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Genome Editing - Fact File

Introduction and Summary

Genome Editing, or Gene Editing, is the use of modern technologies to alter an organism's DNA. Unlike in Genetically Modified (GM) organisms, Gene Editing does not always require the introduction of DNA from other species (though it may be used). Due to this difference, some believe the risks of GE are lower than GM and it should therefore be classified differently to allow for proportionate regulation. Others, however, maintain that all organisms with DNA modified by genetic technologies should be classed as GM.

Gene Editing Technology

CRISPR-Cas9 is the most common Gene Editing Tool, which comprises of two parts. CRISPR is a short RNA template which matches a target sequence, whilst Cas9 is an enzyme which causes a double-stranded break at the target site. Due to the short, simple nature of the CRISPR template, it is faster and less complex than previous technologies – though still takes an average of 79 weeks to obtain an edit. CRISPR-Cas9 can be used for: gene knockout, gene replacement, transcriptional regulation, epigenome editing and even DNA imaging. Following an edit, DNA repair mechanisms can take one of two pathways:

- Non-Homologous End Joining (NHEJ), which is prone to errors and may increase tumour risk.
- Homology-Directed Repair (HDR), which uses a template for repair and can therefore create a precise and predictable sequence.

As mammalian cells preferentially undergo NHEJ, work is underway to understand what drives pathway choice. However, currently it is not possible to select for NHEJ over HDR.

Legislative Background

Gene Editing: The Genetic Technology (Precision Breeding) Bill 2022 is currently in the House of Lords, and will permit the research, development, marketing, and release of "Precision Bred" organisms. Currently, Gene Edited (GE) organisms are regulated via the Environmental Protection Act 1990, which includes the following regulations: The Genetically Modified Organisms (Deliberate Release) Regulations 2002 (amended in 2022) and The Genetically Modified Organisms (Contained Use) Regulations 2014. The government believe these regulations are disproportionate to the risk posed by GE organisms, and the new Bill therefore outlines a simpler regulatory process for PBOs. To ensure safety, PBOs and their products will be reviewed by an expert committee, plus an animal welfare committee if the editing was performed on a vertebrate.
Animal Welfare: The Animals (Scientific Procedures) Act 1986 and the Animal Welfare Act 2006 will underpin GE animal welfare, the former in R&D and the latter for post-R&D.
Intellectual Property: Under the Patents Act 1977/Patents Regulations 2000, GE organisms are patentable as long as they are an "invention" and do not exist in nature. Farmers are permitted to breed from any patented breeding stock purchased for their own agricultural purposes but would not be able to sell the animal or its progeny for the purposes of a commercial reproduction activity.

Potential Uses

Enhanced Research Models: Increased speed and decreased cost of creating animals for disease models; in-vivo editing to reduce the need for germline modification; enhanced in-vivo and in-vitro disease models; creation of complicated multiplex models to allow greater precision in disease modelling.
Gene Therapy: Increased site specificity for gene therapy and reduced reliance on viral transgenes, by using viral vectors instead. Gene therapy may also be possible in somatic cells (trials underway in HIV, TTR and ATR amyloidosis, Sickle Cell Disease/Beta Thalassaemia and for cancer immunotherapy).
Production of Organs for Transplantation: Reduced risk of immune rejection xenotransplanted organs (no regulations currently govern xenotransplantation and it raises many ethical and animal welfare concerns).

Food Security: Increasing yields of both pastoral and arable farming by improving tolerance to novel conditions (e.g., heat, drought, salinity, pH) and increasing animal fertility.
Nutrition: Changing nutritional composition of crops, removal of carcinogens in food e.g., acrylamide in wheat, reducing/removing allergens from foods.
Climate Change: Reducing cattle methane emissions. Improving efficiency of cell lines used to create cultured meat. Creating robust "wild" plants and animals e.g., heat-resistant corals.
Animal Welfare: Polled cattle, gender selection in poultry industry, disease resistance (also in plants).
Pandemic Preparedness: Disease resistance to zoonotic disease, e.g. bird flu may help to prevent pandemics.

Concerns With GE

There remain some technical concerns with Gene Editing, namely: limitations to where a target site can be, unpredicted effects at the target site, failed binding to the target site, off-target binding, template plasmid integration, DNA repair unpredictability, oncogenesis and immunogenesis. Though editing concerns can be negated by screening, it is important to make sure this process is thorough any catches any errors present. There are also concerns with the commercialisation and regulation of GE organisms. The Precision Breeding Bill leaves most regulation to secondary legislation and contains no labelling requirements. As the GE process leaves no markers, traceability may therefore become difficult. Concerns regarding intellectual property rights have repeatedly been raised as, although GE technologies are cheaper to produce, patent fees remain a barrier to market for Small and Medium Enterprises (SMEs). Crop monopolies may therefore become further consolidated and worsen declining crop biodiversity, with patent stacking possibly causing further complexity.

Gene Drive Technology

Gene Drive technology introduces changes to an organism's DNA which will have a greatly increased likelihood of being inherited and therefore spread through a population at an accelerated rate. Proposed applications have included controlling vector-borne diseases or invasive species, introducing disease resistance into wild animal populations or introducing non-toxic pesticides/herbicides. This is still relatively new technology, and many have concerns about the unintended ecological consequences of gene drives as, once released, their spread is uncontrollable, irreversible, and may spread across species boundaries.

Key Stakeholder Opinions

The European Commission: The 2021 EC report on "New Genomic Techniques" (NGTs) acknowledged their potential benefits, but stated concerns regarding safety, environmental impact, labelling and fit with GM-free agriculture. However, the report concluded that previous GMO legislation is not suitable to regulate NGTs, and the EC have since ran a survey and consultation on NGTs to help guide future legislation.
The Royal Society: The Royal Society support of the introduction of new legislation to loosen restrictions on GE, as they believe these technologies "can help address the environmental and societal challenges faced by 21st century agriculture" and help the UK to achieve net-zero.
Nuffield Council on Bioethics: The Nuffield report on GE and animal breeding concluded that GE has the potential to bring benefits to farming but warned that it may allow acceleration of poor breeding practices or enable low-welfare husbandry practices.
BVA: The BVA maintain that GE organisms should remain regulated with GMOs due to the complexity and lack of clarity in DEFRAs proposals, as they believe that GE requires thorough and transparent monitoring.
Public Opinion: In Defra-commissioned market research, consumers had low awareness and knowledge of GE, and many confused it with GM.- though it was generally perceived as safer and more 'natural'. Acceptability was higher in plants than in animals. Public concerns about GE mainly focused on labelling, corporate interests and animal welfare.

Gene Editing: Fact File

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About the Veterinary Policy Research Foundation (VPRF)

The VPRF is a not-for-profit organisation set up by Lord Trees with the purpose of employing a veterinary surgeon as an intern/researcher to facilitate Lord Trees' activities in the House of Lords.

Declarations by the Author

The authors are veterinary surgeons and support scientific, evidence-based discussion over new technologies, especially those which may contribute to the health and welfare of both humans and animals. Professor the Lord Trees is a veterinary surgeon and a crossbench peer. Harriet Davenport is a veterinary surgeon, who has held the role of Parliamentary Veterinary Intern since October 2021. The Parliamentary Veterinary Internship is funded by The Veterinary Policy Research Foundation that receives sponsorship from several veterinary organisations, professional bodies and universities. Further information on the VPRF can be found on our website: https://vprf.wordpress.com/

Contents

Definitions

Base Pairs	Complementary bases on separate DNA strands that bind to form double-stranded DNA.
Chromatid	One half of a duplicated chromosome. Before replication, one chromone is composed of one DNA strand. In replication, the DNA molecule is copied and the two resulting molecules are known as chromatids.
Double Strand Break	A break in double-stranded DNA (dsDNA) in which both strands have been cleaved and are thus separated from each other.
Epigenetics	Modifications to genetic expression, and not the genetic code itself.
Exogenous	Having an external origin.
Indel	The term used for the insertion or deletion of a section of DNA into the genome of an organism. They vary in size, location and can affect the function of certain genes.
Nucleotides	A basic structural component of a nucleic acids found in DNA and other molecules. It consists of a nucleoside linked to a phosphate group – creating the 'backbone' of a DNA strand.
Plasmid	A DNA containing structure within a cell that can replicate independently of the chromosomes. They are typically small circular DNA within the cytoplasm of a bacteria or protozoa. They are commonly used in experimental manipulation of genomes to insert DNA sequences into specific areas of the target genome.
Transcription	The first stage of protein synthesis and gene expression. The process copies a segment of DNA into RNA.
Transgene	A gene that is artificially added into the genome of another organism.
Zygotes	A diploid cell (2 complete sets of chromosomes – one maternal, one paternal) resulting from the fusions of 2 haploid gametes (a sperm/egg cell with a single set of unpaired chromosomes).

How Gene Editing Works

Genome Editing (GE) lets scientists change the DNA of plants, animals and bacteria. Unlike previous Genetic Modification, GE does not **always** require the introduction of DNA from other species, though it is often used. Due to this difference in technique, some believe GE organisms should be classified differently to GMOs, whilst others maintain that all organisms with scientifically modified DNA should be classed as GMOs.

History of Genetic Modification[1]

Homologous Recombination[2]

The earliest form of Genetic Modification. Developed by scientists in the 1970s to replicate natural DNA recombination. In this process, foreign DNA replaces a target gene in an Embryonic Stem Cell to "knock-out" the gene. The foreign DNA has a similar sequence to the target and is flanked by sequences identical to those surrounding the target - the cell recognizes the identical sequences as homologues, causing them to switch. This method allowed scientists to study the function of specific genes but produced a high frequency of off-target effects (OTEs).

Zinc-finger nucleases (ZFNs)

Produced in the 1990s to reduce the frequency of OTEs. ZFNs are proteins which can be engineered to bind to specific DNA sequences in the genome and cut the DNA, allowing scientists to either delete the target sequence or replace it.

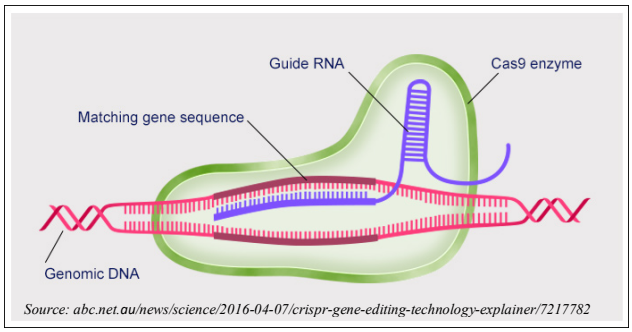
Transcription Activator-Like Effector Nucleases (TALENs)[1]

Created in 2009, these enzymes are engineered from bacterial proteins and bind to specific DNA sequences. Though similar to ZFNs, TALENs are simpler and easier to engineer. However, both remain relatively complex and expensive, and the process is prone to error.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)[1]

CRISPR is seen by many as a more efficient method of gene editing. The CRISPR-Cas9 molecule is comprised of two sections.

- **Guide RNA** – This is a short RNA template which matches the target DNA sequence.
- **Cas9** – An enzyme which cuts the genome at the target site, causing a double-stranded break (DSB).
    - In order to cut to the target DNA, the Cas nuclease must first recognise a sequence bordering the target DNA sequence, known as a protospacer adjacent motif (PAM). This is usually 2-6 base pairs long and is generally found 3-4 nucleotides downstream from the cut site. Target locations for CRISPR editing are therefore limited by the locations of PAM sequences[3].



Source: abc.net.au/news/science/2016-04-07/crispr-gene-editing-technology-explainer/7217782

N.B. Occasionally, a third component – a Homology-Directed Repair (HDR) DNA template – is used to guide the repair of the genome. For further information on HDR, see page 4.

As its binding is dictated by a short guide RNA which does not require complex multi-step protein engineering, CRISPR is seen as faster and more straightforward than previous technologies. However, CRISPR projects still take time. A survey showed that on average, it takes an average of 79 weeks to obtain an edit. This is based on:

- 10 weeks to complete guide design, preparation, optimisation, transfection and analysis
    - Repetition of the above an average of 7 times to obtain the desired edit
- 9 weeks for screening and isolation of edited clones[4]

[1] https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/How-genome-editing-works
[2] https://www.britannica.com/science/homologous-recombination
[3] Synthego. *Importance of the PAM Sequence in CRISPR Experiments.* Available at: https://www.synthego.com/guide/how-to-use-crispr/pam-sequence
[4] https://www.synthego.com/blog/crispr-experiments-challenges#how-long-does-crispr-take-the-real-time-invested-in-the-workflow

	Guide design is rapid and inexpensive	Able to target any DNA sequence	Potential to cause off-target effects
ZFNs	✖	✖	✓
TALENs	✖	✓	✓
CRISPR/Cas9	✓	✓	✓

CRISPR-Cas9 can be used to produce a range of effects at the target site[5]:

- **Gene Knockout/Replacement** - The most widely used application of CRISPR-Cas9. A double-strand break is created at the target site to "cut out" unwanted sequences of DNA. The host DNA is then repaired via one of two mechanisms described in the section below.
- **Base Editing** – Deaminase enzymes are fused to Cas9 and catalyse base edits or substitutions at the target site without causing a double-strand break, resulting in fewer indels.
- **Transcriptional Regulation** – Cas9 is fused to transcription regulators, which can activate (CRISPRa) or inhibit (CRISPRi) transcription of the target gene as desired – this is used in research to interrogate gene function.
- **Epigenome Editing** – this can be used to activate or repress target genes, either temporarily or permanently. This can be done without altering an organism's genome, and was demonstrated in 2021 using a technology known as CRISPRoff.[6]
- **CRISPR Imaging** – Fluorescent molecules are fused to Cas9 to dynamically track specific DNA loci in living cells.

DNA Repair Mechanisms following Gene Knockout/Replacement

- **Non-Homologous End Joining (NHEJ):** In this process, the break-ends are directly ligated with only a short guide sequence. This imprecise repair can result in nucleotide and subsequent gene knockout (due to INDEL formation resulting in loss of function, frameshift mutations, creation of a premature stop codon, etc).[7] These imprecise changes may also cause translocations and telomere fusion – hallmarks of tumour cells, therefore increasing cancer risk.[8]
- **Homology-Directed Repair (HDR):** HDR can either utilize the sister chromatid or an exogenous DNA template for repair, to create a precise and predictable sequence. This allows for the introduction of almost any desired DNA change, and therefore has the potential for wide application and may be safer for clinical use – though would possibly involve transgenic DNA for the template.[6]

N.B. The outcome of gene editing depends on which of the above pathways is favoured by the cell. Mammalian cells preferentially undergo NHEJ, but the mechanism for pathway selection is not yet fully understood. Work is underway to understand how we may manipulate cells to undergo HDR instead of NHEJ and thus increase the precision of GE.[9]

[5] Tycko, J. et al. 2017. *Expanding the CRISPR toolbox.* Nature Methods. Available at: https://media.nature.com/full/nature-cms/uploads/ckeditor/attachments/7742/CRISPR_poster-WEB.pdf

[6] Nuñez, J.K., et al. 2021. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell. DOI: https://doi.org/10.1016/j.cell.2021.03.025

[7] https://invivobiosystems.com/news-annoucements/differences-between-hdr-vs-nhej/

[8] https://www.wikipathways.org/index.php/Pathway:WP438

[9] Yang, H., et al. 2020. *Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks*. International journal of molecular sciences, 21(18), 6461. https://doi.org/10.3390/ijms21186461

How are CRISPR-Cas9 edits made?

CRISPR edits can be made to either somatic (all cells except for germline cells) or germline cells (sperm, ova or embryos).

- **Somatic:** Somatic gene editing targets a certain cell type in an organism e.g., red blood cells, and only affects the edited individual i.e. these changes are NOT heritable. Viral vectors are often used to deliver the CRISPR-Cas9 complex to the target cell type.
- **Germline:** Germline edits are often made to zygotes, so that the edited gene is copied into every cell in the body. These are heritable changes and are often those which face the most controversy.

For more details, see the diagram below:

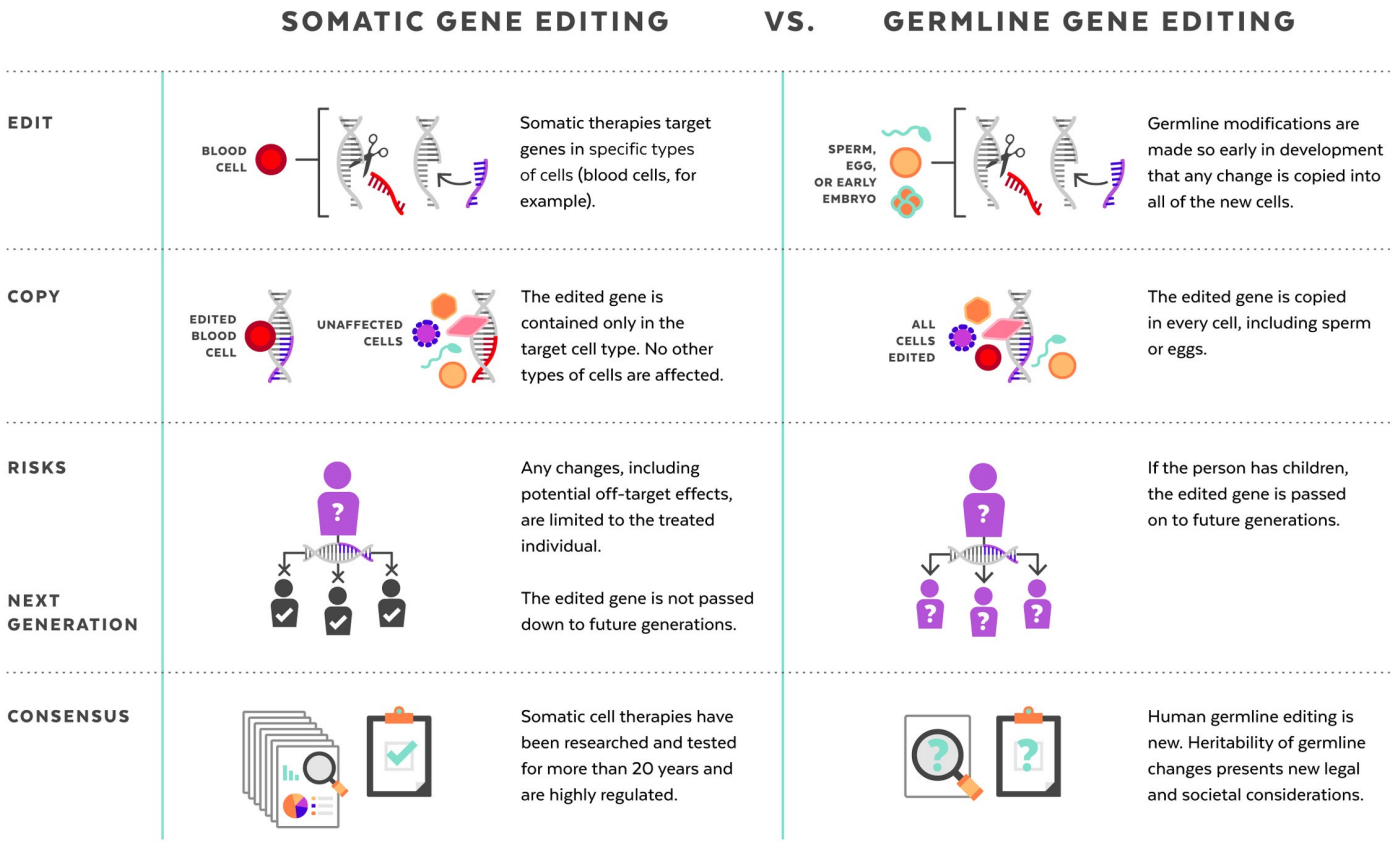


Image Source: https://news.harvard.edu/gazette/story/2019/01/perspectives-on-gene-editing/

Germline editing is the cause of much debate, as it is a much newer technique, which involves making changes that pass to every cell in the edited organism, as well as their offspring. The scientific community has largely pulled together to ensure this area is highly regulated, to ensure the technology is developed with the "*correct oversight and quality controls",* as stated by one of the pioneers of CRISPR technology, Feng Zhang[10].

- A medical ethics paper, published in 2020, highlighted that there have been three ethical/legal problems central to the debate on Germline editing, which must be discussed if this technique is to be developed:
    - *"(i) questions of risk and uncertainty related to the technology and its application, (ii) interference with the human germline and responsibility towards future generations, and (iii) the legitimization of genome editing measures with regard to the concepts of therapy and enhancement."*[11]

[10] Bergman, M. 2019. *Perspectives on Gene Editing.* Harvard Gazette. Available at: https://news.harvard.edu/gazette/story/2019/01/perspectives-on-gene-editing/

[11] Schleidgen, S., Dederer, HG., Sgodda, S. et al. *2020. Human germline editing in the era of CRISPR-Cas: risk and uncertainty, inter-generational responsibility, therapeutic legitimacy.* BMC Med Ethics. https://doi.org/10.1186/s12910-020-00487-1

Legal Environment

UK

GE is a devolved issue in the UK. In England, DEFRA holds lead responsibility for GE regulation and related policy, with the FSA responsible for GMO food and feed in England, Northern Ireland and Wales, and the FSS responsible for this in Scotland. The NI Protocol mandates that NI must align with EU Single Market rules on food/feed, including GMOs.[12] The Advisory Committee on Novel Foods and Processes advises the FSA on the safety of novel foods.[13]

The Genetic Technology (Precision Breeding) Bill 2022

- This Bill was introduced to separate the regulation of Precision Bred Organisms from GM organisms. According to the Bill, a Precision Bred Organism is created by introducing DNA changes using modern biotechnology (for example, Gene Editing with CRISPR), and must not contain any transgenes.
- The term 'precision bred' (PB) was utilised in the Bill to reflect the specific, targeted nature of changes. It does not specify the technique used but focuses on the genetic makeup and characteristics of the final product.
- PB includes technologies, such as gene editing (GE), which can edit DNA more precisely than conventional breeding methods and therefore create targeted changes. It differs from GM in that: "notably, the final organism must not contain functional foreign genes taken from a sexually incompatible species."
- The primary objective is to ensure regulation of PB organisms (PBOs) is proportionate to the risks and therefore simplifies regulation to facilitate market access. The four key changes are:
    - Remove PBO plants and animals from GMO requirements
    - Introduce two PBO notification systems – one for research and one for marketing
    - Establish a proportionate regulatory system for PB animals to ensure welfare is maintained – no changes introduced initially until new system in place
    - Establish a science-based authorisation process for PBO-based food and feed
- UK is a world-leader in genetic research and Defra wish to harness this commercially to drive innovation and investment in the UK. Defra believe the GMO regulations are outdated for current techniques, with ACRE suggesting that PBOs pose no greater risk than conventionally bred organisms.
- England Only
- The intention of the Bill is that the Genetic Modification Inspectorate (GMI) who operate under the Environmental Protection Act 1990 will check compliance with the bill, with the exception of food and feed. An inspector may sometimes need to be accompanied by other subject matter experts – for example, a qualified veterinarian where an inspection relates to animals.
- Food and Feed from PBOs will be monitored and enforced by Trading Standards Officers and Environmental Health Officers
- A new Food Standards Agency (FSA) regulatory framework will be used to regulate food and feed.
- The Genetic Technology (Precision Breeding) Bill 2022 was passed in March 2023. It will permit the research, development, marketing, and release of "Precision Bred" organisms. Currently, Gene Edited (GE) organisms are regulated via the Environmental Protection Act 1990, which includes the following regulations: The Genetically Modified Organisms (Deliberate Release) Regulations 2002 (amended in 2022) and The Genetically Modified Organisms (Contained Use) Regulations 2014. The government believe these regulations are disproportionate to the risk posed by GE organisms, and the new Bill therefore outlines a simpler regulatory process for PBOs. To ensure safety, PBOs and their products will be reviewed by an expert committee, plus an animal welfare committee if the editing was performed on a vertebrate.

Animals (Scientific Procedures) Act 1986[14]

- This Act protects animals for use in research, including Gene Edited animals.
    - A 'regulated procedure' in ASPA is any test, experiment or procedure done to a 'protected animal' which may cause pain, suffering, distress or lasting harm which is equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.
    - 'Protected animals' include *"any living vertebrate other than man"*

[12] *https://www.food.gov.uk/sites/default/files/media/document/consumer-perceptions-of-genome-edited-food.pdf*

[13] UK Government. The Advisory Committee on Novel Foods and Processes. Available at: https://www.gov.uk/government/organisations/advisory-committee-on-novel-foods-and-processes

[14] UK Government. Animals in Scientific Procedures Act 1986. Available at: https://www.legislation.gov.uk/ukpga/1986/14

- A Personal, Project and Establishment licence must be in place for a procedure to be permitted.

The Animal Welfare Act 2006[15]

- This Act protects the welfare of domestic animals, making an individual responsible for an animal and its basic needs. Under the act, it is an offence to cause unnecessary suffering to an animal –
    - *A person commits an offence if—*
        - *(a)an act of his, or a failure of his to act, causes an animal to suffer,*
- GE animals would be afforded the same welfare protections under this Act as any other animal in the UK.

The Genetically Modified Organisms (Contained Use) Regulations 2014[16]

- These Regulations implement the European Directive (EC) No 2009/41, which lays down measures for the contained use of GM micro-organisms, to protect human health and the environment - before contained use can commence, a responsible person must ensure that an assessment of the risks to human health and the environment has been carried out, and they must notify a competent authority about the use.
- This also applies to the contained use of GMOs that are not micro-organisms, known as larger GMOs, but only in relation to risks to human health. Larger GMOs are not covered by the Directive.

The Genetically Modified Organisms (Deliberate Release) Regulations 2002[17]

- Made under the Environmental Protection Act 1990, these regulations control the deliberate release of GMOs into the environment, their marketing and post-market monitoring, by imposing a requirement to obtain consent from the Secretary of State for their release and to comply with any conditions imposed.
- Application fees and compliance measures lead to costs of around £10,000 per field trial.

The Genetically Modified Organisms (Deliberate Release) (Amendment) (England) Regulations 2022[18]

- These amended the 2002 regulations to give an exception to "Qualifying Higher Plants", who have been modified in a way that "*could have occurred naturally",* or "*could have been made using one or more of the techniques set out in regulation 5(2)"* (in vitro fertilisation, natural processes such as conjugation, transduction and transformation, and polyploidy induction)
    - The introduction of these regulations was a first step in paving the way for further legalising GE research by opening up research on certain GE crops
        - Rather than requiring a submitted Risk Assessment and consent from the Secretary of State, the new regulations require only a notice submitted to the Secretary of State containing prescribed information.
        - The House of Lords Secondary Legislation Scrutiny Committee expressed concern that releasing these GE plants could negatively affect the wider environment, especially on commercial crops[19] – though as GE crops must meet the same standards as traditional breeding methods, this is unlikely to increase environmental risk.

Intellectual Property

The Patents Act 1977[20]/ The Patents Regulations 2000[21]

- The 1977 Act states that "*any variety of animal or plant or any essentially biological process for the production of animals or plants, not being a microbiological or other technical process or the product of such a process*" cannot be patented.
- However, the Patents Regulations 2000 amended the Act, clarifying that inventions concerning biological material, including gene sequences, are legitimately the subject of patent applications, as below:

[15] UK Government. The Animal Welfare Act 2006. Available at: https://www.legislation.gov.uk/ukpga/2006/45/
[16] UK Government. The Genetically Modified Organisms (Contained Use) Regulations 2014. Available at: https://www.legislation.gov.uk/uksi/2014/1663/
[17] UK Government. The Genetically Modified Organisms (Deliberate Release) Regulations 2002. https://www.legislation.gov.uk/uksi/2002/2443/
[18] UK Government. The Genetically Modified Organisms (Deliberate Release) (Amendment) (England) Regulations 2022. Available at: https://www.legislation.gov.uk/uksi/2022/347/
[19] Secondary Legislation Scrutiny Committee. 2022. *29th Report of Session 2021–22.* House of Lords. Available at: https://committees.parliament.uk/publications/8865/documents/89203/default/
[20] UK Government. Patents Act 1977. Available at: https://www.legislation.gov.uk/ukpga/1977/37/
[21] UK Government. The Patents Regulations 2000. Available at: https://www.legislation.gov.uk/uksi/2000/2037/

- *An invention shall not be considered unpatentable solely on the ground that it concerns—*
  - *(a)a product consisting of or containing biological material; or*
  - *(b)a process by which biological material is produced, processed or used.*

- These amendments also clarified that farmers are legally able to propagate these organisms on their own farm, though not for commercial purposes:
  - (5) *An act which, apart from this subsection, would constitute an infringement of a patent for an invention shall not do so if:*
    - *(g)it consists of the use by a farmer of the product of his harvest for propagation or multiplication by him on his own holding, where there has been a sale of plant propagating material to the farmer by the proprietor of the patent or with his consent for agricultural use;*
    - *(h)it consists of the use of an animal or animal reproductive material by a farmer for an agricultural purpose following a sale to the farmer, by the proprietor of the patent or with his consent, of breeding stock or other animal reproductive material which constitutes or contains the patented invention*
  - Under these regulations, an agricultural purpose is defined as something which:
    - *(a)includes making an animal or animal reproductive material available for the purposes of pursuing the farmer's agricultural activity; but*
    - *(b)does not include sale within the framework, or for the purposes, of a commercial reproduction activity.*
- As such, patents can be granted to GE organisms if the genetic change **does not already exist in nature,** as this would mean that it is not an "invention"**.**

Ongoing CRISPR-Cas9 legal dispute: Two groups attempted to patent the CRISPR-Cas9 GE system as their own. A group from UC Berkeley, including scientists Jennifer Doudna and Emmanuelle Charpentier, won the Nobel Prize for their work on CRISPR-Cas9 which created a single RNA guide for Cas9 to bind precisely to DNA. The other group from the Broad Institute, including scientist Feng Zhang, who modified the system to work in eukaryotic cells.[22]

- After years in court, the final ruling in March 2022 from the United States Patent and Trademark Office awarded the patent to the Broad Institute[23].
  - Patent battles over CRISPR are likely to continue, adding further complexity to the royalty landscape.

EU

A European Court of Justice (ECJ) ruling in 2018 implied that genome editing techniques alter the genome in such a way that would not occur naturally/by mating and should not be exempt from GMO regulation because they do not have a long safety record.

- These regulations prohibit the use of mutagenic techniques – those which alter the genome without the insertion of foreign DNA - and only allow GMO foods to enter the market, if it has been demonstrated that they are not nutritionally disadvantageous; they do not have adverse effects on health or the environment.[24]
- High energy radiation and alkylating agents were exempt from these rules due to their long history of use - over 3,000 crop varieties have been created this way and listed on the FAO/IAEA Mutant Variety Database. However, these methods are highly inaccurate and create thousands of mutations at once in crops.[25]

Global

In 2018, 13 nations supported an international statement to the World Trade Organisation in favour of science-based and internationally harmonised regulation of genome editing in agriculture. The extent to which countries have so far permitted Gene Editing is shown below:

[22] Hochster, H. S. 2022. *CRISPR Patent Battle: Beautiful Science, Poor Public Policy.* ONCOLOGY 36:5 P263. Available at: https://www.cancernetwork.com/view/journal-crispr-patent-battle-beautiful-science-poor-public-policy

[23] KATZ. 2022. *Decision on the Decision on Priority 37 C.F.R. § 41.125(a).* USPTO. Available at: https://drive.google.com/file/d/1-NxaE-FqWz1spjckk-Q6I_-LuKSzvMg3/view

[24] Court of Justice of the European Union. *Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive.* Press Release No 111/18. Available at: https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf

[25] Jones H. D. (2015). Future of breeding by genome editing is in the hands of regulators. GM crops & food, 6(4), 223–232. https://doi.org/10.1080/21645698.2015.1134405

RATING BY COUNTRY / REGION

Click each column header and arrow to sort the countries / regions

Country / Region	Food / Crops	Animals	Ag Rating
Ecuador	10	10	10
Brazil	10	10	10
Argentina	10	10	10
Paraguay	10	10	10
Japan	8	8	8
Canada	8	8	8
Australia	8	8	8
Israel	10	5	7.5
US	10	4	7
Norway	6	6	6
Central America	6	6	6
Uruguay	6	6	6
India	6	6	6
Chile	10	1	5.5
Colombia	10	1	5.5
Africa	5	5	5
Russia	5	5	5
Switzerland	5	5	5
China	5	5	5
New Zealand	4	4	4
UK	2	2	2
EU	2	2	2
Ukraine	1	1	1
Mexico	1	1	1

COLORS AND RATINGS GUIDE

Regulation Status	Rating
Determined: No Unique Regulations*	10
Lightly Regulated	8
Proposed: No Unique Regulations†	6
Ongoing Research, Regulations In Development	5
Highly Regulated	4
Mostly Prohibited	2
Limited Research, No Clear Regulations	1
Prohibited	0

Lightly Regulated: Some or all types of gene editing are regulated more strictly than conventional agriculture, but not as strictly as transgenic GMOs.

***Determined: No Unique Regulations:** Gene-edited crops that do not incorporate DNA from another species are regulated as conventional plants with no additional restrictions.

†Proposed: No Unique Regulations: Decrees under consideration for gene-edited crops that do not incorporate DNA from another species would no require unique regulations beyond current what is imposed on conventional breeding.

Figures taken from the Gene Literacy Project. *Available at: https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/*

Potential Uses of Gene Editing

Healthcare

Enhanced Research Models

Gene Editing allows scientists to create accurate animal models for disease research, allowing them to study pathological mechanisms, identify potential therapeutic targets, and understand patient responses to drugs. So far, CRISPR has shown multiple advantages in this area:

- The simple two component model of CRISPR-Cas9 has made the creation of GM animals much more efficient, decreasing the time and complexity previously associated with transgenic models where one or more genes have been modified.[26],[27]
- CRISPR has been used for in-vivo gene editing to target specific cells in target tissues, removing the need for germline modified mutant strains of mice.[28]
    - In one study, adeno-associated viral vectors (AVVs) containing Cas9 were used to exclusively target cardiomyocytes to produce a transgenic mouse model for hypertrophic cardiomyopathy.

[26] Shen BZhang JWu H, et al. *Generation of gene-modified mice via Cas9/RNA-mediated gene targeting*. Cell Res 2013;23:720–3.
[27] Shao YGuan YWang L, et al. *CRISPR/Cas-mediated genome editing in the rat via direct injection of one-cell embryos*. Nat Protoc 2014;9:2493–512.
[28] Carroll, K. J. et al. 2016. *A mouse model for adult cardiac-specific gene deletion with CRISPR/Cas9.* Proc. Natl Acad. Sci. USA 113, 338–343.

        - sgRNAs targeting Myh6 were introduced, causing cardiac-specific gene modification at the Myh6 locus, resulting in hypertrophic cardiomyopathy
- CRISPR-Cas9 has been used to create in-vitro and in-vivo organoid tumour models.
    - Modifications were made to tumour suppressor genes and oncogenes in in-vitro models.[29]
    - Scientists have used colonoscopy to introduce viral vectors containing CRISPR components into the distal colon of mice, creating in-vivo tumour organoids.[30]
- As Gene Editing can be performed in multiplex, precision models can be made
    - For example, multiple oncogenes/tumour suppressor genes can be targeted to make precision cancer models e.g. one group created a leukaemia model by targeting over 5 genes at once.[31]
- Neurological disorders are difficult to model in animals due to significant genetic differences and human-specific cell types, however, CRISPR has been used to create an accurate in-vitro model of Alzheimer's Disease.
    - In the study, Human induced pluripotent stem cells (iPSCs) were edited using CRISPR to create accurate model of the disease, with increases in multiple pathological markers, including amyloid-beta and phospho-tau.[32]

Gene Therapy

Traditionally, gene therapy faced issues regarding its lack of site specificity and its reliance on transgenes with viral vectors for delivery. Though CRISPR relies on these vectors for delivery, it offers a simpler and more reliable way of targeting specific sites, though not without drawbacks such as: off-target effects, the requirement for a PAM near to the target site, immunogenic toxicity and possible oncogenesis.[33]

- If these challenges can be overcome, CRISPR could be a key tool in gene therapy. With the first early-phase trials using CRISPR gene therapy underway, currently only in severe diseases, their outcomes are likely to dictate our future use of this technology

Somatic Gene Therapy trials are underway in a handful of conditions:

- HIV: In 2021, a Phase I/II trial was approved by the FDA to develop a CRISPR-based "vaccine" for HIV, whereby CRISPR is used to cut HIV proviral DNA from patient DNA.[34]
- Transthyretin Amyloidosis/ATR amyloidosis (TTA): TTA is a progressive fatal disease, characterised by the build-up of transtyrethrin (TTR) in tissues. In a landmark trial, administration of a CRISPR-based tool (NTLA-2001) was successful in editing TTR in hepatocytes and decreasing production of both wild-type and mutant TTR, with only mild adverse events.[35]
- Blood Disorders, such as Sickle Cell disease and Beta Thalassaemia are also being considered as potential targets for CRISPR-based gene therapy. In both diseases, work is underway to assess whether CRISPR may be used to increase the production of foetal haemoglobin in adults, which is not affected by the sickle cell /Beta thalassaemia mutation.[36]
    - This is being done using ex-vivo editing, whereby stem cells are harvested and edited in a lab, before being returned to the patient – reducing the chance of off-target effects and the long-term presence of CRISPR components in the body.
- Cancer Immunotherapy - CAR-T cells: In another example of ex-vivo gene editing, CRISPR is being explored as a potential way to improve CAR-T cell production.
    - CAR-T cells are human immune cells which have been genetically engineered to recognise cancer, causing the immune system to attack and kill cancerous cells.

[29] Matano, M. et al. 2015 *Modelling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids*. Nat. Med. 21, 256–262

[30] Roper, J. et al. 2018. *Colonoscopy-based colorectal cancer modelling in mice with CRISPR-Cas9 genome editing and organoid transplantation*. Nat. Protoc. 13, 217–234

[31] Heckl, D. et al. 2014. *Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing.* Nat. Biotechnol. 32, 941–946

[32] Israel, Mason A et al. 2012. *Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells.* Nature vol. 482,7384 216-20.

[33] Uddin, F., Rudin, C. M., Sen, T., 2020. *CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future*. Frontiers in Oncology. DOI: https://doi.org/10.3389/fonc.2020.01387

[34] Excision BioTherapeutics. September 2021. *Excision Receives FDA Clearance of IND for Phase 1/2 Trial of EBT-101 CRISPR-Based Therapeutic for Treatment of HIV.* Global News Wire. Available at: https://www.globenewswire.com/news-release/2021/09/15/2297456/0/en/Excision-Receives-FDA-Clearance-of-IND-for-Phase-1-2-Trial-of-EBT-101-CRISPR-Based-Therapeutic-for-Treatment-of-HIV.html

[35] Gillmore, J.D. et al. 2021. *CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis.* New England Journal of Medicine. DOI: 10.1056/NEJMoa2107454

[36] Frangoul, H. et al. 2020. *CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia*. New England Journal of Medicine. DOI: 10.1056/NEJMoa2031054

- CRISPR has the potential to edit T-cells in a much simpler way than the technologies that were used before and has the potential for fewer off-target effects.[37]

Productions of Organs for Transplantation

Currently, there is a shortage of human organs available for transplantation. As such, xenotransplantation (that is, transplanting an organ from one species to another) has been theorised as a solution, but this currently causes acute immune responses in the recipient, resulting in death.

- Scientists have therefore developed organisms edited with CRISPR, which are less likely to stimulate a strong immune response in the recipient. In 2021, the first CRISPR-edited pig-to-human heart transplant occurred in the US.[38]
  - Though the organ was not rejected due to a strong recipient immune response, the patient died shortly after the transplant due to other complications caused by the presence of a porcine cytomegalovirus in the organ.[39]
- There are currently no international legal regulations for this form of xenotransplantation, and many ethical arguments surround the issue[40].
  - There is likely to be strong religious and social arguments against their use.
  - Furthermore, the use of pigs to "grow" these organs raises a number of concerns. The animals will likely need to be kept in a sterile environment, potentially causing harm to their welfare by impairing their ability to experience the five freedoms recommended as the minimum for animal welfare standards[41]. Consideration of animal welfare at the point of organ harvest will also have to be taken into account.

Antimicrobial Resistance

AMR is a widespread global threat, which may require inventive solutions. CRISPR-Cas9 technology is being investigated as one of these solutions.[42] Scientists are working to use CRISPR to edit antibiotic resistance genes in bacteria, to increase their susceptibility to antibiotics and hence prevent AMR.

- This could be used to produce novel antimicrobial drugs, and work is being done to ensure these could be delivered without a viral vector, to reduce OTEs and immune reactions to the therapy.
- However, there are also concerns that CRISPR-Cas9 may **promote** AMR, by conferring resistance genes. A study by Norris et al[43], showed that Recombinetics had created polled cattle using CRISPR and unintentionally conferred genes for antibiotic resistance. This was not picked up by Recombinetics in their screening process, and as such, the Norris study called for more rigorous screening of GE organisms.

Agriculture

Climate Change, Food Security and Productivity

Climate change poses a major risk to global food security. In both nature and agriculture, organisms are having to contend with novel conditions that outpace their natural adaptation capabilities.

- In crops, global decline in yield, productivity and the nutritional value of food has been forecast by the IPCC due to extreme weather events, rising temperatures, and the increase in atmospheric CO_2.

[37] Roberts, R. CRISPR CAR-T cells: Edited T Cells Are Revolutionizing Cancer Treatment. Synthego Blog. Available at: https://www.synthego.com/blog/car-t-crispr-cancer#car-t-therapies-and-crispr-are-fighting-cancer-and-revolutionizing-medicine

[38] Kotz, D. 2021. *University of Maryland School of Medicine Faculty Scientists and Clinicians Perform Historic First Successful Transplant of Porcine Heart into Adult Human with End-Stage Heart Disease.* University of Maryland School of Medicine. Available at: https://www.medschool.umaryland.edu/news/2022/University-of-Maryland-School-of-Medicine-Faculty-Scientists-and-Clinicians-Perform-Historic-First-Successful-Transplant-of-Porcine-Heart-into-Adult-Human-with-End-Stage-Heart-Disease.html

[39] Regalado, A. 2022. *The gene-edited pig heart given to a dying patient was infected with a pig virus.* MIT Technology Review. Available at: https://www.technologyreview.com/2022/05/04/1051725/xenotransplant-patient-died-received-heart-infected-with-pig-virus/

[40] Ryczek, N., Hryhorowicz, M., Zeyland, J., Lipiński, D., & Słomski, R. 2021. *CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research*. International journal of molecular sciences, 22(6), 3196. https://doi.org/10.3390/ijms22063196

[41] RSPCA. *Animal Welfare Act.* Available at: https://www.rspca.org.uk/whatwedo/endcruelty/changingthelaw/whatwechanged/animalwelfareact

[42] Gholizadeh P, et al. 2020. *How CRISPR-Cas System Could Be Used to Combat Antimicrobial Resistance.* Infect Drug Resist. Apr 20;13:1111-1121. doi: https://doi.org/10.2147/IDR.S247271

[43] Norris, A.L., Lee, S.S., Greenlees, K.J. et al. *Template plasmid integration in germline genome-edited cattle*. Nat Biotechnol 38, 163–164 (2020). https://doi.org/10.1038/s41587-019-0394-6

- These issues are also likely to affect livestock. Heat stress can impact feed intake, reduce weight gain, decrease reproductive efficiency, increases mortality and may have other negative health effects. Disease vectors are also likely to increase in range, increasing the number of animals susceptible to vector-borne disease.
- Gene Editing may be able to help in a number of ways - to aid plants and animals adapt to climate change, to mitigate the effects of climate change on agriculture and to mitigate the effects of agriculture on the climate.
    - Such CRISPR edits that have so far been trialled in crops include: increasing drought tolerance, increasing salinity tolerance, changing nutritional composition, enabling production outside of normal growth latitudes, increasing yields and promoting disease resistance.
    - CRISPR edits can also be made to livestock to increase yields and improve thermotolerance.[44] Other suggested uses to improve farming efficiency have included offspring gender selection[45] and the creation of double muscled animals[46].
    - It has also been suggested that livestock could be edited to decrease their greenhouse gas emissions. A study of over 1000 dairy cattle demonstrated that genetics are likely to be a large factor in the determination of a cow's gut microbiome, and that certain microflora are responsible for increased methane production. Therefore, the authors suggest that cattle may be bred to select for a microbiome which produces fewer methane emissions – if the responsible genes are clearly identified, this may be done by CRISPR.[47]
- As food technology develops, there have been reports that some start-ups are looking to CRISPR technology to aid in their development of cultured meat, to improve the efficiency of cell culture and provide meat with a lower carbon cost than conventionally farmed produce[48].
- CRISPR editing may also produce a benefit for wild animals. Climate change is having disastrous effects on coral reefs, and by editing certain genes, we may be able to breed coral with increased heat tolerance, enabling greater resilience for reefs in the face of climate change[49].
    - However, there have been cases of GM fish[50,51] escaping and affecting native populations, and therefore careful thought should be considered to the safeguards surrounding GE animals.
    - CRISPR may prove to be effective in preventing escaped organisms from breeding with wild ones, one lab is already working on creating "sterile parent" GE salmon, who have been engineered to be sterile, a modification which can be temporarily reversed to allow breeding to occur on site.[52]

Herbicide Tolerance

The development of herbicide-resistant crops using CRISPR-Cas9 is already being explored in multiple crops[53], and it is likely that this will be the focus of large seed companies. In a recent study, Corteva held the 7th highest number of patent applications globally[54] – the most of any private company.

[44] Karavolias, N.G. et al. 2021. *Application of Gene Editing for Climate Change in Agriculture.* Frontiers in Sustainable Food Systems. DOI: https://doi.org/10.3389/fsufs.2021.685801

[45] University of California - Davis. 2020*. Meet Cosmo, a bull calf designed to produce more male offspring: Scientists use CRISPR technology to insert sex-determining gene*. ScienceDaily

[46] Gim, G-M. et al. 2021. *Production of MSTN-mutated cattle without exogenous gene integration using CRISPR-Cas9.* Biotechnology Journal: DOI: https://doi.org/10.1002/biot.202100198

[47] Wallace, J.R., et al. 2019. *A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Science Advances.* DOI: 10.1126/sciadv.aav8391

[48] Guedim, Z. 20. 019. *Bill Gates Backed Startup Uses CRISPR to Grow Meat*. Edgy. Available at: https://edgy.app/crispr-to-grow-meat

[49] Cleves, P.A. et al. 2020. *Reduced thermal tolerance in a coral carrying CRISPR-induced mutations in the gene for a heat-shock transcription factor*. PNAS. DOI: https://doi.org/10.1073/pnas.1920779117

[50] Magalhães A.L.B, Brito, M.F.G & Silva, L.G.M. 2022. *The fluorescent introduction has begun in the southern hemisphere: presence and life-history strategies of the transgenic zebrafish Danio rerio (Cypriniformes: Danionidae) in Brazil*, Studies on Neotropical Fauna and Environment. DOI: https://doi.org/10.1080/01650521.2021.2024054

[51] Bolstad, G.H. et al. 2021. *Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon.* Science Advances. DOI: https://doi.org/10.1126/sciadv.abj3397

[52] Abend, L. 2021. *A Sterile Solution: How Crispr Could Protect Wild Salmon.* Undark. Available at: https://undark.org/2021/07/21/crispr-protect-wild-salmon/

[53] Zhang, Y., Massel, K., Godwin, I.D. et al. 2018. *Applications and potential of genome editing in crop improvement.* Genome Biol 19, 210. https://doi.org/10.1186/s13059-018-1586-y

[54] IPStudies. *2020 CRISPR Patent Landscape – Where do we stand.* Available at: https://www.ipstudies.ch/2020/10/2020-crispr-patent-landscape-where-do-we-stand/

- This could provide benefits to farmers who use precision technology to apply herbicides to crops in a targeted way, however, the widespread use of herbicides is controversial and has proven to be harmful for certain organisms such as bees[55].

Food Safety

It has been proposed that GE can make our food more nutritious, and with potentially lower risk of negative health effects.

- Rothamsted have been permitted field trials to explore wheat with lower levels of asparagine – a chemical which is converted to carcinogenic acrylamide in bread baking[56].
- GE also holds potential to decrease allergens in food, with current research focusing on many allergens including peanuts, soy, wheat, chicken and milk.57

Animal Welfare

Both opponents and proponents of the use of gene editing in animals believe it could have a significant impact on animal welfare.

- Proponents believe that it may offer several opportunities to improve animal welfare. Some examples of this include:
    - Day-Old Chicks – Currently in the poultry industry, day-old male chicks are sexed and disposed of via either maceration or asphyxiation, as they are not commercially viable. Though the methods used are believed to be humane, avoiding the process completely by ensuring no male chicks are born would be more humane and more efficient for producers.
        - Gene editing may offer a manner in which to do this. A company called EggXYt have patented their CRISPR-editing method to identify male chicks on the first day of egg incubation, by inserting luciferase into the avian genome[58].
        - However, it's worth noting that day-old chicks are a vital source of feed in the zoo and exotics industry, which will need to be replaced should the industry adopt this technology.
    - Heat Tolerance – The first FDA-approved GE animals for human consumption were cattle edited to have a short, slick hair coat and therefore be more heat-tolerant.
        - Increased heat tolerance is likely to decrease heat stress, improving both animal welfare and productivity.[59]
    - Polled Cattle – Dehorning of cattle is a commonplace practice to reduce the likelihood of injury to farmers and animals, though the process is time-consuming, expensive and may cause pain and distress to the animal. As naturally polled cattle are rare in the dairy industry, gene editing may be able to provide an alternative to de-horning or narrowing the gene pool of dairy cattle. A recently published paper demonstrated the success of CRISPR introduction of a polled gene to a dairy calf.[60]
    - Disease Resistance – see next section
- Others argue that gene editing animals may harm their welfare, and careful safeguards should therefore be taken to ensure that this does not happen.
    - Many opponents of GE fear that that gene editing could be used to improve productivity in animals and increase their tolerance to lower-welfare farming practices e.g. animals edited for disease resistance may be kept at higher stocking density.[61]

[55] Battisti, L. et al. 2021. *Is glyphosate toxic to bees? A meta-analytical review*. Science of The Total Environment. Volume 767. DOI: https://doi.org/10.1016/j.scitotenv.2021.145397

[56] Morrison, O. 2021. '*News of this trial will likely be welcomed by the food industry': Europe's first CRISPR-edited wheat set to be grown.* Food Navigator. Available at: https://www.foodnavigator.com/Article/2021/09/03/News-of-this-trial-will-likely-be-welcomed-by-the-food-industry-Europe-s-first-CRISPR-edited-wheat-set-to-be-grown

[57] Brackett NF, Pomés A, Chapman MD. 2022. *New Frontiers: Precise Editing of Allergen Genes Using CRISPR*. Front Allergy. Jan 17;2:821107. DOI: https://doi.org/10.3389/falgy.2021.821107

[58] Offen, D. 2016. *Methods for gender determination of avian embryos in unhatched eggs and means thereof.* WO 2017/094015 A1. Available at: https://patentimages.storage.googleapis.com/3b/da/53/a9779670a7d446/WO2017094015A1.pdf

[59] FDA. 2022. *FDA Makes Low-Risk Determination for Marketing of Products from Genome-Edited Beef Cattle After Safety Review.* https://www.fda.gov/news-events/press-announcements/fda-makes-low-risk-determination-marketing-products-genome-edited-beef-cattle-after-safety-review

[60] Schuster, F. et al. 2020. *CRISPR/Cas12a mediated knock-in of the Polled Celtic variant to produce a polled genotype in dairy cattle.* Scientific Reports. https://doi.org/10.1038/s41598-020-70531-y

[61] Dalton, J. 2021. *Gene-editing plan 'dark day' for animal welfare and environment, say farming experts*. The Independent.

        - Animal welfare legislation is in place to protect welfare regardless of whether or not an animal has undergone gene editing - this issue is therefore broader than the GE debate.
    - Others believe that the increased speed of genetic changes, seen by some as an advantage of GE, has the ability to compromise animal welfare at an unprecedented rate.[62]
    - The effect of off-target mutations is hard to predict but may affect animal welfare. Previous GE animals have suffered from a range of unexpected mutations, including:
        - Extra thoracic vertebrae in pigs bred with a double mutation in a gene known as MSTN, which was edited to increase muscle growth[63]
        - Though not an off-target effect, CRISPR editing of MSTN in rabbits caused unintended consequences with enlarged tongues[64]
        - Early experiments into cattle edited for heat tolerance led to illness and death in experimental calves, due to common cloning complications – hydrops pregnancy and infection. Understanding and mitigating these complications is vital to ensure both welfare and commercial viability of the technique[65].

Disease Resistance

GE traits for disease resistance are likely to be a major benefit for both livestock and crop farmers.

- In livestock farming, this not only provides farmers with the potential for increased profitability, but also provides a tangible benefit to animal welfare. It may also hold environmental benefits by reducing the morbidity and mortality of livestock.
    - Examples of using GE to promote disease resistance in animals include Porcine Reproductive and Respiratory Syndrome[66] and Bovine TB[67].
    - In African Swine Fever scientists have edited the virus, rather than the animal, to reduce the impact of the disease.[68,69]
- Disease stress in crops poses a major risk to food security. Many studies have been undertaken to promote resistance to fungal, bacterial and viral diseases in crops such as wheat, maize, rice, barley, soy, tomatoes, potatoes, tobacco, brassicas and apples etc.[70]

[62] Veterinary Practice. 2022. *Does Gene Editing Compromise Animal Welfare? Available at: https://www.veterinary-practice.com/article/does-gene-editing-compromise-animal-welfare*

[63] Qian L. et al. 2015. *Targeted mutations in myostatin by zinc-finger nucleases result in double-muscled phenotype in Meishan pigs*. Sci. Rep. DOI: 10.1038/srep14435

[64] Lv, Q., et al. 2016. *Efficient Generation of Myostatin Gene Mutated Rabbit by CRISPR/Cas9.* Scientific reports. https://doi.org/10.1038/srep25029

[65] Laible, G., et al. 2021. *Holstein Friesian dairy cattle edited for diluted coat color as a potential adaptation to climate change.* BMC genomics. https://doi.org/10.1186/s12864-021-08175-z

[66] Burkard, C. et al. 2018. *Pigs Lacking the Scavenger Receptor Cysteine-Rich Domain 5 of CD163 Are Resistant to Porcine Reproductive and Respiratory Syndrome Virus 1 Infection*. Journal of Virology. DOI: https://doi.org/10.1128/JVI.00415-18

[67] Gao, Y., Wu, H., Wang, Y. et al. 2017. *Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects*. Genome Biol 18, 13. DOI: https://doi.org/10.1186/s13059-016-1144-4

[68] Abkallo, H.M. et al. 2021. *Rapid CRISPR/Cas9 Editing of Genotype IX African Swine Fever Virus Circulating in Eastern and Central Africa.* Frontiers in Genetics. DOI: https://doi.org/10.3389/fgene.2021.733674

[69] Hübner, A., Petersen, B., Keil, G.M. et al. 2018. *Efficient inhibition of African swine fever virus replication by CRISPR/Cas9 targeting of the viral p30 gene (CP204L).* Sci Rep 8, 1449. https://doi.org/10.1038/s41598-018-19626-1

[70] Schenke, D. and Cai, D. 2020. *Applications of CRISPR/Cas to Improve Crop Disease Resistance: Beyond Inactivation of Susceptibility Factors.* iScience 23,9. DOI: https://doi.org/10.1016/j.isci.2020.101478

Concerns with Gene Editing and CRISPR-Cas9:

Technical Concerns:

Failed Target Binding: Due to individual genetic variation, it is possible that the guide RNA sequence (which is created to be complementary to a DNA template) may not match recipient DNA, and will not bind to the target gene.

PAM requirement: Although, in theory, CRISPR sequences can be made complementary to any DNA sequence, the specificity of the Cas9 enzyme depends on the presence of the correct neighbouring PAM sequence. This therefore reduces the target site possibilities for the CRISPR-Cas9 complex. Furthermore, as short PAM sequences are more common than longer sequences, off-target mutations arise more frequently when dependent on a shorter PAM sequence. RNA-targeting Cas9 variants have been developed which also broaden the gene targeting spectrum by mitigating PAM requirement restrictions[71].

Oncogenesis: Research in human pluripotent stem cells (hPSCs) has shown that CRISPR often activates p53 (a common tumour suppressor gene) leading to apoptosis of rather than the intended genetic edit. Thus, edits are more likely to be successful in cells in which p53 is suppressed – creating a selection bias for oncogenic cell survival.

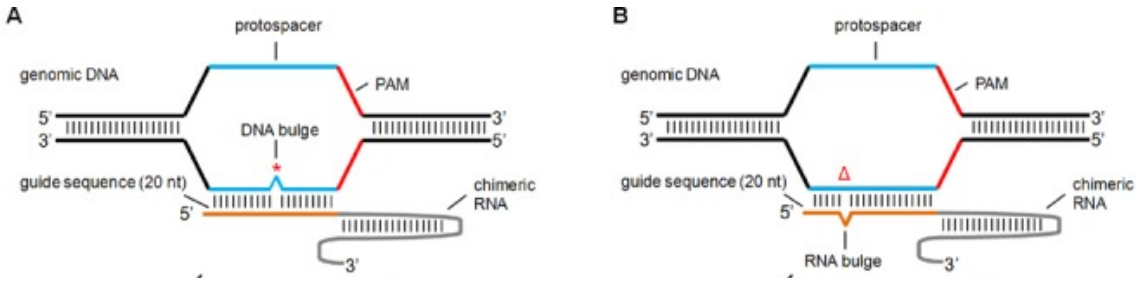
- RNA-targeting CRISPR-Cas9 has been suggested to avoid this issue, but this does not offer the opportunity to create a permanent edit into the hosts genome.[72]

Immunogenesis: The most widely used sources of Cas9 are Staphylococcus aureus and Streptococcus pyogenes – two bacterial species which commonly infect humans and animals. Most humans therefore harbour pre-existing immune responses specific to these bacteria and the Cas9 orthologs derived from them, raising concerns not only for potential inefficacy of the system, but also for potential immunogenic toxicity from CRISPR-Cas9 use.[73]

Vector Delivery: Vectors are used for in-vivo delivery of CRISPR-Cas9, due to their high efficiency in delivering the complex to an organism's DNA. However, issues surrounding their use, including immunogenicity, issues with integration, and off-target effects, such as carcinogenesis, indicate that more work is needed to ensure their safety.[74]

Off-Target Matches: CRISPR-Cas9 complexes do not always bind to their target site and off-target mutations are common - studies have shown that >50% of induced mutations in GE organisms were not intended[75,76].Tolerance mechanisms potentially result in thousands of potential binding sites for the CRISPR-Cas9 complex. There are two mechanisms for this tolerance:

- Base-mismatch tolerance: target sequence binding can tolerate mismatches up to several base pairs[9,77]
- Bulge mismatch: genomic sites can be cleaved even when DNA sequences contain smaller sequences of mismatching DNA ('DNA bulge') or deletions of it ('RNA bulge') compared to the RNA guide strand.[78]



Adapted from: www.researchgate.net/figure/Schematic-of-CRISPR-Cas9-off-target-sites-with-A-1-bp-insertion-DNA-bulge-or-B-1-bp_fig1_262421062z

[71] Ryczek, N., Hryhorowicz, M., Zeyland, J., Lipiński, D., & Słomski, R. (2021). *CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research. International journal of molecular sciences.*. https://doi.org/10.3390/ijms22063196

[72] Uddin, F., Rudin, C. M., Sen, T., 2020. *CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future*. Frontiers in Oncology. DOI: https://doi.org/10.3389/fonc.2020.01387

[73] Charlesworth, C. T., et al. 2019. *Identification of preexisting adaptive immunity to Cas9 proteins in humans*. Nature medicine. https://doi.org/10.1038/s41591-018-0326-x

[74] Mengstie, M. 2022. *Viral Vectors for the in Vivo Delivery of CRISPR Components: Advances and Challenges.* Frontiers In Bioengineering and Biotechnology. DOI: https://doi.org/10.3389/fbioe.2022.895713

[75] Fu Y, et al. 2013. *High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells.* Nature Biotechnology.

[76] Lin Y, et al. 2014. *CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences.* Nucleic Acids Research. 42 (11): 7473–85.

[77] Hsu, P. D., Lander, E. S., & Zhang, F. 2014. *Development and applications of CRISPR-Cas9 for genome engineering*. Cell, *157*(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

[78] Lin, Y. et al. 2014. *CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences.* Nucleic acids research, 42(11), 7473–7485. https://doi.org/10.1093/nar/gku402

- The consequence of OTEs may be severe. OTEs may interrupt genomic stability, disrupt the function of "normal" genes, increase the likelihood of tumours, or have other unintended consequences. Like on-target effects for germline-edited organisms, off-target effects are heritable.[79] We do not understand the impact these effects may have on subsequent generations.
    - Sensitive and comprehensive detection of off-target mutations remains a challenge but will be vital to ensure the welfare of animals undergoing gene editing, as well as the safety of edited crops/genetic therapies.
- Not only do these off-target effects present safety concerns, but they can undermine research by increasing the number of confounding variables, causing misleading or non-reproducible results.
    - Gene editing is still in its infancy, and work is needed to improve the precision and predictability of the technology. Though there is work being done to understand off-target binding[80,81] – why it occurs, how to detect it, and how to reduce it - it is still commonplace.
- It is worth noting that recent research has shown that off-target mutagenesis in **some** experiments *"is not statistically distinguishable from the background rate of de novo mutations occurring due to other processes"* – showing promise for the technology moving forwards[82].

Unpredicted effects at target site: We must fully understand the role of a gene before we alter it. A modification to decrease an animal's susceptibility to one disease may increase its susceptibility to another or cause other unpredictable effects. This can be ironed out easily with appropriate research but should be an important consideration. Further to these unintended effects at target, there can be technical issues with CRISPR at the target site, outlined below:

- **Template Plasmid Integration:** Various publications have demonstrated unintended template plasmid integration in CRISPR/Cas9 GE. A paper by Dickinson et al[83] demonstrated integration of a second copy of the template at the target site, whilst work involving double-stranded DNA repair templates in fish[84] and mice[85] showed that templates may form multimers which integrate at the target site.
    - Two FDA scientists who published a paper on these integration issues in various methods of GE, including TALENs and CRISPR, believe that these errors are *"under reported or overlooked".*[86]

- **Chromosome Removal:** A concern associated with CRISPR-Cas9 germline editing is that DSBs may not repair prior to mitosis leading to subsequent chromosome loss.
    - Studies[87,88] have found that this to be a common occurrence, posing a significant challenge to this area of research.

DNA Repair Unpredictability: The choice of DNA repair pathway is not yet fully understood. With the aforementioned risks associated with NHEJ, it is important that this is understood in greater depth before CRISPR-Cas9 can be used reliably.

[79] Höijer, I. et al. 2022. *CRISPR-Cas9 induces large structural variants at on-target and off-target sites in vivo that segregate across generations*. Nat Commun 13, 627 https://doi.org/10.1038/s41467-022-28244-5
[80] Modrzejewski, D et al. 2020. *Which Factors Affect the Occurrence of Off-Target Effects Caused by the Use of CRISPR/Cas: A Systematic Review in Plants.* Front. Plant Sci. https://doi.org/10.3389/fpls.2020.574959
[81] Naeem, M., Majeed, S., Hoque, M. Z., & Ahmad, I. 2020. *Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing.* Cells, 9(7), 1608. https://doi.org/10.3390/cells9071608
[82] Iyer, V. et al. 2018. *No unexpected CRISPR-Cas9 off-target activity revealed by trio sequencing of gene-edited mice.* PLOS Genetics. DOI: https://doi.org/10.1371/journal.pgen.1007503
[83] Dickinson, D.J., Ward, J.D., Reiner, D.J. & Goldstein, B. 2013. *Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination*. Nat Methods. DOI: https://doi.org/10.1038/nmeth.2641
[84] Gutierrez-Triana, J.A. et al. 2018. *Efficient single-copy HDR by 5' modified long dsDNA donors.* Elife. DOI: https://doi.org/10.7554/eLife.39468
[85] Skryabin, B.V., Gubar, L., Seeger, B., et al. 2020. *Pervasive head-to-tail insertions of DNA templates mask desired CRISPR/Cas9-mediated genome editing events.* Science Advances. DOI: https://doi.org/10.1126/sciadv.aax294
[86] Norris, A.L., Lee, S.S., Greenlees, K.J. et al. 2020. *Template plasmid integration in germline genome-edited cattle*. Nat Biotechnology. https://doi.org/10.1038/s41587-019-0394-6
[87] Zuccaro, M.V . et al. 2020. *Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos. Cell.* DOI: https://doi.org/10.1016/j.cell.2020.10.025
[88] Alanis-Lobato, G., Zohren, J., McCarthy, A. and Niakan, K. 2021. *Frequent loss of heterozygosity in CRISPR-Cas9–edited early human embryos.* PNAS. DOI: https://doi.org/10.1073/pnas.2004832117

- CRISPR systems which induce base edits, rather than DSBs may help to avoid these issues, however, these are still prone to other issues such as off-target effects and are difficult to deliver via viral vectors due to their large size[89].

Other Concerns

Regulation: Adequate and appropriate regulation is vital to good GE governance. Though the Precision Breeding (Genetic Technology) Bill outlines the regulatory processes, there are concerns regarding the amount of regulation which has been left to secondary legislation and negative instrumentation in the Bill.

GE Patenting: Patenting increases the likelihood of a new technology generating profit for the developer, and therefore can incentivise investment and progress. However, concerns have been expressed regarding the patenting of GE organisms.

- Many believe that permitting patents will be beneficial to a few, very large companies, whilst smaller businesses and farmers may suffer.
    - The EU acknowledged this in their report on NGTs, reporting that many stakeholders have expressed concern regarding *"the high cost of patenting innovations and the high patent licence fees as a barrier to market entry for SMEs".*
        - This has already happened following patenting of GMO products, and many believe it will only worsen with GE organisms.
        - Concerns were also raised in the report about patents driving increases in price - increases not relative to the cost of development, but as a direct result of exclusivity of rights.
    - Some state that these patent monopolies have further worsened declining crop diversity - the FAO estimated a 75% loss of crop diversity between 1900 and 2000[90].
- To obtain a patent, the technology you produce must be novel. This may be hard to prove if the law only permits changes which "could have occurred naturally", as it is possible that there may be an organism somewhere with the desired trait.
- Patent Stacking also presents an issue. If patented GE organisms are edited further by other companies, who also file for patents, it could lead to a highly complex system for everyone involved, and a vast amount of royalties for the farmer to pay.[91]

Markers, Traceability and Labelling: Many farmers and consumers have raised concerns regarding the traceability of GE organisms. Currently, GE organisms are not identifiable by any method as the CRISPR system does not leave a marker behind. Therefore, there is an unmet need to ensure the traceability of these organisms and products, not only for consumer and farmer preference, but also to ensure monitoring of their safety and any adverse effects occurring as a result of GE.

- There is no current requirement for GE organisms to be labelled as such. This has not only raised traceability and safety concerns, but places limitations on consumer choice, and puts organic farmers in danger of breeching their certification requirements.

[89] Rees HA, Liu DR. 2018. B*ase editing: precision chemistry on the genome and transcriptome of living cells*. Nat Rev Genet. DOI: 10.1038/s41576-018-0059-1
[90] FAO. Crop Diversity – Use it or Lose it. Available at: https://www.fao.org/news/story/en/item/46803
[91] Plüss, J. and Toruban, P. 2022. *Genome editing's patent problem fuels concern for the future of food.* Swiss Info. Available at: https://www.swissinfo.ch/eng/genome-editing-s-patent-problem-fuels-concern-for-the-future-of-food/47287668

Gene Drive Technology

Perhaps the most controversial potential use for Gene Editing is Gene Drive Technology. The purpose of a Gene Drive is to edit the frequency of a desired allele in a whole population. The edit increases the likelihood of inheritance for a certain allele over others, enabling it to spread rapidly through the generations of a given species *even if* it poses a disadvantage to the affected organism. Though the most heavily researched areas of application are the control of vector borne diseases and invasive species, other speculative applications of the technology can be seen in the figure below, taken from The Royal Society[92].

To have a meaningful effect on the target population, some Gene Drives require the release of a large number of edited individuals (high threshold), whilst others only require the release of a very small number of individuals (low threshold).[93] However, mathematical modelling suggests that even the least effective drive systems are likely to be "highly invasive" in a population[94], and should therefore be fully understood before their release.

Currently, this technology is in its infancy, and therefore many experts have concerns about the unintended consequences that it may bring. Natural Scientists working in the field have already stated that it is difficult to create stable Gene Drives which are expressed at the right time, in the right place and at the right level, without excessive cost to the organism or resistance occurring. Other concerns lie in the fact that once release, their spread will be uncontrollable, irreversible and may even spread to other non-target species, as it is established that genes can spread between species[95].

A survey of natural scientists, social scientists, policy makers, philosophers, ethicists, and NGO workers found that, no matter their background or views on GDT, all interviewees shared concerns on the knowledge gaps in:

- The efficacy and hazards of GDTs in laboratory and cage experiments
- The translation of lab results to the field
- Population dynamics and sizes of natural populations in which GDTs may be used
- The roles of these populations in their ecosystems

Others believe that there has been too much promised by GDT, which both harms the technology (when results are not as expected) and does not allow for a fair comparison to alternatives. As these technologies progress, there will no doubt be considerable debate regarding their use and the relevant ethical considerations, such as how we balance

[92] The Royal Society. 2018. *Gene Drive: Why it matters.* Page 5. Available at: https://royalsociety.org/~/media/policy/Publications/2018/08-11-18-gene-drive-statement.pdf
[93] Bier, E. 2022. *Gene Drives Gaining Speed.* Nature Reviews Genetics. 23, pages 5–22. DOI: https://doi.org/10.1038/s41576-021-00386-0
[94] Noble, C. et al. 2018. *Current CRISPR gene drive systems are likely to be highly invasive in wild populations.* eLife. DOI: https://doi.org/10.7554/eLife.33423
[95] Graham, L. A. and Davies, P. L. 2021. Horizontal Gene Transfer in Vertebrates: A Fishy Tale. Trends in Genetics. Available at: https://pubmed.ncbi.nlm.nih.gov/33714557/

the value and interests of humans, non-human animals, and the environment, and what the role is of humans in nature. [96]

Proposed uses of Gene Drive Technology:

Use in Disease Vectors/Reservoir populations.

- Malaria Control: Multiple research groups have been working on Gene Drives in mosquitos, to prevent the spread of malaria. Different approaches include:
    - Population Control – by producing a Gene Drive resulting in infertility, researchers hope to control the population of mosquitos to prevent the spread of malaria, as well as other vector-borne diseases. However, this is likely to have consequences on the local ecosystem.
    - Anti-parasitic genes – researchers are developing Gene Drives to make mosquitos immune to the malaria parasite, and therefore unable to spread it. Though this won't help to control other mosquito-borne diseases, it may minimize any effects on the local ecosystem.[97]
    - One study found minimal OTEs in mosquitoes edited to be resistant to malaria, highlighting the possibility that these organisms may be suitable for release in the future.[98]
- Dengue: There are also attempts being made to engineer mosquitoes to control dengue. One group were able to develop mosquitoes which *"have significantly reduced viral infection, dissemination, and transmission rates for all four major antigenically distinct DENV serotypes*"[99] – highlighting the potential for GE application in this field.
- Schistosomiasis Control: Researchers are also trying to control schistosomiasis, a disease which affects millions of humans and non-human animals.
    - This may be done by implementing a gene drive in the parasite's intermediate host – the snail – to either reduce the population, or to prevent their susceptibility to schistome infection[100]

Use for Pest Control

New Zealand have expressed a desire to explore Gene Drive Technologies as a means to control invasive species. This may offer an advantage for animal welfare, as the edits made would cause further generations to become infertile, creating a population crash and replacing the need for painful traps and poisons. However, this approach is likely to have global repercussions. If these animals spread to countries in which they are native, not invasive, they may devastate local populations with knock-on effects for entire ecosystems.

- For example, Gene Drives could be used to control rat populations in New Zealand. Edited individuals may stow away on their own, as they have done in the past, on boats and planes but it is also possible that someone may relocate these animals deliberately to try and mitigate the economic damage of rats in another country. In the past, Rabbit Haemorrhagic Disease virus was secretly introduced to New Zealand in an attempt to prevent losses of up to USD $50million per year. Rats create far greater losses - in the US alone these are estimated at USD $19 billion per year. The motivation for such smuggling would therefore be high, but such an event would pose a great threat to ecosystems globally, and could devastate public trust in Gene Drives, preventing their more beneficial applications from being brought to life – such as in the control of VBDs[101].
- Gene Drive Technology has also been suggested in both increasing and decreasing insecticide resistance in insects. There are calls to promote insecticide resistance in pollinators or wild plants in efforts to increase biodiversity and ecosystem health, or to remove resistance genes from agricultural pests[102,103]. Opponents argue that using Gene Drives may greatly increase the use of insecticides in agriculture, with consequences on other, non-edited species.

[96] de Graeff, N., Jongsma, K.R. and Bredenoord, A.L. 2021. *Experts' moral views on gene drive technologies: a qualitative interview study*. BMC Med Ethics. https://doi.org/10.1186/s12910-021-00588-5

[97] Bier, E. 2022. *Gene drives gaining speed.* Nature Reviews Genetics 23, 5–22. https://doi.org/10.1038/s41576-021-00386-0

[98] William T. Garrood, W. T . Kranjc, N. Petri, K., Kim, D.Y. and Simoni, A. 2021. *Analysis of off-target effects in CRISPR-based gene drives in the human malaria mosquito.* PNAS. DOI: https://doi.org/10.1073/pnas.2004838117

[99] Buchman A, Gamez S, Li M, Antoshechkin I, Li HH, et al. 2020. *Broad dengue neutralization in mosquitoes expressing an engineered antibody.* PLOS Pathogens 16(4). DOI: https://doi.org/10.1371/journal.ppat.1008545

[100] Maier, T., et al. 2019. *Gene drives for schistosomiasis transmission control.* PLOS Neglected Tropical Diseases. https://doi.org/10.1371/journal.pntd.0007833

[101] Esvelt, K. and Gemmell, N. 2017. *Conservation demands safe gene drive.* PLOS Biology. DOI: https://doi.org/10.1371/journal.pbio.2003850

[102] Medina, R.F. 2016. *Gene Drives and the Management of Agricultural Pests. Journal of Responsible Innovation.* https://doi.org/10.1080/23299460.2017.1407913

[103] Kaduskar, B., Kushwah, R.B.S., Auradkar, A. et al. 2022. *Reversing insecticide resistance with allelic-drive in Drosophila melanogaster.* Nat Commun 13, 291 (2022). https://doi.org/10.1038/s41467-021-27654-1

Use in Pathogens

Researchers have worked on Gene Drives in *Candida albicans*, preventing biofilm formation and impairing pathogenesis. They also suggested that their work may be expanded to other fungal species, such a C. auris, to reduce multi-drug resistance.[104]

Using CRISPR-Cas9 to combat AMR has been suggested in other pathogens, though it remains in preliminary research at present. Currently, the technology faces challenges including CRISPR delivery to the pathogen and resistance to CRISPR-Cas9 antimicrobials. However, as bacteria often lack the mechanism to undergo NHEJ, targeted efficient bacterial killing via gene editing has been demonstrated in a number of clinically relevant AMR species e.g. *Clostridium species, E. coli,* and *Pseudomonas aeruginosa,* but no work has yet been done in these species targeting genes responsible for AMR.[105]

[104] Shapiro, R. S., et al. 2018. *A CRISPR-Cas9-based gene drive platform for genetic interaction analysis in Candida albicans.* Nature microbiology, 3(1), 73–82. https://doi.org/10.1038/s41564-017-0043-0

[105] Duan, C. et al. 2021. *Harnessing the CRISPR-Cas Systems to Combat Antimicrobial Resistance.* Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2021.716064

Key Reports and Consultations

European Commission

In 2021, the European Commission published a study on the status of "New Genomic Techniques" – those which are used *"to change the genetic material of an organism and that have emerged or have been developed since 2001, when the existing GMO legislation was adopted".* Their findings highlighted that:

- There are many possible benefits from these technologies, including their ability to contribute to food sustainability goals, such as the European Green Deal and the Farm to Fork Strategy.
    - However, concerns were raised about the products of these technologies, with regard to their environmental impact, safety, labelling, and their fit with organic/GM-free agriculture.
- The report acknowledged that both use of, and lack of use of, these technologies raise ethical concerns - most of which are related to how the techniques are used, rather than the techniques themselves.
- There was variation in the views on NGT safety and the need/requirements for risk assessment, but most agreed that this should be reviewed on an individual basis.
    - There were concerns that any regulations imposed would have a disproportionate effect on smaller enterprises seeking to gain market access.
    - Though some technologies may have similar safety profiles to conventional breeding, the study warned that "*indicating that all genomic alterations or allelic combinations generated by CRISPR/Cas9 generally are identical to naturally occurring variations is a misleading oversimplification*".
- The study acknowledged both harms and benefits of patent use in this area. Whilst patents can help to incentivise innovation, they may also cause a barrier to market for smaller businesses and limit access to new technology for others e.g. breeders and farmers.

Regardless of concerns/benefits, the study concluded that the 2001 GMO legislation is *"not fit for purpose for some NGTs and their products… it needs adaptation to scientific and technological progress."* The EC has since completed a survey and consultation on the subject, with results due to be published soon. These are likely to help guide future EC decisions regarding regulation of PBOs.

Nuffield Council on Bioethics[106]

In December 2021, the Nuffield Council on Bioethics published their report into GE in farmed animals.

- The report recognized that GE could bring real benefits to farming, but raised concerns that it may be used to accelerate poor breeding practices.
    - Many of the recommendations in the report summary were non-specific to GE, such as requests for responsible breeding standards, a traffic-light system to monitor and report on both breeding and husbandry, and suggestions for clearer food labelling detailing both GE status and the husbandry practices used for that animal.
    - The Nuffield Council called for strong regulation to guide the development of GE.
- The report called for strong public dialog on the matter, and as such, the Council ran a dialog in early 2022. The results were published in October 2022[107], reporting:
    - In general, the responses were accepting of GE technology – more so for bringing a benefit for the farmed animal, rather than for human consumers
    - For environmental benefits, there was belief that the system should be changed, where possible, rather than manipulating the animal
    - All participants believed that any regulations *"should be used to promote the public good and not just to protect them from harm"*
    - Though GE was perceived as innovative, concerns were raised that it would be used for unsustainable short-term objectives rather than addressing deeper problems in the food system.

DEFRA – Government response to Consultation on Genetic Technologies[108]

[106] Dupré, J. et al. 2021. *Genome editing and farmed animal breeding: social and ethical issues.* Nuffield Council on Bioethics. Available at: https://www.nuffieldbioethics.org/publications/genome-editing-and-farmed-animals

[107] Nuffield Council on Bioethics. 2022. *Public dialogue on genome editing and farmed animals.* Available at: https://www.nuffieldbioethics.org/assets/images/Nuffield-BBSRC-Sciencewise-dialogue-topic-guides-Oct2022.pdf

[108] Defra. 2021. *Government response to gene editing consultation*. Available at: https://www.gov.uk/government/consultations/genetic-technologies-regulation

Ipsos Mori Market Research

As part of their consultation, DEFRA conducted market research into public awareness and attitudes toward GE and GM.

- They found that consumers currently have low awareness and knowledge of GE food – most had not heard the term "genome edited" and many who had confused it with GM food, even if they reported themselves to have a good understanding of what GE is.
- Consumers preferred GE to GM as it was perceived to be safer and more "natural" – a term which often went hand-in-hand with acceptance throughout the interviews. Some felt GE was "unnatural" and closer to GM than conventional breeding.
    - Acceptability of GE was higher in plants than animals.
    - Consumer acceptance of GE increased when consumers felt better informed.
- Consumers concerns included safety risks to humans, corporate interests, and animal welfare/intensive farming.
    - To have the freedom of choice, consumers want transparent labelling (to include the full term "Genome Edited" on any foods containing GE ingredients), and further reassurance via thorough regulation and safety assessments.
    - Consumers had concerns that large corporations may prioritise profit might over benefits for consumers, animals and the environment unless regulated carefully.

ACRE Response

Defra's Advisory Committee for Release into the Environment published their own response to the consultation, stating that GE organisms should have no greater environmental risk than conventionally bred organisms as a result of how they are produced, provided "*gene editing introduces genetic alterations and combinations that are of the type that are selected for in traditional breeding*".

ACRE acknowledged the existence of OTEs and called for strong, scientifically led regulation and the need for continued research to prevent the negative effects of this.

The British Veterinary Association – Response to DEFRA regulation of genetic technologies

The BVA published their response to DEFRAs call for evidence in March 2021. They believe that GE organisms should remain classified as GMOs, due to the complexity of the area and the lack of clarity in the terms used by DEFRA as part of their consultation. They acknowledged the "important role" of GE and similar technologies in food production but believe that they require thorough and transparent monitoring, which GMO legislation already provides.[109]

The Royal Society

In response to the announcement of a genetic technology Bill in the Queen's Speech in 2022, the Royal Society responded in support of such a Bill. In their statement, they stated these technologies *"can help address the environmental and societal challenges faced by 21st century agriculture"* and help the UK to achieve net-zero. With regard to the regulation of GE organisms, they stated their belief that these should be outcomes-based to ensure safety, welfare and environmental impact are considered fairly, and to ensure legislation is future-proof.

The National Academy of Sciences

Food Standards Agency (FSA)

As a result of the new legislation, the FSA have developed a new framework intended to regulate the use of PBOs in food and feed in England. It provides a case-by-case risk assessment and it will ensure that all food safety risks are assessed, managed and communicated to Ministers to inform policy making decisions. PBOs will only be authorised if they are deemed to:

- Not risk human health
- Not mislead customers
- Not have a lower nutritional value than traditionally bred counterparts

They launched a public consultation regarding the framework proposals and expect feedback by early January 2024.

[109] British Veterinary Association. 2021. *Response to DEFRA on the regulation of genetic technologies.* Available at: https://www.bva.co.uk/media/4048/response-to-defra-on-the-regulation-of-genetic-technologies.pdf