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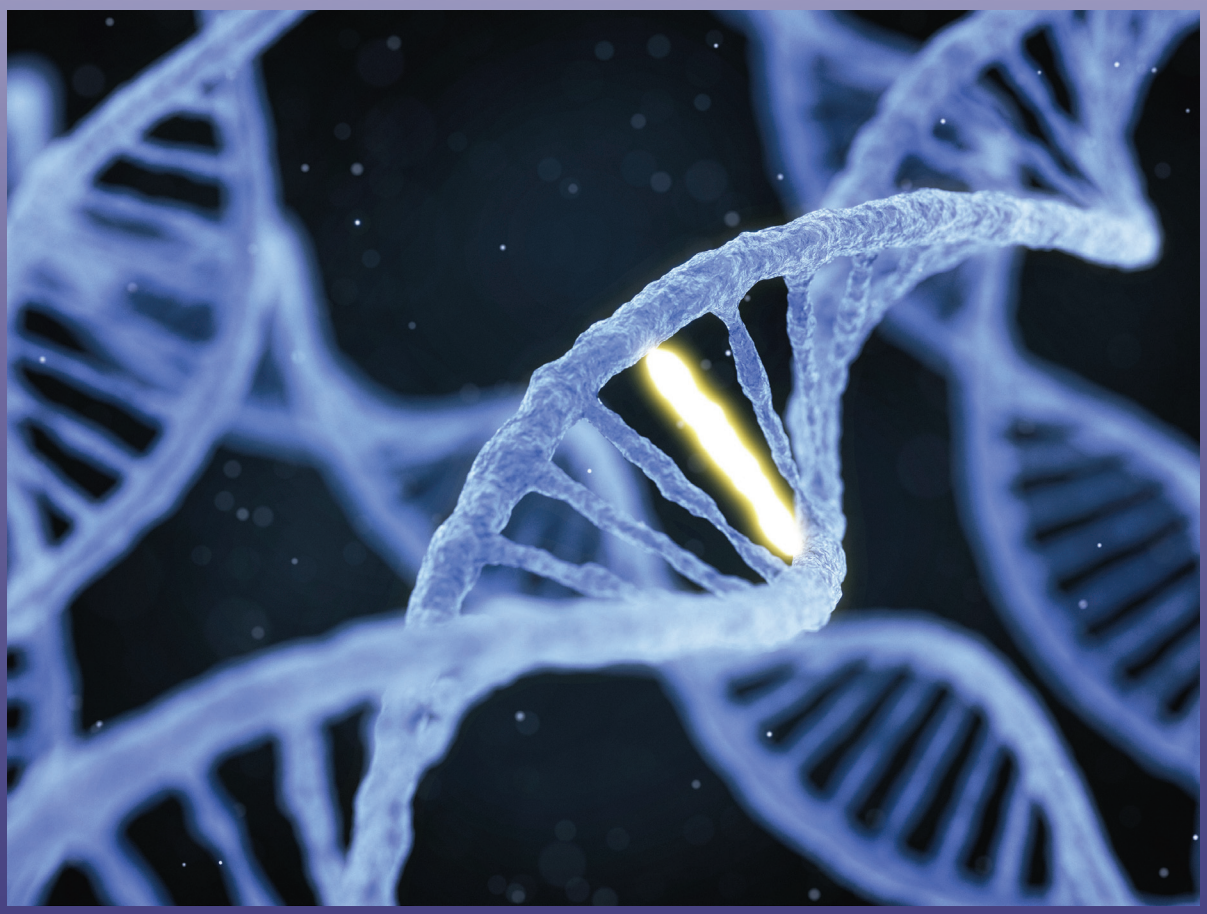
Genome editing: scientific opportunities, public interests and policy options in the European Union



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Science Advice for the Benefit of Europe

EASAC

EASAC – the European Academies' Science Advisory Council – is formed by the national science academies of the EU Member States to enable them to collaborate with each other in giving advice to European policy-makers. It thus provides a means for the collective voice of European science to be heard. EASAC was founded in 2001 at the Royal Swedish Academy of Sciences.

Its mission reflects the view of academies that science is central to many aspects of modern life and that an appreciation of the scientific dimension is a pre-requisite to wise policy-making. This view already underpins the work of many academies at national level. With the growing importance of the European Union as an arena for policy, academies recognise that the scope of their advisory functions needs to extend beyond the national to cover also the European level. Here it is often the case that a trans-European grouping can be more effective than a body from a single country. The academies of Europe have therefore formed EASAC so that they can speak with a common voice with the goal of building science into policy at EU level.

Through EASAC, the academies work together to provide independent, expert, evidence-based advice about the scientific aspects of public policy to those who make or influence policy within the European institutions. Drawing on the memberships and networks of the academies, EASAC accesses the best of European science in carrying out its work. Its views are vigorously independent of commercial or political bias, and it is open and transparent in its processes. EASAC aims to deliver advice that is comprehensible, relevant and timely.

EASAC covers all scientific and technical disciplines, and its experts are drawn from all the countries of the European Union. It is funded by the member academies and by contracts with interested bodies. The expert members of EASAC's working groups give their time free of charge. EASAC has no commercial or business sponsors.

EASAC's activities include substantive studies of the scientific aspects of policy issues, reviews and advice about specific policy documents, workshops aimed at identifying current scientific thinking about major policy issues or at briefing policy-makers, and short, timely statements on topical subjects.

The EASAC Council has 29 individual members – highly experienced scientists nominated one each by the national science academies of EU Member States, by the Academia Europaea and by ALLEA. The national science academies of Norway and Switzerland are also represented. The Council is supported by a professional Secretariat based at the Leopoldina, the German National Academy of Sciences, in Halle (Saale) and by a Brussels Office at the Royal Academies for Science and the Arts of Belgium. The Council agrees the initiation of projects, appoints members of working groups, reviews drafts and approves reports for publication.

To find out more about EASAC, visit the website – www.easac.eu – or contact the EASAC Secretariat at secretariat@easac.eu

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EASAC Secretariat
Deutsche Akademie der Naturforscher Leopoldina
German National Academy of Sciences
Jägerberg 1
D-06108 Halle (Saale)
Germany
tel: +49 (0)345 4723 9833
fax: +49 (0)345 4723 9839
email: secretariat@easac.eu
web: www.easac.eu

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Foreword

In many of the areas in which EASAC, the European Academies' Science Advisory Council, works, where a large and solid body of knowledge is needed to inform the action of our societies, it is important to recognise that there is an intimate mix of science and values involved in discussion. Such discussions are most fruitful when both knowledge and values are well identified. This report presents a broad synthesis of genome editing, one of the newer aspects of the biosciences. It is our hope that presenting clearly the science involved – the duty of academies – will serve the ongoing discussions within society that the report recommends be vigorously pursued.

Genome editing refers to the intentional modification of a targeted DNA sequence in a cell which, by greatly improving our understanding of biological functions, is beginning to revolutionise research.

This powerful new tool has significant potential for application in a wide range of sectors in pursuit of various societal priorities in human and animal health, food and agriculture, the modification of populations in the wild (in particular insect disease vectors) and microbial biotechnology and the bioeconomy. However, alongside the prospective benefits of the technology, safety, ethical and other issues have been raised that need to be explored, and regulatory questions posed that need to be addressed.

It is the purpose of this report from EASAC to take a broad perspective on the research advances and their potential applicability in different sectors to raise awareness of the opportunities and challenges, and to advise on the options to ensure an appropriate framework for managing innovation. It is our view that policy considerations should primarily concentrate on sector-specific product regulation and not on the general principles and practices of genome editing as a technology.

Our work covering the wide range of potential applications builds on previous activity by some of our EASAC member academies and on the ongoing work by our academy colleagues in FEAM, the Federation of European Academies of Medicine, who have focused on genome editing of human cells. Broadly, genome editing is a fast-moving area, not just in research and development but also in terms of the engagement between the scientific and policy communities.

Our report concentrates on recommendations for Europe, but the issues are of great global interest. For example, after our report drafting was complete, the US National Academies of Sciences, Engineering, and Medicine published their final report on the science, ethics and governance of human genome editing[1]. These very recent US recommendations on basic laboratory research and human somatic genome editing are substantially similar to the interim conclusions from the international summit that are discussed in our report. However, the latest US conclusions about human germline (heritable) genome editing extend the potential scope in that they note the possibility of identifying circumstances in which clinical research trials would be permissible for germline genome editing. These circumstances are posited to include a compelling clinical purpose and stringent oversight system. Such recommendations are controversial[2], not least in some of our European Union (EU) Member States, and will require considerable further public engagement by the scientific and medical communities to debate issues and perspectives.

It is not only human genome editing that attracts controversy. Recently, the EU Scientific Advice Mechanism – the newly constituted process to provide the European Commission with high-quality, timely and independent scientific advice on specific policy issues – has started an inquiry[3] on 'New techniques in agricultural biotechnology', and we welcomed the opportunity to contribute our pre-publication findings to this initiative.

This report has been prepared by consultation with a group of experts nominated by our member academies. I thank them and their chairman, Professor Volker ter Meulen, and the EASAC Biosciences Programme Director, Dr Robin Fears, for their expertise, insight and enthusiasm in assessing a wide range of issues and in achieving consensus in the conclusions and recommendations. I also thank our colleagues in FEAM, our independent peer reviewers, our EASAC Biosciences Steering Panel for their guidance, and EASAC council members and their academies for continuing assistance in communicating our messages at the national level as well as to EU institutions.

We believe that our findings are relevant to a wide spectrum of EU and national policy-making. EASAC

[1] The National Academies of Sciences, Engineering, and Medicine 'Human Genome Editing: Science, Ethics and Governance', https://www.nap-edu/download/24623#.
[2] For example, www.sciencemediacentre.org/expert-reaction-to-the-publication-of-new-report-on-gene-editing.
[3] http://ec.europa.eu/research/sam/index.cfm?pg=agribiotechnology.

stands ready to continue contributing to the active debates on contentious points for research and innovation, and on other relevant matters, for example the global implications for biosecurity. Because genome editing is a fast-moving area in many respects, we will be willing to return to our exploration of the topics in this report in due course. To inform our further thinking, we now welcome discussion of any of the points that are raised in our report, or indeed any others that require attention.

Thierry J-L Courvoisier
EASAC President

Summary

Genome editing, the deliberate alteration of a selected DNA sequence in a cell, using site-specific DNA nuclease enzymes, has become a very important tool in basic research. Genome editing has been described by some as a transformative technology and, certainly, in some areas of research and innovation, it is transforming expectations and ambitions. Genome editing can specifically modify individual nucleotides in the genome of living cells and, together with a growing ability to monitor and reduce off-target effects, it brings new opportunities within range. Because of its general applicability (in microbes, and plant, animal and human cells) it has a very wide range of potential uses in tackling societal objectives. These potential applications include, but are not limited to, gene- and cell-based therapies to control diseases and, in reproduction, approaches to avoid the inheritance of disease traits; the control of vector-borne diseases; improved crop and livestock breeding, including improved animal welfare; modification of animal donors for xenotransplantation; and industrial microbial biotechnology to generate biofuels, pharmaceuticals and other high-value chemicals.

The advent of genome editing has evoked enthusiasm but also controversy. Concerns have been expressed, by some non-governmental organisations (NGOs) for example, that genome editing is 'not natural', that there are too many gaps in our knowledge, that impacts are uncertain and may be inequitable, and that regulation cannot keep pace with the speed of technological innovation.

In this report, EASAC takes a broad perspective on the research advances in editing methods and their applications, policy implications and priorities for EU strategy in promoting innovation and managing regulation. Our report draws on previous work by individual academies in Europe and by other international academy collaborations. Our objectives are to raise awareness of the scientific opportunities and public interest issues: to assess what needs to be done to realise those opportunities and take account of societal concerns.

Current knowledge gaps and uncertainties emphasise the need for more basic research. We expect that research advances will fill many of the current knowledge gaps and that progressive refinement of genome editing tools will further increase their efficiency and specificity, thereby reducing off-target effects. We anticipate that the fast pace of change in research and innovation will continue, so EASAC is willing to return to the subject of this report in due course to review its assessments.

EASAC concludes that policy considerations should focus on the applications in prospect rather than the genome editing procedure itself as an emerging technology. It is important to ensure that regulation of applications is evidence-based, takes into account likely benefits as well as hypothetical risks, and is proportionate and sufficiently flexible to cope with future advances in the science. Our recommendations are as follows.

Plants

The increasing precision now possible in plant breeding represents a big change from conventional breeding approaches relying on random, uncontrolled chemical- or radiation-induced mutagenesis and meiotic recombination. In supporting the conclusions from previous EASAC work on new plant breeding techniques, we recommend the following.

- We ask that EU regulators confirm that the products of genome editing, when they do not contain DNA from an unrelated organism, do not fall within the scope of legislation on genetically modified organisms (GMOs).

- We advise that there should be full transparency in disclosing the process used, but that the aim in the EU should be to regulate the specific agricultural trait/product rather than the technology by which it is produced. It follows that new technologies would be excluded from regulation if the genetic changes they produce are similar to, or indistinguishable from, the product of conventional breeding and if no novel, product-based risk is identified.

Animals

Research on animals is already subject to stringent regulation. While most genome-edited animals are currently being generated for basic or biomedical research, the technology also provides opportunities for livestock and aquaculture. It should be appreciated that, in addition to potential increases in production, genome editing brings possibilities to enhance animal health and welfare. For specific applications, we recommend the following:

- Livestock breeding in agriculture should also be governed by the same principle as proposed for plant breeding—to regulate the trait rather than the technology and be open and explicit about what is being done.

- With regard to the modification of large animals to serve as a source for xenotransplantation, we urge EU regulators to prepare for the new opportunities coming into range: this may require further discussion of the mechanism for approving medical products relating to cells and tissues, together with assessment of the implications of whether the edited donor, in the absence of additional transgenes, is regarded as a GMO or not.

Gene drive to modify populations in the wild

Gene drive applications for vector control and other modifications of target populations in the wild offer significant potential opportunities to help address major public health and conservation challenges. As outlined recently by the US National Academies of Sciences, Engineering, and Medicine, a phased approach to research can enable responsible development and offers sufficient time for considering what amendments are needed to current regulatory frameworks to enable the sound evaluation of a gene-drive-based technology. EASAC supports the recommendations by the US National Academies on gene drive approaches:

- It is essential to continue the commitment to phased research to assess the efficacy and safety of gene drives before it can be decided whether they will be suitable for use.

- This research must include robust risk assessment and public engagement.

- EU researchers must continue to engage with researchers and stakeholders in the countries where gene drive systems are most likely to be applied.

Micro-organisms

- We conclude that genome editing in microbes does not raise new issues for regulatory frameworks and is currently subject to the established rules for contained use and deliberate release of GMOs.

- There is a wide range of potential applications, including pharmaceuticals and other high-value chemicals, biofuels, biosensors, bioremediation and the food chain. It is important to recognise this wide range when developing EU strategy for innovation in the bioeconomy.

- Many of the policy issues for microbial genome editing research and innovation fall within the scope of what is regarded as synthetic biology, and we reaffirm the general recommendations from previous EASAC work relating to building research capacity, promoting skills development and recognising the need to achieve a balance between protection of innovation and benefit-sharing.

- Concerns have been raised elsewhere about the possibility for genome editing research to be conducted outside regulated laboratory settings. We recommend that the Global Young Academy should assess the issues raised by the expansion of the Do-It-Yourself (DIY) biology community.

- Concerns have also been expressed elsewhere about the potential biosecurity implications of genome editing. We recommend that the scientific community continues to inform and advise policy-makers during review of the Biological and Toxin Weapon Convention.

Human-cell genome editing

EASAC endorses the emerging conclusions from other collective academy work (International Summit on Gene Editing and FEAM) and the initiatives of EASAC member academies:

- *Basic and clinical research.* Intensive research is needed and should proceed subject to appropriate legal and ethical rules and standardised practices. If, in the process of research, early human embryos or germline cells undergo genome editing, the modified cells should not be used to establish a pregnancy. EASAC recognises that the decision by the European Commission not to fund research on embryos will be unlikely to change at present.

- *Clinical use: somatic gene editing.* There is need to understand the risks such as inaccurate editing and the potential benefit of each proposed genome modification. These applications can and should be rigorously evaluated within existing and evolving regulatory frameworks for gene and cell therapy by the European Medicines Agency and national agencies.

- *Clinical use: germline interventions.* These applications pose many important issues including the risks of inaccurate or incomplete editing, the difficulty of predicting harmful effects, the obligation to consider both the individual and future generations who will carry the genetic alterations, and the possibility that biological enhancements beyond prevention and treatment of disease could exacerbate social inequities or be used coercively. It would be irresponsible to proceed unless and until

the relevant ethical, safety and efficacy issues have been resolved and there is broad societal consensus.

General recommendations for cross-cutting issues

- *Public engagement.* There has to be trust between scientists and the public and, to build trust, there has to be public engagement. Stakeholders, including patients, clinicians, farmers, consumers and NGOs, need to be involved in discussions about risk and benefit, and scientists need to articulate the objectives for their research, potential benefits and risk management practices adopted. There is need for additional social sciences and humanities research to improve public engagement strategies.

- *Enhancing global justice.* There may be risk of increasing inequity and tension between those who have access to the benefits of genome editing applications and those who do not, although the widespread adoption of the technique might facilitate the sharing of benefits. The scientific community must work with others on the determinants to narrow the societal gap: for example, by active knowledge transfer, collaboration between researchers worldwide, open access to tools and education, and education efforts. It is also vital for EU policy-makers to appreciate the consequences, sometimes inadvertent, of EU policy decisions on those outside the EU. There is evidence that previous decisions in the EU (for example, on GMOs) have created difficulties for scientists, farmers and politicians in developing countries. Reforming current regulatory frameworks in the EU and creating the necessary coherence between EU domestic objectives and a development agenda on the basis of partnership and innovation are important for developing countries as well as for Europe.

1 Introduction

Genome editing is the alteration of a targeted DNA sequence, achieved by cutting the DNA molecule at a selected point, which activates the cell's own repair system and thus results in small deletions or insertions[4]. This is commonly used to inactivate a target gene or target sequence. When, at the same time, exogenous DNA is introduced, this can support the repair at the target site and enable a predetermined exchange of single or multiple nucleotides (targeted mutagenesis), for example to replicate or rectify a naturally occurring mutation. In this eventuality, the genome-edited organism would be indistinguishable in this specific place of the genome from an organism in which the mutation occurred naturally. The same method can also be used to insert or exchange fragments of foreign DNA at a predetermined site in the genome, generally then resulting in an organism carrying a transgene.

In this report, EASAC takes a broad perspective on the research advances, applications, policy implications and priorities for EU strategy in promoting innovation and managing regulation. The issues reviewed in our report are relevant for policy-makers at the EU level as well as in Member States: we emphasise the importance of developing consistency and coherence in the principles underpinning policy across the EU, with compatibility between different sectors, in support of research and its translation to innovation.

1.1 What are the prospects for genome editing?

Genome editing to produce selected disruption, correction or integration of genetic material in a cell has significant potential in basic research – including the elucidation of currently poorly understood biological functions of genetic elements – and in wide-ranging fields of application. Genome editing differs from previously employed techniques of genetic engineering in that alterations can be introduced more efficiently and precisely at the molecular level. However, there is more to be done in many cases to understand the biological consequences of those nucleotide changes. Genome editing is a significant scientific advance which, at the same time, may accentuate ethical and social questions associated with some potential applications coming within reach.

The science is advancing rapidly but the technology is already sufficiently mature to warrant assessment of the opportunities and of the challenges for ensuring proportionate, robust and flexible management of research and innovation. There are relevant matters for several EU policy-making departments, relating to the regulation of new products and the avoidance of harm, whether harm is caused inadvertently to human health and the environment, or by intended misuse, with biosecurity consequences.

There are significant strengths in European research in genome editing and it is important that rigorous risk–benefit assessment is part of the regulatory process, that any safety concerns are addressed and that research outputs can be translated into new products and services to fulfil societal needs, underpin the EU bioeconomy[5] and support European competitiveness. Potential benefits include the following: microbial biotechnology, for example in the provision of more efficient pathways for biofuel synthesis, high-value chemicals and pharmaceuticals; new vehicles for drug delivery; sensors and environmental remediation; plant and animal breeding in precision agriculture to tackle issues of food and nutrition security, animal health and a more sustainable agriculture; and a range of other human health applications (Hsu *et al.* 2014; Carroll and Charo, 2015; Barrangou and Doudna, 2016). Tackling disease, genome editing of human cells brings opportunities to treat or avoid monogenic disorders (with recent research in cystic fibrosis, Duchenne muscular dystrophy, diseases affecting the immune system and haemophilia (Prakash *et al*., 2016)) and infectious disease (with first studies in human immunodeficiency virus (HIV)) and diseases that have both a genetic and an environmental component (Porteus, 2015). Examples of prospective benefit and of perceived risks will be discussed later in this report.

1.2 Definition and experimental procedures

Genome editing refers to DNA mutations that are targeted to a specific region of the genome by site-specific nucleases (SSNs). It does not exclude the possibility that mutations in other regions of the

[4] Further scientific detail and the potential for alternative approaches to genome editing are provided in Box 1.

[5] The bioeconomy is regarded strategically as a key component for sustainable growth in the EU (European Commission, 2012). The economic value of genome editing is difficult to forecast and depends, of course, on its eventual contribution to the different fields of application in the bioeconomy (and the share that the EU can appropriate). Currently, the EU's biology-based industries account for 8.5% of the region's work-force, with an annual turnover of more than €2 trillion (El-Chichakli *et al*., 2016). According to the Organisation for Economic Co-operation and Development (OECD), the worldwide export of products related to the bioeconomy in 2014 amounted to about 13% of world trade. Recent comprehensive analysis of the biotechnology sector's contribution to the US economy indicates it is currently about 2% of US gross domestic product (within this 2%, approximately similar proportions are contributed by biotechnology medicines, crops/seeds and industrial products such as biofuels, enzymes and biomaterials (Carlson, 2016)).

genome also occur during the genome editing process: to avoid these unintended consequences, tools are being sharpened to prevent off-target effects.

Two forms of mutagenesis need to be distinguished:

- Simple mutagenesis (non-homologous end-joining), resulting either in base-pair substitutions or small insertions or deletions. This form is indistinguishable from spontaneous or induced random mutagenesis.

- Homologous recombination, in which a template of DNA is supplied with the SSN enabling the replacement of a similar sequence in the genome, or insertion of the added DNA in the genome at a pre-specified place. This form is similar to transfer of genetic material from one species to another after conventional crosses, or in cases of a more distantly related donor of the template DNA, similar to naturally occurring lateral/horizontal gene transfer.

A separate consideration is whether genome editing is achieved by insertion of DNA sequences that code for the editing agent (for example, CRISPR–Cas9) into the genome (and later removed by genetic segregation) or whether the editing agent is introduced transiently as DNA, RNA and/or protein without any integration of foreign DNA sequences into the cell.

Further scientific detail about the recent history of genome editing is provided in Box 1.

1.3 Public interests and values

The outputs from genome editing may have direct or indirect impacts on the well-being and welfare of the public—and the advent of genome editing evokes not only enthusiasm but also controversy. As will be discussed later in this report, when public concerns are elicited, they are usually about the intended use rather than the technology itself. Various queries have been raised about the different applications of genome editing, reflecting field-specific drivers and obstacles, but there are also generic questions that can be asked, as observed in the consultation for the UK Nuffield Council on Bioethics inquiry on genome editing (2015). For example, to what extent can the development of new genome engineering techniques be regarded as distinct from, or continuous with, existing techniques? Does the ease and accuracy of genome editing mean that it is a transformative technology (in either the moral or economic senses) and, therefore, represents a 'tipping point' in the potential of genetic engineering? Should a distinction be made (as it is by some who query these techniques) between directed change and those undirected changes induced, for example, by chemical- or radiation-induced mutagenesis, in conventional plant breeding programmes? There is also a generic technical point that is relevant to the various fields of application. Editing makes only small changes to DNA. At the target site these are easily identified, but off-target changes, which also occur in random mutagenesis, may be difficult to detect without full DNA sequencing. What implications does this have for the regulation of the resulting product?

Potential problems for assessing the products of this emerging technology are compounded in the EU by a legacy of contention and polarisation about the regulation of genetic engineering techniques. Current EU legislative frameworks governing the genetic modification of plants and animals, for example, are controversial; and even when there is an overarching EU policy framework, there is little certainty for researchers and breeders, because individual Member States vary in their implementation or can exercise an 'opt-out'. As critically observed by a recent Member State parliamentary report (UK House of Commons Science and Technology Committee, 2016), '*The regulation of genetic science is an area in which the EU has so far not come close to satisfactorily demonstrating an evidence-based approach to policy making*'.

Responsible innovation requires attending to ethical, legal and societal issues, and seeking to identify common goals important to scientists and the public. Researchers and their funders have a responsibility to engage with the public and to take account of public interests and values. In genome editing these range from the protection of individuals or populations from possible health risks, protection of animals from risks to their health and welfare, to moral and political interests around the acceptable limits to intervening in natural processes (Nuffield Council on Bioethics, 2015).

There is a moral obligation to fight disease and relieve humans and animals from suffering. To the extent that genome editing technologies provide useful tools to achieve such purposes, there is an opportunity cost in using them too late or not at all, particularly if they are safer, more effective and cheaper than alternative technologies. Concerns have been expressed about whether regulation can keep pace with the speed of technological innovation, whether scientists (and society) have fully appreciated the implications of what science can deliver and whether it would be possible to reverse undesirable outcomes. Much of the public debate has focused on human germline modification (which means that genetic changes would be heritable), but ethical issues relating to views of nature and ecosystems are also relevant to applications encompassing non-human targets of genome editing (Charo and Greely, 2015).

Application-specific issues are discussed in our subsequent chapters. General concerns expressed,

Box 1 Summary of the science of programmable nucleases

Genome editing methods take advantage of exogenous programmable nucleases to make double-stranded DNA breaks at selected sites. These breaks activate endogenous repair mechanisms either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). The latter operates when a DNA donor template is provided, and both systems function in all eukaryotic organisms. NHEJ is a more prevalent, error-prone mechanism that often causes mutations (short insertions or deletions), resulting in target gene knockout, when the break is introduced in the coding sequence of a locus; whereas HDR, which functions only in the synthesis (S) and gap 2 (G2) phases of the cell cycle, is the way to knock-in or substitute a desired sequence, for example to replace a mutant DNA fragment for the normal one. The NHEJ efficiency at the site of induced double-stranded DNA break is usually about five- to eight-fold higher than the efficiency of HDR.

The first generation of gene editing tools was based on oligonucleotide-directed mutagenesis (ODM) or microbial meganucleases, possessing long DNA recognition sequences. They were cumbersome to use and often suffered from low efficiency, especially ODM. The desired flexibility in target sequence recognition was achieved with the use of engineered zinc finger nucleases (ZFNs: each finger recognises about three specific nucleotides of DNA) and more recently with transcription activator-like effector nucleases (TALENs: each TALEN recognises short double-stranded specific sequence, typically single nucleotides). In both ZFN (Kim *et al.* 1996) and TALEN (Cermak *et al*., 2011) designs, the DNA recognition module is additionally coupled via a peptide linker to an unspecific DNA cleaving portion, usually the Fok I restriction nuclease domain. As only dimerised Fok I shows DNA cleavage activity, the length of the DNA recognising portion is also doubled by involving two recognition arms, enhancing nuclease specificity. Although TALENs had several advantages over ZFNs, especially in their design, their production is still a laborious process.

Another class of genome editing tool is designer recombinases. Similar to meganucleases, recombinases are difficult to tailor and the generation of enzymes with new DNA-binding specificities is cumbersome and time consuming. However, designer recombinases are highly specific and do not rely on cellular DNA repair as they cut and re-ligate the DNA in a conservative manner. As such, designer recombinases represent interesting alternatives (Karpinski *et al*., 2016), subject to further research.

The revolution in the field of genome editing came in 2012 with the development of the CRISPR–Cas9 system (Jinek *et al*., 2012), which is much easier to design, produce and use. The acronym CRISPR stands for clustered regularly interspersed short palindromic repeats, and it is considered by some to be a distant bacterial analogue of the RNA interference mechanism in eukaryotes; Cas stands for CRISPR-associated protein nuclease. The system is based on the natural defence mechanism against bacteriophages and plasmids evolved by many bacteria and archaea. Unlike protein meganucleases, ZFNs and TALENs, the new system uses RNA for complementary DNA recognition, and Cas9 protein (or related protein) to recognise a matching target sequence in the DNA, flanked by a short protospacer adjacent motif (PAM), and execute DNA cleavage by its two DNase domains. The RNA component is either composed of two molecules, the CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) as in the bacteria it derives from, or, what is more common, these two RNAs are fused by researchers into a single guide RNA (gRNA) which is about 100 nucleotides long.

How does the CRISPR–Cas9 system function? In brief, the Cas9 protein is bound to a gRNA and thereby programmed to recognise a target DNA whose sequence is complementary to a ~20 nucleotide segment in the gRNA. Cas9 binds the PAM motif in the target DNA duplex, separates the DNA strands and facilitates base-pairing between the gRNA and the complementary DNA sequence. Subsequently, Cas9 deploys its two DNase domains, RuvC and HNH, to cleave target DNA, generating a double-stranded break. Then, the DNA repair systems, NHEJ or HDR come into action and DNA is either mutated or replaced. The editing process with CRISPR–Cas9 may be multiplexed to inactivate tens of targets at once (Yang *et al*., 2015).

The important practical issues in genome editing experiments are the delivery of programmable nucleases into cells, their cleavage efficiency and specificity, in terms of avoiding off-target effects. To minimise the off-target effects, new versions of Cas9 and related proteins have been engineered. Recently, a mutation of three or four amino acids in the Cas9 catalytic domain reduced off-target effects dramatically to levels that were hardly noticeable (Klenstiver *et al*., 2016). Furthermore, in addition to Cas9, other bacterial DNases such as Cpf1 (Zetsche *et al*., 2015), which recognise different PAM sequences, can also be used for genome editing and thus increase the range of targetable sequences in genomes.

Besides genome editing, the CRISPR–Cas9 system has been repurposed for sequence-specific regulation of gene expression, either transcription activation or repression, or specific gene imaging using nuclease-deactivated Cas9 termed dCas9 (Dominguez *et al*., 2015). The CRISPR–Cas9 system has also been adapted to recognise and track RNA in living cells (Nelles *et al*., 2016), and a natural RNA-targeting CRISPR system taking advantage of the C2c2 enzyme has been identified (Abudayyeh *et al*., 2016).

for example by some NGOs, that genome editing is not natural, and that there are too many gaps in our knowledge and that impacts are uncertain, as well as there being issues for global justice[6], can probably be applied to all emerging technologies in biology and medicine. It is the role of research and of robust regulatory systems to continue to address the uncertainties and fill the knowledge gaps in a transparent way. A cardinal feature of the accuracy of genome editing is that the functional consequences should be more predictable than when using earlier techniques. Of course, there is continuing need to adopt appropriate safety standards, develop risk assessment techniques and to install effective surveillance, monitoring and disclosure systems, whatever the field of application. The recent report from the Nuffield Council on Bioethics (2016) considers further the range of ethical questions to which the recent advances in

[6] That is, would the societal gap increase between those who are able to use the technologies for their own benefit in medical, agricultural or other applications, and those who are not?

genome editing may give rise. These issues and the implications of the 'slippery slope' argument will be dealt with at various places in our report.

Public interest about science and innovation also often refers to the desirability of open science, benefit-sharing and fair competition. There is controversy about competing patent claims for CRISPR–Cas technology (Egelie *et al.* 2016; Nuffield Council on Bioethics, 2016). At the same time, CRISPR–Cas9 has become an example of open science, where the development of the procedures has resulted in the sharing of tools from more than 80 laboratories.[7] Patent-related aspects were addressed in a recent statement from ALLEA, the All European Academies (2016) which notes that the use of CRISPR–Cas technology does not require any reforms in patent law: '*EU patent law provides the necessary incentives for further development and use across all fields of life sciences*' and that there will be no patents granted which could offend human dignity and/or integrity.

1.4 Previous work by academies of science and medicine

There has already been a significant amount of work by academies on the issues elicited by genome editing and our EASAC report draws on this continuing effort:

- *At the national level in Europe,* the German Academies statement (Leopoldina *et al*., 2015) on opportunities and limits, covers all applications and emphasises the great scientific potential of genome editing in opening up new scope for basic research. This German statement concludes that it is ethically and legally acceptable in many areas (see Chapter 5 of the present report for further discussion, including a moratorium of genome editing for germline interventions[8]) and that new techniques should not automatically be equated with sporadic cases of improper use or with applications whose ethical and legal ramifications have not yet been assessed. While our EASAC study was in progress, KNAW, the Royal Netherlands Academy of Arts and Sciences (2016), published their national position paper on genome editing. This also covers multiple applications and their recommendations are broadly consistent with the recommendations in the present EASAC report.

- *The International Summit on Human Gene Editing* is led by the US National Academies of Sciences, Engineering and Medicine together with the UK Royal Society and the Chinese Academy of Sciences. This consortium is examining the scientific underpinning as well as the clinical, ethical, legal and social implications of the use of human genome editing technologies in biomedical research and medicine, including editing of the human germline (National Academies, 2016a).

- *The US National Academies* have also completed investigations of genome editing and gene drive (National Academies, 2016b), and of genome editing relevant to laboratory animal use.

- *FEAM* organised a workshop in 2016; with support from the InterAcademy Partnership (IAP), to consider the landscape for human genome editing in the EU. This workshop reviewed current scientific and regulatory activity in human genome editing research and clinical applications, to identify where there are significant differences between EU countries and to discuss options for European-level activities (Academy of Medical Sciences, 2016). The report from this workshop was recently published (FEAM, 2017).

The outputs from these other academy activities will be cross-referenced in the following chapters of our report.

1.5 EASAC objectives for this work

In seeking to add value to the work that has already been done, this report draws on the previous academy publications together with advice and information from a group of experts nominated by EASAC member academies (Appendix 1). We take a broad perspective of the science, and our objectives for this report are also wide-ranging in assessing policy and practice:

- To raise awareness across Europe of the scientific opportunities of the new genome editing techniques, and public interest issues, to evaluate what is now needed to realise those opportunities and address those issues, and to consider who should make decisions on governance.

- To identify distinctive aspects confined to particular applications of genome editing, to show where sector-specific outputs are already subject to established policies rules and regulations (at institutional, national and

[7] www.addgene.org/crispr

[8] In Germany, germline therapy and the use of modified germ cells for fertilisation are prohibited under Section 5 of the German Embryo Protection Act. Whether the intervention would be allowed if it served the preservation of the resulting embryo is under debate. The German academies have also published a Statement on progress in molecular breeding and on the possible national ban on cultivation of genetically modified (GM) plants: see https://www.leopoldina.org/uploads/tx_leopublication/2015_03_26_Statement_on_Molecular_Breeding_final.pdf

EU levels) or where changes should now be foreseen.

- To prepare policy-makers to address those issues that have still to be clarified and resolved.

- To serve as an input to global discussions and action on genome editing priorities, alongside the other academy initiatives (that focus on human-cell applications) and for those aspects where global consensus is of particular importance (for example, for biosecurity).

As part of these objectives, we aim to assess what strategic objectives are relevant to the EU level and what is reserved for Member States. EASAC messages are directed to those who make or influence policy in EU institutions, and at Member State level, academies of science in other regions outside the EU, research funding bodies, regulatory authorities, professional societies and others in the scientific community. We recognise the great importance of also engaging with other stakeholders and the community-at-large, and EASAC encourages its member academies to use this report as a resource to disseminate our messages widely.

In the following chapters, we consider particular applications of genome editing and in the final chapter bring together our conclusions and recommendations.

2 Plants and animals

For both plants and animals, genome editing has become an essential tool for basic research, to elucidate gene function and to generate model plants and animals. The scientific advances achieved with genome editing, capitalising also on the progress in genome sequencing that is identifying many genes and alleles of interest for agriculture, enhance the potential for tackling a wide range of applications.

There are major global challenges to be faced in addressing issues for food and nutrition security and agriculture, and the opportunities and challenges are discussed more broadly in an ongoing EASAC project that constitutes the European arm of a worldwide IAP project[9]. Current problems of food and nutrition security are compounded by pressures of growing population, climate and other environmental changes, and by economic inequity and insecurity. Setting priorities for increasing agricultural production must also take account of pressures on other critical resources, particularly water, soil and energy, and the continuing imperative to avoid further loss of ecosystems and biodiversity.

2.1 Plant breeding in agriculture

Plant sciences can do much in continuing to contribute to increased crop quality, for example in developing cultivars with improved water and nitrogen use, better resistance to pests and diseases, or modified crop architecture to reduce waste. Prospects for plant genome editing are discussed widely in the literature (see, for example, Bortesi *et al*., 2016; Quetier, 2016) and in the recent report from the US National Academies (2016c) which notes the potential of genome editing to introduce more complex changes because multiple genes can be edited simultaneously. Genome editing brings new possibilities to improve plant traits, beyond what has been achieved with the previous generation of genetic modification (mutagenesis) approaches. Molecular targets are being selected and tackled to increase yield, stress- and disease-resistance, elevate nutrient use efficiency and reduce allergens, for example, in broad support of the societal objectives for increased food production, conservation of natural resources, less pollution and healthier food. There are many significant research advances described in the US National Academies report and in other recent publications, for example the induction of targeted heritable mutations in barley and brassica (Lawrensen *et al*., 2015) and combatting invading virus DNA in plants (Zhang *et al*., 2015). Of particular interest in breeding is the rapid introduction of known natural alleles (genetic variation) into many different genetic backgrounds.

Research advances in plant breeding are now being translated into novel products. There has been recent progress using genome editing in the commercial development of cold-storable potatoes and no-trans-fat soybean oil, but the first organisms to be allowed by the US Government are CRISPR–Cas9-edited mushrooms (with reduced browning by reducing the activity of the endogenous enzyme polyphenol oxidase) and a waxy corn engineered to contain starch composed exclusively of the branched polysaccharide amylopectin (used in processed foods, adhesives and high-gloss paper). These products do not come within US Department of Agriculture regulations (Waltz, 2016) although they might still be submitted for voluntary review by the US Food and Drug Administration (FDA).

These rapid advances in research and development accentuate a major underlying question for the EU: to what extent will the regulation of plants/food products developed using genome editing be influenced by previous controversies and current legislation on GMOs? The products of genome editing may contain no foreign DNA, and EASAC has previously advised in the Statement on New Breeding Techniques (2015a; encompassing genome editing tools and summarised in

Box 2 Summary of previous EASAC recommendations on new plant breeding techniques

1. EU policy development for agricultural innovation should be transparent, proportionate and fully informed by the advancing scientific evidence and experience worldwide.
2. It is timely to resolve current legislative uncertainties. We ask that EU regulators confirm that the products of new breeding techniques, when they do not contain foreign DNA, do not fall within the scope of GMO legislation.
3. The aim in the EU should be to regulate the specific agricultural trait and/or product, not the technology by which it was produced.
4. The European Commission and Member States should do more to support fundamental research in plant sciences and protect the testing in field trials of novel crop variants against vandalism.
5. Modernising EU regulatory frameworks would help to address the implications of current policy disconnects in support of science and innovation at regional and global levels. At the same time, there is continuing need for wide-ranging engagement on critical issues and this should include re-examination of the appropriate use of the precautionary principle.

Source: EASAC (2015a)

[9] 'Food and nutrition security and agriculture', see www.interacademies.net/News/27419.

Box 2) that such processes should not be regulated in the same way as GMOs, assuming that there is evidence to demonstrate that any transgene has been segregated away in the final product.

The issues are, however, still contentious. For example, if there is a transient transgenic stage during the plant breeding process, some would assert that this makes the final non-transgenic product still a GMO. However, modern whole-genome sequencing methods allow for unambiguous proof that foreign DNA from transgenes has been completely removed. It should also be noted that many of the agricultural sector-specific public concerns raised by NGOs about genome editing were also raised previously in the early days of genetically modified (GM) crops[10] and were addressed systematically then (for example in the UK GM science review (GM Science Review Panel 2003), and see EASAC (2013) for further discussion of the GM crop research evidence base).

A European Commission decision on the status of these products is urgent in view of the accelerating pace of research and development and of the regulatory initiatives being undertaken by individual Member States. For example, an oligonucleotide gene-edited canola strain was assessed as non-GMO in Germany (EASAC 2015a; Huang *et al*., 2016; and see the footnote[11]). The Swedish Board of Agriculture, a national competent authority, also confirmed that some plants in which the genome had been edited using CRISPR–Cas9 do not fall under the EU GMO definition[12]. Discussion in the EASAC Working Group agreed that a strong case can be made for genome-edited crops to be subject only to the rules and regulations that apply to products of conventional breeding, subject to certain guiding principles (Huang *et al*., 2016):

- Minimising the risk of escape of genome-edited crops from laboratories and fields during the research and development (R&D) phase.
- Demonstrating the absence of foreign sequences if genome engineering proteins were introduced as DNA constructs.
- Documenting DNA sequence changes at the target sites.
- In the case of newly introduced DNA, identifying the phylogenetic relationship between donor and recipient.
- Excluding unintended secondary editing events or off-target sites on the basis of available reference genome information.

Even if a trait-based assessment system did not require specific regulation of a new crop variety, there should still be a legal requirement to disclose the process used, with transparency on why a particular process was used. The alternative regulatory options for genome-edited plants, including product-based approaches, are discussed further in detail by Sprink *et al.* (2016).

Recommendations from the European Commission on what is a GMO are delayed, and continuing discussion with the European Commission, European Parliament and Council of Ministers is expected. There is great need for evidence-based proportionate regulation for next-generation plant breeding (Box 2). EU regulatory frameworks should also take account of best practice outside the EU (EASAC 2013, 2015a). For example, reform of the US system for regulation of GMOs and of products using other techniques such as genome editing, which do not currently fall within US GMO regulations, is anticipated in the new US Coordinated Framework for regulating biotechnology. It has been proposed (Strauss and Sax, 2016) that this new US Framework should be product-based not event-based; novelty-based not method-based; and that modifications that are analogous to what occurs in conventional breeding (but which are more precise and better understood than in conventional breeding) should be exempt, unless a novel product-based risk is identified. It would seem reasonable to consider adopting similar criteria in the EU (and compatible with the recommendations in Box 2), while also taking into account essential features of the responsible governance of agricultural biotechnology (Hartley *et al*., 2016), including a commitment to candour, recognition of underlying values and assumptions, and a preparedness to respond to new knowledge or concerns.

Recent proposals from the US Government give some indications of how the revised US regulatory system might function. The US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS, 2017) set out the criteria by which an organism would not be regarded as genetically engineered. For example, it would not be regarded as a genetically engineered organism if the modification were solely a deletion of any size or a single base-pair substitution that could otherwise be obtained through the use of chemical- or radiation-based mutagenesis. It would also not be

[10] These concerns included potential for human toxicity, allergenicity and effects on the environment.
[11] The German Federal Office of Consumer Protection and Food Safety provided an Opinion on the legal classification of New Plant Breeding Techniques, including CRISPR–Cas9, see https://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/Opinion_on_the_legal_classification_of_New_Plant_Breeding_Techniques.pdf?_blob=publicationFile&v=2.
[12] November 2015 'Green light in the tunnel!', Umea Plant Science Centre www.upsc.se.

considered a genetically engineered organism if the modification were solely introducing only naturally occurring nucleic acid sequences from a sexually compatible relative that could otherwise cross with the recipient organism and produce viable progeny through traditional breeding (including, but not limited to, marker-assisted breeding, as well as tissue culture and protoplast, cell or embryo fusion). As part of its broader initiative in biotechnology (see subsequently for issues raised for animals and mosquitoes), the FDA has also very recently invited comments on whether genome-edited plants might present new food safety risks and whether they should follow the same pre-market regulatory review at the FDA as transgenic plants currently do[13]. An accompanying commentary[14] emphasises the FDA principle to maintain product-specific, risk-based regulation.

A second international example is provided by Australia, currently conducting a review and public consultation to provide clarity on whether organisms developed using a range of new technologies (including site-directed nuclease techniques) are subject to regulation as GMOs and to ensure that new technologies are regulated in a manner commensurate with the risks they pose (Australian Government Department of Health, Office of the Gene Technology Regulator, 2016). Four options are identified in this Australian review: (1) no amendment to the current regulations; (2) regulate certain technologies (including all site-directed nuclease techniques); (3) regulate some new technologies on the basis of the process used (excluding site-directed nuclease technologies that do not involve application of a DNA template); and (4) exclude certain new technologies from regulation on the basis of the outcomes they produce: that is, exclude if the genetic changes produced are similar to or indistinguishable from the product of conventional breeding (chemical and radiation mutagenesis and natural mutations). This last option, focusing on product rather than process, would again be similar to the recommendations of EASAC for the EU (Box 2): it is important to achieve international coherence in regulation.

2.2 Animal breeding in agriculture

Genome editing objectives in livestock breeding include improving animal health and improving agricultural traits. Recent examples of research to improve animal health include the following:

(1) To protect from porcine reproductive and respiratory syndrome, economically the most important disease of pigs in Europe, North America and Asia (Whitworth *et al*., 2016);

(2) To edit pig immune-system genes involved in the reaction to the haemorrhagic virus that causes African swine fever (ZFN-mediated in embryo editing of domestic pigs with the warthog RELA orthologue associated with resilience to African swine fever (Lillico *et al*., 2016)), a disease that has been hard to eradicate in sub-Saharan Africa and Eastern Europe (Ainsworth 2015, commenting on work in the UK Roslin Institute).

Other researchers have shown that the prion gene responsible for bovine spongiform encephalopathy (BSE) can be effectively modified by genome editing (Bevacqua *et al*., 2016). There is also significant interest in generating cattle resistant to trypanosome parasites[15], which are responsible for sleeping sickness, a serious problem for farmers in Africa.

Other proposed applications of genome editing of farm animals, addressing goals both to improve animal health and welfare and to improve agricultural traits, include genetic de-horning of dairy cattle for improved husbandry (Carlsson *et al*., 2016). Another opportunity is represented by the Belgian Blue, a natural breed of cattle selected for increased muscle, reduced fat and more tender meat, but where significant inbreeding has led to animal welfare problems. The desired trait arises from a mutation in the myostatin gene, which can be replicated by genome editing, demonstrated for cattle, goats, sheep and pigs (Charo, 2015; Crispo *et al*., 2015; Cyranoski, 2015; Wang *et al.* 2015). Thus, there is potential to avoid the negative effects of inbreeding and, if done in the right breed or in a controlled manner, to avoid problems during labour, which are also typical for the Belgian Blue. Thus, genome editing may enable a much more precise, faster approach to obtain the desired phenotype without other undesired traits co-segregating during natural selection. The genome of most livestock species has been sequenced and the costs of sequencing are becoming more affordable. The genome of a founder animal can, therefore, be fully sequenced to exclude the presence of off-target events as far as possible before release or marketing.

Other research ideas (Reardon, 2016) include the following:

- Generating chicken eggs without allergen, helping children who receive vaccines produced in chicken eggs.

[13] https://www.federalregister.gov/documents/2017/01/19/2017-00840/guidance-genome-editing-in-new-plants.
[14] http://blogs.fda.gov/fdavoice/index.php/2017/01/fdas-science-based-approach-to-genome-edited-products.
[15] https://clippings.ilri.org/2013/10/20/disease-resistant-cattle-for-Africa.

- Editing chickens to make them resistant to infectious diseases (such as avian influenza) and to produce only female offspring. This avoids the culling of male chicks, which are not required for egg production.

- Inactivation of genes for reproductive hormones in farmed fish, rendering them infertile, as a safety measure in case commercially approved, GM salmon or other farmed salmon[16] escape.

- Bees, one of the most important organisms for crop production, can be edited to add hygiene-associated genes so that colonies are less susceptible to mites, fungi or other pathogens.

A broad discussion of methodologies for animal breeding, including TALEN and CRISPR–Cas9, in the European context was published in 2014[17]. This assessment cross-references the Eurobarometer survey of public perceptions of GM animals, and notes societal issues: '*GM animals are perceived as far more problematic than GM crops*' in terms of potential risks, naturalness, usefulness and moral considerations. These problems may not be easily resolved by using newer approaches: '*Genome editing seems easier and quicker than transgenic modification. That does, however, not necessarily mean that genome editing is ethically neutral or will be easily accepted by consumers*'.

When considering the issues for animal welfare and for research objectives, it is important to appreciate that animal research using genome editing is already covered by the strict EU and Member State controls on animal research more generally and it is subject to the widely agreed principles of the '3 Rs' (replacement, reduction, refinement), in particular relating to 'refinement'. The scientific community needs to do more to engage with the public in discussing the issues. It would seem reasonable to conclude that there is a case for considering genome editing in livestock breeding as part of the toolbox for improving agricultural productivity and animal health if concerns about animal welfare or other ethics issues are tackled satisfactorily. The wider range of scientific and societal issues relating to farming animals is being discussed in the current EASAC project on 'Food and Nutrition Security and Agriculture'.

The EU regulation of genome editing in animals will be subject to the forthcoming decisions of Directorate-General for Health and Food Safety (DG Sante) on what is a GMO (see previous section). The advice of EASAC on plant breeding – to regulate the resulting trait rather than technology and to be transparent about what is being done – is also applicable to animal breeding. This view from EASAC is consistent with other recent conclusions (appertaining to genome editing in cattle), '*The products of editing should be subject to the same oversight as other food products, based on the results rather than the process that yields the results*' (Carroll *et al*., 2016). However, in the USA the FDA also recently published a draft revision to its previous guidance relating specifically to the regulation of intentionally altered genomic DNA in animals. In this draft guidance, the FDA proposes pre-market evaluation of genome-edited animals, effectively treating them the same as transgenic animals[18]. This regulation would not apply to non-food species that are raised in contained conditions, such as laboratory animals in research. The FDA proposal is open for public comments until April 2017 and is controversial because of the level of regulation proposed (Maxmen, 2017).

2.3 Other animal work

Other conceivable applications of genome editing in animal breeding (Reardon, 2016), such as to support the re-introduction of extinct species (woolly mammoth, passenger pigeon), or to generate more desirable pets (micro-pigs, koi carp with preferred colours and patterns, dogs with preferred behavioural traits), are beyond the scope of the present report.

2.3.1 Laboratory models

There are also considerable opportunities for using genome editing in developing cellular and animal models of human disease in laboratory research (Hsu *et al*., 2014; Smalley, 2016), including larger animal models[19]. As noted in the Statement by the German Academies (Leopoldina *et al*., 2015), the now feasible concurrent introduction of several targeted mutations can reconstruct complex disease pathways in model organisms and help identify and characterise therapeutic targets. Depending on the genetic modifications required, mouse models that would previously have taken 1–2 years to develop can now be created in months[20]. Advances using genome editing bring potential new models of neurological disorders such as autism, Alzheimer disease and Parkinson's disease in non-human primates, although these opportunities also emphasise the ethical challenges associated with animal welfare (Willyard, 2016).

[16] A recent study (Karlsson *et al*., 2016) shows that wild populations of salmon in areas in Norway with many salmon farms contained higher levels of farmed salmon DNA than those regions with less farming.
[17] Swedish University of Agricultural Sciences, http://www.slu.se/mistrabiotech/GManimalSymposium.
[18] http://www.fda.gov/downloads/Animal/veterinary/GuidanceComplianceEnforcement/GuidanceforIndustry.
[19] For example, the EU-COST action SALAM (Sharing Advances on Large Animal Models), International Society for Transgenic Technologies, http://transtechsociety.org/blog?p=1457. See also Nuffield Council on Bioethics (2016) and Barrangou and Doudna (2016) for discussion.
[20] Burton H, 3 February 2016 www.phgfoundation.org/blog/17136.

The US National Academies of Sciences, Engineering and Medicine of Science organised a workshop on gene editing under their initiative on Science and Welfare in Laboratory Animal Use (Institute for Laboratory Animal Research, ILAR). This wide-ranging workshop covered species-specific use of genome editing technologies in laboratory animals, regulatory issues, ethical issues and various stakeholder perspectives[21].

2.3.2 Xenotransplantation

Another application of genome editing in livestock is in xenotransplantation, the transfer of tissues and organs from animals to treat loss or dysfunction in humans. Research and societal interest in xenotransplantation has quite a long history and the fundamental issues were covered comprehensively in the Nuffield Council on Bioethics report of 1996. A project funded by Framework Programme 6, 'Xenome'[22], included a survey of public perceptions of xenotransplantation in several European countries.

There is clinical need for xenotransplants for patients with end-stage organ failure (heart, kidney, liver), but also for a variety of cell types, some of which are already being investigated as possible xenotransplants, such as liver cells (Nagata *et al.*, 2007), neurons (Leveque *et al.*, 2011), cornea (Hara and Cooper, 2011) and pancreatic islets (Elliott *et al.*, 2011).

A recent comprehensive review (Perota *et al*., 2016) discusses the immunological barriers to xenotransplantation[23], which especially apply to whole-organ transplantation. By elimination of a sugar epitope that is not present in humans (Gal-epitope) (Phelps *et al.*, 2003; Kang *et al*., 2016), the initial obstacle of hyperacute rejection (occurring within minutes) could be surmounted. It was the major factor behind recent successes with xenografted hearts (which survived more than 2 years) and kidneys (which survived up to 136 days) in non-human primates (Iwase *et al.*, 2015; Murthy *et al.*, 2016). Protection against delayed rejection (occurring within weeks), however, requires further modifications of the source animals, including expression of xeno-relevant transgenes and removal of xenoreactive non-Gal epitopes. The latter can be realised through genome editing (Li *et al.*, 2015), which also offers new opportunities to reduce the load of porcine endogenous retroviruses (Yang *et al.*, 2015). Further research is required to assess the long-term efficacy and safety of whole-organ xenotransplants, but tissue transplants such as porcine islets, which can be encapsulated, could soon enter the clinic. The first clinical trials using encapsulated neonatal porcine islets to treat type 1 diabetic patients have already been performed (Elliott *et al.*, 2011).

Regarding relevant legislation and regulations, clinical trials conducted within the EU using xenogeneic medicinal products are regulated by the European Medicines Agency (EMA). EMA guidelines on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) came into effect in 2010. Detailed requirements for islet transplantation have recently been published (Cozzi *et al.*, 2016). The World Health Organization (WHO) had its first consultation on regulatory requirements for clinical xenotransplantation trials in Changsha, China, in 2008 and a second consultation in 2011 in Geneva, Switzerland, where it was concluded that principles and guidance contained in the Changsha Communiqué[24] remain valid and fully actionable. Further general EU expertise that may be relevant to assessing the products of genome editing in xenotransplantation is accruing from the Framework Programme 7 projects Translink (assessing risk factors associated with animal-derived bioprosthetic heart valve; www.translinkproject.com) and Xenoislet (developing transgenic pigs to treat type 1 diabetes; http://xenoislet.eu).

Recently it has been proposed that human organs destined for transplantation could be grown within pigs. Here too, genome editing is an important tool (Reardon 2015b; Perkel 2016) in possibly realising this objective. If a gene essential for the development of an organ, such as pancreas or heart, were inactivated by genome editing in the early embryo, the developing foetus would lack the organ. But if (induced) pluripotent stem cells were introduced into the embryo, then these could participate in foetal development, compensate for the defect in the host embryo and produce the organ. This type of complementation has been achieved with embryos and stem cells of the same species (pig; Matsunari *et al.*, 2013) and between closely related species (mice and rats; Kobayashi *et al.*, 2010). The resulting animal is a chimaera consisting of cells from the embryo and the injected stem cells. It remains to be seen whether such a scheme would work with more distantly related species such as humans and pigs. Preliminary experiments to investigate this are currently underway in various laboratories around the world[25]. Because of the ethical issues, a vital part of this work is devising the means to restrict the developmental potential of the injected human induced pluripotent stem cells to avoid any contribution to the chimaera beyond the organ to be transplanted. This restriction potentially can be achieved by inactivation of specific developmental genes through genome editing.

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[21] December 2015 ILAR Roundtable http://nas-sites.org/ilar-roundtable/roundtable-activities-gene-editing-to-modify-animal-genomes-for-research/webcast.

[22] *Xenotransplantation between medicine and society*, http://www.observa.it/gli-xenotrapianti-tra-medicina-e-societa/?lang=en.

[23] These include hyperacute rejection, acute humoral xenograft rejection, immune cell-mediated rejection and instant blood-mediated inflammatory rejection.

[24] The Changsha Communique 2008 on http://www.who.int/transplantation/xeno/ChangshaCommunique.pdf.

[25] Curie J. *US lab attempting to grow pig embryos with human pancreases*, Bionews 13 June 2016; http://www.bionews.org.uk/page_658075.asp.

3 Gene drive in modification of populations in the wild

3.1 Use of gene-drive-based technologies

Gene drive is a process of biased inheritance that allows a gene to be transmitted from parent to offspring at an increased rate. As a result, the gene can increase in frequency in a population over multiple generations. Gene drive systems are hence 'self-sustaining': this is the key differentiating characteristic from other forms of genetic modifications, which are applied either only to one generation or are eventually selected out, if disadvantageous, over a few generations.

Currently gene drive studies are focused on the genetic modification of wild populations of some particularly harmful species, such as disease vectors. Gene drive has been proposed as an efficacious tool to address several major public health challenges, including the transmission of malaria, Zika and dengue fever by mosquitoes (Gantz *et al*., 2015; Alphey, 2016; Hammond *et al*., 2016). Other potential applications for gene drive (Reardon, 2016) include editing ticks so that they are unable to transmit bacteria that cause Lyme disease, and editing aquatic snails to prevent them from transmitting the parasitic disease schistosomiasis.

There is considerable interest in the potential benefits of gene drive systems. A recent UK House of Lords Science and Technology Committee report on GM insects (2015) highlights the possible value of gene drive systems to eradicate disease-carrying vectors that affect crops and people. Potential applications (in addition to malaria, Zika and dengue) are suggested to include containment of chikungunya, West Nile fever and Chagas disease, together with various applications for sustaining agriculture (e.g. tackling bluetongue disease, equine infectious anaemia, infectious salmon anaemia, Mediterranean fruit fly). The UK is a leader in research and innovation on GM insects, including gene drives (with growing competition by the USA and China making considerable recent investment), but public awareness of the scope and potential of these technologies is yet to be mapped. This parliamentary report calls for increased public investment in GM insect field trials to test the science, promote public engagement and lead international developments.

While the potential and promise of gene drive technology is significant, the research is still at a relatively early stage. Some groups have expressed concerns about the potential risks of using gene-drive-based technology[26]. However, as noted by the recent report by the US National Academies of Sciences, Engineering and Medicine (National Academies, 2016b) in their review of gene drive opportunities and challenges, it is essential to continue research to establish the efficacy and safety of gene drives before it can be decided whether they are suitable for use. The report concluded that the significant potential of this application justifies proceeding with phased research and testing so that benefits and risks can be properly assessed. The US National Academies recommendations are summarised in Box 3.

3.2 Challenges and limitations to use of gene-drive-based technologies

There are some specific issues that could hinder the efficacy of a gene drive system in some populations. For example, efficacy would be compromised if genetic diversity in natural populations provides sources of natural resistance to the gene drive (Deredec *et al*., 2008; Unckless *et al*., 2015). Researchers will also need to examine the possible risk of resistance stemming from the genetic modification as a possible limit on long-term

Box 3 Summary of US National Academies recommendations on gene drive

1. Funders of gene drive research should coordinate to reduce gaps in knowledge about the molecular biology of gene drives and other critical research areas including population genetics, evolutionary biology, ecosystem modelling, ecological risk assessment and public engagement.
2. Funders of gene drive research should establish open access repositories of data and standard operating procedures for gene drive research: to share knowledge, and guide both risk assessment frameworks and research design and monitoring standards.
3. Key characteristics of gene drives – including their intentional spread and potential irreversibility of environmental effects – should be used to frame societal appraisal of the technology.
4. Robust ecological risk assessment must be part both of field trials and environmental release of gene drive-modified organisms.
5. Conducting risk assessment and making policy decisions must involve public engagement.[27]
6. Selecting sites for field testing and environmental releases should be guided by scientific judgement, feasibility of risk assessment and opportunity for community engagement. Preference should be accorded to locations in countries with existing scientific capacity and governance frameworks to conduct and oversee safe investigation of gene drives.

Source: adapted from National Academies (2016b).

[26] For example, the International Union of Conservation of Nature (IUCN) has recently called for a moratorium on gene drive research until further assessment of the impact on conservation can be made: http://portals.iucn.org/congress/motion/095. In other developments, a recent meeting of the United Nations Convention on Biological Diversity rejected calls for a global moratorium on gene drives but instead encouraged caution in field testing the products of synthetic biology, including gene drives, with better risk assessment of potential effects (Callaway, 2016c).

[27] See, for example, Anon. (2015) and Reardon (2016).

efficacy of specific applications of gene drive approaches. More research is also needed to assess genetic stability in the wild: that is, the impact of alternative DNA repair pathways (Alphey, 2016), and there are efforts to engineer practical gene drive systems designed to select against the emergence of drive-resistant alleles (Noble *et al*., 2016).

In addition to efficacy questions, the recent increased interest in gene drive has led to questions about the potential safety of such a technology. Concerns have been raised that the spread of gene drive constructs may be difficult to control and might have ecological consequences attributable to reduction in the population of the target species (which is a question relevant to all vector control interventions) or spread of genes to other species beyond those intended. These questions will need to be addressed through safety studies and a risk assessment for each application of gene drive. Prior modelling of the manipulation of natural populations is likely to be an essential part of research studies, and there will need to be extensive risk assessment to consider the possible consequences for ecosystems and to substantiate use of remediation measures. Given the variety of ways gene drive could be applied, safety concerns need to be related to a specific product and cannot be realistically assessed on general terms. Ultimately all products should be subject to a thorough risk assessment that will take into account the characteristics of the product developed, its intended use and the conditions of use.

The second concern often expressed is about the risks linked to an accidental escape of a gene drive organism. This concern is not unique to gene drive research but the self-sustaining nature of the technology makes it an important consideration. Several control and containment measures have been suggested to curtail the accidental spread of a modified organism if escaped from laboratory research containment (Akbari *et al*., 2015). In addition, ecological containment – whereby laboratory research is performed where there is no natural population of the same insect in the region, so that interbreeding is not possible if the modified insect escapes from the research facility – offers additional safeguards. Some have suggested the possibility of using molecular confinement methods (DiCarlo *et al*., 2015). It has been suggested that it could be possible to develop a drive system to overwrite a previous one, which would act as a safeguard mechanism (Wu *et al*., 2016), but this has not yet been fully explored.

3.3 Regulation of gene-drive-based technologies

One of the main challenges to the development of gene-drive-based products is regulatory. Some of the novel aspects of the technology may require clarifications and adjustments in current regulatory frameworks. Several reports, including those of the US National Academies of Sciences, Engineering and Medicine and the UK House of Lords, have mentioned this topic. Options for legal regulation of gene drives are also discussed in detail in several publications (for example, Oye *et al*., 2015; Champer *et al*., 2016)[28].

The report from the UK House of Lords noted that the existing regulatory regime for GMOs could be a basis for regulating GM insects, with ongoing monitoring of advances in research needed to ensure the framework remains fit for purpose. The House of Lords report noted two challenges: the importance of integrating the consideration of benefits into risk assessments; and the new question of persistence posed by the application of gene drive technology, which would require specific consideration and the stipulation of monitoring requirements. Outside the UK and USA, other countries have begun to review their regulatory frameworks to ensure they are fit for purpose. For example, Australia began a review process in December 2016.

The EASAC Working Group observed that, because gene drive is further into the future than some of the other fields of application of genome editing, there is time to consider the issues while R&D continues under frameworks that consider the potential risks in a stepwise fashion and are built on extensive stakeholder engagement. Research groups such as Target Malaria are already following these recommendations and are reaching out to stakeholders as a core pillar of their activities[29].

There are many possible applications of gene drive technology and it will be important to consider for each the cost/benefit of the proposed application and to compare it with other methods aimed at controlling the targeted species. At the present time, the research is largely focused on addressing key public health issues such as malaria, where the current harm inflicted by the target species would be an important consideration in assessing the use of a gene-drive-based technology. It is also important to note that efforts to construct adaptable governance policies can draw on existing guidelines, particularly the WHO Guidance Framework for Testing of GM Mosquitoes, to facilitate the necessary international coordination and collaboration. Gene drive should be regarded as complementary to other approaches to controlling infectious diseases and invasive pests, helping to provide an additional tool for improving public health and conservation.

[28] In January 2017 the US FDA also provided draft guidance for industry on mosquito-related products, seeking to clarify whether such products should be regulated as 'new animal drugs', while also emphasising the FDA principle to maintain product-specific, risk-based regulation (see footnote 14).

[29] Target Malaria (http://targetmalaria.org) and the discussion of gene drive approaches for controlling malaria vectors in Africa (http://aasciences.ac.ke/updates/events/using-gene-drive-approaches).

4 Micro-organisms

Genome editing augments, and might simplify, the existing and extensive technology already available for the genetic alteration of micro-organisms. However, it offers genetic access to prokaryotic species and some parasites and fungi that have been more refractory in this respect. Homologous recombination has led to extensive natural and laboratory-generated gene exchange between micro-organisms (involving transformation of DNA, by transduction or conjugation). In the view of the EASAC Working Group, therefore, genome editing in microbes raises no new ethical issues or issues for regulatory frameworks. Generally, the EU regulation of genome-edited microbes will be subject to the established rules for contained use and deliberate release of GMOs, and dependent on the ongoing legal analysis by DG Sante of what is a GMO.

4.1 The bioeconomy

There are various applications of genome editing in microbes already underway or envisaged as a basis for programmable and high-throughput functional genomics (Selle and Barrangou, 2015). The following are some examples:

- Applications in producing third-generation biofuels in bacteria, fungi and microalgae (Liao *et al*., 2016), exemplified by modified yeast degradation of wood xylose for biofuel as discussed in the German Academies statement (Leopoldina *et al*., 2015).

- Modified yeasts may also be employed in food production, for example to enhance flavour in beer, but again there are implications for doing this according to whether the edited-yeast beer would be counted as a GM food (Callaway, 2016b).

- Potential opportunities for microbial modification in bioremediation, although uses of modified microbes outside contained facilities may raise environmental concerns.

- Genome editing of microbes in contained use to underpin novel approaches to generating pharmaceuticals or other high-value chemicals (Smanski *et al*., 2016), potentially reinvigorating drug discovery pipelines and establishing new routes for synthesising complex chemicals. For example, editing to increase mevalonate production in yeast facilitates a key step in synthesising anticancer drugs (Jakociunes *et al*., 2015).

- Application of CRISPR–Cas may also be valuable in generating novel antimicrobial agents, conferring abilities to avoid drug resistance and the indiscriminate killing of harmless, or even beneficial, bacteria (Citorik *et al*., 2014; Barrangou and Doudna, 2016). As well as novel antivirals and antibacterials, there are opportunities for vaccines and drug discovery to tackle intracellular parasites such as *Plasmodium* and *Toxoplasma* species (Carrasquilla and Owusu, 2016).

- Potential applications of edited microbes as sensors of human disease signals such as inflammation (Tauxe, 2015).

- Enabling the recording of defined biological events into stable genetic memory, with proof of principle demonstrated for CRISPR–Cas-edited *Escherichia coli* (Shipman *et al*., 2016). Expanding DNA data storage capacity provides a strategy to generate intrinsic devices within various cells that autonomously record the timing of complex and inaccessible processes such as gene dysregulation in cancer. Linking DNA memories with the power of cells to sense and act on their environment could lead to considerable advances in synthetic biology (Borkowski *et al*., 2016).

4.1.1 Synthetic biology

Many of the regulatory issues for microbial genome editing research and innovation fall within the scope of what is regarded as synthetic biology. A previous EASAC report on synthetic biology (2010) covers some relevant general points for regulation, codes of conduct, models of open science and benefit-sharing, skills development and the EU bioeconomy, although it predates the newest phase of genome editing. In the Working Group's opinion, the advance of genome editing does not alter the conclusions reached in that earlier report. The European Commission's scientific committees recently completed their advice on synthetic biology following extensive public consultations (SCENIHR, SCHER and SCCS 2016). This advice covers microbial genome editing, concluding, with respect to environmental risks, that the increasing speed of modification of micro-organisms by genome editing might pose challenges to risk assessment capacity while not in itself creating new risks. The recent discussions in the Convention on Biological Diversity (CBD) about synthetic biology have also encompassed genome editing, including gene drive (UNEP, 2015),

within the terms of the operational definition[30]. These ongoing CBD discussions are highly relevant to global governance of the environmental aspects of genome editing.

4.2 Biosafety

Concerns have been raised about the possibility that genome editing research could be conducted outside regulated laboratory settings, for example by 'biohackers' in the DIY biology community (Ledford, 2015a). The equipment and reagents are readily available but there is no evidence that genome editing is much used yet by DIY biologists (Kuiken, 2016). Moreover, it has been said that there is no *a priori* reason to expect the DIY community to cause more harm when using genome editing than anyone else, and DIY biologists must similarly conform to established biosafety legislation. The DIY community has been active in developing norms and a code of conduct[31] to support a proactive culture of personal responsibility (Kuiken, 2016). EASAC supports a proposal made in previous IAP discussion that the Global Young Academy (https://globalyoungacademy.net) should assess the issues for DIY research that is being conducted outside conventional laboratory settings.

4.3 Biosecurity

In the recent report by the Nuffield Council on Bioethics (2016), the technology of genome editing was described as transformative. While it can be argued that this might be the case for the modification of eukaryotic organisms, in the view of the Working Group genome editing merely augments and simplifies technology already available for the modification of microbes. Therefore, it is questionable to what extent it leads to new concerns about deliberate misuse of genome editing in state-sponsored research or for terrorism. For example, the recent annual threat assessment of the US intelligence community[32] includes genome editing in a discussion of weapons of mass destruction and proliferation, observing, '*Given the broad distribution, low cost, and accelerated pace of development of this dual-use technology, its deliberate or unintentional misuse might lead to far-reaching economic and national security implications*'. PCAST, the group of science and technology advisers to the US President, recently recommended developing a new biodefense strategy, in part because of perceived dangers posed by new technologies such as CRISPR[33].

It remains to be ascertained whether microbial genome editing raises significant new issues for harm to human, animal or plant health relevant to the Biological and Toxin Weapons Convention (BWC)[34]. The accuracy of genome editing may accentuate some current issues for the scientific underpinning of the BWC. For example, article VII of the BWC stipulates that mutual aid should be given in cases of suspected attacks with modified organisms. Genome editing might, therefore, have implications for developing adequate microbial forensics to detect, characterise and track infectious disease outbreaks to distinguish between deliberately induced and natural epidemics. Examples of genome editing have been reviewed by the IAP Biosecurity Working Group[35] in their discussions of science and technology developments relevant to the BWC, and it is important that the scientific community continues to advise policy-makers during the current process of review of the BWC: EASAC aims to continue supporting discussion of biosecurity and other aspects of genome editing.

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[30] Definition recommended by CBD Subsidiary Body on Scientific, Technical and Technological Advice (https://www.cbd.int/doc/recommendations/sbstta-20/sbstta-20-rec-08-en.doc); '*Synthetic biology is a further development of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems*'.

[31] https://diybio.org/codes.

[32] Clapper JR, 9 February 2016, on http://www.dni.gov/files/documents/SASC_Unclassified_2016_ATA_SFR_Final.pdf.

[33] https://www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST/pcast_biodefense_letter_report_final.pdf.

[34] There might also be biosecurity concerns arising from intended misuse of gene drive systems (Oye *et al*., 2015; Champer *et al*., 2016), deliberately spreading human, animal or plant diseases. There have been calls for a restriction on access to information on gene drives to prevent misuse for malicious purposes (Gurwitz, 2014), but this would probably be both ineffective and counter-productive in hampering attempts to enhance biosecurity (Oye and Esvelt, 2014).

[35] See www.iapbwg.pan.pl/index.php and https://royalsociety.org/topics-policy/projects/biological-toxin-weapons-convention ('The Biological and Toxin Weapons Convention. Implications of advances in science and technology') for discussion of the issues at the Warsaw 2015 meeting.

5 Human health: somatic and germline cell applications

Applications of genome editing described in the previous chapters are all potentially relevant to human health objectives. However, much of the debate elsewhere on genome editing with regard to human health has focused on gene editing in human cells, which will be the focus of the present chapter. Considerable progress is being made in basic research in taking a genome-wide and cell-systems approach in the use of genome editing to elucidate causal linkages between genetic variation and biological function and to perform functional genomic screens (Hsu *et al*., 2014). It is not possible in our report to review all such research advances, but their breadth is illustrated in research using the CRISPR–Cas system that includes the following:

- Identification of essential genes in human cells (and tumour-specific vulnerabilities) (see, for example, Hart *et al*., 2015; Osario *et al.* 2015).
- Reprogramming of adult cells into stem cells (Howden *et al*., 2015).
- Prevention of flavivirus reproduction without disrupting the host (Zhang *et al*., 2016).
- Studying the influence of epigenetics on regulatory functions and cellular reprogramming (Ledford, 2015b; Kungulovski and Jettsch, 2016) including in the brain (Bailus *et al*., 2016).

When considering these opportunities and the requisite regulatory framework, there is critical need to distinguish between the use of genome editing in the basic research context and in the clinical application, and between its use in somatic cells and in germline cells. However, one general problem perceived when reviewing country policies towards genome-related technologies (Isasi *et al*., 2016) is the vagueness encountered in basic definitions and in distinguishing between clinical and research applications. For example, in some countries there is considerable uncertainty about whether existing bans on genetic engineering in embryos and other germline cells for clinical purposes also encompass prohibition to conduct basic research (Isasi *et al*., 2016). The conclusions from the FEAM workshop (Academy of Medical Sciences, 2016; FEAM, 2017) also emphasised the need to develop and share common definitions: for example, the definition of 'embryo' varies across Europe, which may relate to varying value assumptions.

As emphasised in the Statement by the German Academies (Leopoldina *et al*., 2015), support for putative applications in human germline interventions that have an impact on the genome of offspring requires more research both to understand complex interactions between genes and to understand the molecular mechanisms involved in editing, in order to increase efficiency, selectivity and safety. As discussed in the Statement of the Hinxton Group (2015; an international, interdisciplinary consortium on stem cells, ethics and law), safety research is important to clarify both the extent and impact of off-target events (unintended genetic alterations)[36], interaction between individual gene functions, and mosaicism (genetic variation across cells). This knowledge is required to improve even further the fidelity of genome editing. Such research also requires improving *in silico* tools to predict off-target effects and whether they are likely to be deleterious, and to guide design in genome editing.

Recent findings in the USA from a Pew Research survey (Funk *et al*., 2016) epitomise the current mix of excitement and concern in the general public. Almost 70% of respondents to the survey said that they were 'very' or 'somewhat' worried about use of genome editing technologies *in utero* to reduce a child's risk of serious disease, with about 50% indicating they were enthusiastic about such a use: three in ten respondents were both enthusiastic and worried. Patient-group representatives in Europe are eager to see genome editing progress (FEAM, 2017). For example, in a survey by Genetic Alliance UK, more than 75% of respondents, those with a genetic condition or family members, supported the use of genome editing technology but made a clear distinction between tackling medical conditions (where it was supported) and the enhancement of physical or cognitive attributes in healthy people (where it was not supported)[37].

5.1 Slippery slope, risk and proportionality

Does genome editing represent a 'slippery slope'? In general terms, the slippery slope describes how a technology may be introduced that seems morally acceptable or even laudatory in dealing with a problem but the technique is then extended to further areas or problems, ending up by application in a way that is

[36] Recent research (see Box 1 and Kleinstiver *et al*., 2016; Slaymaker *et al*., 2016) suggests that engineered Cas9 nucleases as alternatives to CRISPR–Cas9, for example eSpCas9, SpCas9–HF1, may significantly reduce 'off-target' editing. Furthermore, recent research has crafted a genome editing 'toolbox' capable of targeting multiple genes while limiting unintended effects, by turning the Cas9 system off once it has accomplished its intended task and before editing off-target sites (Cao *et al*., 2016)

[37] 'Genome editing technologies: the patient perspective', 23 November 2016; on https://www.geneticalliance.org.uk/media/2623/nerri_finalreport15112016.pdf.

morally objectionable. To prevent this, some contend that the technique should not be applied in the first instance. However, this argument is based on two assumptions: (1) that the slope is slippery such that extension of the technique cannot be prevented; and (2) that the end of the slope is ethically objectionable. Therefore, it is vital to ensure that ethical evaluation of the final state is robust and it is also essential to consider whether the slope can be made less slippery. In the case of genome editing, the nature of the slippery slope may encompass the difficulty in defining multiple boundaries, between basic, translational and clinical research as well as boundaries between tackling severe or other diseases, non-therapeutic purposes (biological enhancement) and eugenics.

Assessment of risk–benefit may be an important factor in deciding whether to embark on what may be perceived as a slippery slope, and in knowing when to stop. However, there are again difficulties in terminology in assessing risk and benefit, as discussed in other contexts in previous EASAC work (EASAC, 2015b). 'Risk' is sometimes used synonymously with 'negative outcome', sometimes with 'the likelihood of a negative outcome'. Furthermore, because of gaps and uncertainties in our knowledge, the comparison of risk and benefit may involve incommensurate elements. Multi-stakeholder dialogue is one way of assessing the risks and benefits while taking account of differing perspectives in valuing risk and benefit, but such assessment should not be made 'once and for all'—we must be prepared to revise assessments if the evidence or values change.

The need to apply the principle of proportionality when considering risks of emerging technologies, and the relationship between the proportionality and precautionary principles, is discussed in detail by Hermeren (2012). As noted by EASAC work in other contexts (EASAC, 2015b), if considering applying the precautionary principle, it is equally necessary to understand the risks of not embarking on new work, namely the benefits that may be lost to society by deterring research and innovation. When assessing the proportionality of an approach, three questions should be asked (Hermeren, 2012):

1. Is the approach relevant to bring about or help achieve the goal?

2. Is it the most favourable option; that is, could there be a less controversial or risky means to attain the goal?

3. Are the means excessive in relation to the intended goal?

Although these considerations may be relevant to all applications of genome editing, when interpreted in terms of human health outcomes they may be regarded as most tangible for human-cell editing. Thus, the issues are raised here as the prelude to discussion of human-cell modification.

5.2 Biomedicine/somatic changes

Potential somatic cell applications include gene- and cell-based therapies. The new approach to gene therapy has expected advantages over previous, vector-mediated, gene delivery, for example by circumventing concerns about the safety of the viral vector. Further detail on the range of clinical research in somatic cells (and in autologous induced pluripotent cells) is provided in the FEAM review of the current landscape (Academy of Medical Sciences, 2016) and the outputs from the International Summit on Human Gene Editing (National Academies, 2016a) and the FEAM workshop (FEAM, 2017).

One of the first clinical examples of genome editing (using the ZFN technique) was modification of the *CCR5* gene in T cells to treat patients with HIV. A subsequent example, the treatment of a child with acute lymphoblastic leukaemia using TALEN-modified donor immune cells, has aroused significant public interest. Research is now moving from the study of individual responses to controlled clinical trials.

Although *in vivo* human genome editing trials started in 2016, for example on factor IX therapy of haemophilia B (Reardon, 2015a; and see Academy of Medical Sciences, 2016), it is currently easier to envisage *ex vivo* treatment (modification of the patient's cells in the laboratory and returning them after propagation to the patient) because direct delivery of genome editing tools to tissue within the body presents challenges for specific and efficient targeting (Caroll and Charo, 2015). The first phase I CRISPR–Cas9 trial has started in China, enrolling patients with metastatic non-small-cell lung cancer (where chemotherapy, radiation therapy and other treatments have failed) (Cyranoski, 2016). T cells are extracted from the blood of enrolled patients and CRISPR–Cas9 is used to knock out the gene that encodes PD-1 protein (normally acting as a constraint on the cell's capacity to launch an immune response) before returning the T cells to the patient. A related study proposal (to treat myeloma, melanoma and sarcoma, but with other edited modifications in addition to PD-1 knockout) has been approved in the USA by the National Institutes of Health (NIH) Recombinant DNA Research Advisory Committee.

In the EU, the general regulatory procedures for such clinical research are clear. The European Commission Regulation (EC) 1304/2007 on advanced therapy medicinal products (gene, cellular and tissue based) sets out EU requirements for therapies and standards for

clinical trials. A single centralised assessment procedure run by the EMA covers safety, efficacy and quality of products developed: further detail of the regulatory frameworks is provided in the FEAM review (Academy of Medical Sciences, 2016). However, the output from the FEAM workshop (FEAM, 2017) noted that preparation of specific regulatory guidance would require ongoing dialogue between regulators and researchers from both the academic and commercial sectors.

As with other innovation in healthcare, these advances raise questions as to whether benefits will be distributed equitably (or differently from existing treatments) and in what ways the interests of people in vulnerable groups may be affected.

5.3 Reproduction/germline changes

Genome editing of the germline (this includes germ cells and early embryos) has potential applicability to avoid inherited genetic disease. Although there are already some other options for preventing familial disease – in particular pre-implantation genetic diagnosis – there are circumstances in which these other, established, methods would not be effective (Nuffield Council on Bioethics, 2016). Monogenic diseases may individually be rare but in aggregate there are many thousands of rare diseases (www.omim.org) and the WHO estimates that the prevalence of all single-gene disorders at birth is approximately 1% worldwide[38].

Making human genetic changes heritable is not currently allowed by national legislation in any Member State[39], nor may it be financed by EU research. The European Commission should, nonetheless, take note of what is being discussed and proposed outside the EU.

Recent Chinese research on human embryos, including modification of the gene of beta-globin responsible for the blood disorder beta-thalassemia via the CRISPR–Cas 9 system (see Academy of Medical Sciences, 2016 for further detail), has stimulated extensive discussion on what research and applications should be allowed. There have been various proposals for a moratorium, for example, from the United Nations Educational Scientific and Cultural Organization (UNESCO) International Bioethics Committee[40], and the FEAM review (Academy of Medical Sciences, 2016) provides a comprehensive account of European and other international statements on human germline genome editing. The European Group on Ethics in Science and Technology (EGE, 2016) also concludes that there should be a moratorium on gene editing of human embryos or gametes that would result in the modification of the human genome. The EGE cautions on whether a clear distinction can be made between basic and translational research, and this difficulty in defining boundaries has implications for what research may be permitted or would fall within the scope of a moratorium.

The German Academies statement (Leopoldina *et al*., 2015) endorses suggestions for an international moratorium on all forms of human germline engineering that could have an impact on the genome of offspring. From these Academies' perspective, the moratorium would provide an opportunity to discuss unresolved questions and develop recommendations for regulation, but it should not constitute a general restriction on methodological developments and limit any promising new genome editing approaches. In some EU Member States, research can be conducted on germ cells and human embryos up to 14 days

Box 4 Some ethical considerations in human germline applications

1. Safety.
2. Dignity, with regard to the boundary between treatment and design. Although this distinction is not always clear cut, designing enhanced functions might be perceived to jeopardise the genetic integrity of all human beings, bring concerns for the welfare of the child, and may accentuate equity and proportionality concerns.
3. Justice, with regard to equity in the sharing of benefits.
4. Proportionality: see section 5.1.
5. Autonomy: the right of individuals to decide as long as nobody else is harmed.

Sources: UNESCO, Hinxton Group and European Group on Ethics in Science and Technology, Nuffield Council on Bioethics[41] and EASAC Working Group discussion.

[38] http://www.who.int/genomics/public/geneticdiseases/en/index2.html.

[39] UK regulations allowing mitochondrial replacement therapy, to correct faulty mitochondrial DNA, came into force in October 2015. However, in the passage of the enabling regulations, the government minister explicitly asserted that the UK Government did not regard the procedures as producing 'genetic modification' (Earl Howe, Hansard, HL Deb 5 February 2015; cited as footnote 181 in Nuffield Council on Bioethics, 2016).

[40] 'Updating its reflection on the human genome and human rights' calls for a moratorium on germline applications and hereditary modifications. In surveying the legislative position worldwide, 29 of 39 countries reviewed by UNESCO had a ban on editing the human germline. In 25 countries, the ban was legally binding, 4 had guidelines, not laws (China, Japan, Ireland, India) while rules in the remaining 10 countries were ambiguous.

[41] In their wide-ranging analysis, the Nuffield Council on Bioethics (2016) broadly identify additional key moral perspectives that inform attitudes to different potential applications of genome editing. These include the following: science as a moral enterprise, moral conservatism, and moral norms and human rights (see their report for further detail).

after fertilisation of the egg cells[42], when justified and supported by rigorous scientific and ethical review. After this period, embryos are discarded and there are no genetically engineered offspring. Genome editing research in human embryos is now approved in the UK (Academy of Medical Sciences, 2016) and Sweden (Callaway, 2016a). The recent success of research on culturing human embryos up to 13 days (Shahbazi *et al*., 2016) indicates the possibility of further research on human embryo development and may re-open the debate on whether legislation should be amended to allow embryo research *in vitro* to continue for longer than the current legal limit of 14 days.

Although ethical and legal aspects (see Box 4) are a national/local responsibility for EU Member States, the EU Clinical Trials Directive 2001/20/EC and Clinical Trials Regulation EU No. 536/2014 (effective after May 2016) include the provision '… *no gene therapy trials may be carried out which result in modifications to the subject's germ line genetic identity*'. The ethics committee of the French national biomedical research agency INSERM (Institut national de la santé et de la recherche médicale) recently called for a review of the ban on all genetic modifications to the human germline as part of a wider initiative that should also act to promote open debate on the societal aspects of genome editing technologies (Hirsch *et al*., 2017).

Although germline clinical applications are currently not allowed, further consideration of the issues for deciding future options has to take account of the wide spectrum of possible interventions: from avoidance of serious disease-causing mutations to biological enhancement. Where might the boundary be for any moral obligation to treat/avoid disease? It should also be noted (Mathews *et al*., 2015) that use of genome editing, if permitted in basic research on human sperm, eggs and embryos, could yield insight, for example, on how cell types are specified in the early human embryo, understanding biology and genetics of stem cell lines, and on the role of specific genes in the differentiation of sperm and eggs and the development of diseases.

Some germline modification objectives will be more controversial than others (even in a well-regulated context): technical and safety concerns may be resolved by scientific research, but moral considerations require ethics and other humanities research and public debate. It has been suggested (Mathews *et al*., 2015) that national academies are well placed to take the lead on efforts to ensure that debates on applications of genome editing are geographically and demographically inclusive and inform policy discussions.

Active discussion in this area raises some practical questions for the scientific and policy-making communities. It is of great importance that the issues identified in discussion on somatic and germline cell genome editing by the academy initiatives (International Summit on Gene Editing (National Academies, 2016a) and the FEAM review (Academy of Medical Sciences, 2016); FEAM, 2017; and the work by individual member academies of EASAC) should reach a wider audience. Although these academy-led activities are not yet complete, EASAC endorses the interim conclusions from the International Summit on Gene Editing (National Academies, 2016a)[43], which include the following:

- *Basic and preclinical research.* Intensive research is clearly needed and should proceed subject to appropriate legal and ethical rules and oversight. If, in the process of research, early human embryos or germline cells undergo genome editing, the modified cells should not be used to establish a pregnancy. In view of the divergent views at the national level across the EU on the acceptability of embryo research, it is acknowledged that the decision by the European Commission not to fund research on embryos will be unlikely to change at present (FEAM, 2017).

- *Clinical use: somatic gene editing.* There is need to understand the risks, such as inaccurate editing, and the potential benefits of each proposed genetic modification. These applications can and should be appropriately and rigorously evaluated within existing and evolving regulatory frameworks for gene therapy—in the EU by the EMA and national agencies.

- *Clinical use: germline interventions.* These applications pose many important issues,

[42] Making genetic changes in early embryos, for example to study disease processes or to improve outcomes of in vitro fertilisation depends on the law of the Member State. Where it is allowed, it is subject to rigorous scientific and ethical review. Research on surplus embryos is allowed (normally to a 14-day limit) in 16 Member States, forbidden in 4 and undefined in 8: answer given by Commissioner Moedas to question in the European Parliament, E-003329/2016; 28 June 2016; http://www.europarl.europa.eu/sides/getDoc.do?type=WQ&reference=E-2016-003329&language=EN. Where such research is permitted, the use of research material in humans even for treating patients is expressly prohibited. Most Member States have ratified the Oviedo Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine which, *inter alia*, prohibits intentional human germline modification and the creation of human embryos for research purposes. The FEAM review (Academy of Medical Sciences, 2016) provides a comprehensive account of the current situation in Member States with respect to national regulatory frameworks covering the use of embryos in genome and other research.

[43] After the first Summit in December 2015, the National Academies have organised further meetings (February–July 2016) to engage with stakeholder groups, discuss governance perspectives, the issues for race and genetics in US history and the intersection of moral views and public policy. Material from the presentations in these subsequent discussions is available on http://nationalacademies.org/gene-editing.

including the risks of inaccurate or incomplete editing, the difficulty of predicting harmful effects, the obligation to consider implications for both the individual and future generations who will carry the genetic alterations, and the possibility that 'enhancements' to subsets of the population could exacerbate social inequities or be used coercively. It would be irresponsible to proceed unless and until the relevant safety and efficacy issues have been resolved and there is broad societal consensus about the appropriateness of the proposed application.

- *Need for an ongoing forum.* The international community should strive to establish norms for acceptable uses of human germline editing and to harmonise regulation. EASAC welcomes the opportunity to help in taking the discussion forward and engaging with additional audiences.

6 Conclusions and recommendations

Genome editing, the deliberate alteration of a selected DNA sequence in a cell, is a very important toolset in fundamental research to understand biological processes and disease. Genome editing has been described by some (for example, the Nuffield Council on Bioethics, 2016) as a transformative technology and, certainly, in some areas of research and innovation, it is transforming expectations and ambitions. Genome editing has the potential to deliver precise nucleotide changes. Taken together with the growing ability to monitor and avoid off-target effects, it brings new opportunities within range. Because of its general applicability (in microbes, and plant, animal and human cells) it has a very wide range of potential uses to tackle societal objectives and to accelerate innovation in the bioeconomy. These potential applications include gene- and cell-based therapies to control diseases and in reproduction to avoid the inheritance of disease traits, the control of vector-borne diseases, improved crop and livestock breeding, including improved animal health and welfare, modifying animal donors for xenotransplantation, and industrial microbial biotechnology to generate biofuels, pharmaceuticals and other high-value chemicals, among other possibilities.

Present knowledge gaps and uncertainties emphasise the need for more basic research. We expect that research advances will fill many of the knowledge gaps referred to previously in our report and that progressive refinement of genome editing tools will further increase their efficiency and specificity, thereby reducing off-target effects. Given the increasingly widespread use of genome editing, the research community should consider how best to maintain an accessible database of modifications undertaken – although it would be a challenge to be comprehensive – and what the necessary quality control procedures are to inform future research. We anticipate that the fast pace of change in research and innovation will continue, and EASAC is willing to return to the subject of this report in due course to review our assessments.

EASAC concludes that policy considerations should focus on the applications in prospect rather than the genome editing procedure itself as an emerging technology. It is important to ensure that regulation of applications is evidence-based, proportionate and sufficiently flexible to cope with future advances in the science. In the following paragraphs we summarise our main sector-specific recommendations from the preceding chapters and add some general conclusions.

Plants

The increasing precision now possible in plant breeding represents a big improvement compared with conventional breeding approaches relying on random, uncontrolled chemical- or radiation-induced mutagenesis and on intra- or interspecific crossings with random distribution of genes or alleles. We reaffirm our recommendations from the previous EASAC work on new plant breeding techniques:

- We ask that EU regulators confirm that the products of genome editing, when they do not contain DNA from an unrelated organism, do not fall within the scope of GMO legislation.

- There should be full transparency in disclosing the process used, but the aim in the EU should be to regulate the specific agricultural trait/product rather than the technology by which it is produced. It follows that new technologies would be excluded from regulation if the genetic changes they produce are similar to, or indistinguishable from, the product of conventional breeding and if no novel, product-based risk can be identified.

Animals

Research on animals is already subject to stringent regulation and it should be appreciated that genome editing brings opportunities to enhance animal health and welfare as well as to improve agricultural traits. With regard to specific applications, we recommend the following:

- Livestock breeding should also be governed by the same principle as proposed for plant breeding—to regulate the trait rather than the technology and be open and explicit about what is being done.

- With regard to the modification of animals to serve as a source for xenotransplantation, EU regulators should actively prepare for the new opportunities coming into range: this may require further discussion of the mechanism for approving medical products relating to cells and tissues, together with assessment of the implications of whether the edited donor is regarded as a GMO or not.

Gene drive to modify populations in the wild

EASAC supports the recommendations recently published by the US National Academies:

- It is essential to continue the commitment to phased research to assess the efficacy and safety of gene drives before it can be decided whether or not they will be suitable for use.
- This research must include robust risk assessment and public engagement.
- EU researchers must continue to engage with researchers and stakeholders in the countries where gene drive systems are most likely to be applied.

Micro-organisms

- Genome editing in microbes does not raise new issues for regulatory frameworks and is currently subject to the established rules for contained use and deliberate release of GMOs.
- There is a wide range of potential applications, including pharmaceuticals and other high-value chemicals, biofuels, biosensors, bioremediation and the food chain. It is important to recognise this wide range when developing EU strategy for innovation in the bioeconomy.
- Many of the policy issues for microbial genome editing research and innovation fall within the scope of what is regarded as synthetic biology and we reaffirm the general recommendations from previous EASAC work (EASAC, 2010; and discussed further in the global context on http://www.interacademy.net/File.aspx?id=23974d). These previous recommendations for synthetic biology covered issues, for example, for building research capacity and delivering training on interdisciplinary skills in higher education.
- Concerns have been raised elsewhere about the potential for genome editing research to be conducted outside regulated laboratory settings. We recommend that the Global Young Academy should assess the issues raised by the expansion of the DIY biology community.
- Concerns have also been expressed elsewhere about the potential biosecurity implications of genome editing. We recommend that the scientific community continues to inform and advise policy-makers during review of the BWC.

Human-cell genome editing

EASAC endorses the emerging conclusions from the other collective academy work (International Summit on Gene Editing and FEAM) and the initiatives by individual national member academies:

- *Basic and clinical research.* Intensive research is needed and should proceed subject to appropriate legal and ethical rules and oversight. If, in the process of research, early human embryos or germline cells undergo genome editing, the modified cells should not be used to establish a pregnancy. EASAC recognises that the decision by the European Commission not to fund research on embryos will be unlikely to change at present.
- *Clinical use: somatic gene editing.* There is need to understand the risks such as inaccurate editing and the potential benefit of each proposed genetic modification. These applications can and should be rigorously evaluated within existing and evolving regulatory frameworks for gene and cell therapy by the EMA and national agencies.
- *Clinical use: germline interventions.* These applications pose many important issues including the risks of inaccurate or incomplete editing, the difficulty of predicting harmful effects, the obligation to consider both the individual and future generations who will carry the genetic alterations, and the possibility that biological enhancements beyond prevention and treatment of disease could exacerbate social inequities or be used coercively. It would be irresponsible to proceed unless and until the relevant scientific, ethical, safety and efficacy issues have been resolved and there is broad societal consensus.

General recommendations for cross-cutting issues

- *Public engagement.* There has to be trust between scientists and the public, and, to build trust, there has to be public engagement. As observed in the previous chapters, stakeholders (such as patients, clinicians, farmers, consumers and NGOs) need to be involved in discussions about risk and benefit, and scientists need to articulate the objectives of their research, potential benefits and risk management practices adopted. This is not a special responsibility for genome researchers, as all scientists have the responsibility to be open and candid about

their work (IAP–IAC, 2012; Nuffield Council on Bioethics, 2016). There is need for additional social science and humanities research to improve public engagement strategies.

- *Enhancing global justice.* As noted previously, there may be risk of increasing inequity and tension between those who have access to the benefits of genome editing applications and those who do not, although the widespread adoption of the technique might facilitate sharing of the benefits. The scientific community must work with others on the determinants to narrow the societal gap: for example, by active knowledge transfer, collaboration between researchers worldwide, open access to tools and education, and education efforts. It is also vital for EU policy-makers to appreciate the consequences, sometimes inadvertent, of EU policy decisions on those outside the EU. There is evidence that previous decisions in the EU (for example, on GMOs) have created difficulties for scientists, farmers and politicians in developing countries (EASAC, 2013). Reforming current regulatory frameworks in the EU and creating the necessary coherence between EU domestic objectives and a development agenda on the basis of partnership and innovation is important for developing countries as well as for Europe.

Appendix 1 Working Group composition and procedures

The report was prepared by consultation with a Working Group of experts acting in an individual capacity and nominated by member academies of EASAC:

Volker ter Meulen (Chair, Germany)
Austin Burt (UK)
Baerbel Friedrich (Germany)
Goran Hermeren (Sweden, nominated by ALLEA)
Wlodzimierz Krzyzosiak (Poland)
Cecilia Leao (Portugal)
Joseph Martial (Belgium)
Bert Rima (Ireland)
Radislav Sedlacek (Czech Republic)
Bruno Studer (Switzerland)
Miikka Vikkula (Belgium, nominated by FEAM)
Kirmo Wartiovaara (Finland)
Anna Wedell (Sweden)
Detlef Weigel (Germany)
Robin Fears (secretariat, UK)

The Working Group met in June and October 2016 in Brussels, together with external guests Johannes Fritsch (Germany) and, at the first meeting Tim Sykes (Switzerland, in place of Bruno Studer), and at the second meeting with Angelika Schnieke (Germany) and Siegrid Weiland and Jeremy Bray (European Commission Scientific Advice Mechanism). EASAC thanks the Working Group members and guests for their insight, commitment and support, and thanks members of the EASAC Biosciences Steering Panel for their advice and guidance.

The draft report was subject to peer review by experts nominated by EASAC member academies.

Abbreviations

ALLEA	All European Academies
APHIS	Animal and Plant Health Inspection Service
BSE	Bovine spongiform encephalopathy
BWC	Biological and Toxin Weapons Convention
Cas	CRISPR-associated protein nuclease
CBD	Convention on Biological Diversity
CRISPR	Clustered regularly interspersed palindromic repeats
DIY	Do-It-Yourself
DNA	Deoxyribonucleic acid
EASAC	European Academies' Science Advisory Council
EGE	European Group on Ethics in Science and Technology
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
FEAM	Federation of European Academies of Medicine
GM	Genetically modified
GMO	Genetically modified organism
HDR	Homology-directed repair
HIV	Human immunodeficiency virus
IAP	InterAcademy Partnership
ILAR	Institute for Laboratory Animal Research
INSERM	Institut national de la santé et de la recherche médicale
IUCN	International Union for Conservation of Nature
NGO	Non-governmental organisation
NHEJ	Non-homologous end-joining
NIH	National Institutes of Health
ODM	Oligonucleotide-directed mutagenesis
OECD	Organisation for Economic Co-operation and Development
PAM	Protospacer adjacent motif
R&D	Research and development
RNA	Ribonucleic acid
SSN	Site-specific nuclease
TALEN	Transcription activator-like effector nuclease
UNESCO	United Nations Educational Scientific and Cultural Organization
WHO	World Health Organization
ZFN	Zinc finger nuclease

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For further information:

EASAC Secretariat
Deutsche Akademie der Naturforscher Leopoldina
German National Academy of Sciences
Postfach 110543
06019 Halle (Saale)
Germany

tel +49 (0)345 4723 9833
fax +49 (0)345 4723 9839
secretariat@easac.eu

EASAC Brussels Office
Royal Academies for Science and the
Arts of Belgium (RASAB)
Hertogsstraat 1 Rue Ducale
1000 Brussels
Belgium

tel +32 (2) 550 23 32
fax +32 (2) 550 23 78
brusselsoffice@easac.eu

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