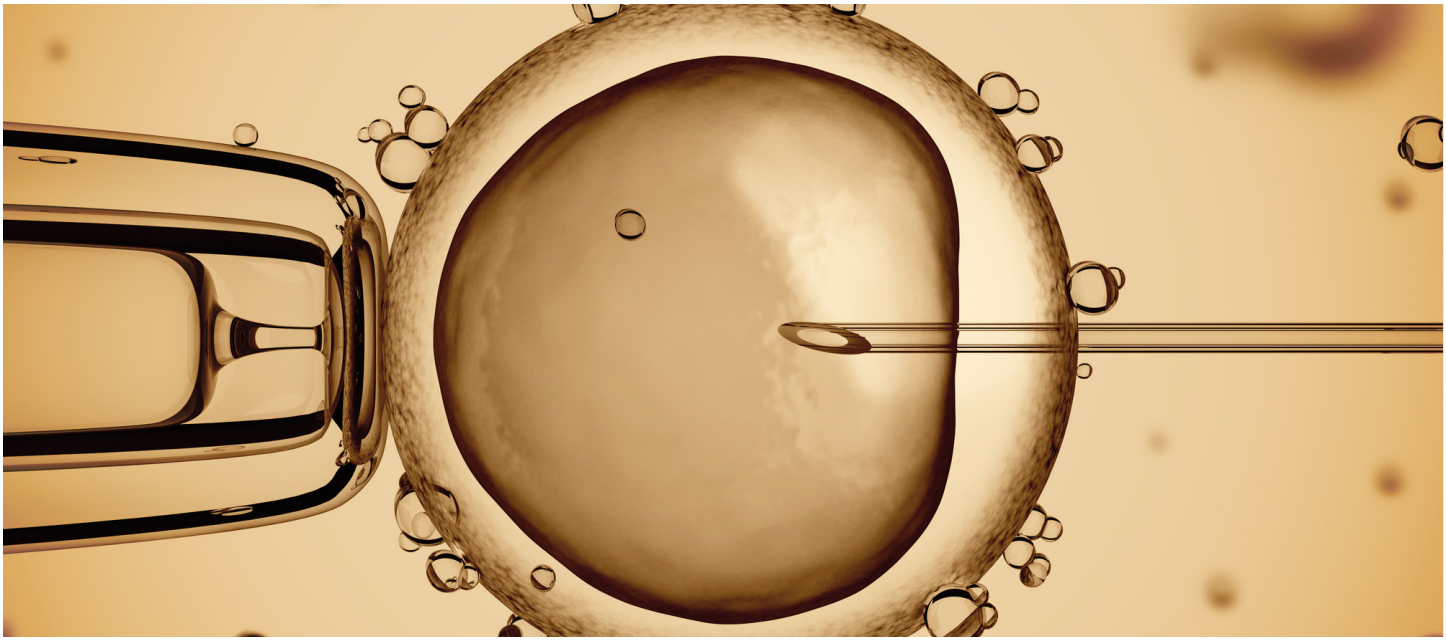
In the last 50 years, the landscape of animal agriculture in the U.S. has changed dramatically.[32] The idyllic image of diversified family farms where animals and crops coexist has been replaced with large-scale intensive 'factory' farms where large numbers of animals — often tens of thousands — are kept confined indoors in conditions that often prioritize profit over animal well-being.[33] The vast majority of animals raised for food are produced within this dominant model, broadly referred to as CAFOs.[34] CAFOs are a by-product of the industrialization of agriculture designed for more streamlined processing and product uniformity.[35]

The intensive animal farming model has had negative consequences for animal, human and environmental health.[36] Close quarters, large quantities of manure and the widespread application of antibiotics and synthetic hormones all contribute to the mounting threats of the system. In the U.S., approximately 335 million tons of animal waste per year[37] containing compounds such as ammonia, nitrogen and phosphorus as well as pathogens and other odorous compounds[38] has contributed to air, water and land pollution.[39] Animal agriculture is also a leading cause of climate change, accounting for 16.5 percent of global greenhouse gas emissions.[40]

Industrial animal farming also contributes to the growing threat of antibiotic resistance in humans. Resistance to antibiotics kills at least 23,000 Americans each year, according to the Centers for Disease Control and Prevention.[41] In 2011, around 70 percent of medically important antibiotics in the U.S. were sold for use in farm animals, not in human medicine.[42] The routine use of antibiotics in farm animals, to pre-empt the spread of animal diseases and to accelerate animal growth, allows bacteria to develop resistance to antibiotics.[43] Both antibiotics and antibiotic resistant bacteria can escape from farms into the environment through feces, air, water, soil, meat and even workers.[44] Once antibiotics are in the environment, they contribute to the development of antibiotic-resistant bacteria.[45]

Animals on intensive farms are subject to problematic conditions and practices. For example, pregnant pigs are kept in gestation crates where they are unable to turn around or lie down, and broiler chickens[46] raised for meat have been selectively bred over generations for hyper-production so that many struggle to move or even stand. There are minimal federal laws regulating the treatment of the nearly 10 billion[47] animals raised on farms for food in the U.S.[48]

In response to the current problems created by intensive animal farming and facilitated by the availability of new genetic engineering techniques, such as gene editing, researchers are developing a new generation of genetically engineered farm animals. These include pigs resistant to certain diseases and cows without horns (see *Gene editing in farm animals*). However, current problems in animal farming could be exacerbated by the commercialization of genetically engineered farm animals. For example, animals genetically engineered to be resistant to various diseases could further facilitate the crowded and unsanitary conditions common in CAFOs, and the spread of other, additional diseases.



Genetically engineering techniques use cloning or microinjection of genetic material into an egg cell— both of which are problematic.

What is a genetically engineered animal?

Genetic engineering is very different from conventional (often called selective) breeding. Genetic engineering does not rely on mating to obtain desired traits. Instead, researchers directly alter the genetic material (usually DNA) of an organism using laboratory techniques. It is this direct alteration of genetic material by humans that defines genetic engineering in the U.S.[49] and underpins the definition of a genetically modified organism in the United Nations[50] and the European Union[51].

Standard (or first generation) genetic engineering — as devised in the 1970s — inserts genes (made up of DNA) at a random location into an organism's own DNA, or genome. The inserted genes generally confer a trait (e.g. a growth hormone in the case of the genetically engineered AquAdvantage salmon)[52]. If those genes are from a different organism (often called "foreign" genes), then the resulting genetically modified organism (GMO) is transgenic. For example, the genetically engineered AquAdvantage salmon (see *Status of genetically engineered animals*) is transgenic because genes from other species of fish have been inserted.[53]

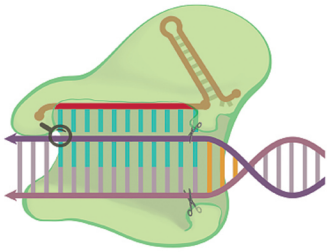
Genetic engineering does not always result in the desired outcomes. The insertion of genes at random sites of an animal's genome has been described as "ham-fisted"[54] and the expression of the inserted genes is unpredictable[55]. Consequently, although there are exceptions, such as the genetically engineered AquAdvantage salmon, first-generation genetic engineering techniques have not, in general, been successful in producing healthy genetically engineered animals that expressed the new trait consistently over multiple generations[56]. However, in the last few years, new (or second-generation) techniques of genetic engineering, such as gene editing, have increased the technical feasibility of producing commercial genetically engineered animals (see *What is gene editing?*).

What is gene editing?[57]

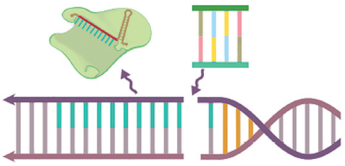
Gene editing (also called genome editing) is a set of new genetic engineering techniques,[58] principally used for altering the genetic material of plants and animals. Gene editing has only recently become commercially feasible, with the most talked about technique, clustered regularly interspaced short palindromic repeats (CRISPR)[59], developed around 2012[60,61]. All gene editing techniques use a synthetic molecular guide with the goal of changing DNA while it is present in the organism, i.e., in situ. With gene editing, as with first-generation genetic engineering techniques, the change in the organism's genetic material is not achieved through the breeding process as it would be

How gene editing works

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



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



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

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

in conventional breeding. Instead, the genetic material is changed directly and artificially, by humans using laboratory techniques. This means that gene editing, like other forms of genetic engineering, produces GMOs.

Gene editing differs from standard or first-generation genetic engineering techniques in that, with gene editing, genes do not necessarily have to be inserted into the organism to produce a new trait. Instead, genetic material, usually a nuclease (enzyme) that cuts DNA,[62] is introduced into the organism. This nuclease cuts the organism's DNA. The cut to the DNA activates the cell repair mechanisms which repair the DNA. There are many possible changes to the DNA that CRISPR can achieve, depending on how the DNA is repaired.[63] Without any controls on DNA repair, gene editing can be used to disrupt a target gene or genes (to "knock out" genes). However, a synthetic DNA repair template is often used to direct a particular change in the DNA. Alternatively, genes conferring a particular trait can be inserted using gene editing during the DNA repair.[64] The resulting gene-edited organism may or may not produce a novel protein as part of the novel trait, as most current commercialized GMOs do — it may knock out a gene instead. In fact, many, if not most, of the current genetically engineered animals under development using gene editing have knocked-out genes, with relatively few containing inserted genes.

Developers hope that, because genetically engineered animals can be produced by gene editing without inserted genes (e.g. from a different species), they may be more acceptable to consumers and be viewed more leniently by regulators than first-generation genetically engineered animals.[65] Genetic changes introduced by the gene editing are often referred to by the developers as "tweaks".[66] However, these "tweaks" can cause substantial genetic errors that could affect food safety (see *Genetic errors created by the genetic engineering process*).

Gene editing techniques, in particular CRISPR, are reported to give more predictable results than first-generation genetic engineering techniques for animals, increasing the technical feasibility of producing genetically engineered animals.[67] This has resulted in a deluge of "proof of concept" studies of genetically engineered animals created by gene editing (see *Gene editing in farm animals*). However, there are concerns regarding genetic errors with gene-edited organisms[68] (see *Genetic errors created by the genetic engineering process*), particularly in combination with other commonly used techniques such as animal cloning (see *Cloning as part of the genetic engineering process*).

Cloning as part of the genetic engineering process

Unlike plants, which can entirely regenerate from single cells, animals can only develop from an embryo. Hence, genetically engineered animals can only be developed by cloning methods or by the direct manipulation of the fertilized egg cell (zygote) by microinjection.

Although cloning is often used as part of the genetic engineering process[69], genetically engineered animals are different from cloned animals. With cloned animals, the aim is to create an identical copy of a whole animal, made by copying genetic information within a single cell from an adult animal. The DNA from a cell of an adult donor animal is transferred into an egg cell, which is then implanted into the womb of an adult female.[70] In the womb, the embryo develops into an animal that is genetically identical to the donor animal (i.e. a clone). The world's first cloned animal — Dolly the sheep — was produced in 1996.[71] At the stage of DNA transfer, the DNA can be altered (genetically engineered), in which case the embryo will carry the genetic modification and become a genetically engineered animal. Similar concerns regarding animal welfare and food safety apply to both clones and genetically engineered animals, especially as genetically engineered animals are also often clones.[72]

With cloning, the DNA from a cell of an adult donor animal is transferred into an egg cell, which is then implanted into the womb of an adult female. The concept is that the embryo develops into a genetically identical animal to the donor animal, unless the transferred DNA has been genetically engineered, in which case it will also carry the genetic modification.[73]

Cloning typically achieves a success rate of only about 10-25 percent,[74] meaning that most embryos transferred into host's wombs do not result in a full-term pregnancy and are aborted. For example, a study on gene-edited, cloned cattle found that, of 147 genetically engineered embryos resulting in 50 pregnancies, only 23 calves were born and only just over half (13) survived longer than 6 months.[75] A similar study on cloned, gene-edited cattle found that out of 83 pregnancies, 20 calves were born, with 11 calves surviving longer than three months.[76] For those cloned animals that survive, birth defects are common.[77] Defects include premature death, pneumonia, liver failure and obesity. For example, a study on cloned mice found that up to 4 percent of the genes were malfunctioning during pregnancy.[78]

Although the U.S. Food and Drug Administration (FDA) has concluded that products from cloned animals are safe to eat,[79] the problems of birth defects, abortions and early postnatal death, in addition to the necessary use of euthanasia in cloned animals[80] has led to a high level of concern regarding their welfare (see *Ethical and welfare concerns for genetically engineered animals*).[81] Indeed, animal welfare concerns are so prominent that, in 2015, the European Union voted to ban the cloning of all farm animals, their descendants and products derived from them — including imports into the EU.[82]

Using microinjection to genetically engineer animals means that cloning is not necessary. With microinjection, either the genes to be inserted (the transgene) or the gene editing complex are injected into embryos. Microinjection can give rise to genetic "mosaicism" where, if the embryo is already more than one cell, some of the cells will be genetically engineered, and some not.[83] If mosaicism occurs, the genetic engineering may not be effective and the engineered change to DNA might not be transmitted to offspring of the genetically engineered animal.[84] Although mosaicism is more of a technical difficulty than a food safety, animal health/welfare or environmental concern, it represents a stumbling block for the gene editing of animals without resorting to cloning. This means that, despite advances in microinjection, cloning is still widely used to created gene-edited animals.[85]

AquAdvantage salmon is the only genetically engineered animal approved as food, but this could soon change.

Status of genetically engineered animals

Currently, there are no commercialized genetically engineered farm animals (e.g. pigs, sheep, cows, chickens) anywhere in the world. In the EU, no genetically engineered farm animals, or their products, have been approved for marketing, nor have there been any applications for marketing genetically engineered animals in the EU.[86] The only genetically engineered animal approved for human consumption is a genetically engineered salmon, called the AquAdvantage salmon, approved only in the U.S. and Canada[87] and currently only for sale in Canada[88]. The salmon was approved in the U.S. in 2015.[89] It was only approved after long deliberations, as many scientists[90] and environmental groups[91] raised serious concerns regarding the risks of escape of the genetically engineered salmon and potential negative impact on wild salmon populations, and concerns regarding food safety. Despite the FDA's approval, 80 grocery retailers with nearly 16,000 stores in the U.S. have made commitments to not sell genetically engineered salmon.[92]

The FDA, which oversees both the environmental and food and drug aspects of genetically engineered animals, has previously approved a few applications for genetically engineered animals. Prior to the genetically engineered salmon approval, these approvals have not been for food use, but for drug production, e.g. a goat engineered to produce a human pharmaceutical in its milk[93], also approved in the EU[94], and a chicken engineered to produce a human pharmaceutical in its eggs[95]. In 2003, the FDA decided that a novelty genetically engineered fish (GloFish), marketed as a pet, was not a food or drug, and saw "no reason to regulate these particular fish" as the GloFish did not "pose any more threat to the environment than their unmodified counterparts".[96] However, there was no risk assessment upon which to base the FDA's claim.

The landscape of genetically engineered animals may be about to change, as new genetic engineering techniques, such as gene editing, appear to be technically more successful in creating genetically engineered animals than first-generation genetic engineering techniques.

This report focuses on genetically engineered farm animals in agriculture. However, several other types of genetically engineered animals are either under consideration or in development, which are not detailed in this report. These include:[97]

- More genetically engineered "pharm" animals to produce particular drugs or pharmaceuticals
- Other species of genetically engineered fish, e.g. trout
- Genetically engineered animals for research purposes, e.g. "knock out" mice with certain genes disabled
- Animals that have been genetically engineered to be sources for cells, tissues or organs for transplantation into humans (xenotransplantation)
- Novel genetically engineered pets, similar to the Glofish. For example, micro-pigs or koi carp engineered with altered size, patterns and colors
- 'De-extinction' animals, created by genetically engineering modern species to closely resemble their extinct counterparts, e.g. genetically engineered pigeons which are designed to be similar to extinct passenger pigeons[98]
- Mosquitoes that have been genetically engineered to be "self-limiting" (in that the offspring do not reach adulthood), in order to reduce populations of mosquitoes. Pilot projects by a company, Oxitec, have taken place in Brazil, Panama and the Cayman Islands[99], although the trial in the Cayman Islands has ceased because it was

not successful in reducing the size of the mosquito population[100]. Oxitec has also applied to release the genetically engineered mosquitoes in Florida, U.S.[101], while Target Malaria is planning releases of self-limiting mosquitoes in Burkina Faso in Africa,[102] and possibly other African countries such as Mali and Uganda[103].

If dehorning of cattle is unnecessary, then so too is gene editing to produce hornless cattle.

Gene editing in farm animals

Gene editing, particularly CRISPR/Cas, has been applied to several farm animals in experimental proof of concept studies (see examples below).[104] The goals of the gene editing generally fall into three categories: increased yield, increased cost effectiveness in raising animals (e.g. disease resistance that facilitates living in overcrowded and unsanitary conditions) and changes the composition of the milk, meat or eggs (e.g. changed nutrition).

Potential gene-edited animals for increased yield include:

- "Super-muscly" cows, sheep, goats and pigs to produce a higher yield of meat per animal[105]
- Increased wool and hair length in sheep[106] and goats[107]

Potential gene-edited animals for increased effectiveness include:

- Hornless (polled) cattle[108]
- Pigs resistant to different diseases, e.g. porcine reproductive and respiratory syndrome virus (PRRSV)[109], African swine fever[110] or transmissible gastroenteritis virus (TGEV)[111]
- Cows with human genes inserted into them to increase antibacterial properties of their milk, reducing susceptibility to mastitis[112]
- Cows with increased resistance to tuberculosis[113]

Potential genetically engineered animals for changed nutrition include:

- Gene-edited chickens that could potentially produce eggs without a certain egg white protein that some people are allergic to.[114] However, the eggs produced by the gene-edited chicken have yet to be tested
- Pigs engineered to produce high levels of omega-3 fatty acids, potentially providing health benefits[115]

Most, if not all, of the examples of gene-edited animals are proof of concept studies. Proof of concept studies report only that the intended genetic change has been achieved. However, such studies don't mean that they will be on the market anytime soon, or even at all. For example, the study of the genetically engineered pig with high omega-3 acids was published in 2007, but there have not been any applications for commercial production. There's a great deal of difference between a research study and a commercial venture.

As yet, there have been no applications to regulators to commercialize animal products from gene-edited farm animals anywhere in the

world. However, some countries, such as China, are investing heavily into developing gene-edited animals.[116] For example, the gene-edited sheep and goats with increased wool and hair length were developed by Chinese scientists, supported by a grant from the Chinese Agriculture Ministry in the case of sheep.[117] Similarly, gene-edited super-muscly sheep and goats, cows with reduced susceptibility to mastitis and cows resistant to tuberculosis have all been developed by Chinese scientists, supported by government grants.[118]

Some private companies are also investing in gene editing for farm animals. For example, a UK company, Genus, has funded much of the research to date on gene-edited pigs resistant to PRRSV[119], and a U.S. company, Recombinetics, has led development of gene-edited hornless cattle[120]. Genus and Recombinetics have also collaborated to produce super-muscly sheep and cattle.[121]

Proof of concept studies rarely assess any unexpected effects created by the genetic engineering process, nor assess food safety or any potential environmental effects. In short, although the desired change may be achieved through genetic engineering, there is no, or very little, information on what else might have inadvertently changed in the organism in these proof of concept studies.

Gene editing for disease resistance would make it easier to raise pigs in unsanitary, crowded conditions common in factory farms.

Are genetically engineered animals necessary in agriculture?

In many cases, the types of genetically engineered traits for agricultural proposals will be ones that are sought after only within a paradigm of intensive livestock farming, e.g. super-muscly animals. Therefore, it is critical to ask questions about what problems genetically engineered animals are seeking to solve, and whether there are less risky and more sustainable and humane solutions to that problem.

Many of the examples of gene-edited farm animal proposals, as described in *Gene editing in farm animals*, are intended to maximize profits in animal farming. For example, super-muscly animals increase the amount of meat from an individual animal, but there are already animal welfare issues with conventionally bred "double-muscled" farm animals. (See *Ethical and welfare concerns for genetically engineered animals*)[122]

Proposals for gene-edited pigs that are resistant to diseases such as PRRS are trying to treat the symptoms of intensive farming, rather than addressing the root cause of the problem. In pigs, PRRS is a modern disease, dating from the late 1980s, and is associated with keeping pigs in industrial farms with a high stocking density.[123] Infection is affected by husbandry practices such as early age of weaning.[124] In addition, the gene editing approach may not produce long-term resistance to the PRRS virus, because there are many strains of the virus, and the virus keeps evolving to overcome resistance.[125]

It is critical to ask questions about what problems genetically engineered animals are seeking to solve, and whether there are less risky and more sustainable and humane solutions to that problem.

Other gene-edited pig applications propose to make pigs that are rich in omega-3. However, this can be achieved without genetic engineering. A Scandinavian firm has produced a pig rich in omega-3 naturally, simply by adding (non-GMO)

rapeseed oil to the pig's diet.[126] The genetic engineering of pigs to be enriched with omega-3 has been criticized as unnecessary for reasons beyond food safety issues: omega-3 is available in many foodstuffs (such as rapeseed oil); there are welfare issues associated with unnecessary research on animals; the proposed genetic engineering avoids addressing conventional, intensive pig farming problems; and genetically engineered pigs will not move food systems toward a healthier natural diet. [127]

> *"Offering us GM [genetically modified] pork to provide us with a plentiful nutrient is an obvious attempt to drum up a need that justifies the science... We are altering the genome of an animal to enable consumers to continue with their self-destructive eating habits. What does this say about us if that is reason enough to manipulate sentient life?* Fiester (2006)[128]

Another proposed application of genetic engineering is dehorned cattle. The practice of physically dehorning and disbudding cattle is performed in order to protect animals and handlers from accidental injury while cattle are packed into small, contained spaces whether on-farm or during transport (such as in trailers).[129] Alongside incurring additional costs for farmers, dehorning is painful for the animals and raises animal welfare concerns.[130] It is questionable whether the practice of dehorning is necessary, as it is associated with the close packing of cattle.[131]

If hornless cattle are required, there are alternatives to genetic engineering. For example, cattle can be, and have been, bred to be without horns using conventional (selective) breeding.[132] However, this is seen as problematic for the popular dairy breed in Europe and the U.S., the Holstein, mainly because the genetic makeup of polled cattle results in lower milk production[133]. However, this genetic gap in milk production levels is closing[134] due to advanced conventional breeding methods, such as marker-assisted selection and genomic selection.[135] Such advanced conventional breeding techniques are currently being used to breed hornless cattle in Australia.[136]

Many of the 'solutions' offered by genetically engineered (including gene-edited) animals are in response to problems caused by current intensive animal farming systems. A more ecological (and humane) way of farming would address the root cause, which is intensive animal farming.

Intensive farming practices for cattle cause numerous problems, and are linked to disease, for example, to a higher risk of tuberculosis in cattle.[137] Research has shown that the risk of herd infection for tuberculosis doubles with herds of 150 cattle or more, compared to those with 50 or fewer cattle. Fewer hedgerows and the use of silage, typical of more intensive farming practices, were among the list of additional factors that contributed to increased risk of tuberculosis infection. As with hornless cattle, genetic engineering is unable to address the root causes of this problem. In addition to moving away from intensive cattle farming practices, cattle resistant to tuberculosis are being developed through advanced conventional breeding techniques such as marker-assisted selection.[138]

Recent advances in conventional animal breeding show that genetically engineered animals are not necessary in agriculture. As gene editing is a relatively new area of research for scientists, many of the current studies are performed to show what is technically possible,[139] not necessarily what is needed.

Many of the "solutions" offered by genetically engineered (including gene-edited) animals are in response to problems caused by current intensive animal farming systems. While some proponents propose there may be welfare benefits associated with some of the engineered traits, such as disease resistance or hornless cattle, this welfare benefit is within the frame of intensive animal farming practices.[140] For example, increased incidents of mastitis during lactation are found in CAFOs, and it's thought this is because the animals are exposed to more bacteria because of poor cleanliness.[141] A more ecological (and humane) way of farming would

address the root cause, which is intensive animal farming.

Large herds or flocks kept in confined areas, e.g. within CAFOs, are more vulnerable to the rapid spread of animal diseases.[142] In the case of ruminants, allowing animals to graze on well-managed pastures will help to prevent the poor health that can arise from high-density, sometimes unhygienic conditions in CAFOs, thereby reducing the need for antibiotics.[143] In the case of chickens and pigs, healthier, less stressed animals that are allowed to be free range in farming systems optimized for animal welfare farming systems will manifest fewer of the health problems so common in intensive farms.[144]

Ethical and welfare concerns for genetically engineered animals

There are already considerable ethical[145] and welfare[146] concerns regarding the raising of farm animals in CAFOs (see *Current problems with the intensive farming of animals*). As explained below, genetic engineering could magnify these concerns in two principal ways: the effect the new trait has on animal welfare and the physical process of genetic engineering.

The physical process of genetic engineering raises ethical concerns related to farm animals, regardless of whether cloning is used or not.[147] Ethical concerns that have been documented in respect to the genetic engineering of animals include: the treatment of animals solely as instruments for human benefit and interests; infringement of the integrity of the animal by causing fundamental alterations to its DNA and the patenting of genetically engineered animals as technological products.

During the genetic engineering process, large numbers of animals are required as "mothers" for implantation of genetically engineered embryos. It is estimated that an average of 24 embryos are needed to produce one gene-edited pig using microinjection instead of cloning.[148] This is five times fewer animals than required by cloning,[149] but still subjects many animals to dangerous procedures[150]. This is compounded by the fact that although research institutions are federally regulated, protections for animals in research or agriculture are minimal,[151] enforcement by United States Department of Agriculture (USDA) is lax[152] and animals have no recourse under the law.

Genetic engineering of animals can perpetuate poor animal management, particularly in intensive farming operations.

Genetic engineering of animals can perpetuate poor animal management, particularly in intensive farming operations (see *Are genetically engineered animals necessary in agriculture?*), compounding existing welfare concerns. For example, gene editing for disease resistance could facilitate the raising of pigs in less hygienic conditions, or cattle without horns could be kept in more crowded enclosures.[153]

> *"It could be argued that benefits of GM [genetic modification] or genome editing for animal welfare are only relevant if animal management is not downgraded as a consequence; for example, if more resistant animals are kept in less hygienic stables or polled animals are kept in more crowded enclosures"* Eriksson et al. (2018)[154]

The introduced trait may itself cause, or increase, existing welfare problems in genetically engineered animals. For example, concerns already exist over the welfare of (conventionally bred) "double-muscled" pigs and cattle, which may have problems calving and have high mortality rates.[155] Such problems could also occur in other gene-edited super-muscly farm animals.

> *"Many ethical concerns can be expected to arise by promoting double-muscling through genome editing. Difficult delivery abounds in Belgian Blue cattle because the active expression of MSTN starts in pregnancy and frequently necessitates Caesarean section. Belgian Blue calves can suffer from leg problems (due to their heavier weight), breathing complications, and enlarged tongues. Some people would consider that*

The introduced trait may itself cause, or increase, existing welfare problems in genetically engineered animals.

If genetic engineering of animals affects their health or welfare, it could affect nutritional aspects of their meat, eggs and dairy products.

> *animals that are destined to acquire double-muscling through genome editing lose their "purpose as a creature."* Ishii (2017)[156]

In addition to welfare issues arising from the introduced trait, welfare issues can arise from any genetic errors created by the gene editing process, for example those caused by off-target effects (see *Genetic errors created by genetic engineering processes*). These genetic errors could cause malfunctioning of one or more parts of the cell machinery and lead to health problems in the genetically engineered animal.[157] Importantly, such genetic errors can occur as an unintended consequence of genetic engineering, even if genes (e.g. from a different species) are not inserted into the animal, as might be the case with gene-edited animals (see *What is gene editing?*). For example, researchers found that gene editing for super-muscly animals resulted in rabbits, pigs and a goat having enlarged tongues and pigs having an extra spinal vertebra (see *Unexpected effects with gene editing: on-target*), even though no DNA had been inserted.

> *"Some off-target mutations could be deleterious mutations that negatively affect animal health; this may lead to concerns over animal welfare. For example, missed off-target mutations could affect animal health if such unintended genetic changes lead to tumor formation due to mechanisms such as the disruption of a tumor suppressor gene. As the history of cloned animals suggests, the investigation of off-target mutations seems vital to the use of genome editing in livestock breeding from the viewpoint of animal welfare."* Daley et al. (2010)[158]

If animals are in sub-optimal health, e.g. as a result of poor welfare, this can affect the composition of their meat, eggs and dairy products. A long-term U.S. study found that pasture-grazed meat is healthier for people than grain-fed meat as it has less overall fat, a more desirable fatty acid profile, with a better ratio of omega-3 fatty acids to omega 6 fatty acids, higher in precursors for vitamins A and E and certain cancer-fighting antioxidants.[159] Similarly, studies in the UK found that organic meat and milk are more nutritious than their conventionally (non-GMO) produced counterparts, for example containing 50 percent more beneficial omega-3 fatty acids,[160] primarily because the animals were pasture-grazed. Therefore, if genetic engineering of animals affects their health and welfare, either directly because of the genetic engineering process, or indirectly, e.g. because it further intensifies animal farming, it could affect nutritional aspects of meat, eggs and dairy products derived from genetically engineered animals.

Like the GMO salmon, food from gene-edited farm animals could soon be given the green light but without any safety assessment.

Consumer acceptance of genetically engineered animals

> *"Technology that was already controversial in a crop context is perceived as even more problematic when applied to sentient organisms, such as farmed livestock."* Ishii (2017)[161]

There is already widespread consumer rejection of genetically engineered crops around the

world, as demonstrated by the labeling of GMO-derived food in 64 countries[162] and the calls for labeling of food derived from GMOs in the U.S.[163] In Europe, GMO-derived food is absent from shops[164], with the cultivation of genetically engineered crops banned in nearly two-thirds of EU countries[165]. A recent poll found that a majority of U.S. adults believe that engineering animals "to increase protein production" is "taking technology too far."[166] Societal concerns such as animal welfare suggest that many people are likely to have even more concerns about genetically engineered animals[167] than for genetically engineered crops[168] and are likely to reject genetically engineered animals on ethical and welfare grounds, regardless of their trust in the regulatory system to address food safety and environmental concerns.[169]

Societal concerns such as animal welfare suggest that many people are likely to have even more concerns about genetically engineered animals than for genetically engineered crops and are likely to reject genetically engineered animals on ethical and welfare grounds.

Could gene drive systems be applied to farm animals?

Gene editing techniques have facilitated the possibility of "gene drives." Gene drive systems enable biased inheritance of a genetic element so that offspring within a population have an increased chance of inheritance of a given trait.[170] This means a few gene-edited organisms could potentially "drive" new genes through populations of a species, even the entire global population. As yet, no gene drive system has been field tested or deployed.[171] It's not known whether gene drive systems would actually work in real situations, as organisms might evolve to be resistant to them[172], although researchers are currently working on ways this resistance could be overcome[173]. However, serious concerns have already been voiced regarding the potential adverse effects of gene drive systems on biodiversity, ecological and agricultural systems and the humans that depend on them (see *Concerns regarding gene drive systems in farm animals*).[174]

For farm animals, a hypothetical gene drive system intended to drive a desired trait though a herd or population of a farm animal has been outlined.

Although the main focus of research on gene drive systems is on mosquitoes,[175] agricultural insect pests[176] and invasive species,[177] a gene drive-type mechanism has recently been developed for mammals[178]. For farm animals, a hypothetical gene drive system intended to drive a desired trait though a herd or population of a farm animal has been outlined (Fig. 1)[179]. The intention is that a gene drive system could increase the speed of spreading a gene-edited trait through a population compared to spreading the gene-edited trait through selective breeding. For example, computer scenarios with pigs showed that the gene drives spread a trait through pig populations 1.5 times more quickly than with gene editing alone.[180]

Concerns regarding gene drive systems in farm animals

Although still at the hypothetical stage, risks identified with gene drive systems intended to drive a particular trait through a herd or population of farm animals include the following:[181]

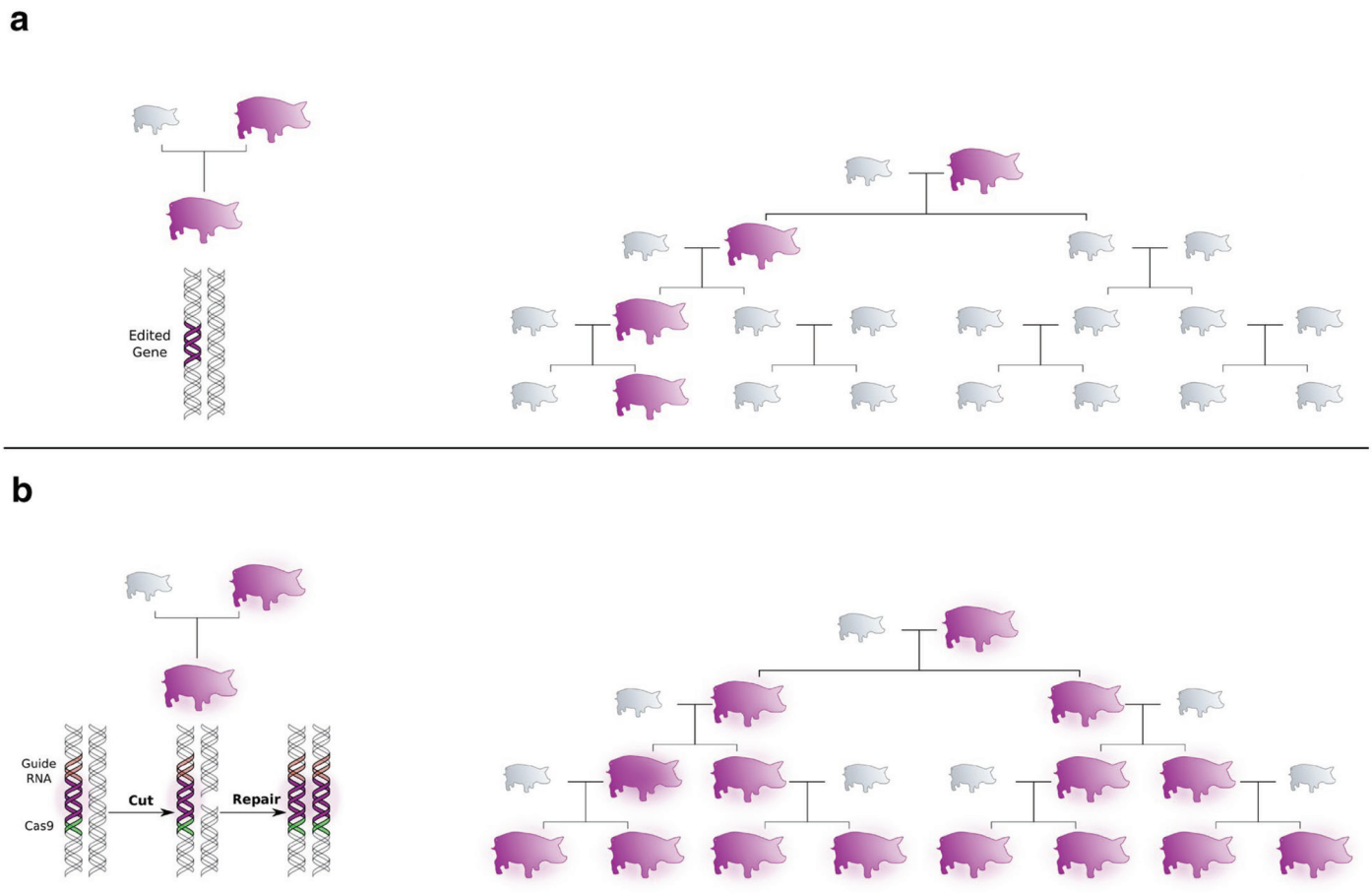
- The gene chosen to spread may turn out not to confer the desired trait. This could occur because of the wrong choice of gene, unforeseen environmental changes that influence gene expression or changes in the genetic background. All these aspects could have adverse effects on the animals that gained the trait.
- Accidental spread of gene drives from a farmed population to a natural population, which could affect biodiversity and potentially entire ecosystems
- The genes inserted to perform the gene

drive could mutate and no longer produce the desired effect, but could give rise to off-target effects instead (see *Unexpected effects with gene editing: off target*).

Scientists and others are already warning[182] that the consequences of gene drives could be severe should any unexpected effects from the gene editing process (e.g. from off-target effects, see *Unexpected effects with gene editing: off target*)[183] or other, e.g. ecological, unintended consequences arise[184]. Although the primary focus of these discussions is currently gene drive systems in insects, the concerns also extend to farm animals. A global agreement at the United Nations Convention on Biological Diversity[185] in 2018 agreed that, prior to any gene drive release (including experimental releases), a thorough risk assessment must be carried out and safety measures put in place to prevent potential adverse effects. Governments must also seek or obtain the approval of potentially affected

indigenous peoples and local communities prior to considering any release of gene drives. The decision also acknowledges that more studies and research on impacts of gene drives are needed to develop guidelines to assess gene drive organisms before they are considered for release.

Fig. 1: How gene editing combined with gene drive system could drive a trait through a herd of animals, from Gonen et al 2017[186]. **(a)** Inheritance with genome editing and (**b)** inheritance with genome editing with gene drives.



Complexity of Animal Genomes

The old notion in molecular biology of "one gene, one function" became invalid.

Animal genomes are complex. Genomes contain genes, made up of DNA, which are "read" and processed by the cell components (e.g. mRNA)[187] to produce proteins, but each gene can contribute to multiple proteins. One of the big surprises of the DNA sequencing of the human genome in the early 2000s was the small number of genes it contained for such a complex organism.[188] The implication of a relatively small number of genes in humans means that genes must be able to code for multiple proteins.[189] The old notion in molecular biology of "one gene, one function" became invalid.[190]

It is now known that genes achieve the production of multiple proteins from a single gene by a process called "alternative splicing."[191] During the alternative splicing process, parts (exons) of a gene are read to produce a protein. By skipping different exons, different proteins are produced. In this way, genes produce multiple proteins. Alternative splicing is regulated by the cell and is essential to its proper functioning.[192] It occurs not only in humans, but in all multi-celled animals and plants, but to a greater extent in animals than higher plants.[193] This means that any disruption to alternative splicing could have a greater effect in animals compared to plants.

Unintended 'Skipping'

Genetic engineering, including gene editing, can change the way genes are alternatively spliced. If genes are inserted during the genetic engineering process, in addition to the intended function of the inserted gene, the exons in the inserted genes could be read along with exons from the animal's own genes, to unintentionally produce an altered, or even an entirely novel protein. Gene editing (such as CRISPR) is known to cause unintended exon skipping (see Fig. 2) and, in experiments, has produced unintended proteins[194] (see *Unexpected effects with gene editing: on-target*). As allergens are proteins, the disruption to alternative splicing is of concern as it can compromise animal health and welfare and can also affect food safety (see *Concerns for food safety and consumer's health*).

Disabling even a single gene (often called a "genetic tweak")[195] can have important consequences. Genetically engineered animals used for laboratory research, e.g. mice or zebrafish, with a certain gene disabled ('knocked out') do not always behave as expected.[196] This is because of a multitude of factors, including interactions between genes, persistence of some of the supposedly eliminated genetic material and the existence of multiple pathways for a trait, which can compensate for the disabled gene.[197] For example, one concern regarding gene-edited PRRS-resistant pigs is that the gene that has been knocked out (*CD163*) is known to have important other functions, e.g. in defending against infections and regulation of blood composition.[198] More insight regarding these other functions of the gene would be needed before any assurances could be given that knocking out this gene wouldn't compromise the health and welfare of the gene-edited pig, or its safety as a food product.

Genetic errors created by genetic engineering processes

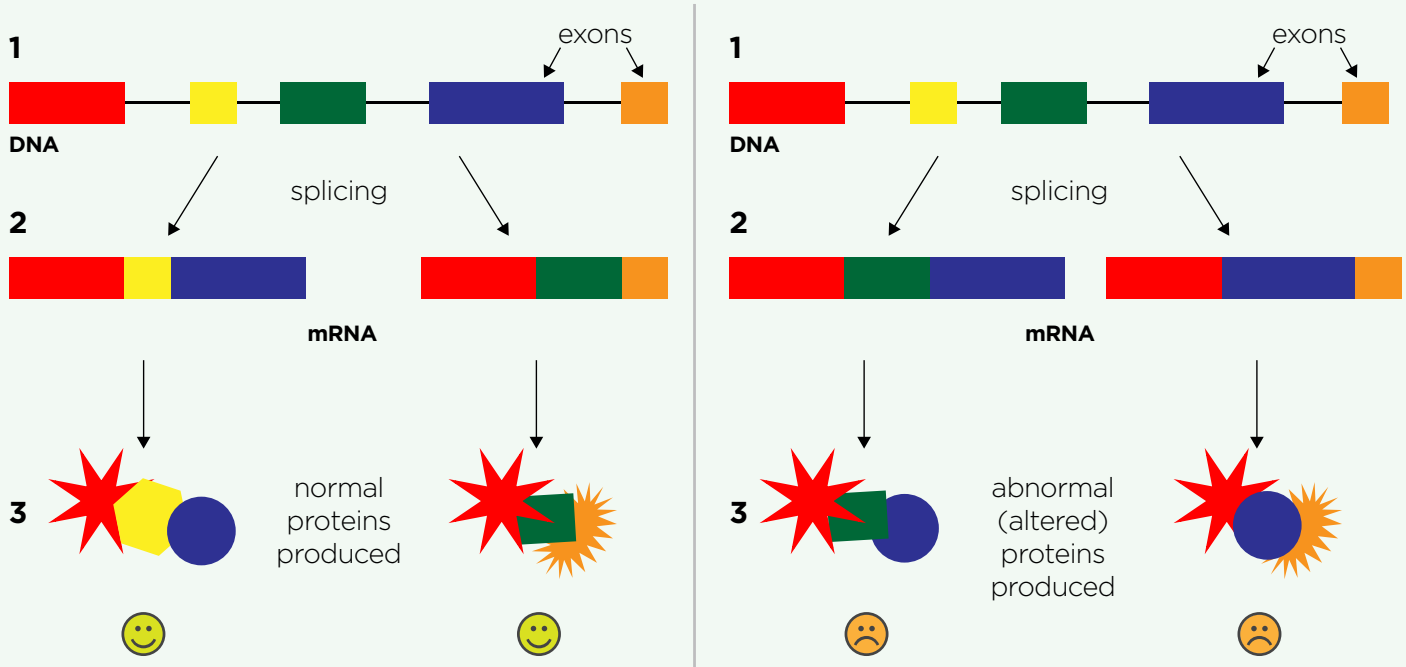
Second-generation genetic engineering, using gene editing techniques, is promoted as being more precise than first-generation genetic engineering, which suffered from technical difficulties.[199] Although precision may have improved beyond the random insertion of genes in first-generation genetically engineered animals, the reality is that gene editing can produce genetic errors. These genetic errors can result in unexpected and unintended effects in the resulting GMO. These could cause changes in the protein and composition profiles that could affect food safety (see *Concerns for food safety and consumer's health*). So far, most of the proof of concept papers have only examined gene-edited animals for changes in their DNA (and sometimes only for the intended, rather than any unintended change to DNA). None of these studies have carefully examined the gene-edited animal for possible production of unintended or altered proteins.

Genetic errors in first-generation genetically engineered animals

The insertion of DNA can cause sections of the animal's own DNA to become rearranged,[200] as has often happened with standard genetically engineered crops.[201] Although these genetic errors have been observed in genetically engineered plants, they are far less well known in animals because detailed studies have largely not been performed. However, unexpected effects can occur. One study trying to eliminate a known allergen in cow's milk through genetic engineering involving gene insertion found it

Fig. 2:

Disruption of the alternative splicing mechanism can produce abnormal proteins in gene-edited animals



A) During normal gene expression, multiple proteins can be produced from one gene by alternative splicing

1) Parts (exons) of a gene (DNA) can be 'read' alternatively, omitting different exons.
2) These give rise to different sequences of mRNA (intermediary product between genes and proteins)
3) In turn, different proteins are produced. In this way, several proteins can be produced from one gene.

B) Gene editing can unintentionally disrupt alternative splicing, leading to the production of abnormal proteins

1) Gene editing is known to cause unintended exon skipping.
2) This changes the way genes are alternatively spliced.
3) Unintended exon skipping can lead to the production of new or altered proteins. This abnormal protein may be inactive, or it could be active in a way that affects the genetically modified animal's health and welfare, or could be allergenic to humans, affecting food safety.

Far from being "precise", gene editing can unintentionally alter additional genes leading to unexpected effects.

also affected levels of all the other milk proteins, and one calf was even born without a tail, although the exact cause of this unexpected effect is not known.[202]

Unexpected effects with gene editing: off-target

With gene editing, although genes may not be inserted, genetic errors can still be generated. One of the main ways that gene editing can be imprecise and create genetic errors is by causing "off-target" effects — changes to other genes that were not intended. Most studies looking at potential gene-edited animals in farming consider off-target effects to be both a major challenge and a major concern.[203] With gene editing, off-target effects have been detected in animals such as pigs[204], as well as model animals used in research, such as rats and mice[205]. However, the implications of these off-target effects to animal welfare or food safety have rarely been examined.

The detection of off-target effects can be confounded by genetic variation, meaning that some off-target effects may go undetected.[206] Off-target effects could unintentionally alter important genes, causing changes in chemistry or protein production — both of which are important for animal welfare (see *Ethical and welfare concerns for genetically engineered animals*) and food safety (see *Concerns for food safety and consumer's health*).

> *"Due to off target mutations, there may be loss of function of a gene, adverse events, even fetal abnormalities."* Rodriguez (2017)[207]

Unexpected effects with gene editing: on-target

Studies on gene-edited animals or laboratory cell cultures have found that CRISPR can inadvertently cause extensive deletions and complex re-arrangements of DNA.[208] These deletions and re-arrangements of DNA by CRISPR may cause parts of the gene (exons) to be "missed" when the DNA is read, altering the alternative splicing process (see *Complexity of animal genomes*).[209] This misreading of DNA has the potential to produce altered proteins. Indeed, one of the studies, using a laboratory culture of human cells, found an altered protein produced in error by the misreading of DNA caused by the gene editing process.[210] The authors concluded:

> *"Although most indel [insertion or deletion] mutations are likely to produce a true knockout, we have here shown that at least in some cases, they may result in altered splicing and even expression of an aberrant protein."* Kapahnke et al. (2016)[211]

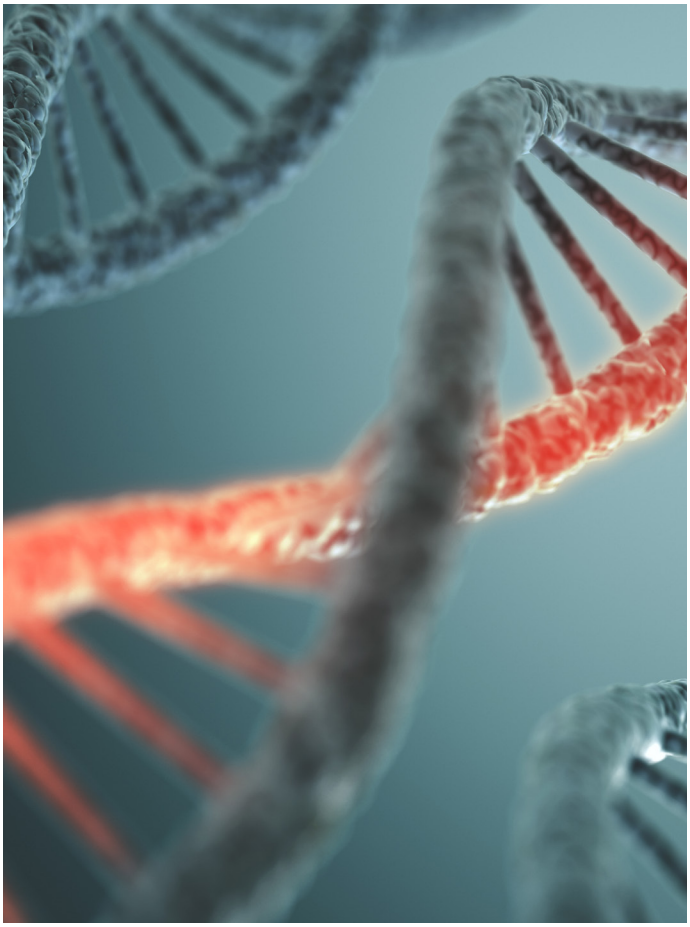
The multi-functional aspects of genes in animals (see *Complexity of animal genomes*) means that "tweaking" one gene can have unintended consequences. Indeed, unexpected effects from on-target alterations have been identified in gene-edited animals and have impacted animal health. In particular, gene-edited super-muscly animals are associated with abnormalities that lead to severe health problems. These have commonly led to aborted pregnancies, stillbirths and infant deaths.

Examples of unexpected effects in gene-edited animals that impact animal health include early death in gene-edited super-muscly pigs due to increased susceptibility to stress and umbilical hernia (damage in the navel area that can cause the small intestine to bulge through).[212] The study's authors recommended further studies to determine how super-muscly gene edits affect the health of pigs. Another study found that all gene-edited super-muscly pigs of a certain breed died after only four days, and some had "remarkably enlarged tongues"[213]. A further study found enlarged tongues in nearly half of gene-edited rabbits and also in a gene-edited goat, the only gene-edited goat to survive longer than eight months.[214] The tongue is a muscle, and it appears that the super-muscly trait is expressed in tongue muscle. Other unexpected effects have been seen in gene-edited pigs. Super-muscly pigs were found to have an extra vertebra compared to control (non gene-edited) pigs. Although pigs can have slightly different numbers of vertebrae, the underlying mechanism of this change isn't known, but is thought likely to be associated with the muscly gene.[215]

Health problems also exist in conventionally bred well- or double-muscled animals. For example, Porcine Stress Syndrome (PSS) can develop in either heavily muscled or lean breeds.[216] However, there are a greater number of incidents of

abnormalities such as enlarged tongues and infant death with gene-edited animals,[217] and these have been attributed to gene editing,[218] especially if both copies of the gene are edited (homozygous knock outs), e.g. in the offspring of two gene-edited parents.[219]

> *"[The muscly gene] Mstn KO caused abnormalities in gene edited animals, which suggested that Mstn KO may not be an ideal way to improve the muscle mass in rabbits, and also in animals, such as pigs and goats... This safety issue must be studied further before applied to animal reproduction processes."* Guo et al. (2016)[220]



Interference with gene regulation caused by gene editing

In addition to altering an organism's DNA, gene editing may have unintended impacts on an organism's ability to express or suppress other genes. Within an organism, genes are switched on (expressed) and off in different parts of the organism at different times as the organism grows, functions and reproduces. In addition, genes interact with each other, either suppressing or reinforcing their expression. The orchestration of gene function in an organism is part of a complex regulatory network. However, the precise way that this regulatory network operates is intricate and still poorly understood, as exemplified by recent advances in our knowledge of how gene expression is regulated (see, e.g. *Complexity of animal genomes*).[221]

There have already been reports of an unexpected response from the cell regulatory network during gene editing. For example, in experiments with human cells, the cuts in DNA created by CRISPR were unexpectedly found to kill cells or stop them from growing.[222] 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. Thus, gene edits to DNA may unintentionally affect the operation of the organism's genetic regulatory network. This could result in the organism's own (unedited) genes not being expressed as they should be. For example, they could be over or under expressed or expressed at the wrong time or wrong place, leading to unexpected effects.

In summary, gene editing can cause unexpected effects in a number of different ways: through genetic errors caused by the insertion of DNA (if inserted) or the gene editing process (both off-target and on-target effects) and through interference with gene regulation. These can give rise to food safety, environmental and animal welfare concerns.

Gene editing can cause unexpected effects in a number of different ways: through genetic errors caused by the insertion of DNA (if inserted) or the gene editing process (both off-target and on-target effects) and through interference with gene regulation. These can give rise to food safety, environmental and animal welfare concerns.

Food safety and environmental concerns of genetically engineered farm animals

There are considerable concerns regarding the environmental and food safety of genetically engineered farm animals intended for human consumption. These concerns are in addition to the concerns regarding animal welfare (see *Ethical and welfare concerns for genetically engineered animals*).

Broadly, the concerns fall into two categories: those related to the novel trait and those related to the genetic engineering process. The novel trait conferred by the genetic engineering process could have impacts on food and environmental safety. For example, the increased antibacterial properties in milk from cows that have been genetically engineered to reduce susceptibility to mastitis might affect or impair human gut bacteria. Hence, the effect of the novel trait needs to be carefully considered. However, the overarching concern related to food and environmental safety of all genetically engineered organisms (both plants and animals and including gene-edited organisms) is that they can exhibit unexpected and unpredictable effects as a result of the genetic engineering process (see *Unexpected effects with gene editing: on-target*). Any unexpected or unpredictable effects could result in unintended alterations to physiological processes in the genetically engineered animal, potentially altering the composition and chemistry of the edible parts of animals, or how it interacts with the environment. Very few studies have looked at the food or environmental safety of genetically engineered animals, so this area needs more scientific research.

The lack of foreign genes and novel protein doesn't make gene-edited animals safe to eat. Disabling a gene could disrupt protein production, potentially resulting in the production of unintended novel or altered proteins and affecting food safety.

Concerns for food safety and consumer's health

The U.S. FDA recognizes that one of the primary concerns regarding the food safety of GMOs is that any novel or altered proteins created by the genetic engineering process (whether intentionally or inadvertently created) might give rise to allergies when eaten by people.[223] All allergens are proteins, so any new or altered proteins must be carefully examined.[224] In addition, the FDA is also concerned with whether the genetic change has altered any physiological processes in the genetically engineered animal that might result in an increased food consumption risk.[225] For example, the changes in the protein profile in milk from genetically engineered cows[226] would need to be evaluated to see if they posed any food consumption risk. However, neither issues such as the overall nutritional value of food derived from the genetically engineered animal products nor the implications of how peoples' eating habits may change (and how this might be important for consumers' health) are explicit in the FDA guidance.

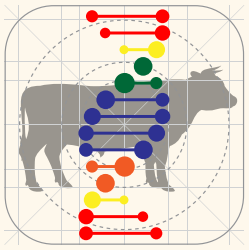
Genetically engineered animals produced by first-generation genetic engineering techniques had genes inserted into them in order to produce a novel product (usually a protein), e.g. the AquAdvantage genetically engineered salmon contains a gene from Chinook salmon that produces a growth hormone (a type of small protein).[227] This gives rise to food safety concerns regarding whether the new protein produced might be allergenic, whether there might be any adverse effects on consumers from the growth hormone and whether the nutritional profile has been altered in any way.[228]

Many of the examples of gene-edited animals listed (see *Gene editing in farm animals*) have a gene that has been disabled (or knocked out) by the gene editing process, e.g. cattle without horns and tuberculosis-resistant cattle. Such gene-edited animals with knocked-out genes are not intended to produce a novel protein. However, the lack of foreign genes and novel protein doesn't make gene-edited animals safe to eat. As described earlier (see *Complexity of animal genomes*), disabling a gene could disrupt protein production, potentially resulting in the production

of unintended novel or altered proteins and affecting food safety.

Gene editing is prone to creating genetic errors in the resulting GMO (see *Unexpected effects with gene editing: off-target, Unexpected effects with gene editing: on-target* and *Interference with gene regulation caused by gene editing*). These genetic errors can give rise to unexpected and unpredictable effects in the resulting GMO. For example, CRISPR has been shown to produce an unintended protein in human cells from the misreading of DNA (see *Unexpected effects with gene editing: on-target*).[229]

The concerns over food safety of genetically engineered animals mean that they need to be scrutinized extremely carefully before being marketed to consumers. However, there are concerns that the FDA could approve the marketing of genetically engineered animals without reviewing any food or environmental data. (see *Regulation of genetically engineered animals in the U.S.*).



SAFE TO EAT?

Genetic engineering can create altered proteins, potentially creating new allergens

SAFE FOR THE ANIMAL?

- Genetic engineering can impact the health of the animal
- Cloning, often used in the genetic engineering process can result in birth defects and premature deaths
- Genetically engineered traits may perpetuate factory farming

SAFE FOR THE ENVIRONMENT?

- Unknown environmental impacts
- Super-muscly genetically engineered animals might need a protein-rich diet, requiring more feed
- Genetically engineered traits may perpetuate factory farming

Contamination of food from experimental genetically engineered animals

Although there are very few genetically engineered animals commercially available (see *Status of genetically engineered animals*), the contamination of food or animal feed with experimental genetically engineered animals has occurred. There have been four recorded incidents, all occurring between 2001-2005, where experimental genetically engineered pigs entered into the food or feed supply unauthorized.[230] These were either accidentally commingled with non-genetically engineered farm animals at the slaughterhouse, sometimes due to mislabelling or — in one case — were deliberately stolen. These experimental GMOs had undergone no food safety assessment whatsoever.

Environmental issues associated with genetically engineered farm animals

The environmental risks associated with genetically engineered farm animals are not well defined because there are, as yet, no commercially available genetically engineered farm animals and very few studies on what the potential risks might be have been performed. However, risks include escape into the wider environment, the use of antibiotic marker resistance genes (if used) and the further intensification of animal agriculture.

Escape of genetically engineered animals into the wider environment

Although, in general, genetically engineered farm animals may not have the same potential as genetically engineered plants, fish or insects to escape and form feral populations (i.e. to form populations living in the wild, but derived from farm escapes)[231] pigs, goats, horses or rabbits are all described as having a high ability to become feral with a moderate likelihood of escape from captivity[232]. If such genetically engineered animals were to escape from the farm environment, this could result in them joining existing escaped (feral) or wild populations or forming new populations. The genetic trait could spread through these populations, which could, potentially, act as a gene pool — transferring the genetic trait back to farm animals via mating.

This could result in unauthorized genetically engineered farm animals, possibly without the farmer's knowledge. These unauthorized genetically engineered farm animals could then end up on peoples' plates, or even spread their genes through the herd, again without the farmer's knowledge. Some genetically engineered animal species could also exchange genes with wild populations, with unknown consequences for biodiversity and the environment.

Use of antibiotic resistance marker genes

The use of antibiotic resistance marker genes raises concerns for the future use of antibiotics. Antibiotic resistance marker genes are sometimes inserted alongside the functional genes during the transgenic genetic engineering process to let researchers know the inserted genes have been integrated. Although the use of these types of genes in genetically engineered animals is diminishing, if they are used, they could lead to antibiotic resistance in bacteria, leading to a reduction in the efficacy of antibiotics for some bacterial infections.[233]

Impacts of farming genetically engineered animals on the environment

One of the main environmental concerns of genetically engineered farm animals is that they further embed the paradigm of unsustainable industrial agriculture. This has already been seen with genetically engineered herbicide-tolerant crops, e.g. Roundup Ready crops tolerant to the herbicide glyphosate, which make up nearly 90 percent of genetically engineered crops globally[234]. Genetically engineered herbicide-tolerant crops are designed for ease of use in monoculture crop systems, leading to fields clear of all vegetation apart from the genetically engineered herbicide-tolerant crop. In the case of Roundup Ready genetically engineered crops, this leads to increases in the use of glyphosate[235] and diminished biodiversity in agricultural fields[236].

The farming of gene-edited animals could change the type of feed required. For example, conventionally bred double muscle cows have a reduced capacity for feed intake, cannot utilize low energy foods efficiently and are often fed high-energy diets.[237] Gene-edited super-muscly cows might be expected to have similar dietary demands. Higher energy diets typically use increased quantities of crops such as soya and corn, with less input from pasture grass. That is, they are animals more suited to intensive CAFO operations.

If gene-edited farm animals become widespread, this could increase the availability of cheaper meat, fueling increased meat consumption, and hence increased demand for animal feed.[238] Increased demand for animal feed would lead to an increased need for resources such as crop land, water, fertilizers and pesticides, adding to existing pressure on ecosystems, biodiversity and the climate.[239] A common focus of gene editing in animals is to develop disease resistance, allowing large herds of animals to be kept in the intensive conditions that spread disease in the first place. Just like genetically engineered crops, genetically engineered animals are largely designed for unsustainable, industrial agricultural systems. Many studies have concluded there is an urgent need to shift to more ecological ways of farming,[240] with less of an environmental and social impact. However, instead of instigating this shift, genetically engineered animals could further embed the paradigm of unsustainable industrial agriculture.

In contrast to intensive CAFOs, when managed responsibly, small and mid-scale high-welfare animal production (including intensive and holistic grazing systems) can generate important ecological benefits, including carbon sequestration, water savings and reduced dependence on fossil fuels.[241] In addition, sustainable animal farming methods support the integration of farm animals with crop production, using manure to improve soil fertility and animals to control weeds, thus decreasing dependence on fossil fuel-intensive fertilizers and pesticides. On well-managed pastures, animal waste provides vital organic nourishment for soils and crops, producing less methane (a greenhouse gas) than manure stored in vats on intensive farms.[242] Rotational and holistic grazing systems can also capture and store more water below the ground.[243]

Many studies have concluded there is an urgent need to shift to more ecological ways of farming, with less of an environmental and social impact. However, instead of instigating this shift, genetically engineered animals could further embed the paradigm of unsustainable industrial agriculture.

Regulation of genetically engineered animals in the U.S.

Patents on genetically engineered animals

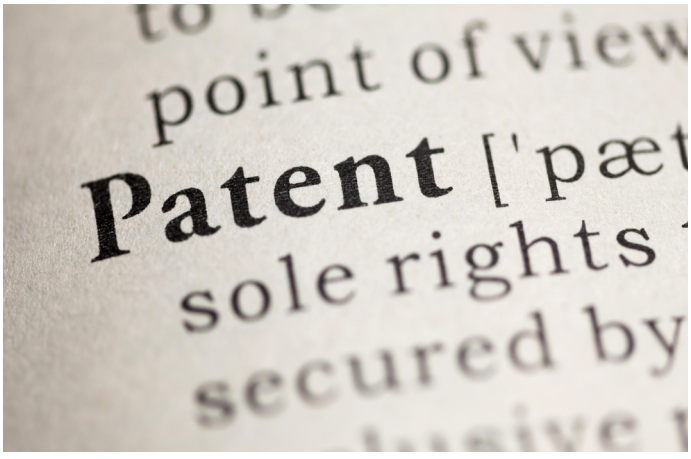
The issue of patents on genetically engineered animals has not yet been fully addressed, especially for gene-edited animals.[244] For genetically engineered crops, the company that developed the crop generally holds a patent on the genetically engineered crop and all seed produced from it. This has caused problems for farmers, who have been sued for saving seed.[245] With a gene-edited cow, would the farmer or the developer own the offspring? Would this be the same if no novel DNA has been inserted? Could farmers be sued for the usual practice of raising offspring for meat? Aside from the ethical issues of patenting animals and other lifeforms,[246] the legal issues remain challenging.

Environmental and food safety oversight of genetically engineered animals in the U.S.

In the U.S., the FDA should oversee both the environmental and food safety aspects of genetically engineered animals. However, there are no specific regulations or guidance that cover the environmental aspects of genetically engineered animals.[247] Instead potential applicants are requested to contact the FDA as the risk assessment would *"depend on the animal product, claim, and conditions of use"*.[248] By contrast, the European Food Safety Authority (EFSA) of the EU has issued comprehensive guidance for the environmental risk assessment of genetically engineered animals[249], despite the fact that no applications to commercialize genetically engineered animals have been made in the EU. An environmental risk assessment would be required for all genetically engineered (including gene-edited) animals, whether intended for food or not, according to the EU GMO regulations[250]. No guidelines exist for the food safety assessment of genetically engineered animals in the EU and, most likely, would only be developed if there was an application to market a genetically engineered animal as food.

The FDA has examined the one genetically engineered animal intended for food, the AquAdvantage salmon[251] and this included both an environmental and food safety risk assessment. However, the FDA approval was lambasted as the *"first-ever approval of laboratory-created food animal [that] violated laws and ignored risks to wild salmon and fishing communities."*[252]

For gene-edited animals, the FDA has recently launched its new Plant and Animal Biotechnology Innovation Action Plan.[253] This new plan aims to *"avoid unnecessary barriers to future innovation in plant and animal biotechnology"*. It plans, in 2019, *"to clarify the FDA's regulatory approach to the regulation of intentional genomic alterations in animals, including through genome editing."* It will include an option to *"exercise enforcement discretion"* regarding data requirements,[254] meaning that an approval to market genetically engineered animals could go ahead without the FDA reviewing any food or environmental data. Already, pressure is building from the developers of gene-edited animals, with requests to the FDA to make them largely free of regulatory oversight,[255] and, in June 2019, Trump signed an executive order which directs federal



agencies, including the FDA, to "streamline" the regulations for genetically engineered organisms. The result may be that genome-edited animals will be able to enter the food chain with little or no regulatory oversight.[256]

There is already an example of how genetically engineered animals could evade regulatory oversight. The lack of a request for an environmental risk assessment for the Glofish (see *Status of genetically engineered animals*) exposed a gap in the U.S. regulatory system for genetically engineered animals[257]. This regulatory gap could persist, as the FDA may exert *"enforcement discretion,"* meaning that future non-food genetically engineered animals, such as pets, are not likely to require any data to be submitted prior to marketing approval.[258]

The ability for the FDA to exert *"enforcement discretion"* is of serious concern, as the USDA has already decided that it will not regulate gene-edited plants[259] that are not classified as plant pests, nor developed from plant pests and could have been developed by standard (conventional) breeding techniques.[260] This policy has led to about 30 genetically modified organisms, mostly plants, bypassing the USDA regulatory system between 2011 and 2017.[261] The concern is that gene-edited animals could similarly evade regulatory oversight in the U.S under enforcement discretion. This particularly applies to knockout gene-edited animals, with or more genes disabled, as these could, at least theoretically, have been developed by conventional breeding. In Japan and Australia, these types of gene-edited plants and animals appear likely to go unregulated,[262] setting a dangerous precedent. However, it is clear that even the "tweaking" (knocking out) of a single gene in animals can result in genetic errors that could impact food safety.

Given all the proof of concept studies on gene-edited animals (see *Gene editing in farm animals*), there could be one or more applications to market genetically engineered farm animals. It's essential that all genetically engineered animals, including those produced by gene editing, are subject to robust regulatory oversight. Otherwise, food from gene-edited animals could end up on our plates in the near future without any meaningful safety assessment.

In addition to regulatory oversight, societal concerns such as ethics and the welfare of genetically engineered animals also need to also be considered. So far, there is a disjunction between the academic, regulatory and public debates concerning genetically engineered animals.[263] Social sciences and workers in the fields of ethics and philosophy are unrepresented in the academic and regulatory debates, which also lack comparison with alternatives to gene-editing, such as ecological farming methods.[264]

It's essential that all genetically engineered animals, including those produced by gene editing, are subject to robust regulatory oversight. Otherwise, food from gene-edited animals could end up on our plates in the near future without any meaningful safety assessment.

Conclusion

Scientific studies are increasingly demonstrating that the genetic engineering of animals may result in negative impacts related to food safety, animal health and welfare, and the environment. This report details two primary concerns. First, that gene editing technologies are less precise than purported, leading to unintended consequences. Second, that many emerging applications of these genetic engineering technologies could result in further entrenching the intensive animal farming model, rather than generating true solutions to the serious animal welfare, public health and environmental problems it creates.

Emerging science shows that genetic engineering technologies are not as precise or predictable as imagined. Studies are finding CRISPR may cause genetic disruption, such as off-target effects, large unintended deletions and rearrangements of DNA, and interference with gene regulation. One concern for food safety is that gene editing animals can result in unexpected effects and impacts on protein production that could result in new allergies. Despite this, the U.S. FDA is proposing a new plan that could substantially weaken the regulations surrounding genetically engineering, and, in particular, gene-edited animals, meaning they could evade regulatory oversight. If this were to happen, food from gene-edited animals could end up on consumers' plates without a meaningful safety assessment. And although still hypothetical, gene drive systems for farm animals — a genetic engineering technology being developed to drive a desired trait though a herd or population — could have unpredictable consequences, ranging from genetic errors arising from the gene editing process (e.g. off-target effects) to impacts on wildlife.

Given the uncertainties and risks from gene editing, it is critical that robust oversight and regulation of all gene-edited animals be established to ensure the safety of animals, consumers and the environment.

Of the many genetically engineered food animals under development, many of the traits being researched would facilitate engineering animals to better fit intensive factory animal farming. Some examples are "super-muscly" animals and pigs resistant to the respiratory disease PRRSV. Traits that appear to offer solutions — such as disease resistance or hornless cattle — will in fact engineer animals to withstand the unsanitary and crowded living conditions of factory farms, raising serious ethical and welfare concerns.

We need true solutions to the problems posed by industrial animal agriculture. Decades of research demonstrate that agroecological models of production, including diversified organic and well-managed pasture-based systems, provide a host of benefits. These include higher animal welfare, improved nutritional profiles of the food produced, reduced risk of antibiotic-resistant bacteria, carbon sequestration, soil fertility, water savings and reduced dependence on pesticides and fossil fuels. These ecosystem benefits are increasingly important in light of recent reports by the United Nations that emphasize the need to rapidly transition away from industrial agriculture and reduce consumption of factory farmed meat and dairy.

It is increasingly clear that genetic engineering of farm animals is unnecessary. Instead of creating genetically engineered animals to fit into factory farms, we must develop sustainable and ecological animal agriculture systems that support animal welfare, preserve and restore biodiversity and protect public health.

Sustainable and ecological agriculture, without genetic engineering, can support animal welfare and enrich biodiversity while protecting public health.

References

1 Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. (2019). Global biodiversity assessment. Summary for policymakers. Retrieved from https://www.ipbes.net/news/ipbes-global-assessment-summary-policymakers-pdf; Secretariat of the Convention on Biological Diversity (2014) Global Biodiversity Outlook 4. Retrieved from https://www.cbd.int/gbo/gbo4/publication/gbo4-en.pdf; International assessment of agricultural knowledge, science and technology for development (2009) Global report. McIntyre, B.D. (ed.) Island Press, Washington D.C. Retrieved from https://www.weltagrarbericht.de/reports/Global_Report/Global_content.html

2 Lebacq, T., Baret, P.V. & Stilmant, D. (2013) Sustainability indicators for livestock farming. A review. Agronomy for Sustainable Development 33: 311–327; Horrigan, L., Lawrence, R.S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental Health Perspectives 110: 445-56.

3 Wang, X., Niu, Y., Zhou, J. et al. (2016) Multiplex gene editing via CRISPR/ Cas9 exhibits 4 desirable muscle hypertrophy without detectable off-target effects in sheep. Scientific Reports 6: 32271. Retrieved from https://www.doi.org/10.1038/srep32271; Crispo, M., Mulet, A.P., Tesson, L. et al. (2015) Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes, PLoS One 10: e0136690. Retrieved from https://doi.org/10.1371/journal.pone.0136690; Retrieved from Cyranoski, D. (2015) Super-muscly pigs created by small genetic tweak. Nature (news) Retrieved from 523: 13-14; Proudfoot, C., Carlson, D.F., Huddart, R. et al. (2015) Genome edited sheep and cattle. Transgenic Research 24: 147-53.

4 Burkard, C., Opriessnig, T. Mileham, A.J., Stadejek, T., Ait-Ali, T., Lillico, S.G., Whitelaw, C.B.A. & Archibald, A.L. (2018) Pigs lacking the scavenger receptor cysteine-rich domain 5 of CD163 are resistant to PRRSV-1 infection. Journal of Virology 92: e00415. Retrieved from https://doi.org/10.1128/JVI.00415-18; Burkard C, Lillico SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T, Whitelaw, C.B.A. & Archibald, A.L. (2017) Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathogens 13: e1006206. Retrieved from https://doi.org/10.1371/journal.ppat.1006206; Whitworth, K.M., Rowland, R.R., Ewen, C.L., Trible, B.R., Kerrigan, M.A., Cino-Ozuna, A.G., Samuel, M.S. Lightner, J.E., McLaren, D.G., Mileham, A.J., Wells, K.D. & Prather, R.S. (2016) Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology 34: 20-22.

5 Oishi, I., Yoshii, K., Miyahara, D., Kagami, H. & Tagami, T. (2016) Targeted mutagenesis in chicken using CRISPR/Cas9 system. Scientific Reports.6: 23980. Retrieved from https://doi.org/10.1038/srep23980; Park, T.S., Lee, H.J., Kim, K.H., Kim, J.S. & Han, J.Y. (2014). Targeted gene knockout in chickens mediated by TALENs. Proceedings of the National Academy of Sciences 111: 12716-12721.

6 Baltzegar, J., Cavin Barnes, J. Elsensohn, J.E., Gutzmann, N., Jones, M.S., King, S. & Sudweeks, J. (2018) Anticipating complexity in the deployment of gene drive insects in agriculture. Journal of Responsible Innovation 5: S81S97. Retrieved from https://doi.org/10.1080/23299460.2017.1407910

7 Unckless, R.L., Clark, A.G. & Messer, P.W. (2017) Evolution of resistance against CRISPR/Cas9 gene drive. Genetics. 205: 827-841.

8 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.

9 Lebacq, T., Baret, P.V. & Stilmant, D. (2013) Sustainability indicators for livestock farming. A review. Agronomy for Sustainable Development 33: 311–327; Horrigan, L., Lawrence, R.S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental Health Perspectives 110: 445-56.

10 United Nations Food and Agriculture Organization (2018) Global livestock environmental assessment model. Version 2.0, revision 5. http://www.fao.org/gleam/en/

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

12 Rana, P. & Craymer, L. (2018) Big tongues and extra vertebrae: the unintended consequences of animal gene editing. The Wall Street Journal, December 14. https://www.wsj.com/articles/deformities-alarm-scientists-racing-to-rewrite-animal-dna-11544808779?mod=e2tw; 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. https://doi.org/10.1038/srep14435; Guo, R., Wan, Y., Xu, D. et al. (2016) Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. Scientific Reports 6: 29855 https://doi.org/10.1038/srep29855

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

14 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.

15 Ormandy, E.H., Dale, J. & Griffin, G. (2011) Genetic engineering of animals: Ethical issues, including welfare concerns. Canadian Veterinary Journal 52: 544-50; Kirkden, R.D. & Broom, D.M. (2012) Welfare of genetically modified and cloned animals used for food. Report commissioned by Compassion in World Farming. Retrieved from https://www.ciwf.org.uk/research/cloning-genetics/. See also other reports by Compassion in World Farming on cloning and genetics at https://www.ciwf.org.uk/research/cloning-genetics/.

16 Tan, W., Proudfoot, C., Lillico, S. G., & Whitelaw, C. B. A. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273–287.

17 National Institutes of Health (2017) What are the potential drawbacks of cloning animals? National Human Genome Research Institute. Retrieved from https://www.genome.gov/25020028/cloning-fact-sheet/#al-6; Van Eenennaam, A.L. (2017) Genetic modification of food animals. Current Opinion in Biotechnology 44: 27-34; Keefer, C.L. (2015) Artificial cloning of domestic animals. Proceedings of the National Academy of Sciences 112: 8874–8878.

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

19 Begley, S. (2018) CRISPR-edited cells linked to cancer risk in 2 studies. Scientific American June 12. Retrieved from https://www.scientificamerican.com/article/crispr-edited-cells-linked-to-cancer-risk-in-2-studies/; 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.

20 E.g. Springmann, M., Clark, M., Mason-D'Croz, D. et al. (2018) Options for keeping the food system within environmental limits. Nature 562: 519-525; International Panel of Experts on Sustainable Food Systems (2016) From uniformity to diversity: a paradigm shift from industrial agriculture to diversified agroecological systems. Retrieved from http://www.ipes-food.org/_img/upload/files/UniformityToDiversity_FULL.pdf; Foley, J.A., Ramankutty, N., Brauman, K.A. et al. (2011) Solutions for a cultivated planet. Nature 478: 337-42; International assessment of agricultural knowledge, science and technology for development (2009) Global report. McIntyre, B.D. (ed.) Island Press, Washington D.C. Retrieved from https://www.weltagrarbericht.de/reports/Global_Report/Global_content.html.

21 National Research Council (2002). Environmental concerns. In: Animal biotechnology: science-based concerns. Committee on defining science-based concerns associated with products of animal biotechnology. US National Academies Press, Washington DC. Ch.5

22 Pew Research Center (2018) Most Americans accept genetic engineering of animals that benefits human health, but many oppose other uses. Retrieved from https://www.pewresearch.org/science/2018/08/16/most-americans-accept-genetic-engineering-of-animals-that-benefits-human-health-but-many-oppose-other-uses/

23 Friends of the Earth U.S. (2017) Companies with policies to not sell genetically engineered seafood. Retrieved from https://1bps6437gg8c169i0y1drtgz-wpengine.netdna-ssl.com/wp-content/uploads/2017/09/GE-free-seafood-company-policy-chart_June2017.pdf

24 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187, pg. 27. Retrieved from: https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

25 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187, pg. 27. Retrieved from: https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

26 Mallapaty, S. (2019) Australian gene-editing rules adopt 'middle ground'. Nature (news) April 23. Retrieved from: https://www.nature.com/articles/d41586-019-01282-8

27 Weber, K. T., & Gokhale, B. S. (2011) Effect of grazing on soil-water content in semiarid rangelands of southeast Idaho. Journal of Arid Environments 75: 464–470. Savory Institute (2015) Climate change, healthy soils and holistic planned grazing: a restoration story. Boulder, Colorado, U.S. Retrieved from https://www.savory.global/wp-content/uploads/2017/02/2015-climate-a-restoration-story.pdf

28 Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. (2019) Global biodiversity assessment. Summary for policymakers. Retrieved from https://www.ipbes.net/news/ipbes-global-assessment-summary-policymakers-pdf; Intergovernmental Panel on Climate Change (2018) Global Warming 1.5°C. Retrieved from: https://report.ipcc.ch/sr15/pdf/sr15_spm_final.pdf

29 Greger, M. & Koneswaran, G. (2010) The public health impacts of concentrated animal feeding operations on local communities. Farm Community Health 33: 373-382.

30 Lebacq, T., Baret, P.V. & Stilmant, D. (2013) Sustainability indicators for livestock farming. A review. Agronomy for Sustainable Development 33: 311–327; Horrigan, L., Lawrence, R.S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental Health Perspectives 110: 445-56.

31 Rana, P. & Craymer, L. (2018) Big tongues and extra vertebrae: the unintended consequences of animal gene editing. The Wall Street Journal, December 14. Retrieved from https://www.wsj.com/articles/deformities-alarm-scientists-racing-to-rewrite-animal-dna-11544808779?mod=e2tw; Johnson, C.Y. (2018) Gene-edited farm animals are coming. Will we eat them? The Washington Post, December 17. Retrieved from https://www.washingtonpost.com/news/national/wp/2018/12/17/feature/gene-edited-farm-animals-are-coming-will-we-eat-them/?noredirect=on&utm_term=.6660b3e1e0d3

32 MacDonald, J.M., Hoppe, R.A. & Newton, D. (2018) Three decades of consolidation in U.S. agriculture. Economic Information Bulletin No. (EIB-189), USDA, Economic Research Service. Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=88056

33 Hartung J. (2013) A short history of livestock production. Ch. 1 in: Banhazi, A.A.T. (ed.) Livestock housing: modern management to ensure optimal health and welfare of farm animals. Wageningen: Wageningen Academic Publishers, The Netherlands.

34 USDA (2019) 2017 Census of Agriculture; U.S. Summary and State Data. Retrieved from https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf; Sentient Media (2019) 99% of U.S. Farmed Animals Live on Factory Farms. Sentient Media. Retrieved from https://sentientmedia.org/u-s-farmed-animals-live-on-factory-farms/

35 Casey, J.A., Kim, B.F., Larsen, J., Price, L.B. & Nachman, K.E. (2015) Industrial Food Animal Production and Community Health. Current Environmental Health Reports 259-271.

36 Lebacq, T., Baret, P.V. & Stilmant, D. (2013) Sustainability indicators for livestock farming. A review. Agronomy for Sustainable Development 33: 311–327; Horrigan, L., Lawrence, R.S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental Health Perspectives 110: 445-56.

37 USDA (2013) National Program 214. Agricultural & industrial byproducts: accomplishment report 2009-2013. Agricultural Research Service. Retrieved from https://www.ars.usda.gov/ARSUserFiles/np214/NP214AccomplishmentRpt2009-2013FINAL.pdf

38 Casey, J.A., Kim, B.F., Larsen, J., Price, L.B. & Nachman, K.E. (2015) Industrial Food Animal Production and Community Health. Current Environmental Health Reports 259-271.

39 Lebacq, T., Baret, P.V. & Stilmant, D. (2013) Sustainability indicators for livestock farming. A review. Agronomy for Sustainable Development 33: 311–327; Horrigan, L., Lawrence, R.S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental Health Perspectives 110: 445-56.

40 United Nations Food and Agriculture Organisation (2018) Global livestock environmental assessment model. Version 2.0, revision 5. Retrieved from http://www.fao.org/gleam/en/

41 U.S. Department of Health and Human Services (2013) Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

42 Human use of antibiotics in 2011 was 3,289,176 kg U.S. Department of Health and Human Services (2011) Drug Use Review, Table 1, pg. 5. Retrieved from https://www.fda.gov/downloads/Drugs/DrugSafety/ InformationbyDrugClass/UCM319435.pdf; Animal use for medically important antibiotics in 2011 was 8,255,697 kg. U.S. FDA (2015) Summary report on antimicrobials sold or distributed for use in food-producing animals, Table 10, pg 42. Retrieved from https://www.fda. gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/UCM476258.pdf

43 U.S. Department of Health and Human Services (2013) Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf; MacDonald, J.M. & McBride, W.D. (2009) The transformation of U.S. livestock agriculture: scale, efficiency, and risks. USDA Economic Research Service, Economic Information Bulletin No. 43. Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=44294

44 Brooks, J. P., Mclaughlin, M., Scheffler, B. & Miles, D. (2010) Microbial and antibiotic-resistant constituents associated with biological aerosols and poultry litter within a commercial poultry house. Science of the Total Environment 408: 4770-4777; McEachran, A.D., Blackwell, B.R., Hanson, D., Wooten, K.J., Mayer, G.D., Cox, S.B. & Smith, P.N. (2015) Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environmental Health Perspectives 123: 337-343; Barza, M. & Gorbach, S.L. (eds.) (2002) The need to improve antimicrobial use in agriculture: ecological and human health consequences. Clinical Infectious Diseases 34 (Suppl. 3): S71-144.

45 McEachran, A.D., Blackwell, B.R., Hanson, D., Wooten, K.J., Mayer, G.D., Cox, S.B. & Smith, P.N. (2015) Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environmental Health Perspectives 123: 337-343.

46 The American Society for the Prevention of Cruelty to Animals (2015) A growing problem. Selective breeding in the chicken industry: the case for slower growth. Retrieved from https://www.aspca.org/sites/default/files/chix_white_paper_nov2015_lores.pdf

47 USDA -APHIS (2018) Overview of U.S. livestock, poultry, and aquaculture production in 2017. Retrieved from https://www.aphis.usda.gov/animal_health/nahms/downloads/Demographics2017.pdf. Estimate of 9.7 billion farm animals includes cattle, pigs and hogs, goats, sheep, horses and other equine species, chickens and turkeys.

48 Animal Welfare Institute. (2019) Legal Protections for Animals on Farms. Retreived from https://awionline.org/sites/default/files/uploads/documents/FA-AWI-LegalProtections-AnimalsonFarms-110714.pdf; Perzigian, A.B. (2003) Governing laws in the United States and the EU. In: Detailed discussion of genetic engineering and animal rights: the legal terrain and ethical underpinnings. Part IV. Animal Legal and Historical Center, Michigan State University College of Law, U.S. Retrieved from https://www.animallaw.info/article/detailed-discussion-genetic-engineering-and-animal-rights-legal-terrain-and-ethical

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

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

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

52 FDA (2017) AquAdvantage salmon fact sheet. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm473238.htm

53 FDA (2017) AquAdvantage salmon fact sheet. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm473238.htm

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

55 Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

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

57 For more information on gene editing, see Cotter, J. & Perls, D. (2018) Gene-edited organisms in agriculture: risks and unexpected consequences. Friends of the Earth USA. Retrieved from http://foe.org/wp-content/uploads/2018/09/FOE_GenomeEditingAgReport_final.pdf

58 Gene editing techniques include CRISPR (clustered regularly interspaced short palindromic repeat); TALEN (transcription activator-like effector nucleases); ODM (oligonucleotide directed mutagenesis) and ZFN (zinc-finger nucleases).

59 Although the abbreviation CRISPR is commonly used, the full terminology is CRISPR/Cas, with Cas being an abbreviation for CRISPR associated protein. Currently the principal Cas used is Cas9, often written as CRISPR/Cas9.

60 Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821.

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

62 Most gene editing techniques employ nucleases. One exception is oligonucleotide directed mutagenesis, which introduces a short stretch of DNA that causes a change in the DNA of the organism. However, this has so far been primarily applied to plants rather than animals.

63 For more information on gene editing, see Cotter, J. & Perls, D. (2018) Gene-edited organisms in agriculture: risks and unexpected consequences. Friends of the Earth USA. Retrieved from http://foe.org/wp-content/uploads/2018/09/FOE_GenomeEditingAgReport_final.pdf

64 West, J., & Gill, W. W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science, 41: 1–6; Sander, J.D. & Joung, J.K. (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotechnology 32: 347–355.

65 Ainsworth, C. (2015) A new breed of edits. Nature (outlook) 528: S15-S16; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. 2016. Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; Van Eenennaam, A.L. (2017) Genetic modification of food animals. Current Opinion in Biotechnology 44: 27–34.

66 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

67 Ishii, T. (2017) Genome-edited livestock: ethics and social acceptance. Animal Frontiers 7: 24–32; Nuffield Council on Bioethics (2016) Genome editing: an ethical review. Retrieved from http://nuffieldbioethics.org/wp-content/uploads/Genome-editing-an-ethical-review.pdf; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; West, J., & Gill, W. W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

68 Yum, S-Y., Youn, K-Y., Choi, W.J. & Jang, G. (2018). Development of genome engineering technologies in cattle: from random to specific. Journal of Animal Science and Biotechnology 9: 16. Retrieved from https://doi.org/10.1186/s40104-018-0232-6; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; West, J., & Gill, W. W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

69 Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

70 National Institutes of Health (2017) How are animals cloned? Cloning. National Human Genome Research Institute. Retrieved from https://www.genome.gov/25020028/cloning-fact-sheet/#al-6

71 The Roslin Institute (2018) The life of Dolly. University of Edinburgh. Retrieved from https://www.ed.ac.uk/roslin/about/dolly/facts/life-of-dolly

72 Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

73 National Institutes of Health (2017) How are animals cloned? Cloning. National Human Genome Research Institute. Retrieved from https://www.genome.gov/25020028/cloning-fact-sheet/#al-6

74 Keefer, C.L. (2015) Artificial cloning of domestic animals. Proceedings of the National Academy of Sciences 112: 8874–8878.

75 Wu, H., Wang, Y., Zhang, Y., Yang, M., Lv, J., Liu, J. & Zhang, Y. (2015) TALE nickase-mediated *SP110* knock in endows cattle with increased resistance to tuberculosis. Proceedings of the National Academy of Sciences 112: E1530–E1539.

76 Gao, Y., Wu, H., Wang, Y., Liu, X., Chen, L., Li, Q., Cui, C., Liu, X., Zhang, J. & Zhang, Y. (2017) Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects. Genome Biology, 18: 13. Retrieved from https://doi.org/10.1186/s13059-016-1144-4

77 National Institutes of Health (2017) What are the potential drawbacks of cloning animals? National Human Genome Research Institute. Retrieved from https://www.genome.gov/25020028/cloning-fact-sheet/#al-6; Van Eenennaam, A.L. (2017) Genetic modification of food animals. Current Opinion in Biotechnology 44: 27-34; Keefer, C.L. (2015) Artificial cloning of domestic animals. Proceedings of the National Academy of Sciences 112: 8874–8878.

78 Humpherys, D., Eggan, K., Akutsu, H., Friedman, A., Hochedlinger, K., Yanagimachi, R., Lander, E.S., Golub, T.R. & Jaenisch, R. (2002) Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. Proceedings of the National Academy of Sciences 99: 12889-12894.

79 FDA (2018) Animal Cloning and Food Safety. Retrieved from https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm148768.htm

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

81 Yum, S-Y., Youn, K-Y., Choi, W.J. & Jang, G. (2018). Development of genome engineering technologies in cattle: from random to specific. Journal of Animal Science and Biotechnology 9: 16. Retrieved from https://doi.org/10.1186/s40104-018-0232-6; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; National Institutes of Health (2017) How are animals cloned? Cloning. National Human Genome Research Institute. Retrieved from https://www.genome.gov/25020028/cloning-fact-sheet/#al-6; Ormandy, E.H., Dale, J. & Griffin, G. (2011) Genetic engineering of animals: Ethical issues, including welfare concerns. Canadian Veterinary Journal 52: 544-50; Kirkden, R.D. & Broom, D.M. (2012) Welfare of genetically modified and cloned animals used for food. Report commissioned by Compassion in World Farming. Retrieved from https://www.ciwf.org.uk/research/cloning-genetics/. See also other reports by Compassion in World Farming on cloning and genetics at https://www.ciwf.org.uk/research/cloning-genetics/.

82 European Parliament (2015) EP wants animal cloning ban extended to offspring and imports. Press release 8th September. Retrieved from http://www.europarl.europa.eu/news/en/press-room/20150903IPR91517/ep-wants-animal-cloning-ban-extended-to-offspring-and-imports

83 Yum, S-Y., Youn, K-Y., Choi, W.J. & Jang, G. (2018). Development of genome engineering technologies in cattle: from random to specific. Journal of Animal Science and Biotechnology 9: 16. Retrieved from https://doi.org/10.1186/s40104-018-0232-6; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

84 Singh, P., Schimenti, J.C. & Bolcun-Filas, E. (2015) A mouse geneticist's practical guide to CRISPR applications Genetics 199: 1-15.

85 Yum, S-Y., Youn, K-Y., Choi, W.J. & Jang, G. (2018). Development of genome engineering technologies in cattle: from random to specific. Journal of Animal Science and Biotechnology 9: 16. Retrieved from https://doi.org/10.1186/s40104-018-0232-6; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87 (Table 1).

86 EFSA (2018) Genetically modified animals. Retrieved from https://www.efsa.europa.eu/en/topics/topic/genetically-modified-animals

87 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398.

88 Government of Canada (2016) AquAdvantage Salmon. Retrieved from https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/aquadvantage-salmon.html

89 FDA (2019) AquAdvantage Salmon Fact Sheet. Retrieved from https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/aquadvantage-salmon-fact-sheet

90 Devlin, R.H., D'Andrade, M., Uh, M. & Biagi, C.A. (2004) Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions. Proceedings of the National Academy of Sciences 101: 9303–9308; Sundström, L.F., Lõhmus, M., Tymchuk, W.E. & Devlin, R.H. (2007) Gene-environment interactions influence ecological consequences of transgenic animals. Proceedings of the National Academy of Sciences 104: 3889–3894.

91 See petitions and responses to public comments relating to the FDA approval of AquAdvantage Salmon at https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm280853.htm; Friends of Earth (2017) Genetically engineered fish: an unnecessary risk to the environment, public health and fishing communities. Issue Brief. Retrieved from https://1bps6437gg8c169i0y1drtgz-wpengine.netdna-ssl.com/wp-content/uploads/2017/legacy/Issue_brief_Genetically_engineered_fish.pdf

92 Friends of the Earth U.S. (2017) Companies with policies to not sell genetically engineered seafood. Retrieved from https://1bps6437gg8c169i0y1drtgz-wpengine.netdna-ssl.com/wp-content/uploads/2017/09/GE-free-seafood-company-policy-chart_June2017.pdf

93 FDA (2009) Approval Letter – Atryn. Available via FDA Atryn website. Retrieved from https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/ucm134042.htm

94 European Medicines Agency (2016) ATryn. European Public Assessment Report - summary for the public. Retrieved from https://www.ema.europa.eu/documents/overview/atryn-epar-summary-public_en.pdf.

95 FDA (2015) FDA approves first drug to treat a rare enzyme disorder in pediatric and adult patients. Press release December 8. Retrieved from http://wayback.archive-it.org/7993/20170111094705/http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm476013.htm

96 FDA (2014). Statement Regarding Glofish. Retrieved from https://wayback.archive-it.org/7993/20170404230909/https:/www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/ucm413959.htm; FDA (2017). Animals with intentional genomic alterations - consumer Q&A. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm113672.htm

97 Readon, S. (2016) The CRISPR Zoo. Nature (news feature) 531: 160-163; FDA (2017). Animals with intentional genomic alterations - consumer Q&A. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm113672.htm; Lievens, A., Petrillo, M., Querci, M. & Patak, A. (2015) Genetically modified animals: options and issues for traceability and enforcement. Trends in Food Science & Technology 44: 159-176.

98 Readon, S. (2016) The CRISPR zoo. Nature (news feature) 531: 160-163; FDA (2017) Animals with intentional genomic alterations - consumer Q&A. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm113672.htm; Lievens, A., Petrillo, M., Querci, M. & Patak, A. (2015) Genetically modified animals: options and issues for traceability and enforcement. Trends in Food Science & Technology 44: 159-176.

99 Oxitec (2018) Programmes. Retrieved from https://www.oxitec.com/friendly-mosquitoes/

100 Whittaker, J. (2018) Minister: no more funds for genetically modified mosquito program. Cayman Compass (Cayman Islands' news website), November 25. Retrieved from https://www.caymancompass.com/2018/11/25/minister-no-more-funds-for-genetically-modified-mosquito-program/

101 Environmental Protection Agency (2018) EPA reopens public comment period on application for experimental use permit to combat mosquitoes. May 10. Retrieved from https://www.epa.gov/pesticides/epa-reopens-public-comment-period-application-experimental-use-permit-combat-mosquitoes

102 Target Malaria (2018) Burkina Faso is getting ready for its next stage of research – sterile male mosquito release. Blog, November 23. Retrieved from https://targetmalaria.org/burkina-faso-is-getting-ready-for-its-next-stage-of-research-sterile-male-mosquito-release/

103 Target Malaria (2018) Where we operate. Retrieved from https://targetmalaria.org/

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

105 Wang, X., Niu, Y., Zhou, J. et al. (2016) Multiplex gene editing via CRISPR/ Cas9 exhibits desirable muscle hypertrophy without detectable off-target effects in sheep. Scientific Reports 6: 32271. Retrieved from https://www.doi.org/10.1038/srep32271; Crispo, M., Mulet, A.P., Tesson, L. et al. (2015) Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes, PLoS One 10: e0136690 https://doi.org/10.1371/journal.pone.0136690; Cyranoski, D. (2015) Super-muscly pigs created by small genetic tweak. Nature (news) 523: 13-14; Proudfoot, C., Carlson, D.F., Huddart, R. et al. (2015) Genome edited sheep and cattle. Transgenic Research 24: 147-53; He, Z., Zhang, T., Jiang, L., Zhou, M., Wu, D., Mei, J. & Cheng, Y. (2018) Use of CRISPR/Cas9 technology efficiently targetted goat myostatin through zygotes microinjection resulting in double-muscled phenotype in goats. Bioscience Reports 38. Retrieved from https://doi.org/10.1042/BSR20180742

106 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. FEBS Journal 284: 2764-2773.

107 Wang, X., Cai, B., Zhou, J. et al. (2016) Disruption of FGF5 in cashmere goats using CRISPR/Cas9 results in more secondary hair follicles and longer fibers. PLoS One. 11: e0164640. Retrieved from https://doi.org/10.1371/journal.pone.0164640

108 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; Betchel, W. (2018) Partnership aims to improve animal welfare with genetics. Agweb (news). Retrieved from https://www.agweb.com/article/partnership-aims-to-improve-animal-welfare-with-genetics/

109 Burkard, C., Opriessnig, T. Mileham, A.J., Stadejek, T., Ait-Ali, T., Lillico, S.G., Whitelaw, C.B.A. & Archibald, A.L. (2018) Pigs lacking the scavenger receptor cysteine-rich domain 5 of CD163 are resistant to PRRSV-1 infection. Journal of Virology 92: e00415. Retrieved from https://doi.org/10.1128/JVI.00415-18; Burkard C, Lillico SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T, Whitelaw, C.B.A. & Archibald, A.L. (2017) Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathogens 13: e1006206. Retrieved from https://doi.org/10.1371/journal.ppat.1006206; Whitworth, K.M., Rowland, R.R., Ewen, C.L., Trible, B.R., Kerrigan, M.A., Cino-Ozuna, A.G., Samuel, M.S. Lightner, J.E., McLaren, D.G., Mileham, A.J., Wells, K.D. & Prather, R.S. (2016) Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology 34: 20-22.

110 Xie, Z., Pang, D., Yuan, H. et al. (2018) Genetically modified pigs are protected from classical swine fever virus. PLoS Pathogens 14: e1007193. Retrieved from https://doi.org/10.1371/journal.ppat.1007193

111 Whitworth, K.M., Rowland, R.R.R., Petrovan, V. et al. (2019) Resistance to coronavirus infection in amino peptidase N-deficient pigs. Transgenic Research 28: 21–32; Hübner, A., Petersen, B., Keil, G.M., Niemann, H., Mettenleiter, T.C. & Fuchs, W. (2018) Efficient inhibition of African swine fever virus replication by CRISPR/Cas9 targeting of the viral p30 gene (CP204L). Scientific Reports 8: 1449. Retrieved from https://doi.org/10.1038/s41598-018-19626-1

112 Liu, X., Wang, Y., Tian, Y., et al. (2014) Generation of mastitis resistance in cows by targeting human lysozyme gene to b-casein locus using zinc-finger nucleases. Proceedings of the Royal Society B: Biological Sciences. 281: 20133368. Retrieved from https://doi.org/10.1098/rspb.2013.3368

113 Wu, H., Wang, Y., Zhang, Y., Yang, M., Lv, J., Liu, J. & Zhang, Y. (2015) TALE nickase-mediated *SP110* knock in endows cattle with increased resistance to tuberculosis. Proceedings of the National Academy of Sciences 112: E1530–E1539; Gao, Y., Wu, H., Wang, Y., Liu, X., Chen, L., Li, Q., Cui, C., Liu, X., Zhang, J. & Zhang, Y. (2017) Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects. Genome Biology, 18: 13. Retrieved from https://doi.org/10.1186/s13059-016-1144-4

114 Oishi, I., Yoshii, K., Miyahara, D., Kagami, H. & Tagami, T. (2016) Targeted mutagenesis in chicken using CRISPR/Cas9 system. Scientific Reports.6: 23980. Retrieved from https://doi.org/10.1038/srep23980; Park, T.S., Lee, H.J., Kim, K.H., Kim, J.S. & Han, J.Y. (2014). Targeted gene knockout in chickens mediated by TALENs. Proceedings of the National Academy of Sciences 111: 12716-12721.

115 Lai, L., Kang, J.X., Li, R. et al. (2006) Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nature Biotechnology 24: 435–436.

116 Reardon, S. (2016) The CRISPR Zoo. Nature (news feature) 531: 160-163.; Larson, C. (2015) China's bold push into genetically customized animals. Scientific American. Retrieved from https://www.scientificamerican.com/article/china-s-bold-push-into-genetically-customized-animals/

117 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. FEBS Journal 284: 2764-2773; Wang, X., Cai, B., Zhou, J. et al. (2016) Disruption of FGF5 in cashmere goats using CRISPR/Cas9 results in more secondary hair follicles and longer fibers. PLoS One. 11: e0164640. Retrieved from https://doi.org/10.1371/journal.pone.0164640

118 Wang, X., Niu, Y., Zhou, J. et al. (2016) Multiplex gene editing via CRISPR/ Cas9 exhibits desirable muscle hypertrophy without detectable off-target effects in sheep. Scientific Reports 6: 32271. Retrieved from https://www.doi.org/10.1038/srep32271; Liu, X., Wang, Y., Tian, Y., et al. (2014) Generation of mastitis resistance in cows by targeting human lysozyme gene to b-casein locus using zinc-finger nucleases. Proceedings of the Royal Society B: Biological Sciences. 281: 20133368. Retrieved from https://doi.org/10.1098/rspb.2013.3368; Wu, H., Wang, Y., Zhang, Y., Yang, M., Lv, J., Liu, J. & Zhang, Y. (2015) TALE nickase-mediated *SP110* knock in endows cattle with increased resistance to tuberculosis. Proceedings of the National Academy of Sciences 112: E1530–E1539; Gao, Y., Wu, H., Wang, Y., Liu, X., Chen, L., Li, Q., Cui, C., Liu, X., Zhang, J. & Zhang, Y. (2017) Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects. Genome Biology, 18: 13. Retrieved from https://doi.org/10.1186/s13059-016-1144-4; He, Z., Zhang, T., Jiang, L., Zhou, M., Wu, D., Mei, J. & Cheng, Y. (2018) Use of CRISPR/Cas9 technology efficiently targetted goat myostatin through zygotes microinjection resulting in double-muscled phenotype in goats. Bioscience Reports 38. Retrieved from https://doi.org/10.1042/BSR20180742

119 Genus (2015). PRRSv resistance development programme progressing as planned. Retrieved from https://www.genusplc.com/about-us/our-history/ and see funding sources in: Burkard, C., Opriessnig, T. Mileham, A.J., Stadejek, T., Ait-Ali, T., Lillico, S.G., Whitelaw, C.B.A. & Archibald, A.L. (2018) Pigs lacking the scavenger receptor cysteine-rich domain 5 of CD163 are resistant to PRRSV-1 infection. Journal of Virology 92: e00415. Retrieved from https://doi.org/10.1128/JVI.00415-18; Burkard C, Lillico SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T, Whitelaw, C.B.A. & Archibald, A.L. (2017) Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathogens 13: e1006206. Retrieved from https://doi.org/10.1371/journal.ppat.1006206; Whitworth, K.M., Rowland, R.R., Ewen, C.L., Trible, B.R., Kerrigan, M.A., Cino-Ozuna, A.G., Samuel, M.S. Lightner, J.E., McLaren, D.G., Mileham, A.J., Wells, K.D. & Prather, R.S. (2016) Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology 34: 20-22.

120 Recombinetics (2018) Our story. Retrieved from https://web.archive.org/web/20181014224448/http://recombinetics.com/our-story/; 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.

121 Proudfoot, C., Carlson, D.F., Huddart, R. et al. (2015) Genome edited sheep and cattle. Transgenic Research 24: 147-53.

122 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398.

123 Dietze, K., Pinto, J., Wainwright, S., Hamilton, C. & Khomenko, S. (2011) Focus on porcine reproductive and respiratory syndrome (PRRS) - virulence jumps and persistent circulation in Southeast Asia. Focus On 5: 1-8. Retrieved from http://www.fao.org/3/a-al849e.pdf; Velasova, M., Alarcon, P., Williamson, S. & Wieland, B. (2012) Risk factors for porcine reproductive and respiratory syndrome virus infection and resulting challenges for effective disease surveillance. BMC Veterinary Research 8:1 84. Retrieved from https://doi.org/10.1186/1746-6148-8-184.

124 Velasova, M., Alarcon, P., Williamson, S. & Wieland, B. (2012) Risk factors for porcine reproductive and respiratory syndrome virus infection and resulting challenges for effective disease surveillance. BMC Veterinary Research 8:1 84. Retrieved from https://doi.org/10.1186/1746-6148-8-184.

125 Reiner, G. (2016) Genetic resistance – an alternative for controlling PRRS? Porcine Health Management 2: 27. Retrieved from https://doi.org/10.1186/s40813-016-0045-y; Tait-Burkard, C., Doeschl-Wilson, A., McGrew, M.J., Archibald, A.L., Sang, H.M., Houston, R.D., Whitelaw, C.B. & Watson, M. (2018) Livestock 2.0 – genome editing for fitter, healthier, and more productive farmed animals. Genome Biology 19: 204. Retrieved from https://doi.org/10.1186/s13059-018-1583-1

126 Flodins (n.d.) Flodins omega-3 pork. Retrieved from https://web.archive.org/web/20180902203939/http://www.flodinsfood.asia/omega-3-pork/

127 Fiester, A. (2006) Why the omega-3 piggy should not go to market. Nature Biotechnology 24: 1472-1473.

128 Fiester, A. (2006) Why the omega-3 piggy should not go to market. Nature Biotechnology 24: 1472-1473.

129 Reardon, S. (2016) The CRISPR Zoo. Nature (news feature) 531: 160-163; 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.

130 Reardon, S. (2016) The CRISPR Zoo. Nature (news feature) 531: 160-163; 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.

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

132 Windig, J.J., Hoving-Bolink, R.A. & Veerkamp, R.F. (2015) Breeding for polledness in Holstein cattle. Livestock Science 179: 96–101.

133 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; Windig, J.J., Hoving-Bolink, R.A. & Veerkamp, R.F. (2015) Breeding for polledness in Holstein cattle. Livestock Science 179: 96–101; 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.

134 Windig, J.J., Hoving-Bolink, R.A. & Veerkamp, R.F. (2015) Breeding for polledness in Holstein cattle. Livestock Science 179: 96–101.

135 Gaspa, G., VeerkampR.F., Calus, M.P.L. & Windig, J.J. (2015) Assessment of genomic selection for introgression of polledness into Holstein Friesian cattle by simulation. Livestock Science 179: 86–95.

136 Commonwealth Scientific and Industrial Research Organisation, Australia (2017) Breeding hornless cattle. Retrieved from https://www.csiro.au/en/Research/AF/Areas/Livestock/Hornless-Cattle

137 Winkler, B. & Mathews, F. (2015) Environmental risk factors associated with bovine tuberculosis among cattle in high-risk areas. Biology Letters 11: 20150536. Retrieved from http://dx.doi.org/10.1098/rsbl.2015.0536

138 Roslin Institute (2018) Breeding for tuberculosis resistant cattle. Retrieved from https://www.ed.ac.uk/roslin/research/isp/control-infectious-diseases/genetic-basis-of-host-resistance/breeding-tuberculosis-resistant-cattle; Raphaka, K., Matika, O., Sánchez-Molano, E., Mrode, R., Coffey, M.P., Riggio, V., Glass, E.J., Woolliams, J.A., Bishop, S.C. & Banos, G. (2017) Genomic regions underlying susceptibility to bovine tuberculosis in Holstein-Friesian cattle. BMC Genetics 18: 27. Retrieved from https://doi.org/10.1186/s12863-017-0493-7.

139 Ormandy, E.H., Dale, J. & Griffin, G. (2011) Genetic engineering of animals: ethical issues, including welfare concerns. Canadian Veterinary Journal 52: 544-50.

140 de Graeff, N., Jongsma, K.R., Johnston, J., Hartley, S. & Bredenoord, A.L. (2019) The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. Philosophical Transactions of the Royal Society B 374: 20180106. Retrieved from https://doi.org/10.1098/rstb.2018.0106

141 Arnott, G., Ferris, C.P. & O'Connell, N.E. 2017. Welfare of dairy cows in continuously housed and pasture-based production systems. Animal 11 261–273.

142 MacDonald, J.M. & McBride, W.D. (2009) The transformation of U.S. livestock agriculture: scale, efficiency, and risks. USDA Economic Research Service, Economic Information Bulletin No. 43. Retrieved from https://www.ers.usda.gov/publications/pub-details/?pubid=44294; Arnott, G., Ferris, C.P. & O'Connell, N.E. 2017. Welfare of dairy cows in continuously housed and pasture-based production systems. Animal 11 261–273.

143 Compassion in World Farming & World Society for the Protection of Animals (2013) Zoonotic diseases, human health and farm animal welfare. Retrieved from https://www.ciwf.org.uk/media/3756123/Zoonotic-diseases-human-health-and-farm-animal-welfare-16-page-report.pdf

144 Compassion in World Farming & World Society for the Protection of Animals (2013) Zoonotic diseases, human health and farm animal welfare. Retrieved from https://www.ciwf.org.uk/media/3756123/Zoonotic-diseases-human-health-and-farm-animal-welfare-16-page-report.pdf

145 Marie, M. 2006. Ethics: The new challenge for animal agriculture. Livestock Science 103: 203-207.

146 See, e.g. Compassion in World Farming U.S.A. (2018) Farm Animals. Retrieved from http://www.ciwf.com/farm-animals/

147 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; Bruce, A. (2017) Genome edited animals: Learning from GM crops? Transgenic Research 26: 385–398; Ishii, T. (2017) Genome-edited livestock: ethics and social acceptance. Animal Frontiers 7: 24–32; Rodriguez, E. (2017) Ethical issues in genome editing for non-human organisms using CRISPR/Cas9 system. Journal of Clinical Research & Bioethics 8: 1000300. Retrieved from https://doi.org/10.4172/2155-9627.1000300; Ormandy, E.H., Dale, J. & Griffin, G. (2011) Genetic engineering of animals: ethical issues, including welfare concerns. Canadian Veterinary Journal 52: 544-50; Opinion of the Group of Advisers on the Ethical Implications of Biotechnology to the European Commission (1996) No. 7. Ethical aspects of genetic modification of animals. Retrieved from http://ec.europa.eu/environment/chemicals/lab_animals/pdf/genetic_modification.pdf

148 Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

149 Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. 2016. Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87.

150 Rodriguez, E. (2017) Ethical issues in genome editing for non-human organisms using CRISPR/Cas9 system. Journal of Clinical Research & Bioethics 8: 1000300. Retrieved from https://doi.org/10.4172/2155-9627.1000300

151 Animal Welfare Institute. (2019) Legal Protections for Animals on Farms. Retreived from https://awionline.org/sites/default/files/uploads/documents/FA-AWI-LegalProtections-AnimalsonFarms-110714.pdf; Perzigian, A.B. (2003) Governing laws in the United States and the EU. In: Detailed discussion of genetic engineering and animal rights: the legal terrain and ethical underpinnings. Part IV. Animal Legal and Historical Center, Michigan State University College of Law, U.S. Retrieved from https://www.animallaw.info/article/detailed-discussion-genetic-engineering-and-animal-rights-legal-terrain-and-ethical

152 Animal and Plant Health Inspection Service (APHIS) (2019) Animal Care Enforcement Summary (AWA and HPA). USDA. Retrieved from https://www.aphis.usda.gov/aphis/ourfocus/business-services/ies/ies_performance_metrics/ies-ac_enforcement_summary; Brulliard, K. (2018) USDA's enforcement of animal welfare laws plummeted in 2018, agency figures show. Washington Post. Retrieved from https://www.washingtonpost.com/science/2018/10/18/usdas-enforcement-animal-welfare-laws-plummeted-agency-figures-show/?utm_term=.33f75b2d9216

153 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398; 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.

154 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.

155 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398.

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

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

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

159 Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A. & Larson, S. (2010) A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9: 10. Retrieved from https://doi.org/10.1186/1475-2891-9-10

160 Srednicka-Tober, D., Baranski, M., Seal, C.J., et al. (2016) Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta- and redundancy analyses. British Journal of Nutrition 115: 1043-1060; Srednicka-Tober, D., Baranski, M., Seal, C.J. et al. (2016) Composition differences between organic and conventional meat; a systematic literature review and meta-analysis. British Journal of Nutrition 115: 994-1011.

161 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398.

162 Center for Food Safety (2018) International labeling laws. Retrieved from https://www.centerforfoodsafety.org/issues/976/ge-food-labeling/international-labeling-laws

163 U.S.A. Agricultural Marketing Service (2018) National bioengineered food disclosure standard. Retrieved from https://www.regulations.gov/document?D=AMS_FRDOC_0001-1709

164 USDA Foreign Agricultural Service (2016) EU-28: Agricultural Biotechnology Annual. Retrieved from https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Agricultural%20Biotechnology%20Annual_Paris_EU-28_12-22-2017.pdf

165 USDA Foreign Agricultural Service (2016) EU-28: Agricultural Biotechnology Annual. Retrieved from https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Agricultural%20Biotechnology%20Annual_Paris_EU-28_12-22-2017.pdf

166 Pew Research Center (2018) Most Americans accept genetic engineering of animals that benefits human health, but many oppose other uses. Retrieved from https://www.pewresearch.org/science/2018/08/16/most-americans-accept-genetic-engineering-of-animals-that-benefits-human-health-but-many-oppose-other-uses/

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

168 Bruce, A. (2017) Genome edited animals: learning from GM crops? Transgenic Research 26: 385–398; Ishii, T. (2017) Genome-edited livestock: ethics and social acceptance. Animal Frontiers 7: 24–32.

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

170 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

171 Baltzegar, J., Cavin Barnes, J. Elsensohn, J.E., Gutzmann, N., Jones, M.S., King, S. & Sudweeks, J. (2018) Anticipating complexity in the deployment of gene drive insects in agriculture. Journal of Responsible Innovation 5: S81S97. Retrieved from https://doi.org/10.1080/23299460.2017.1407910

172 Unckless, R.L., Clark, A.G. & Messer, P.W. (2017) Evolution of resistance against CRISPR/Cas9 gene drive. Genetics. 205: 827-841.

173 Marshall, J.M., Buchman, A. & Sánchez, C.H.M. & Akbari, O.S. Overcoming evolved resistance to population-suppressing homing-based gene drives. Scientific Reports 7: 3776. Retrieved from https://doi.org/10.1038/s41598-017-02744-7

174 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.

175 Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K., Nolan, T. & Crisanti, A. (2018) A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nature Biotechnology 36:1062-1066.

176 See, e.g. 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; 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

177 Kofler, N., Collins, J.P. & Kuzma, J. (2018) Editing nature: local roots of global governance. Science 362: 527-529; International Union for Conservation of Nature (2018) Development of an IUCN policy on synthetic biology. Retrieved from https://www.iucn.org/theme/science-and-economics/our-work/other-work/synthetic-biology-and-biodiversity-conservation/development-iucn-policy-synthetic-biology

178 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.

179 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. Retrieved from https://doi.org/10.1186/s12711-016-0280-3

180 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. Retrieved from https://doi.org/10.1186/s12711-016-0280-3

181 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. Retrieved from https://doi.org/10.1186/s12711-016-0280-3

182 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

183 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.

184 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

185 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; Convention on Biological Diversity (2018). Synthetic Biology. Retrieved from https://www.cbd.int/doc/c/043c/a200/78251e44a6f7ceed13b44312/cop-14-wg-02-crp-20-en.pdf

186 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. Retrieved from https://doi.org/10.1186/s12711-016-0280-3

187 For more detail, see Cotter, J. & Perls, D. (2018) Gene-edited organisms in agriculture: risks and unexpected consequences. Friends of the Earth USA. Retrieved from http://foe.org/wp-content/uploads/2018/09/FOE_GenomeEditingAgReport_final.pdf

188 National Institutes of Health (2016) An overview of the human genome project. National Human Genome Research Institute. Retrieved from https://www.genome.gov/12011238/an-overview-of-the-human-genome-project/; Ezkurdia, L., Juan, D., Rodriguez, J.M., Frankish, A., Diekhans, M., Harrow, J., Vazquez, J., Valencia, A. & Tress, M.L. (2014) Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. Human Molecular Genetics 23: 5866–587; Hidalgo, O., Pellicer, J., Christenhus, M., Schneider, H., Leitch, A.R. & Leitch, I.J. (2017) Is there an upper limit to genome size? Trends in Plant Science 22: 567-573.

189 Ezkurdia, L., Juan, D., Rodriguez, J.M., Frankish, A., Diekhans, M., Harrow, J., Vazquez, J., Valencia, A. & Tress, M.L. (2014) Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. Human Molecular Genetics 23: 5866–587.

190 Wang, Y., Liu, J., Huang, B. et al. (2015) Mechanism of alternative splicing and its regulation. Biomedical Reports 3: 152–158.

191 Wang, Y., Liu, J., Huang, B. et al. (2015) Mechanism of alternative splicing and its regulation. Biomedical Reports 3: 152–158; Nilsen, T.W. & Graveley, B.R. (2010) Expansion of the eukaryotic proteome by alternative splicing. Nature 463: 457-463; see also Grigoryev, Y. (2017) What is alternative splicing, and why is it important? Retrieved from https://bitesizebio.com/10148/what-is-alternative-splicing-and-why-is-it-important/

192 Wang, Y., Liu, J., Huang, B. et al. (2015) Mechanism of alternative splicing and its regulation. Biomedical Reports 3: 152–158

193 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.

194 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.

195 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

196 Housden, B. E., Muhar, M., Gemberling, M., Gersbach, C. A., Stainier, D. Y. R., Seydoux, G., Mohr, S.E., Zuber, J. & Perrimon, N. (2017) Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. Nature Reviews Genetics 18: 24–40; Eisener-Dorman, A.F., Lawrence, D.A. & Bolivar, V.J. (2009) Cautionary insights on knockout mouse studies: the gene or not the gene? Brain, Behavior and Immunity 23: 318–324.

197 Housden, B. E., Muhar, M., Gemberling, M., Gersbach, C. A., Stainier, D. Y. R., Seydoux, G., Mohr, S.E., Zuber, J. & Perrimon, N. (2017) Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. Nature Reviews Genetics 18: 24–40; Eisener-Dorman, A.F., Lawrence, D.A. & Bolivar, V.J. (2009) Cautionary insights on knockout mouse studies: the gene or not the gene? Brain, Behavior and Immunity 23: 318–324.

198 Reiner, G. (2016) Genetic resistance - an alternative for controlling PRRS? Porcine Health Management 2: 27. Retrieved from https://doi.org/10.1186/s40813-016-0045-y

199 See, e.g. Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; Burkard, C., Lillico, S.G., Reid, E., Jackson, B., Mileham, A.J., Ait-Ali, T, Whitelaw, C.B.A. & Archibald, A.L. (2017) Precision engineering for PRRSV resistance in pigs: macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathogens 13: e1006206. Retrieved from https://doi.org/10.1371/journal.ppat.1006206; Voytas, D.F. & Gao, C. (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS Biology 12: e1001877.

200 European Food Safety Authority (2013) Guidance on the environmental risk assessment of genetically modified animals. EFSA Journal 11: 3200. Retrieved from https://doi.org/10.2903/j.efsa.2013.3200

201 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.

202 Jabed, A., Wagner, S., McCracken, J., Wells, D.N. & Laible, G. (2012) Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk. Proceedings of the National Academy of Sciences 109: 16811–16816.

203 Yum, S-Y., Youn, K-Y., Choi, W.J. & Jang, G. (2018). Development of genome engineering technologies in cattle: from random to specific. Journal of Animal Science and Biotechnology 9: 16. Retrieved from https://doi.org/10.1186/s40104-018-0232-6; Tan, W., Proudfoot, C., Lillico, S.G. & Whitelaw, C.B. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Research 25: 273-87; West, J. & Gill, W.W. (2016) Genome editing in large animals. Journal of Equine Veterinary Science 41: 1–6.

204 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 Retrieved from https://doi.org/10.1186/s40104-017-0228-7

205 Anderson, K. R., Haeussler, M., Watanabe, C. et al. (2018) CRISPR off-target analysis in genetically engineered rats and mice. Nature Methods. Retrieved from https://doi.org/10.1038/s41592-018-0011-5; Shin, H. Y., Wang, C., Lee, H. K., et al. (2017) CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nature Communications 8: 15464.

206 Wang, G., Du, M., Wang, J., & Zhu, T. F. (2018) Genetic variation may confound analysis of CRISPR-Cas9 off-target mutations. Cell Discovery 4 Retrieved from https://doi.org/10.1038/s41421-018-0025-2

207 Rodriguez, E. (2017) Ethical issues in genome editing for non-human organisms using Crispr/Cas9 system. Journal of Clinical Research & Bioethics 8: 1000300. Retrieved from https://www.longdom.org/open-access/ethical-issues-in-genome-editing-for-nonhuman-organisms-using-crisprcas9-system-2155-9627-1000300.pdf

208 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.

209 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. Retrieved from doi.org/10.1186/s13059-017-1237-8; Lalonde, S., Stone, O.A., Lessard, S., Lavertu, A., Desjardins, J., Beaudoin, M., Rivas, M., Stainier, D.Y.R. & Lettre, G. (2017) Frameshift indels introduced by genome editing can lead to in-frame exon skipping. PLoS ONE 12: e0178700; Kapahnke, M., Banning, A. & Tikkanen, R. (2016) Random splicing of several exons caused by a single base change in the target exon of CRISPR/Cas9 mediated gene knockout. Cells 5: 45. Retrieved from https://doi.org/10.3390/cells5040045

210 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. Retrieved from https://doi.org/10.3390/cells5040045

211 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. Retrieved from https://doi.org/10.3390/cells5040045

212 Kang, J-D., Kim, S., Zhu, H-Y. et al. (2017) Generation of cloned adult muscular pigs with myostatin gene mutation by genetic engineering. RSC Advances 7: 12541-12549.

213 Wang, K., Tang, X., Xie, Z., Zou, X., Li, M., Yuan, H., Guo, N., Ouyang, H., Jiao, H. & Pang, D. (2017) CRISPR/Cas9-mediated knockout of myostatin in Chinese indigenous Erhualian pigs. Transgenic Research 26: 799-805; Wang, K., Ouyang, H., Xie, Z., Yao, C., Guo, N., Li, M., Jiao, H. & Pang D. (2015) Efficient generation of myostatin mutations in pigs using the CRISPR/Cas9 System. Scientific Reports 5: 16623. Retrieved from https://doi.org/10.1038/srep16623; Tait-Burkard, C., Doeschl-Wilson, A., McGrew, M.J., Archibald, A.L., Sang, H.M., Houston, R.D., Whitelaw, C.B. & Watson, M. (2018) Livestock 2.0 - genome editing for fitter, healthier, and more productive farmed animals. Genome Biology 19: 204. Retrieved from https://doi.org/10.1186/s13059-018-1583-1

214 Guo, R., Wan, Y., Xu, D. et al. (2016) Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. Scientific Reports 6: 29855. Retrieved from https://doi.org/10.1038/srep29855

215 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. Retrieved from https://doi.org/10.1038/srep14435

216 Pig Progress (n.d.) Porcine Stress Syndrome (PSS). Retrieved from https://www.pigprogress.net/Health/Health-Tool/diseases/Porcine-Stress-Syndrome-PSS/

217 Guo, R., Wan, Y., Xu, D. et al. (2016) Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. Scientific Reports 6: 29855. Retrieved from https://doi.org/10.1038/srep29855

218 Guo, R., Wan, Y., Xu, D. et al. (2016) Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. Scientific Reports 6: 29855. Retrieved from https://doi.org/10.1038/srep29855

219 Tait-Burkard, C., Doeschl-Wilson, A., McGrew, M.J., Archibald, A.L., Sang, H.M., Houston, R.D., Whitelaw, C.B. & Watson, M. (2018) Livestock 2.0 - genome editing for fitter, healthier, and more productive farmed animals. Genome Biology 19: 204. Retrieved from https://doi.org/10.1186/s13059-018-1583-1

220 Guo, R., Wan, Y., Xu, D. et al. (2016) Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. Scientific Reports 6: 29855. Retrieved from https://doi.org/10.1038/srep29855

221 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; Wang, Y., Liu, J., Huang, B. et al. (2015) Mechanism of alternative splicing and its regulation. Biomedical Reports 3: 152–158.

222 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.

223 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

224 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. Retrieved from https://doi.org/10.1186/2045-7022-4-11.

225 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

226 Jabed, A., Wagner, S., McCracken, J., Wells, D.N. & Laible, G. (2012) Targeted microRNA expression in dairy cattle directs production of β-lactoglobulin-free, high-casein milk. Proceedings of the National Academy of Sciences 109: 16811–16816.

227 FDA (2017) AquAdvantage salmon fact sheet. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm473238.htm

228 FDA (2017) Food safety evaluation. AquAdvantage salmon fact sheet. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm473238.htm

229 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. Retrieved from https://doi.org/10.3390/cells5040045

230 Price, B., Cotter, J., (2014) The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. International Journal of Food Contamination 1: 5. Retrieved from https://doi.org/10.1186/s40550-014-0005-8

231 National Research Council (2004) Bioconfinement of animals: fish, shellfish, and insects. In: Biological confinement of genetically engineered organisms. US National Academies Press, Washington DC.

232 Houdebine, L-M. (2014) Impacts of genetically modified animals on the ecosystem and human activities, Global Bioethics 25: 3-18; National Research Council (2002). Environmental concerns. In: Animal biotechnology: science-based concerns. Committee on defining science-based concerns associated with products of animal biotechnology. US National Academies Press, Washington DC. Ch.5

233 National Research Council (2002). Applications of biotechnology techniques. In: Animal biotechnology: science-based concerns. Committee on defining science-based concerns associated with products of animal biotechnology. US National Academies Press, Washington DC. Ch. 2

234 International Service for the Acquisition of Agri-biotech Applications (ISAAA) (2017). Global status of commercialized biotech/GM crops in 2017. ISAAA Brief No. 53. Table 36, including herbicide tolerance stacked with insect resistance, 2017 data. Retrieved from http://www.isaaa.org/resources/publications/briefs/53/download/isaaa-brief-53-2017.pdf

235 Schütte, G. Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassalli, S., Ruohonen-Lehto, M., Saucy, A-G.W. & Mertens, M. (2017) Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. Environmental Science Europe 29: 5. Retrieved from https://doi.org/10.1186/s12302-016-0100-y Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28: 3. Retrieved from https://doi.org/10.1186/s12302-016-0100-y

236 Heard, M.S., Hawes, C., Champion, G.T. et al. (2003) Retrieved from https://doi.org/10.1186/s12302-016-0070-0. Weeds in fields with contrasting conventional and genetically modifies herbicide-tolerant crop – I. Effects on abundance and diversity. Philosophical Transactions of The Royal Society London B 358: 1819-1832; Heard, M.S., Hawes, C., Champion, G.T. et al. (2003) Weeds in fields with contrasting conventional and genetically modifies herbicide-tolerant crops. II. Effects on individual species. Philosophical Transactions of The Royal Society London B 358: 1833-1846; Roy, D.B., Bohan, D.A., Haughton, A.J. et al. (2003) Invertebrates and vegetation of the field margins adjacent to crops subject to contrasting herbicides regimes in the Farm Scale Evaluations of genetically modified herbicide –tolerant crops. Philosophical Translations of The Royal Society London B 358: 1879-1898.

237 Fiems, L. 2012. Double muscling in cattle: genes, husbandry, carcasses and meat. Animals 2: 472-506.

238 Benz-Schwarzburg, J. & Ferrari, A. (2016) Super-muscly pigs: trading ethics for efficiency. Issues in Science and Technology 32: 29–32.

239 United Nations Food and Agriculture Organisation (2018) World livestock: transforming the livestock sector through the Sustainable Development Goals. FAO, Rome. Retrieved from http://www.fao.org/3/CA1201EN/ca1201en.pdf

240 Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (2019) Global biodiversity assessment. Summary for policymakers. Retrieved from https://www.ipbes.net/news/ipbes-global-assessment-summary-policymakers-pdf; Secretariat of the Convention on Biological Diversity (2014) Global Biodiversity Outlook 4. Retrieved from https://www.cbd.int/gbo/gbo4/publication/gbo4-en.pdf; Springmann, M., Clark, M., Mason-D'Croz, D. et al. (2018) Options for keeping the food system within environmental limits. Nature 562: 519-525; Foley, J.A., Ramankutty, N., Brauman, K.A. et al. (2011) Solutions for a cultivated planet. Nature 478: 337-42; International assessment of agricultural knowledge, science and technology for development (2009) Global report. McIntyre, B.D. (ed.) Island Press, Washington D.C. Retrieved from https://www.weltagrarbericht.de/reports/Global_Report/Global_content.html

241 Savory Institute (2015) Climate change, healthy soils and holistic planned grazing: a restoration story. Boulder, Colorado, U.S. Retrieved from https://www.savory.global/wp-content/uploads/2017/02/2015-climate-a-restoration-story.pdf

242 Weber, K. T., & Gokhale, B. S. (2011). Effect of grazing on soil-water content in semiarid rangelands of southeast Idaho. Journal of Arid Environments 75: 464–470.

243 Savory Institute (2015) Climate change, healthy soils and holistic planned grazing: a restoration story. Boulder, Colorado, U.S. Retrieved from https://www.savory.global/wp-content/uploads/2017/02/2015-climate-a-restoration-story.pdf

244 Webber, P. (2014) Does CRISPR-Cas open new possibilities for patents or present a moral maze? Nature Biotechnology 32: 331-333.

245 See, e.g. U.S. Supreme Court 2013. Bowman v. Monsanto Co., 569 U.S. 278. Justia. Retrieved from: https://supreme.justia.com/cases/federal/us/569/278/

246 Ormandy, E.H., Dale, J. & Griffin, G. (2011) Genetic engineering of animals: Ethical issues, including welfare concerns. Canadian Veterinary Journal 52: 544-50.

247 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187. pg. 27. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

248 FDA (2017) Regulation of genetically engineered animals. Draft revised guidance no. 187. pg. 27. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

249 EFSA (2013) Guidance on the environmental risk assessment of genetically modified animals. Panel on Genetically Modified Organisms. EFSA Journal 11: 3200. Retrieved from https://doi.org/10.2903/j.efsa.2013.3200

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

251 FDA (2017) AquAdvantage salmon fact sheet. Retrieved from https://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/BiotechnologyProductsatCVMAnimalsandAnimalFood/AnimalswithIntentionalGenomicAlterations/ucm473238.htm

252 Earthjustice (2016) Lawsuit challenges FDA's approval of genetically engineered salmon. Press release March 31. Retrieved from https://earthjustice.org/news/press/2016/lawsuit-challenges-fda-s-approval-of-genetically-engineered-salmon

253 FDA (2018) Statement from FDA Commissioner Scott Gottlieb, M.D., and Deputy Commissioner Anna Abram on the FDA's new plan to advance plant, animal biotechnology innovation. Press announcement October 30. Retrieved from https://www.fda.gov/animal-veterinary/cvm-updates/fda-announces-plant-and-animal-biotechnology-innovation-action-plan

254 FDA (2018) Plant and animal biotechnology innovation action plan. Retrieved from https://www.fda.gov/downloads/Safety/Biotechnology/UCM624517.pdf

255 Ledford, H. (2019) Creators of gene-edited animals bypass US market. Nature (news) 566: 433-434.

256 Executive Office of the President (2019) Modernizing the regulatory framework for agricultural biotechnology products. Executive order 13874, June 11. Retrieved from https://www.federalregister.gov/documents/2019/06/14/2019-12802/modernizing-the-regulatory-framework-for-agricultural-biotechnology-products

257 Knight, J. (2003) GloFish casts light on murky policing of transgenic animals. Nature (news) 426: 372; Anon (2004) The one that got away. Nature Biotechnology (editorial) 22: 1.

258 FDA (2015) Guidance for industry - regulation of genetically engineered animals containing heritable recombinant DNA constructs. Retrieved from https://wayback.archive-it.org/7993/20161022003714/http:/www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf; FDA (2017) guidance for industry -regulation of intentionally altered genomic DNA in animals. Draft guidance. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

259 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

260 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

261 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.

262 Mallapaty, S. (2019) Australian gene-editing rules adopt 'middle ground'. Nature (news) April 23. Retrieved from https://www.nature.com/articles/d41586-019-01282-8; Normile, D. (2019) Gene-edited foods are safe, Japanese panel concludes. Science (news) March 19. https://doi.org/10.1126/science.aax3903

263 de Graeff, N., Jongsma, K.R., Johnston, J., Hartley, S. & Bredenoord, A.L. (2019) The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. Philosophical Transactions of the Royal Society B 374: 20180106. Retrieved from https://doi.org/10.1098/rstb.2018.0106

264 de Graeff, N., Jongsma, K.R., Johnston, J., Hartley, S. & Bredenoord, A.L. (2019) The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. Philosophical Transactions of the Royal Society B 374: 20180106. Retrieved from https://doi.org/10.1098/rstb.2018.0106