CRISPR & Animals

Implications of Genome Editing for Policy and Society

Policy Report

CGM/180501-01

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Design: Avant la lettre, Utrecht

Infographics: Fallen Serenity Productions

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DATUM 01 May 2018
KENMERK CGM/180501-01
ONDERWERP Policy report 'CRISPR & Animals:
Implications of Genome Editing for Policy and Society'

Dear Mrs Van Veldhoven,

Please find attached the policy report 'CRISPR & Animals: Implications of Genome Editing for Policy and Society'.

Summary:
This report describes the scientific developments and policy implications of genome editing in animals. Genome editing techniques such as CRISPR-Cas can be used to make small or large changes at specific locations in the DNA of animals with ease and efficiency. Potential applications are in farm animals, pets and laboratory animals as well as in medicine (xenotransplantation) and population control (gene drives in insects and animals in the wild, and even bringing back extinct animal species). Environmental and ecosystem applications are probably not geographically restricted and will require international consultation and cooperation.

Given the international character of scientific research and the trade in animals and their gametes and in products of animal origin, it is inevitable that the Netherlands, along with Europe as a whole, will be confronted with the consequences of these techniques. A complicating factor is that changes in the DNA of organisms made using the CRISPR-Cas technique are difficult or impossible to detect and distinguish from naturally occurring mutations. This presents challenges both to policy (detection and regulatory enforcement) and to society (consumer choice). Besides benefiting people, some new applications may bring relative benefits or harm the animals concerned. This can alter the balance of risks, benefits and ethical considerations.

Given the accelerating pace of technological change, the government and stakeholders should waste no time in adopting a position on the possible importation of genome-edited animals and products derived from them. For this they must first consult with scientists, breeders, industry and societal stakeholders. In this policy report COGEM gives advice and suggestions on organising this process.

The full text of the report is attached.

Yours sincerely,

Professor Sybe Schaap
Chair of COGEM

c.c. H.P. de Wijs, Head of the GMO Office
J.K.B.H. Kwisthout, Ministry of Infrastructure and Water Management

Summary

Genome editing techniques such as CRISPR-Cas can be used to make small or large changes at specific locations in the genetic material (DNA) of organisms (targeted or site-directed mutagenesis) with ease and efficiency. These emerging genome editing techniques remove a number of technical barriers to modifying the genetic makeup of plants, animals and humans, shifting attention to the ethical and governance issues, such as the possibility in future of human genome editing, about which COGEM published a policy report in 2017.

Genome editing has also given a new impetus to animal biotechnology. It opens up a broad range of potential applications in farm animals, pets and laboratory animals as well as in medicine, such as xenotransplantation, and the control of insect and animals populations in the wild.

Most of the work on genome editing techniques in animals is being done outside Europe. Given the international character of scientific research, animal breeding and the trade in animals, animal gametes and animal products, it is inevitable that the Netherlands, along with Europe as a whole, will at some time be confronted with the consequences of these techniques. A complicating factor is that changes in the DNA of organisms made using the CRISPR-Cas technique are often difficult or impossible to detect and distinguish from naturally occurring mutations. This means that thought must be given to the political, policy, professional, scientific and societal implications of these developments in the broadest possible terms.

Applications of genome editing in animals are highly diverse

The aim of most genome editing applications in farm animals (cows, pigs, goats, sheep and chickens) is largely the same as in current selective breeding practices: increasing yields, conferring disease resistance and improving product quality. A number of genome editing applications are almost ready for market. Some are, in principle, relevant to Dutch livestock farming (such as those for increasing yields and improving product quality) or are even urgently needed (such as those for disease resistance). However, introducing a new trait into existing populations (while necessarily retaining variability and other carefully selected traits) is expected to take at least five to ten years from the first introduction. Besides, obtaining authorisation for placing on the market of genome editing in animals is itself an unpredictable process. No market authorisations have been granted for genetically modified (GM) animals in Europe. In the United States the authorisation process for the first GM animal for food production purposes (a GM salmon) took almost 20 years.

Laboratory animals are used in scientific research and safety studies around the world. Making specific and more complex disease models in laboratory animals for research purposes is easier, quicker and cheaper using genome editing techniques. This could result in an increase in the absolute number of GM laboratory animals used or a shift towards the use of alternative species as test animals. Also, differences in legislation between jurisdictions may encourage 'moral freeriding': making use in the Netherlands of products, applications or information from experiments that are themselves not permitted in the Netherlands.

Genome editing has also given new impetus to research into xenotransplantation – the transplantation of organs from animals to humans – by opening up possibilities for resolving some technical problems surrounding immune reactions and the possible transmission of animal diseases. These developments are still at an early experimental stage and no indication can be given of when clinical and commercial applications may be expected. Work is also progressing on new techniques, such as growing human organs in animals (chimerism) so that they can later be transplanted to a patient. In both cases, the use of animals for the production of organs for transplantation raises moral as well as technical issues. Clinical xenotransplantation is prohibited in the Netherlands as long as there are insufficient guarantees that the techniques are safe. Should these applications become available elsewhere and the prohibition on their use in the Netherlands remains in force, medical tourism will become a distinct possibility.

Genome editing makes it easy to create efficient gene drive systems. Gene drive techniques increase the rate of inheritance of certain traits so that, in principle, all offspring possess the introduced trait. In theory, gene drives can be used as tools for ecological engineering and population control, not only to combat infectious diseases, pests and invasive exotics, but also to restore and preserve endangered or other species. In some cases, these techniques may offer efficiency and animal welfare advantages over existing practices, for example as alternatives to controlling invasive exotics with poisons or traps. On the other hand, there are concerns about possible negative, unforeseen or irreversible consequences at the population and ecosystem levels, as well as potential cross-border environmental impacts. Using gene drives to control invasive exotics that are not considered to be pests in other countries, such as their country of origin, could therefore be problematic. National policy obviously has its limitations and international cooperation on the use of gene drives is required.

Genome editing also offers possibilities (although still theoretical) for reintroducing extinct animal species and the restoration or improvement of endangered animal species. Using these techniques to 'resurrect' extinct animal species is a niche application that is still in the exploratory research phase. Such applications are controversial and some consider that other areas of research should be given higher priority for funding. The ability to reverse the extinction of animal species could also undermine the perceived urgency of nature conservation efforts.

New applications alter the substance of the public debate

Genetic modification of animals is not new, but genome editing will bring forward the realisation of applications that have been talked about for a long time. The discussions about such applications and the questions they raise are therefore now no longer purely academic, making it necessary to adopt a position and decide on the direction future developments should take. The issues involved are: whether or not genome editing in animals for purposes such as conferring disease resistance and increasing yields is desirable, or indeed necessary; the changing uses of laboratory animals; the further instrumentalisation of farm animals; and the use of animals for organ transplantation (xenotransplantation). Moreover, genome editing is not limited to domesticated animals, which are usually kept in controlled environments, such as a laboratory or production facility. Applications are also being developed to control wild populations and reintroduce animal species that are not restricted to the level of the cell or organism, but operate at the system level. This adds a whole new dimension to the issues surrounding the use of animals, such as telos (the goal or purpose of an animal), integrity, naturalness, instrumentalisation, animal welfare and alternatives to existing practices, for example in livestock farming.

Scope of national legislation on animals limited in an international context

The laws and regulations relevant to genome editing in animals include specific rules on genetic modification and general regulations on the use of animals for various purposes. The Dutch regulatory system is largely derived from EU law. Genetic modification is subject to authorisation and in the EU these activities must first be assessed for their potential risks to human health and the environment. The extent to which the legislation governing genetically modified organisms (GMOs) should or should not apply to genome editing applications is under discussion, both within Europe and elsewhere. The European Court of Justice has been asked to clarify the precise scope of the exemption from the provisions of the EU GMO Directive 2001/18 for organisms modified by mutagenesis in order to determine whether or not site-directed mutagenesis techniques, such as CRISPR-Cas, are covered by this exemption. The questions put to the Court were made in connection with site-directed mutagenesis in plants, but it is not yet known whether or not the decision will also apply to other organisms, such as animals. The decision is expected in the course of 2018.[a]

In addition, various national laws are applicable to animals. The Animals Act applies to all animals kept for commercial, hobby or other purposes and is largely concerned with animal

a This report was compiled before the judgement of the European Court of Justice was published on 25 July 2018. The Court ruled that organisms obtained by new mutagenesis techniques (such as genome editing) are subject to the obligations laid down by the GMO Directive and that these organisms are not exempted from those obligations because there is no proven safety record. This is in contrast to organisms obtained by traditional mutagenesis techniques (radiation, chemicals) which are exempted from the GMO directive.
URL: http://curia.europa.eu/juris/documents.jsf?num=C-528/16

health and wellbeing. The Experiments on Animals Act, which applies to all vertebrates and some invertebrates, aims to facilitate important scientific research and as far as possible prevent or minimise any distress to laboratory animals. The Nature Conservation Act applies to both plants and animals and aims to conserve, protect and restore wild and cultivated nature, and contains lists of endangered species and invasive exotics. Besides this legislation, in recent years various initiatives have been started with the aim of making agreements on the future of livestock breeding and other relevant activities.

Dialogue and consultation with national and international stakeholders

Developments outside the Netherlands are moving fast and it is expected that they will soon reach the Netherlands in the form of imported laboratory and other animals, or their gametes, and the products of such animals (food, medicines and other products such as leather and wool). Opinions on the use of animals and the applications of genetic modification differ widely and it is unlikely that these can be resolved into a consistent vision. Nevertheless, decisions will have to be taken on the use and regulation of genome editing in animals.

COGEM has identified six themes of relevance to either the substance or the process of the public dialogue on genome editing in animals:

1. **Speed:** International developments are moving fast and the existing technical barriers to the commercial development of GM animals are being broken down by the efficiency, accuracy, simplicity and broad applicability of CRISPR-Cas.
2. **Enforceability:** It is theoretically possible to detect genetic modifications made by genome editing, but only if it is known where in the genome to look. However, some genome editing applications cannot be detected because the modification falls within the range of natural variation within the species.
3. **Complexity**: Environmental and ecosystem applications (e.g. gene drives for controlling infectious diseases or invasive exotics) are probably not geographically restricted and so the spread of gene drive organisms will not stop at national borders.
4. **Mobilisation potential:** The combination of genetic modification, animals and ecosystem applications may lead to mounting public disquiet and debate.
5. **Naturalness:** The nature of these applications makes the idea of 'naturalness' more ambiguous in both legal and everyday contexts.
6. **Proportionality:** Some new applications not only bring benefits to people, but also relative benefits for animal welfare, while other applications facilitate further instrumentalisation of animals which can result in physical harm to the animals concerned. This can alter the balance of risks and benefits.

The Dutch government wants biotechnology and relevant government policies to meet society's needs and address its problems. Stakeholder engagement is one way it tries to achieve this, for example through public surveys and by organising meetings and focus

groups. However, these initiatives often present further challenges because such gatherings are seldom successful and do not bring proponents and opponents any closer together.

COGEM observes that the problems surrounding stakeholder engagement may not lie with the available methods, but with the stage preceding the choice of a particular method. The first phase of problem structuring and formulation is crucial for successful and productive stakeholder engagement, but often there is confusion about the goal of stakeholder participation (to refine the substance or improve the process), the facts of the matter at hand, the nature of the engagement (participation, consultation or representation) and who the stakeholders are. Transparency about the goal and the process and the importance of reciprocity between stakeholders are therefore essential conditions for success. In this policy report, COGEM makes suggestions on how to improve the process of stakeholder engagement.

Conclusions and observations

- EU and Dutch legislation cannot regulate or control international developments in genome editing in animals because some applications are so subtle that it is extremely hard or impossible to distinguish them from naturally occurring mutations. International trade and differences between regulatory systems make it almost inevitable that applications of genome editing in animals will, intentionally or otherwise, find their way into Europe, and therefore the Netherlands. This presents challenges both to policy (detection and regulatory enforcement) and to society (consumer choice).
- Besides benefiting people, some new applications may provide relative benefits or potentially harm to the animals concerned. This can alter the balance of risks, benefits and ethical considerations.
- Given the speed of technological change, the government and stakeholders must waste no time in adopting a position on the possible importation of genome-edited animals and products derived from them. For this they must first consult with scientists, breeders, businesses and societal stakeholders. In this policy report COGEM makes suggestions on organising this process. If the Netherlands wants to retain its compulsory ethical review of animal biotechnology for non-medical applications, it will be essential to re-evaluate the purpose and remit of any commission responsible for this review in the light of developments concerning genome editing in animals and the associated shifts in the public debate.
- Environmental and ecosystem applications (e.g. gene drives for controlling infectious diseases or invasive exotics) are probably not geographically restricted. This means that any EU or Dutch policy initiatives will have little effect and international consultation and cooperation will be needed when such applications become available in neighbouring countries.

Contents

1. Introduction

This policy report[b] identifies the issues and consequences of genome editing in animals for the Dutch government, researchers, professional groups and society.

1.1 Genome editing advances the creation of GM animals

The introduction of genome editing techniques (such as ZFN, TALENs and CRISPR-Cas[c]) has in recent years given a huge impetus to biotechnological research.[1,2,3,4] This applies to all fields in which genetic research and biotechnological techniques are applied, such as agriculture, industry and medicine. Genome editing is a form of genetic modification which makes it possible to efficiently, accurately and easily introduce small or large changes into the genetic material (DNA) of every type of cell in any organism (microorganisms, plants, animals and humans) (**see text box 'Genome editing and genetic modification'**). The CRISPR-Cas technology in particular has in recent years enabled rapid improvements in the efficiency and accuracy of genetic modification.

Genome editing and genetic modification

Genetic modification is the alteration of the DNA of an organism in a way that is not possible in natural reproduction or by natural recombination. This involves either the introduction of 'new' genes (insertions) or making small changes (mutations or deletions) in the DNA of an organism (**see Figure 1**).

Over the years various techniques have been developed to bring about mutations in organisms (**see Figure 2**).

In classical mutagenesis, tissues or cells are exposed to chemical agents or radiation to induce mutations. Radiation-induced mutagenesis has been in use since the 1920s. Chemical mutagens, such as ethyl methane sulphonate, were introduced later because they were easier to use and needed less advanced equipment.[5] The mutations induced by classical mutagenesis are random. Based on their long history of safe use, the products of mutagenesis were exempted from the GMO legislation when this was enacted.

Various methods have also been developed to introduce DNA sequences into cells, for example by injection, bombardment with macro- or nanoparticles coated with DNA, or in viral vectors (**see Figure 3**).

The first genetically modified (GM) mouse was created in 1981 by microinjecting DNA into the cell nucleus.[6] In 1987 a technique was developed in which target cells are bombarded at very high velocity with DNA-coated microparticles (e.g.

b This policy report is about the application of genome editing in animals. These techniques involve making genetic modifications in the gametes (germline cells) of animals so that the modifications are passed on to subsequent generations. This report does not cover modifications of somatic cells (gene therapy in humans or animals), germline editing in humans, or plant genome editing.

c Zinc Finger Nuclease (ZFN), Transcription Activator Like Effector Nuclease (TALEN), Clustered Regularly Interspaced Short Palindromic Repeat s/CRISPR-associated protein 9 (CRISPR)/Cas9), see Chapter 3.

Figure 1: Three types of alterations to DNA sequences: mutations, deletions and insertions.

gold particles). This technique, known as **particle bombardment** or **biolistics**, was mainly used in plants, but also has some applications in animals. It is hardly used nowadays. **Viral vectors** for genetic modification infect animal host cells and integrate all or part of their genome into the genome of the host cell, which results in the desired trait being expressed for longer.

With new techniques developed in recent years it is now possible to make precise, targeted changes to the genome. Nucleases (enzymes that can cut RNA or DNA) can be used to make alterations at specific locations in the host DNA. These **genome editing** techniques include 'meganucleases', ZFNs and TALENs. To make precisely targeted alterations to DNA sequences, the nucleases must bind with a specific DNA sequence, rather like a key fitting into a lock, which makes the production of site-directed nucleases very complicated and time-consuming. Around 2012 there was another breakthrough in genome editing techniques when it was revealed that CRISPR-Cas is able to cut DNA at the site of a specific sequence. The 'guide RNA' in the CRISPR-Cas complex that recognises the specific site on the DNA to be cut is easy to alter so that it recognises a new target DNA sequence, making this technique very flexible and easier to use than the other nuclease techniques.

In the past, COGEM has published various policy, advisory and other reports on genetic modification in animals.[3,7,8,9,10,11,12,13] The genetic modification of animals is not new, but genome editing will bring forward applications that have been talked about for a long time. The debate about such applications is therefore now no longer academic and positions will need to be adopted and decisions taken about future developments. The issues to be

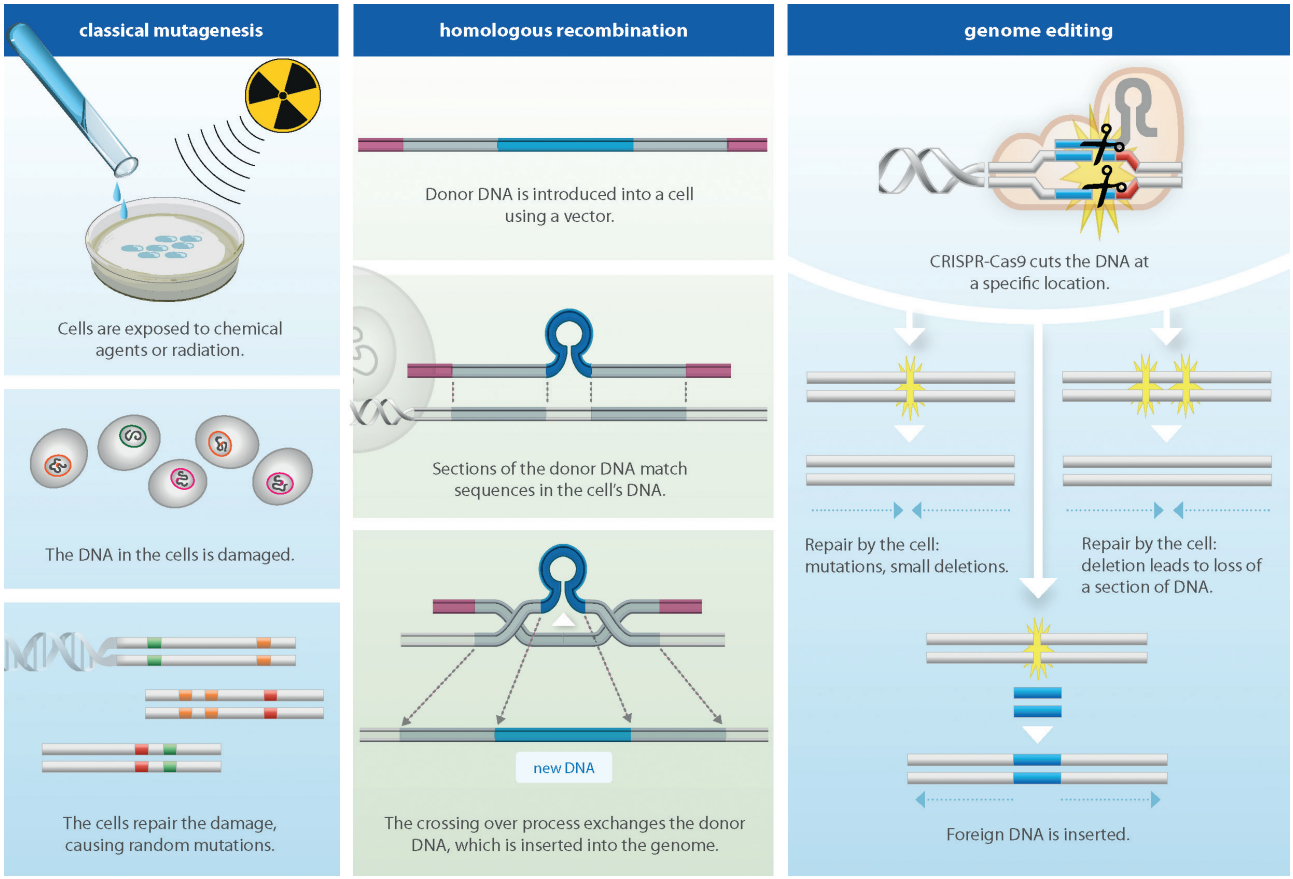

Figure 2: Different modification techniques.

addressed include whether or not genome editing in animals to introduce disease resistance and increase yields is desirable or even necessary, the changing uses of laboratory animals, the further instrumentalisation of farm animals and the use of animals for organ transplantation (xenotransplantation). Also, genome editing applications are not limited to domesticated animals held in controlled environments (e.g. a laboratory or production facility); applications are being developed that will make changes in nature at the ecosystem level by altering species populations.

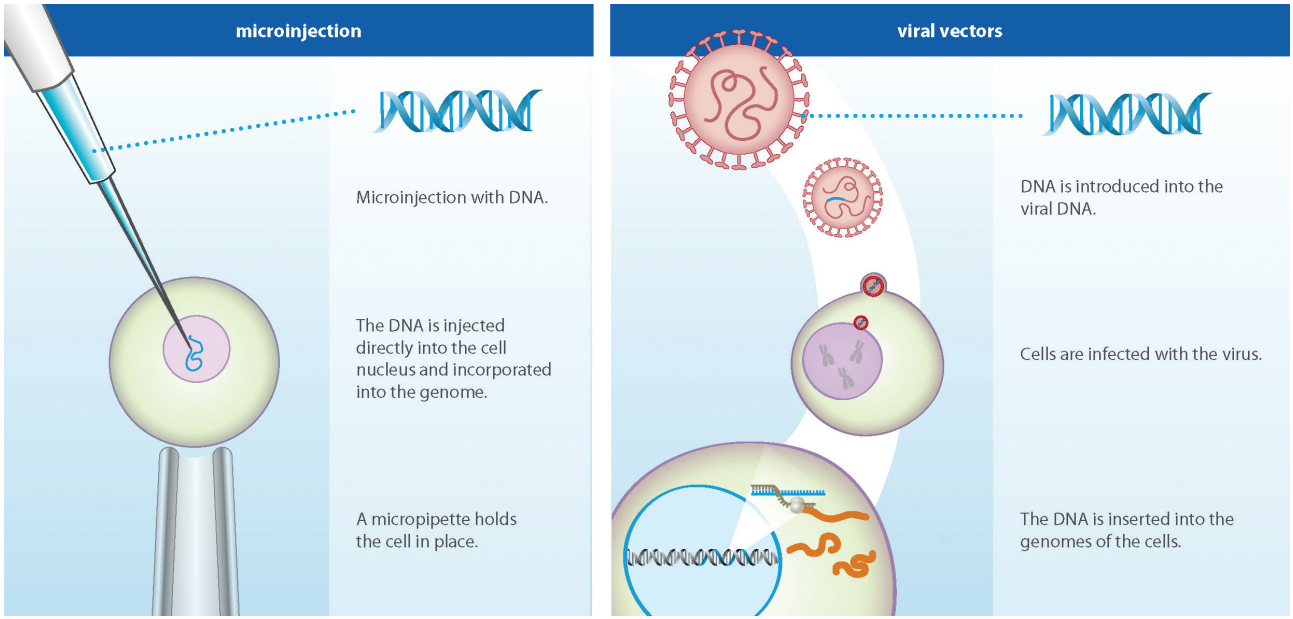

Figure 3: Methods for modifying cells (egg cells, embryonic stem cells or somatic cells) to create a GM animal.

1.2 The Netherlands permits research but not commercial applications

The Netherlands has a strict set of regulations governing biotechnology in animals[d] (**see Chapter 4**) which effectively rule out commercial applications. In the Netherlands, animal biotechnology is strictly limited to medical or other scientific research, mostly on mice and to a lesser extent on rats, zebra fish and rabbits. Just a few medicines derived from GM animals have been authorised for placing on the market in Europe, and may therefore be imported.[14,15]

Cloning animals is not genetic modification, but it does fall within the scope of animal biotechnology. There are known to be cloned animals in the Netherlands, such as a dog called Pipo and several sport horses.[16,17] It is also possible and legally permitted to import products derived from cloned animals or their offspring (e.g. meat) for sale in the Netherlands. In several North and South American countries and in China, cloning valuable sires and dams is gradually becoming normal practice.[18,19,20,21] It is virtually impossible to identify the offspring of cloned animals.

d Animal biotechnology is a broader field than genetic modification. Among other things, it includes animal cloning, which involves making a genetic copy of an animal.

1.3 International developments: growing numbers of GM animals

More use is made of animal biotechnology in other countries, both for research and in several commercial applications. A GM salmon was approved for sale and consumption in the United States and Canada[e] after a procedure lasting almost 20 years.[22,23] Without an authorisation for placing on the market (import) in Europe, this salmon will not be offered for sale in the Netherlands.

Modern biotechnology is a dynamic field and new techniques are continually emerging. Genome editing, and CRISPR-Cas in particular, is the latest example. The introduction of CRISPR-Cas would appear to remove the remaining technical barriers to developing GM animals, owing to:

- its effectiveness: the efficiency and accuracy of the technique;
- its accessibility: the simplicity and broad applicability of the technique;
- its rapid uptake in the field: the scale and worldwide distribution of its use.

The development of genome editing techniques in animals occurs mostly outside the Netherlands and Europe. Given the international character of scientific research, animal breeding and the trade in gametes (sperm and egg cells) and embryos as well as animals and animal products, it is probable that the Netherlands and Europe as a whole will in any event be confronted with the consequences of these techniques.

1.4 Issues surrounding genome editing in animals

In 2017 COGEM held an international symposium to review the advances in and consequences of genome editing in animals (**see Appendix A**). The outcome of that symposium, as well as the expertise among COGEM members and a literature study, formed the basis for this policy report, which identifies the possible consequences of the advances being made in genome editing in animals.

The public and ethical debates surrounding the relationship between people and animals are neither new nor specific to biotechnology or the genetic modification of animals. Animals play a part in our lives in many different ways: as pets, for our livelihoods (livestock), as food or a supplier of other products (leather, wool, medicines), as service providers (pollination by insects, guide dogs for the blind), for pleasure (zoos, petting zoos, circus), and also less visibly as laboratory animals. The debate about animal biotechnology involves a mix of general and specific issues. **Chapter 2**, therefore, sets out the general societal issues

e In 2015 a GM salmon was given approval for marketing as a food in the US, but in practice it has not been produced or eaten there due to a number of additional requirements. Until official labelling requirements have been adopted, the salmon, the eggs of the salmon and food products derived from the salmon are subject to an import ban. The GM salmon is produced and eaten in Canada, where it has also been approved for marketing as a food.

in the debate about animals. **Chapter 3** identifies and discusses the influences of genome editing techniques on the development of modified animals, focusing on genome editing, or germline modification, in animals, which results in the animal passing the genetic changes on to its offspring. The emergence of genome editing highlights existing legislative and regulatory differences between jurisdictions around the world. In **Chapter 4** the existing legal frameworks governing the application of genome editing in animals are reviewed, identifying where these new developments may lead to problems. Having identified and described the scientific developments and legal frameworks, **Chapter 5** examines how developments in genome editing in animals can influence the public debate on modified animals. Finally, Chapter 6 explores how a dialogue and public debate on genome editing in animals can be organised, and by whom, in the interests of safety, innovation and progress in the fields of medicine and animal welfare, and to secure its social acceptance.

2. Wider societal considerations about the use of animals

Discussions about the relationship between people and animals and the use of animals for human benefit are characterised by a number of overarching themes and concepts. The debate on genome editing in animals also inevitably raises broader questions about the context of the current system in which animals are held, but this policy report does not go into the acceptability or desirability of existing systems for things like food production and the use of laboratory animals. Given the complexity and connectedness of the arguments, the general themes and concepts in the debate are mentioned here for completeness and we refer the reader to them throughout the report where relevant.

The context within which animals are used varies and can be described as the entirety of the physical, social and cultural circumstances within which people intentionally relate to and treat animals. The relationship between people and animals varies according to the context, sometimes also for the same animal in different contexts.[24] This complex relationship often does not depend directly on the animal species or application concerned, but on both. This means that using the same animal for a different purpose may be considered either acceptable or unacceptable, or may meet with resistance. For example, we keep mice and rats as pets, use them for research and control them when they become pests. We keep dogs as household pets, for personal assistance (guide dogs for the blind) or protection (guard dogs and police dogs), as a laboratory animal (toxicological and pharmaceutical research), as working animals (huskies) and in Asia it is not uncommon to keep them for food. The use of animals may also differ within the same context, such as the differences in livestock farming between countries and cultures and the different animal husbandry regimes on conventional and organic farms. People's ideas about the role of animals and what is acceptable or desirable can vary considerably depending on the country, culture or individual. How we view these relationships revolves around three core questions: 1) Should we keep animals? 2) For what purposes may we keep animals? 3) Under what conditions should we keep animals?[f]

Although individuals hold different opinions about the desirability and acceptability of using animals, the regulatory system should reflect the overall level of public acceptance or support for the use of animals. On this basis, it can be concluded that keeping animals is considered to be acceptable. The legislation imposes minimum standards on the purposes for which animals may be kept and the conditions under which they may be kept. These

f See also the Assessment Model for Policy on Animals in the report by the Council on Animal Affairs, 'Moral issues and public policy on animals' (2010) (available from the RDA website: https://english.rda.nl/publications/publications/2010/07/20/moral-issues-and-public-policy-on-animals).

rules differ depending on the type of animal and for each purpose. For example, the rules for laboratory animals are different from those for farm animals and there is another set of rules for household pets. Furthermore, the legislation is not static and is amended in response to shifts in public opinion. Examples of this are the Dutch ban on the use of wild animals in circuses,[g] which came into force in 2015, and the ban on keeping animals for fur production,[h] which came into force in 2013.

The law governing the use of animals and animal products in the Netherlands is the Animals Act,[i] which consolidates and amends a range of laws on animal health and welfare. The principle underlying this law is that 'the intrinsic value of animals, being sentient beings, is acknowledged and that any infringement of the integrity or welfare of animals beyond what is reasonable and necessary must be prevented.' Besides integrity and animal welfare, naturalness and instrumentalisation are also fundamental to the debate about the use of animals. These and other concepts are discussed further in this chapter.

2.1 Telos: the intrinsic and extrinsic value of animals

The classical Greek word telos (τελος) means an end, aim or purpose. In bioethics it mainly refers to the species-specific goal or inherent nature of an organism (animal or plant) to pursue its unique form of life, complete its reproduction cycle and fulfil its ecosystem services. A niche, biotope or ecosystem can also be said to have its own telos. An organism can even have multiple teloi. An animal can have a telos in its capacity as an organism and also have a separate purpose ascribed to it by humans.

These teloi can be linked to the intrinsic and extrinsic value of animals. An animal's telos is based on the recognition that plants and animals have certain species-specific 'ends' or 'goals'.[j] Animals can have a value in themselves as autonomous organisms within an ecosystem, an intrinsic value the animal earns by virtue of the fact that it exists. Another aspect, integrity, refers to the nature, wholeness and completeness of an organism. In addition, animals and plants can have a telos imposed on them by humans, for example for the pro-

g Decree of 28 August 2015 revising the Keepers of Animals Decree in connection with the prohibition on participation by wild mammals in circuses and other performances and on the transportation of animals for these purposes [*Besluit van 28 augustus 2015, houdende wijziging van het Besluit houders van dieren in verband met het verbod op deelname met zoogdieren behorende tot wilde diersoorten aan circussen en andere optredens en op vervoer van die dieren ten behoeve daarvan*].

h Act on the Prohibition of Fur Farming 2013 (*Wet verbod pelsdierhouderij*).

i The Animals Act 2013 (Wet dieren) replaces the Animal Health and Welfare Act and several other laws containing rules on keeping animals.

j See also the classification of species-specific behaviour and mental health categories by the Council on Animal Affairs in its report 'Breeding and Reproductive Technologies' (2015). Available from the RDA website (in Dutch only): https://www.rda.nl/publicaties/zienswijzen/2016/02/29/zienswijze-fokkerij-en-voortplantingstechnieken-uitgebreid-verslag-pdf

duction of food. This telos represents the extrinsic value of the animal, its value for human purposes.

These two forms of telos – intrinsic and extrinsic – can be in opposition or in conflict with each other; the more an animal is domesticated, the more its own intrinsic telos is sacrificed in order to realise the telos imposed by humankind.[25] The two teloi may also be able to coexist, and in the best case scenario may even be in harmony. Where the acceptable or desired balance between the two teloi lies depends on how people see themselves in relation to nature.[k]

The extremes can best be illustrated by means of examples. The use of bees and bumble bees for pollination by fruit growers can be seen as the realisation of both teloi; it is the nature of these insects to pollinate. The use of pigs for xenotransplantation, the generation of organs for transplantation into people, will probably be seen as a major sacrifice of the animal's own telos in favour of a purpose imposed by humans. At the same time, however, it is possible to hold the opinion that the purpose – organ transplantation for critically ill people – justifies the sacrifice of the animal's intrinsic telos. The degree to which an animal's integrity is damaged depends on human actions and the intentions behind those actions.[26]

2.2 Integrity

What is meant by the integrity of an animal is its intrinsic value, not only as a means of achieving an end, but also in itself and for its own sake. Integrity refers to the wholeness and completeness of an animal and is related to its ability to behave independently (without human assistance) in a manner inherent and unique to its species (realise its telos).[27] Species identity refers to the genotypic and phenotypic traits and the behaviour of animals.

For some people, integrity is an absolute norm and any form of physical or genetic modification is a violation of the animal's integrity; for others, the effect of the modification should also be taken into account. For example, the production of a foreign protein in the milk of an animal can be seen as a less objectionable violation of the animal's integrity than the introduction of foreign DNA that alters the physical appearance or behaviour of the animal. In other words, if a genotypic alteration is expressed in the physical (phenotypic) traits of the animal, the violation of species identity is often considered to be more serious. Tail docking of dogs for aesthetic reasons is widely considered to be a violation of the animal's integrity. However, if a dog's tail is docked for medical reasons, this is no longer considered to be a violation, or at least a much lesser one. Further, the concept of integrity and the violation of integrity are linked specifically to human interference with the animal.

k In environmental philosophy a distinction is made between different human–animal relationships, such as master (anthropocentric), steward (anthropocentric, nature is on loan to us and we are responsible for it), partner (nature and the environment are central, cooperation) and participant (nature is central, humans are part of a whole).

2.3 Naturalness

The more an animal is able to realise its own telos, the more this is generally considered to be a 'natural' situation. When there is a greater emphasis on the extrinsic value of an animal – the value of the animal for human purposes – this is usually considered to be a less 'natural' situation.

'Naturalness' is a difficult but frequently used term in relation to biotechnology. It is a difficult term, because it has the status of a dogma and is taken as a given in discussions, but at the same time people interpret it in very different ways. What is natural for one person is unnatural for someone else. Moreover, the term is not value-free: 'natural' is often associated with 'good' and 'pure', while unnatural, synthetic and artificial generally have negative associations.

2.4 Instrumentalisation

The use of animals for a human purpose or goal is called instrumentalisation. Instrumentalisation is not necessarily animal unfriendly or deleterious to animal welfare, because this is related to the previous point about the extent to which an animal retains the ability to realise its own telos. Some people are of the opinion that every instrumental use of animals violates their integrity and is therefore unacceptable, whereas for others using animals for certain purposes, either subject to conditions or not, is acceptable. From this perspective, it is usually accepted that the more important or worth pursuing a goal is, the higher the permissible cost (harm to the animal).

Dutch legislation is based on the principle that animals may be used for certain purposes, but are not solely of instrumental value. This is why laboratory animals may not be used as a resource or a means without good reason; depending on the context, certain minimum welfare standards apply to all animals. For example, from the Animals Act (Section 4: Biotechnology) it can be concluded that biomedical research (exempt) is a worthier purpose than altering animals for sports or leisure purposes (prohibited).[8]

2.5 Animal welfare

Generally speaking, animal welfare refers to the physical and emotional wellbeing of animals, as expressed in their health and behaviour. However, animal welfare is not a well-defined or clear-cut concept. For some time there has been a debate (both philosophical and operational) among scientists and ethicists about what animal welfare is and what methods can be used to measure it.[28] As a rule, animal welfare is measured by applying the Five Freedoms test:

1. freedom from hunger and thirst;
2. freedom from discomfort;

3. freedom from pain, injury and disease;
4. freedom from fear and distress;
5. freedom to behave normally.

These Five Freedoms are based on the findings of the Brambell Committee, which in 1965 was charged by the British government to determine the conditions under which animals should be kept.[29] These conditions do not apply to animals that are not kept, such as wild animals and animals free to roam in nature reserves and other protected areas. In 1976 these principles formed the basis for the European Convention for the Protection of Animals Kept for Farming Purposes, which eventually led to various EU directives and regulations.[1]

Some of the principles underlying animal welfare have been incorporated into legal requirements (minimum standards) for keeping animals (**see Chapter 4**). However, these standards may be put into practice in different ways, an example being the differences between conventional and organic farms. Also, the interpretation of terms such as 'discomfort', 'distress' and 'normal behaviour' depends on the context, the prevailing culture and individual beliefs and preferences.

2.6 Current practice and alternatives

The debate about the use of animals is dominated by existing practices. It is not always clear what these really involve or which practice should be taken as the basis for constructing a framework for assessment or evaluation. For example, should assessments of new applications in farm animals be based on conventional or organic livestock farming practices? Do we assume a society in which eating meat every day is normal or one in which our diet is largely or entirely vegetarian?

Current practice is often seen as the natural state of affairs (including the problems, risks, benefits and harms it entails), whereas new developments are viewed in a critical light. However, not everyone is comfortable with current practice and view new techniques that maintain the status quo as problematic, as technological fixes that do not address the real, underlying problem: existing practices.

The legislation determines the legal limits to the use of animals under current practice, but how people view the acceptability or desirability of these practices can differ considerably. This also goes for alternatives to current practice – what one person thinks is a good alternative can be out of the question for another. Individuals can put their views into practice by exercising their freedom of choice as consumers of goods and services. Each new possibility or application that appears on the horizon prompts renewed discussion of the acceptability or otherwise of existing practices, making this debate an inherent part of the appraisal and acceptance of new developments.

1 European Convention for the Protection of Animals kept for Farming Purposes, Strasbourg, 10.III.1976.

2.7 Sub-conclusions

- Concepts such as telos, integrity, animal welfare, naturalness and instrumentalisation, and the role of current practice and alternatives are all fundamental to the debate about the use of animals.
- People's ideas about animals and the uses we make of them can vary considerably depending on the country, the prevailing culture or the individual concerned.
- People's attitudes to animals are ambiguous. Relationships between people and animals vary depending on the context and can even vary between contexts for the same animal: using the same animal for a different purpose may be considered acceptable or unacceptable, or meet with resistance.
- The legislation on keeping animals reflect this situation. There are different laws and regulations for different types of animal uses, such as experiments, breeding, production and pets.
- The thresholds of acceptability for the instrumentalisation or violation of the integrity of animals depend on the type of use.
- The debate on genome editing in animals inevitably raises questions about the current system in which animals are held.

3. Possibilities and applications of genome editing

The efficiency and widespread applicability of CRISPR-Cas have made some types of genome editing in animals much easier. Using CRISPR-Cas it is possible in a short time to make simple or complex modifications and the technique is easier to use, not only in the traditional laboratory animals (rats and mice) but in other animal species as well. This chapter begins with a brief technical introduction to genetic modification in animals and then discusses the possibilities of genome editing and applications in domesticated animals, for xenotransplantation, for ecological engineering (population control) and in laboratory animals.

Genetic modification techniques often make use of homologous recombination to insert transgenes at a specific site in the cell's DNA. This involves introducing the transgene into the cell along with a DNA sequence that closely resembles the DNA sequence in the host cell. Various methods can be used to do this, such as microinjection, viral transfection and electroporation – a technique in which an electrical field is applied to cells to increase the permeability of the cell membrane to allow DNA to enter. The overlapping sections of DNA sequences (homologous regions) flanking the transgene and the cell's own DNA ensure that the different DNA strands bind together and the exchange of sequences can take place. Replacing the original sequence with the introduced sequence (containing the transgene) in this manner allows new genes to be inserted or genes to be switched off or deleted (knock-outs). Homologous recombination is not very efficient[m] as the transgene becomes integrated into just 1% of the cultured cells.[30,31]

The inefficiency of this mechanism meant that the use of older techniques for genetic modification in single-celled zygotes (fertilised egg cells) – to ensure the desired genetic trait will be present in all body cells – was often not a practical proposition (many zygotes are needed). Since 1986, therefore, embryonal stem cells are frequently used for genetic modification in animals because these cells are capable of propagating themselves indefinitely and are able to differentiate into a range of different cell types (pluripotency).[32] To genetically modify an animal in this way, embryonic stem cells of the animal are modified and then introduced into a developing embryo. The resulting animal is a chimera consisting partly of genetically modified cells and partly of wild type cells. If the animal's gametes develop from the genetically modified part of the animal, a fully genetically modified animal can be obtained by repeated back-crossing.

m When induced by electroporation. Transfection by microinjection is more efficient, but also more labour intensive because it can only be used on one cell at a time.

Mouse embryonic stem cells are relatively easy to obtain and to culture, but the procedure is much more complicated in other animal species, especially in larger mammals. Despite the rapid progress that has been made in obtaining and culturing rodent and human embryonic stem cells, researchers have not yet been able to obtain embryonic stem cells from farm animals.[30] In 2006 a technique was developed for converting adult (differentiated) mice cells into pluripotent cells, called induced pluripotent stem cells (iPSCs).[n] This tech-

n The researchers responsible were awarded the Nobel Prize for this in 2012.

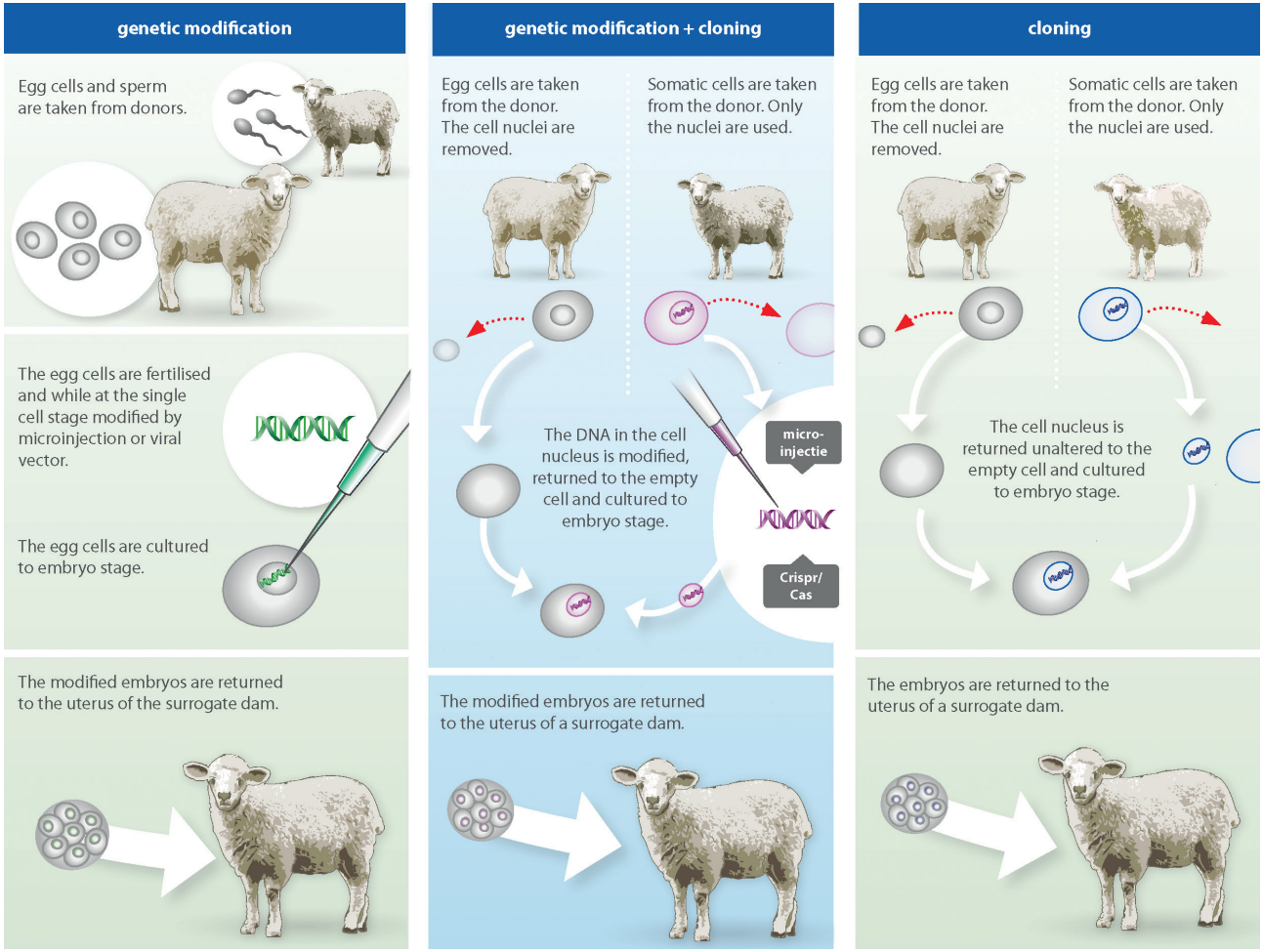

Figure 4: Different strategies for creating GM animals, either by direct modification of gametes or by modification of somatic cells or stem cells in combination with cloning.

nique proved to be applicable to humans as well, but has had only very limited success in farm animals.[33] An alternative method to embryonic stem cells and pluripotent stem cells (iPSCs) is somatic cell nuclear transfer (SCNT), in which the nucleus of an egg cell is replaced by a nucleus from a normal body cell (somatic cell) that has already been genetically modified (**see Figure 4**).

The emergence of this method has made genetic modification possible in larger mammals, including sheep, goats, pigs and cows.[34,35,30] Although SCNT is technically complicated and requires considerable expertise, it is the principal method for creating GM farm animals.[30] Nevertheless, genetic modification remains problematic in some animal species (e.g. ferrets and primates), partly due to the low success rates of SCNT.[o] Genome editing offers a solution for these animals, because it is a more efficient and accurate method for genetically modifying zygotes (fertilised egg cells). In 2015, CRISPR-Cas was used to introduce mutations in ferrets that cause a similar clinical presentation to that in humans.[36]

CRISPR-Cas is easier to use and less labour intensive than conventional techniques and is preferred to earlier genome editing techniques that used proteins for binding to the target sequence in the DNA (**see text box 'Genome editing: ZFNs, TALENs and CRISPR-Cas'**).[37] CRISPR-Cas makes it possible to introduce multiple mutations in a single procedure and so make complex modifications in animals.[38] A study on goats has shown that CRISPR-Cas can be used to switch off several genes at the same time. Deletions were made in four genes: myostatin (MSTN), nucleoporin 155 (NUP), prion protein (PrP) and beta-lactoglobulin (BLG).[39]

Genome editing: ZFNs, TALENs and CRISPR-Cas

Genome editing involves two main steps: the cutting of the DNA at the desired location or locations by a DNA nuclease introduced into the cell, followed by the repair of the cut DNA by a cellular repair mechanism. Depending on which of the two repair mechanisms present in cells is used (homology directed repair (HDR) and non-homologous end-joining (NHEJ)) and on the introduced DNA fragment, a new or existing DNA fragment can be inserted at the site of the cut or mutations can be generated around the site of the cut. In recent years various nucleases have been developed for cutting the DNA at the desired location (**see Figure 5**).

Z*inc* **F***inger* **N***uclease (ZFN)*: Zinc fingers are frequently occurring protein structures containing zinc ions (Zn2+) that can bind to DNA. The use of zinc fingers in genome editing techniques was developed in 1996.[40] The zinc finger nucleases (ZFNs) created for this purpose consist of several DNA-binding zinc finger protein domains,[p] each of which binds to a series of three nucleotides, coupled with a cleavage protein (from the endonuclease Fokl). To get a ZFN to bind to a specific site in the DNA various combinations of zinc finger domains first have to be tested, which makes the development of ZFNs a very complicated, time-consuming and expensive business.

o In January 2018, Chinese researchers were able to clone two crab-eating macaques for the first time using the SCNT technique.

p Proteins consist of polymer chains of amino acids. A protein motif or protein domain is a recurring sequence of amino acids in a protein. These domains are highly stable and often folded independently of the rest of the protein chain.

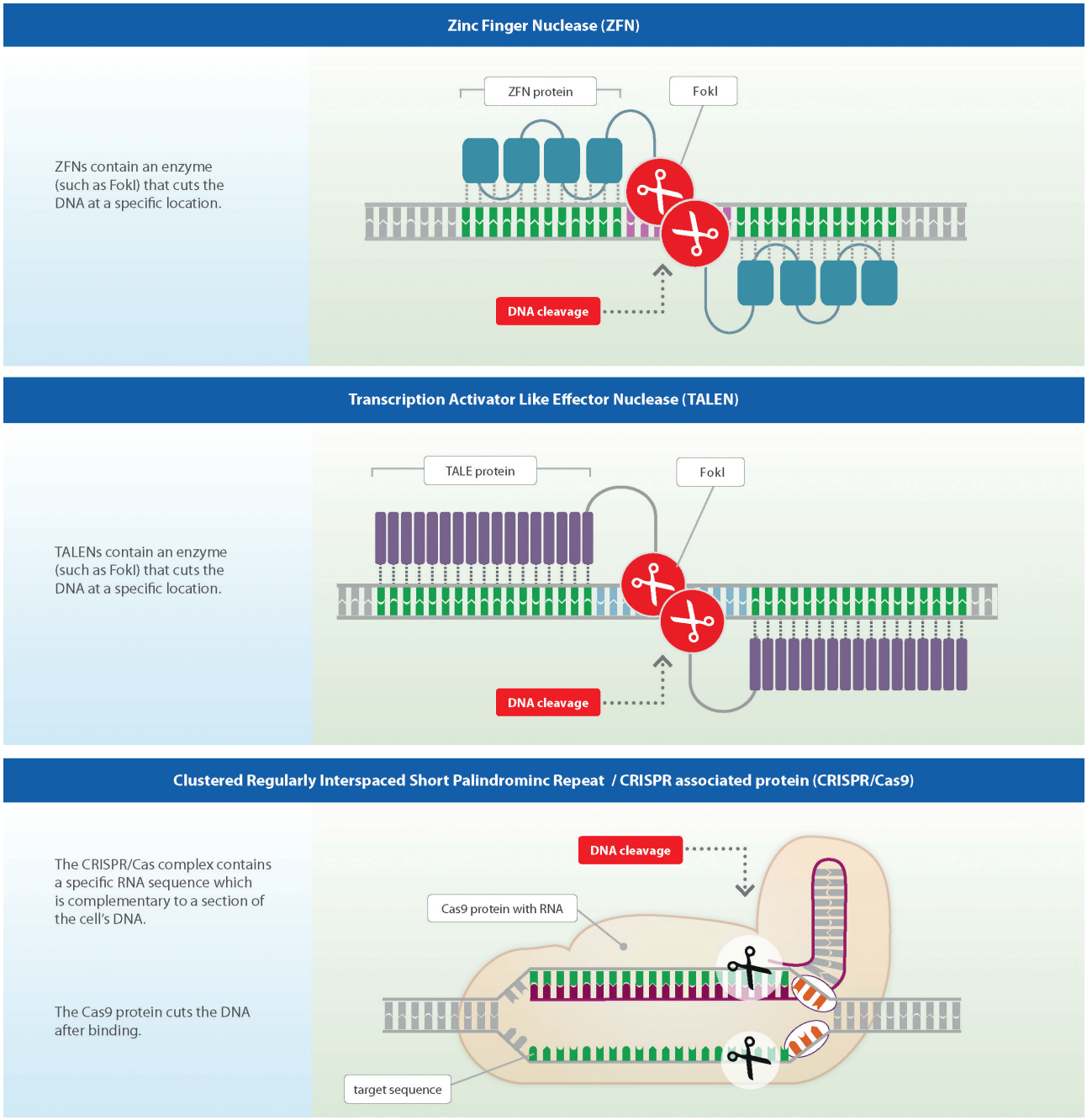

Figure 5: Different genome editing techniques: ZFNs, TALENs and CRISPR-Cas.

T*ranscription* **A***ctivator* **L***ike* **E***ffector* **N***uclease (TALEN):* The TALEN genome editing technique was introduced in 2010.[41] This technique makes use of the same nuclease as the ZFN technique, but the nuclease is combined with a DNA-binding protein domain derived from Transcription Activator Like Effector (TALE) proteins from plant pathogenic bacteria of the genus Xanthomonas. TALENs consist of repeated amino acid sequences of about 34 amino acids in which the amino acids in positions 12 and 13 differ in each repeat. The combination of TALE domains determines which are the binding sequences. Even though this direct relation between the variable amino acids and the bound nucleotides makes the development of TALENs easier than ZFNs, the process is still time-consuming. Also, TALENS are often too large or complex to be transmitted to the host cell by viruses.

C*lustered* **R***egularly* **I***nterspaced* **S***hort* **P***alindromic* **R***epeats / CRISPR-associated protein 9 (CRISPR/Cas9):* This technique is based on an immune system present in many bacteria species (>40%) and confers resistance to 'foreign' (e.g. viral) DNA. Its name is derived from the use of 'clustered regularly interspaced short palindromic repeats' (CRISPR) to recognise the foreign DNA. These are short non-repetitive nucleotide sequences (called protospacer sequences) which are situated between repeating nucleotide sequences in the bacterial genome. Associated with these sequences are diverse bacterial proteins capable of modifying DNA or RNA: the CRISPR-associated (Cas) proteins. CRISPR DNA codes for short CRISPR RNA (crRNA) molecules which the Cas proteins send to a specific sequence (target sequence) in the foreign DNA, where the Cas9 protein (which has nuclease activity) creates a double strand break in this DNA. The target sequence is recognised by the 20-nucleotide long protospacer sequence in the crRNA.[42]

Genome editing makes use of the Cas9 protein and a modified guide crRNA. This sequence is selected and synthesised such that the Cas9 protein cuts the DNA at the desired location. Various CRISPR nucleases have been developed to increase the specificity of CRISPR-Cas and much research is being conducted to further improve the system.[43,44] The DNA-binding domain in CRISPR-Cas thus consists of a small 'guide' RNA which is relatively easy to modify so that it recognises the target sequence, in contrast to the proteins in ZFNs and TALENs. In recent years the number of publications on CRISPR-Cas genome editing techniques has increased exponentially and CRISPR has largely replaced the other techniques.[45]

Work on genetic modification in birds began later than in other animals. The usual methods for creating transgene mammals, such as genetic modification of embryonic stem cells and injecting DNA into the nucleus of a single-celled zygote, are not possible in birds because of their reproductive system, which is why primordial germ cells (PGCs) are often used for genetic modification in birds.[46] PGCs are stem cells that will later develop into gametes (sperm or egg cells). Mammalian PGCs migrate through the embryonic tissue and into the developing gonads, but in birds the PGCs are transported via the blood. This characteristic has been used to bring about considerable advances in the genetic modification of chickens. In 2006 it was first demonstrated that PGCs could be isolated, cultured and transformed, and then replaced in the bird embryo. The adult bird will then pass the genetic modification on to its offspring (germline transmission).[47] In 2013 the first study was published on the development of GM birds by transforming the PGCs in vivo.[48] The development of new targeted genome editing techniques, including CRISPR-Cas, has also given a boost to avian biotechnology.

In this chapter various possible applications of genome editing in farm animals, pets and laboratory animals as well as for xenotransplantation and ecological engineering are discussed in

the light of recent scientific advances. At the moment, there are no commercial applications of genome editing in animals, with the exception of laboratory animals for medical research.

3.1 Domesticated animals

Domesticated animals live in or near the home and are fed and cared for by people. A distinction can be made between farm animals (such as cattle, pigs, sheep, goats, horses and poultry) and pets (such as dogs, cats, birds and rodents). Breeding serves collective interests such as increasing yields and improving product quality, but also has environmental and biodiversity goals, which sometimes go beyond the scope of the individual breeder's competence. For this reason breeding goals are formulated at the population level by breeders associations and registered through breed registries such as herd or stud books. To bring about the desired changes, choices are then made at the level of the individual animal.

Research into the genetic modification of farm animals has been going on for many years. The first GM animal in the Netherlands, the bull Herman, was created as early as 1990. The DNA of this animal, made by the biotechnology company Pharming, contained a gene that coded for lactoferrin, a human anti-inflammatory protein. Sheep and goats have for a long time been popular animals for genetic modification and other biotechnological techniques, such as cloning. Dolly the Sheep was the first cloned mammal,[q] produced in 1996, and one of the first transgene farm animals, produced in 1997, was also a sheep.[49] The first GM animal from a which a product has been put on the market (antithrombin in the milk) was a goat. Genome editing applications in domesticated animals have so far been limited almost exclusively to farm animals – with just a few exceptions (**see text box 'Pets and biohackers'**).

Pets and biohackers

A few examples of genome editing in pets are mentioned in the literature. 'Micropigs' – pigs that weigh no more than 15 kg and are as big as a dachshund – have been created in China. These pigs were originally created for research purposes, but were later offered for sale as pets[50,51] – a decision that was subsequently reversed. The same thing happened with GM zebra fish containing a gene for fluorescence. These 'glowing fish' were also created for research purposes, but are now sold in some countries to aquarium enthusiasts and sometimes illegally imported into the Netherlands. It is illegal to use the fish for commercial purposes because no application has been made for marketing authorisation.

In 2015, also in China, two dogs (beagles) were genetically modified to switch off the myostatin gene, resulting in a doubling of muscle mass. The researchers argued that this increase in muscle mass would improve the animal's speed, making it ideal for use by the police or military.[52,53] Genome editing in dogs holds prospects of repairing genetic defects found in certain breeds (caused by inbreeding and selective breeding for outward appearance). A dog breeder in America built a small genetic lab in his back yard and bought a genome editing kit and DNA online. This 'biohacker' (do-it-yourself scientist) intended to cure various dog breeds of their specific genetic abnormalities (such as bladder problems in Dalmatians caused by kidney stones).[54,55] However, the American Food & Drug Administration (FDA) put a stop to this by amending the regulations on the use of genome editing technologies in animals.[56]

q The cloning of frogs was described as early as 1952 (Briggs and King, PNAS 38:455 (1952)).

Farm animals are bred by selecting traits that confer resistance to disease, increase yields or improve the quality of the product. Genome editing research also aims to produce these traits, especially for livestock and poultry farming (**see Figure 6**).[57,58] There is a global trade in genetic material (egg cells, sperm and embryos). The United States and Canada are major players in the trade in genetic material for cattle breeding, followed by the Netherlands.[59]

3.1.1 Increasing yields

Genetic modifications that increase the productivity of farm animals are geared mainly to increasing meat yield, but can also be used for other products such as wool. Yields can also be increased by traditional breeding methods. Selecting animals for breeding that have the most favourable traits keeps these traits in the population. However, such traits are sometimes also associated with an increased risk of behavioural, physical or immunological problems.[60] The explanation for such problems may be that the genes for these traits are located close to each other on the chromosome and difficult to separate during meiosis,[r] that the gene products interact with each other, or that a single gene influences several different traits.[60] Genome editing techniques may be able to provide a solution to these problems because they can be used to make precisely targeted modifications to the genome.[61] However, other potential adverse effects, such as off-target effects, pleiotropy[s] and epigenetic effects, cannot be ruled out entirely. In addition, increasing production levels or growth rates, whether by selection or genetic modification, can be inherently problematic if the skeletal and muscular structure of the animal do not change accordingly (**see text box 'Health problems caused by selecting for increased muscle mass'**).[28] This is a well-known problem in the animal breeding industry.[62]

Health problems caused by selecting for increased muscle mass

Studies have been performed on various farm animals kept for meat production (cattle,[63] pigs,[64,65] sheep[63,66] and goats[39,67]) to determine how to switch off the myostatin gene, which plays an important role in regulating muscle mass. The protein myostatin inhibits muscle development and so animals in which this gene has been switched off make more muscle tissue and meat production is increased. This trait also occurs naturally in animals. The beef breed Belgian Blue has a mutation in the myostatin gene that occurred spontaneously and has been sustained through selective breeding. Introducing myostatin mutations into beef breeds through conventional breeding is very time-consuming, though, and it took some decades to fully develop the Belgian Blue breed. Increasing muscle mass can also harm the health of the animals. Belgian Blues are known for their difficult births and most calves are delivered by caesarean section. Calves often have problems with their legs or experience breathing problems due to their enlarged tongues.[68] These health problems are associated with a specific mutation in the myostatin gene. Researchers are trying to prevent these health problems by using genome editing to introduce other mutations into the myostatin gene that will still lead to increased muscle mass, but will have fewer or less severe adverse effects than the mutations currently present in the Belgian Blue breed.[61,69]

r This is a reductional division in which the number of chromosomes in the cell is halved for the production of reproductive cells.

s Pleiotropy is when one gene has an effect on two or more phenotypic traits. Modifying the gene to alter one trait can therefore have one or more additional positive or negative effects.

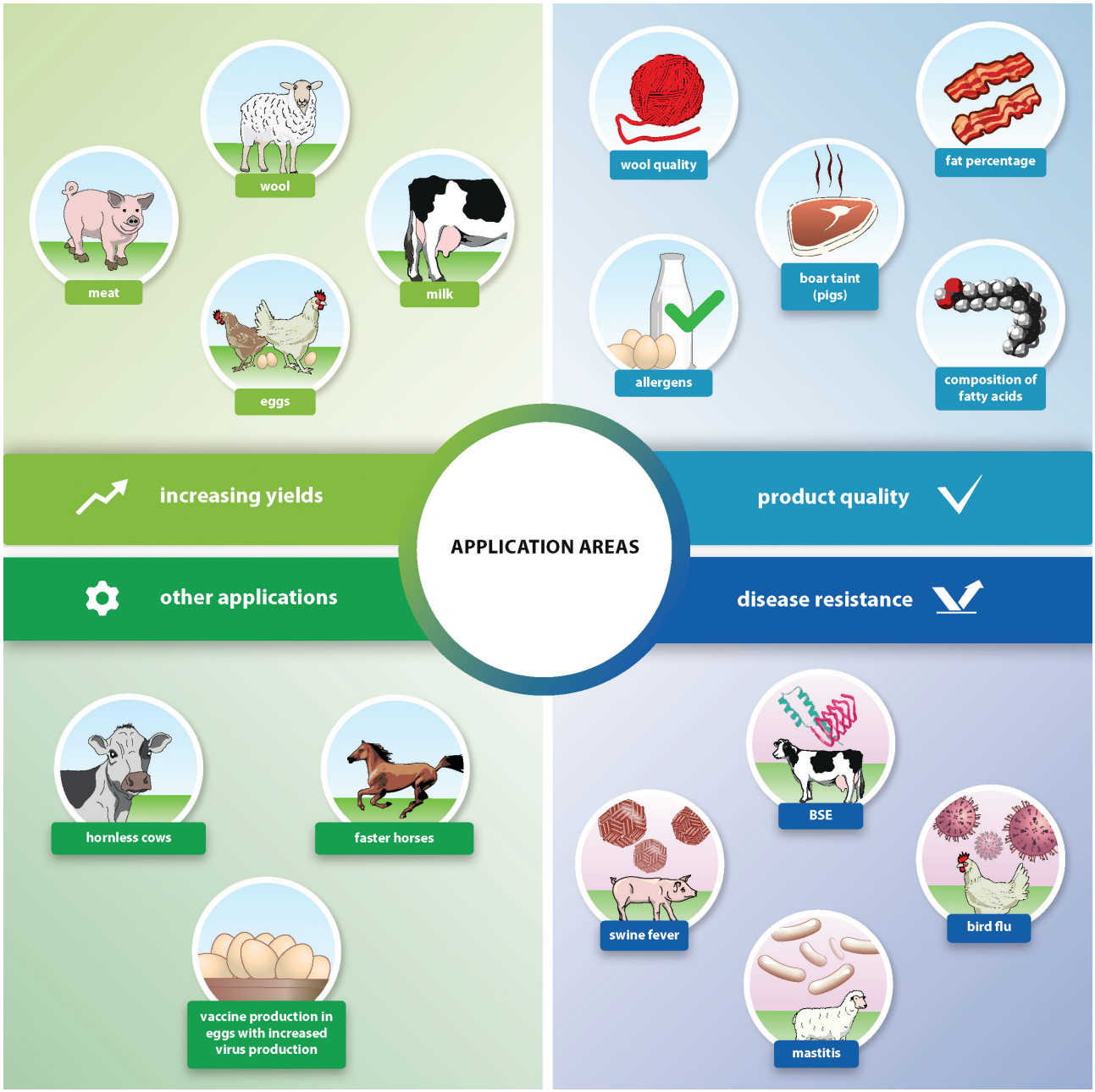

Figure 6: Research on genome editing in farm animals focuses mainly on increasing yields, improving product quality and introducing disease resistance.

In addition to research into the possibilities of genome editing to increase yields and improve product quality in cattle, research is ongoing into applications in other production animals, such as sheep and goats. In South America and China, researchers are working to create GM sheep in which two traits from different breeds are combined. Sheep bred for their high quality wool, such as Merino sheep, produce relatively little meat. Other sheep breeds, such as the Texel sheep, are heavily muscled but produce unexceptional wool yields. The researchers succeeded in removing the myostatin gene with CRISPR-Cas9, leading to an increase in the muscle mass of the sheep.[66] Research is currently focused on improving wool production by switching off the FGF5 gene, which inhibits hair growth.[70] The aim is to combine these traits in a single animal so that it can be used to produce both wool and meat.

In China, research is being conducted on Cashmere goats, which are known primarily for their high quality wool, but are also kept for their meat. Researchers want to increase not only wool production, but also meat production in this breed by modifying the myostatin gene and the FGF5 gene using CRISPR-Cas9.[67] The first Cashmere goats with both genes modified are already being kept at a university in China.

Besides increasing wool and meat production, research on genetic modification in sheep also aims to improve reproductive success. Previous studies have shown that mutations in the BMPR-IB gene in other species lead to a shorter ovulation cycle and more offspring.[71] More research is needed before any conclusions can be drawn about the effect of this genetic modification in adult sheep.

Research on genome editing to increase yields in farm animals is still in the pioneering stage. The results are promising, but many obstacles still need to be overcome before the technique can be made more efficient. For example, in sheep and goats genome editing can still only be used to introduce mutations and make deletions, but not to insert transgene sequences in the DNA.[35,71]. In pigs there have been some successful attempts to insert genes.[72,73]

3.1.2 Disease resistance

Genetic modification to increase or confer disease resistance is focused primarily on infectious diseases that can cause widespread or severe damage to the herd. Animal diseases can be caused by viruses, bacteria or other pathogens, such as prions (abnormal and infectious proteins). Depending on their severity and incidence, diseases of farm animals have the potential to cause considerable economic loss. For example, mastitis, a painful bacterial infection of mammary glands in cattle, can have major financial consequences, which in the Netherlands can run into more than 100 million euros each year.[74,75] The damage is mainly due to the loss of production (milk from sick animals and animals given antibiotics is not fit for consumption), treatment costs and sometimes the costs of culling chronically infected cows. Mastitis can usually be treated with antibiotics. Cows that do not respond to antibiotic treatment present a danger to other animals and measures have to be taken

to prevent the infection spreading to other animals. Culling is often the only option for chronically infected cows that do not respond to treatment.[74]

In a 2014 study on cows, ZFNs were used to insert a human transgene that gives resistance to the bacteria that cause mastitis.[76] The milk from these transgene cows contains an enzyme, human lysozyme, that prevents the growth of the bacteria. Among the 20 transgene cows that were exposed to various bacteria, not a single mammary gland became infected, in contrast to the control group.

Research is also being conducted into resistance to tuberculosis and the prion disease bovine spongiform encephalopathy (BSE) in cattle.[77,78] BSE ('mad cow disease') is caused by prions, which can be transmitted to people by eating meat from infected animals and can cause Creutzfeldt-Jakob disease, a very serious condition.[79] Researchers have succeeded in disrupting the prion gene PRNP with CRISPR-Cas so that no more prions can be produced.

Outbreaks of contagious animal diseases must be notified to the authorities because of their potentially serious economic consequences. The economic damage resulting from an outbreak of classical swine fever in the Netherlands in 1997–1998 was estimated to be more than two billion euros.[80] The symptoms of this viral disease are fever, intestinal problems and pneumonia; it causes death in young piglets and is highly contagious.[81] Because treatment is not possible, all animals on infected farms have to be culled, as well as those on neighbouring farms as a precautionary measure to prevent the spread of the disease. In addition to the economic consequences, culling infected animals and preventive clearances of additional large numbers of animals leads to public consternation and debate. Research is therefore being carried out into developing resistance to contagious diseases such as swine fever. Several such studies have already been published in which genome editing techniques have been used in pigs and pig embryos to develop resistance to the African swine fever virus.[82,83] Studies are also underway to induce resistance in pigs to porcine reproductive and respiratory syndrome virus (PRRSV), which causes respiratory and fertility disorders leading to significant economic losses.[84,85]

Bird flu (avian influenza) not only has major economic consequences for the poultry sector, but also presents a health risk to people (in humans the virus can cause mild to severe illness). Wild birds present a risk to the poultry sector because they are a reservoir for bird flu. They can carry the virus and excrete it in their droppings, but do not always become ill themselves. There are two variants of bird flu, a mild form and a serious form. The serious form can lead to acute death and the fatality rate can be as high as 100%.[86] The mild form induces few if any symptoms, but can mutate into the dangerous form, which is why in the Netherlands poultry farms are cleared in the event of an outbreak of either the serious or the mild form of bird flu.[87] In addition, farms in the vicinity of an infected farm have to take measures to prevent infection (such as keeping the birds indoors). In 2014, bird flu caused 10 million euros worth of damage each week.[88] It is possible to vaccinate poultry against bird flu, but the vaccine is expensive to manufacture, it is not always effective

and there is resistance from abroad to accepting products from vaccinated animals.[89] Vaccination is therefore seldom used. Genome editing techniques are being used in studies to identify the genes involved in infection with and resistance to the virus in chickens[90] and related birds (such as ducks, which often carry the virus but display no symptoms).[91] In 2011 researchers in England created transgene chickens that can become infected but do not pass on the virus.[92,93]

Besides avian influenza, research is being conducted into developing resistance to avian leucosis, caused by the avian leucosis virus (ALV), which also causes cancer in chickens and for which there are no treatments. The genes and proteins involved in the viral infection have been identified[94] and genome editing has been used to disrupt the target gene to make chicken cells resistant to ALV.[95]

3.1.3 Product quality

Genetic modifications to improve product quality aim to change the composition of animal products (such as the fat percentage), remove traits that can lead to unwanted effects (such as allergens) or add new traits (such as producing substances associated with health benefits, such as omega fatty acids).

Some animal proteins (chicken protein and cows' milk protein) can induce allergic reactions in people. About 12% of all young children in the Netherlands develop a cows' milk allergy, but the vast majority of these children (85%) grow out of this before their fifth year and just a small percentage remain allergic.[96,97] About two per cent of all children have allergic reactions to chicken proteins. Fewer than one per cent of all adults remain allergic.[98] These allergic reactions lead to respiratory and gastrointestinal complaints; in severe cases the reaction can cause a life-threatening anaphylactic shock.

In 2016 a study investigated whether or not CRISPR-Cas could be used to reduce the expression of two protein genes, ovalbumin and ovomucoid (which are associated with allergic reactions in people) in chickens.[99] It might then be possible in future to create chickens that can lay hypoallergenic eggs. These could then also be used in the production of vaccines for certain viruses that takes place in eggs (**see also section 3.1.4**).

In cows and goats, the gene that codes for the allergen beta-lactoglobulin (BLG gene) has been deleted using various genome editing techniques to obtain hypoallergenic milk. The resulting animals produced less or even no beta-lactoglobulin.[100] In 2011 in China, a herd of more than 300 transgene cows were created that contain human genes and whose milk is 80% similar to human mother's milk.[101] This milk has so far not been put on the market.

As well as deleting sequences that can cause allergic reactions, researchers are working on adding sequences that will improve product quality. A research group in America has created transgene goats that express a human lysozyme in their milk which can be used to treat

diarrhoea.[102,103] The researchers see this as the first step in the development of foods that promote human health, especially for use in developing countries.

Genome editing is also being used to improve meat quality in pigs. In China, CRISPR-Cas9 has been used to create GM pigs that contain a gene not found naturally in pigs. The presence of this gene increases the animal's rate of fat metabolism, which the researchers claim makes the animal better able to withstand low temperatures. According to the researchers, this improves the efficiency of feed conversion and these new traits will help to improve the quality of the meat because the animals will store less fat.[104,105] Researchers are also studying whether or not genome editing can be used to reduce the problem of 'boar taint', an unpleasant smell or taste that can be evident when cooking and eating pork products from non-castrated male pigs and which is caused by changes in the animals' hormonal system. Nowadays boar taint occurs in only three to four per cent of non-castrated boars.[106] To prevent boar taint, male piglets are castrated at a young age. This is costly and the piglets are not always given anaesthetics or painkillers, although this is changing in the Netherlands.[107] Researchers have now developed a method that uses genome editing to prevent male pigs going through puberty, thus obviating the need for chemical or physical castration.[108] The possible effects on feed conversion efficiency, animal welfare and the quality of the meat have yet to be investigated.

Less obvious traits are also being studied. Chinese researchers claim that their study in which pigs are genetically modified to serve as a human disease model (for Von Willebrand disease, a blood clotting disorder) also has potential in the food industry. In these animals a gene involved in blood clotting has been switched off, disrupting the blood clotting process. Because blood remaining in meat provides an ideal medium for bacteria to flourish, these GM pigs could be slaughtered more efficiently because their blood would drain from the meat more quickly.[109]

3.1.4 Other applications

Besides increasing yields, introducing disease resistance and improving product quality, genome editing can also be used to modify other traits in animals. A few examples are mentioned below.

Dehorning cows is standard practice in the interests of safety, for both the animals and people, and to make it easier to provide the animals with comfortable housing, for example in cubicle sheds. Horned cows that are high in the social hierarchy can wound weaker animals sharing the shed when they are competing for feed or water. Cows with horns need more room because otherwise they would harm each other and they may also get stuck in equipment or feeders.[110] Horn growth can be prevented by killing or burning the horn-producing tissue and so dehorning is usually done on calves by applying a caustic substance or hot iron to the horn bud. This method is less invasive than surgical removal (mainly in adult animals), but is still a stressful and painful procedure. In Europe about 80% of dairy cows are dehorned, but just a small proportion of dairy farms (about 20%) use pain relief.[111] Some

cattle are naturally hornless (polled) as a result of a regularly occurring mutation. Hornless cows could therefore also be obtained through conventional breeding, but this is a time-consuming process and can be detrimental to meat and milk production.[112] Researchers have succeeded in creating hornless cows by introducing the polled gene (taken from naturally hornless cows) into embryonal cells using the TALENs genome editing technique.[113] The researchers claim that two healthy homozygote hornless calves called Spotigy and Buri show how genome editing techniques can be successfully applied in livestock rearing.

In addition to the production of hypoallergenic eggs, genome editing is also used to increase the level of vaccine production in eggs. Several vaccines are produced in eggs, but yields are limited due to a number of technical factors. The genes responsible for inhibiting the virus replication in chickens have been identified and modified using CRISPR-Cas9. The resulting fertilised chicken eggs can produce much higher vaccine titres, reducing the number of eggs needed and the costs of producing the vaccines.[114,115]

There are also reports in the media of genome editing in sport horses. Using CRISPR-Cas technology, researchers in Argentina have produced horse embryos in which the myostatin gene has been disrupted to promote muscle development. The researchers hope this will improve the performance of the horses. They expect the first horses modified by genome editing will be born in 2019.[116,117]

The genetic modification of farm animals to increase yields, confer disease resistance and improve product quality raises both technical and practical as well as ethical questions (**see text box A**). The example of hornless cows has been widely reported in the media, but this is just one of the limited number of traits that are controlled by a single gene (monogenic). Many traits are polygenic[t] and are therefore a complicated target for genetic modification or genome editing.

A number of genome editing applications in farm animals are almost ready for market. However, introducing a new trait into existing populations (while necessarily retaining variability and other carefully selected traits) is expected to take at least five to ten years from the first introduction. Besides, obtaining authorisation for placing on the market of GM animals is itself an unpredictable process.

A: Societal issues

Genome editing can be used in production animals to make changes that also deliver animal welfare benefits, such as disease resistance. However, modifying genetic traits to increase yields often increases the risks of adverse effects from an animal health and welfare perspective. Moreover, questions may be raised about modifying traits to facilitating the existing system of industrial livestock farming, which some people find objectionable. On the other hand, genome editing can also open up possibilities for improving animal health and welfare.

t Polygenic traits are characteristics controlled by the integrated action of multiple independent genes, in contrast to monogenic traits which are controlled by a single gene.

3.2 Laboratory animals

Laboratory animals are used in experiments on the effects of medicines and chemicals and in food safety studies, as well as in fundamental research into the causes and the course of diseases and disorders. Disease models used to be made by chemical and radiation mutagenesis, but now genetic modification is used to generate specific disease models for research into human medical conditions. In 2015, 26.7% of the laboratory animals used in the Netherlands were genetically modified (a total of 462,179 GM animals).[118] Most of these animals were bred specially for the purpose by the research institute concerned (355,799 animals) and a much smaller number were bred by an animal breeder (17,157) or came from elsewhere (e.g. from other European or international research institutes). Specialised laboratories that produce GM animal models on a commercial basis for research purposes can be found around the world.[u] Use is made of these laboratories in the Netherlands as well.

3.2.1 Disease models for complex conditions

As the technology for mapping the complete human genome has progressed, a growing number of the combinations of genes responsible for increasingly complex conditions (caused by mutations in several genes) are being identified. As CRISPR makes it possible to make multiple mutations at the same time in organisms, these complex conditions can now be mimicked in animals. The mutations do not always have to be introduced into embryos, but can also be induced locally (in the animal). CRISPR-Cas is also used for tissue-specific studies, for example in oncological research.[119] Tumours can be induced in specific tissues by genome editing to mimic patient-specific clinical presentations. These laboratory animals can then be used in personalised studies to identify possible treatment options.

3.2.2 More rapid development of disease models

Until recently, developing a GM laboratory animal was an expensive and time-consuming process. Producing the desired traits required a relatively large number of laboratory animals because of the inaccuracy and inefficiency of the techniques used, and modification often remained limited to a single or just a few genes. Mimicking complex conditions involving multiple genes or developing stable homozygous lines often required tricky manipulations in embryonic stem cells over several generations. Animals with a relatively short reproductive cycle (fruit flies, mice) were therefore most suitable for these types of experiments.

Whereas previously a year was needed to developed a specific GM mouse (plus a possible 6 to 18 months if no embryonic stem cells of the desired laboratory strain were available and had to be back-crossed), the same mouse can now be developed in 6.5 to 8 months using CRISPR-Cas technology.[120] Besides cutting costs, this also reduces the number of laboratory animals that have to be used.[121] As the complexity of the mutation increases, for example

u The Jackson Laboratory in the US, for example, has more than 7,000 GM mice available and each year delivers about 3 million mice to 50 countries.

because of the size of the fragment that has to be inserted into the genome, the smaller the cost and time savings become. However, because CRISPR-Cas can be used directly to modify the genome (without any need for additional back-crossing), CRISPR-Cas is still preferred to older methods for complex modifications.

3.2.3 Shift in preferred laboratory animals

The choice of laboratory animal to be used for research depends on a range of considerations. First and foremost, of course, is the representativeness of the animal as a disease or development model for humans. Practical considerations, such as reproductive rate, lifespan, costs and space also play a role, as do ethical considerations. The main animals used for research are mice, rats, zebra fish and rabbits. Other animal species are often associated with certain types of research, such as pigs for xenotransplantation, dogs for toxicological research and primates for life-threatening human diseases. Before the introduction of CRISPR-Cas, generating specific disease models was also limited by the degree to which genetic modification of laboratory animals was technically possible (**see introduction to Chapter 3**). CRISPR-Cas makes it easier to create disease models in the less common laboratory animals, which may lead to a shift in the species of laboratory animals used and could intensify the debate about the use of laboratory animals (**see text box B**).[122]

In some fields of research, primates are preferred to rats or mice as laboratory animals, especially in the neurosciences, not only because of their genetic similarities to humans, but also because of the similarity in their behaviour in response to introduced diseases and disorders (such as autism, Alzheimer's disease and schizophrenia).[123,124] Genetically modifying primates was impossible before CRISPR-Cas (the first transgene monkey was born in 2001), but subsequent developments in this field have been very slow due to limitations of the technique as well as ethical objections.[125]

Various primate species have now been successfully modified using genome editing techniques. In China and Japan various GM Java and rhesus monkeys have been created, among other purposes to introduce mutations associated with autism in people and which in monkeys can also induce behaviour characteristic of autism in humans.[126,127,128] Some people expect that the availability of CRISPR-Cas will lead to an increase in the use of primates for research purposes. Research centres are said to have been established in China where large groups of GM primates are produced for research purposes.[129] On the other hand, in some cases genome editing can reduce the need to use primates as laboratory animals. An example is the GM mouse with the human gene for the polio virus receptor, which obviates the need to use primates for this research.

Although CRISPR-Cas has made it easier to genetically modify primates, this application is still in its infancy.[119] Obstacles include the limited availability of correct genome sequences of primate species, their long generation time, the costs and the limited availability of primate embryos. A long generation time is a disadvantage because crossing is sometimes

necessary to obtain homozygous[v] GM primates. This could be avoided by using a higher CRISPR-Cas concentration in primate embryos, but studies show that this harms the development of the embryo.[119]

B: Societal issues

In the Netherlands, and in Europe as a whole, the aim is to minimise the use of laboratory animals and various initiatives are underway to develop alternatives and reduce the number of laboratory animals. Under the existing legislation, the use of laboratory animals in studies for which there are no alternatives is tolerated as a necessary evil. Nevertheless, the fact remains that the use of laboratory animals is controversial and some groups are vehemently opposed to it. Genome editing facilitates not only the use of GM laboratory animals, but also the use of other animal species as laboratory animals, which may serve to heighten the debate about the use of laboratory animals. The need to use primates as laboratory animals is again being debated in the Netherlands and at the European level.[130,131] At the same time, other countries, such as China, appear to be embracing the possibilities offered by genome editing for generating GM primates as an opportunity for new research. Using the knowledge and products arising from research carried out in other countries but which is prohibited in the Netherlands can be seen as a form of moral freeriding.

3.3 Xenotransplantation and chimeric animals for organ transplantation

The main use of GM animals is still as laboratory animals or disease models, primarily for medical research. However, there is a wider range of potential medical uses of GM animals. Research is ongoing to produce organs and tissues in animals for transplantation into humans. There are two main methods for doing this: transplanting animal organs into people (xenotransplantation) and growing human organs in animals (chimerism) so that they can later be transplanted into a patient (**see Figure 7**).

Various experiments on xenotransplantation from animals to people have been done in the past (**see text box 'Experiments with xenotransplantation'**). Clinical xenotransplantation is prohibited by law in the Netherlands for as long as there are insufficient guarantees of its safety (**see Chapter 4**).

Experimental xenotransplantation

In the past, experimental xenotransplantations have been done from pigs to primates and even from primates to people. In 1963 and 1964 six American kidney patients received chimpanzee kidneys, but this was not successful. In 1984 an American baby was given a baboon heart while waiting for a human donor heart. The baby died of heart failure caused by rejection phenomena 21 days after the transplantation. A human donor heart was not found in time.[132] A 35 year old American who received a baboon liver in 1992 also died after two difficult months in a hospital bed. A new technique was developed in the 1990s and used to genetically modify pigs so that their organs would induce fewer rejection reactions in people.[133] Limited success has been achieved with this method; hearts, kidneys, corneas and livers have

v Homozygous individuals have the desired gene on both chromosomes (identical alleles at the same locus of a chromosome pair), in contrast to heterozygous individuals who have two different alleles at the same locus on each chromosome.

been transplanted into monkeys, which survived for up to 900 days (under immunosuppression treatment).[134] The use of this technique in people remains a long way off.

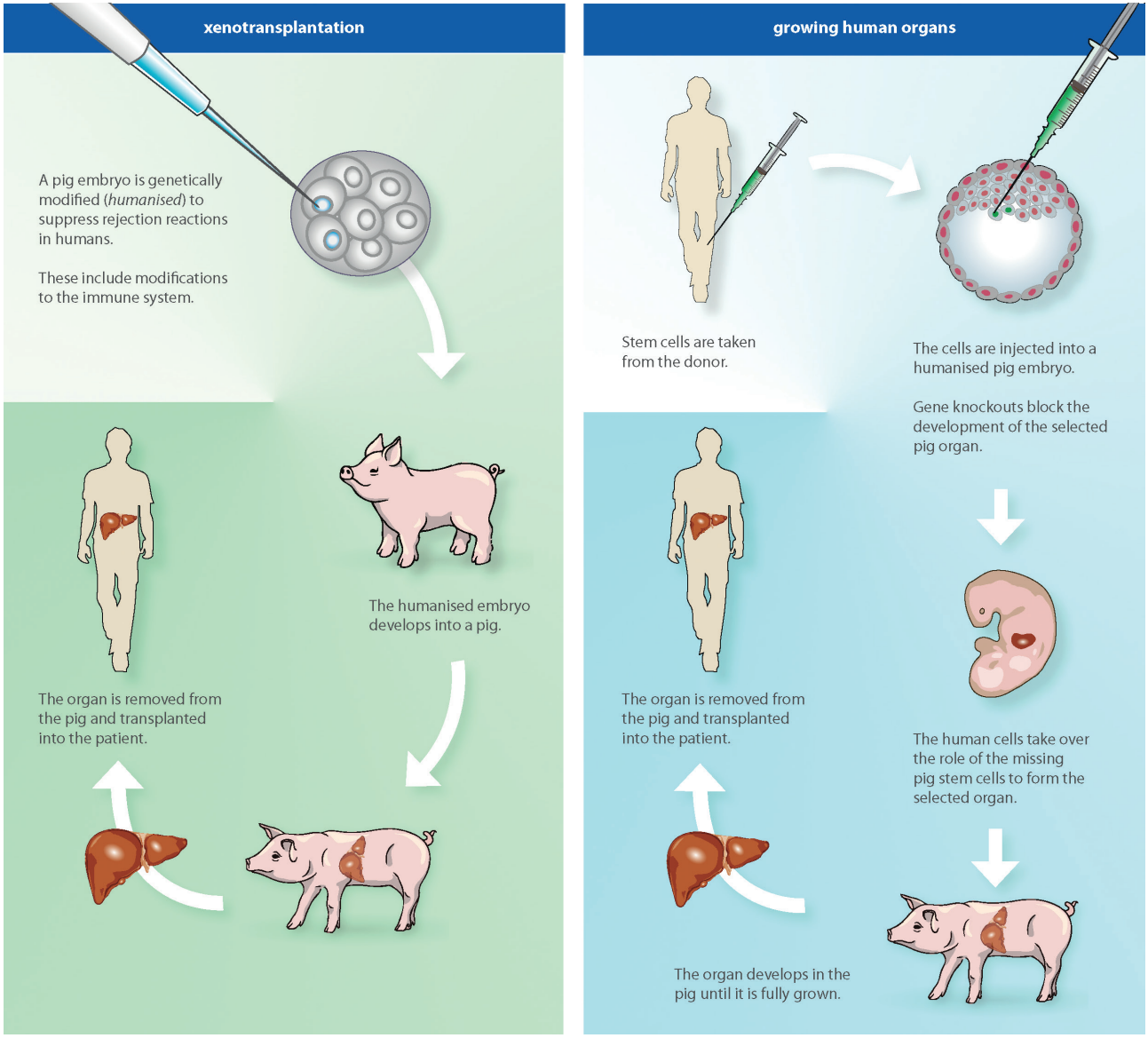

Figure 7: The two most researched methods for obtaining organs for xenotransplantation: humanised animal organs and growing human organs in animals.

3.3.1 Xenotransplantation

In the period from 2012 to 2016 in the Netherlands, on average 1,100 patients were on a waiting list for an organ transplant. In 2016 there were 689 organ transplants (postmortal, from deceased donors), but despite the increasing number of living organ donors, there is a continual shortage of donors.[135] Research into xenotransplantation as a possible answer to the shortage of organ donors has been going on for a long time. Xenotransplantation research is usually done on pigs, because the size of pig organs is comparable with the size of human organs and because pigs can reproduce relatively quickly and have many offspring.[136] However, two factors make xenotransplantation a difficult process: the strong rejection reaction to organs from other animal species and the possible increased chance of zoonosis (the transmission of viruses from animal to human). Because pigs are less related to humans than primates, the chance of the transmission of pathogens from pigs to humans is smaller than from primates, but cannot yet be ruled out.

It is claimed that genome editing can help to solve these problems and increase the efficiency of xenotransplantation.[137,138,139] In recent years there has been renewed interest in genetically modifying of pigs so that their organs induce a weaker immune reaction following transplantation[140,141] and scientific studies have been done on rejection reactions to tissues or cells from GM pigs.[142,143] It has also been shown that protoviruses and viruses integrated into pig DNA (and which may be transmitted to the human patient following transplantation) can be inactivated or removed using CRISPR-Cas9, thus reducing the chance of their being transmitted to the patient.[144]

Type I diabetes is usually treated with insulin injections, but an alternative treatment on offer is transplantation of the islets of Langerhans.[w] Because the islets cannot be donated by living donors, there is a donor shortage. It has recently been shown that pigs could be an alternative source of islets of Langerhans. However, the insulin produced by pig islets differs from human insulin by a single amino acid, which is potentially enough to induce a rejection reaction in humans. Using genome editing, researchers have succeeded in developing a pig that expresses human insulin.[145]

These applications have again raised expectations.[146] In November 2017, United Therapeutics Corporation invested 24 million euros in research into the transplantation of pig organs at the University of Maryland. United Therapeutics is working on the creation of a 'more human' pig so that its organs will induce fewer rejection reactions. The company is also working with Smithfield Foods, one of the biggest pig processors worldwide, which is going to build special housing for modified pigs bred to produce organs for transplantation.[147]

3.3.2 Chimeric animals for organ and tissue transplantation

A relatively new development is growing human organs in animals. Combining cells from different animal species or from humans and animals is not genetic modification, because

w These are specialised cells in the pancreas that make insulin.

the DNA of the organisms is not altered, but 'humanised' GM animals are often used to prevent immune reactions to 'foreign' cells.

Much research has been done into the conversion of differentiated cells into pluripotent cells, because pluripotent cells can develop into each type of cell involved in the development of an organism. In 2015 researchers in America developed the concept of xeno-pluripotency, in which pluripotent stem cells belonging to one animal species are capable of adapting to the embryonal development process of another animal species. This concept makes it possible to develop chimeric animals (animals whose bodies consist not only of their own cells but also contain tissues or organs of another animal).[148] In the future it may be possible to use cells taken from a patient to create a chimeric pig, for example, in which the genes for the development of a specific organ in the animal are switched off and human pluripotent stem cells are used to grow the equivalent human organ in the animal instead.[149] Switching off the development of a specific organ is relatively simple, but getting pluripotent stem cells from another animals species to take this process over is still very difficult indeed.[150]

Recently researchers developed a rat–mouse chimera by introducing pluripotent stem cells from a rat into a mouse embryo (in the blastocyst[x] stage), where they became part of the inner cell mass (ICM) from which the embryo was formed.[151] The rat stem cells took part in the development of the gall bladder in the mouse, an organ which rats do not have. Apparently, the rat stem cells in the mouse can be stimulated to form cells specific to the gall bladder, a process which is suppressed in the development of normal rat embryos. Most rat–mouse chimera have been able to reach the adult stage and a few chimeras have lived for two years (the average maximum lifetime of a mouse). However, it has so far not proved possible to grow pluripotent stem cells from mice or rats in a pig embryo.

Researchers have inserted human pluripotent stem cells into the ICM of a pre-implanted pig and cow blastocyte to determine whether or not the human cells can participate in the development of the animal. The results indicate that, with variable efficiency, the human pluripotent stem cells were able to survive and integrate into the pig or cow blastocyst. Although this technique is still inefficient (just 1 in 100,000 cells in the human–pig chimera came from the human),[152] it offers hope for organ transplantation and regenerative therapies in future.[148,153] Japanese researchers recently reported the results of a study in which a human pancreas was grown in a pig from human stem cells.[154]

Besides research into organ transplantation, chimeric animals have also been created for use in fundamental research into the development of diseases and disorders. In a recently published article, researchers reported the successful implantation of human brain

x The blastocyte is a stage in the development of the embryo. It is a hollow sphere of cells with a thickening on the inner wall which develops into the embryo (the inner cell mass, ICM). The other cells form the extraembryonic tissue (such as the placenta and amniotic sac).

organoids[y] into a mouse brain. Although people previously thought that the brain is too complex an organ to permit functional connections with exogenous stem cells, the human stem cells were able to develop further in the mouse.[155,156] This study aimed to provide more understanding of human brain disorders. The use of animals for the production of organs for transplantation raises ethical as well as technical issues (**see text box C**).

C: Societal issues

Using animals as organ donors represents a high degree of instrumentalisation at the cost of the intrinsic value or telos of the animal (e.g. keeping a pig for xenotransplantation in a sterile laboratory environment). Clinical xenotransplantation is prohibited by law for as long as there is doubt about safety. Should these applications become available outside the Netherlands and the prohibition on their use in the Netherlands remains in force, medical tourism will be a distinct possibility.

Generating chimeric animals raises questions about the boundaries between animals and humans, especially in the light of recent developments involving the use of human brain tissue.[150] These questions revolve around the issue of potential changes in consciousness and how these could be measured. When is the mouse still a mouse and when is it partly 'human'? This type of research raises important ethical issues around which an international debate is emerging.[157] The moral acceptability of growing human organs in animals was put on the political agenda in the Netherlands following a question in the Dutch House of Representatives about initiatives with the potential for reducing the shortage of organs for transplantation.[158]

3.4 Ecological engineering: population control

The rise of CRISPR-Cas has advanced genetic modification applications at the population level (ecological engineering). These techniques aim to change the genetic composition of animal populations, introduce or reintroduce animal populations, control animal populations or, in the most extreme cases, exterminate them. This could provide an alternative to existing practices for controlling the vectors of infectious diseases and invasive exotics by hunting, setting traps and poisoning.[159,160] A little used method is to introduce a virus or a pathogenic microorganism to control the invasive population.[161,162] Genome editing applications for population control are aimed at pest insects or disease vectors, such as mosquitoes and rodents.

3.4.1 Controlling infectious diseases

Various methods have been developed for controlling infectious diseases, for example by creating transgenic mosquitoes that can survive only under certain conditions (**see text box 'Genetically modified *Aedes aegypti*'**). Pests and diseases can also be controlled by releasing sterile insects into the environment. These insects can be created either by genetic

y Organoids are three-dimensional cell cultures that form simplified and miniature versions of organs and tissues and contain all the types of cells that go to make up real organs.

modification or by radiation. The sterile insect technique (SIT)[z], in which radiation is used to sterilise the insects, has been in use for some time (**see Figure 8**).

Genetically modified *Aedes aegypti*

Dengue fever is a viral disease transmitted by female mosquitoes. The mosquito species involved are also responsible for infections with chikungunya, yellow fever and Zika viruses.[163] Most infections with dengue virus are asymptomatic (40–80%).[164] Mild symptoms include fever, headache, muscle and joint pain, nausea and vomiting, and usually do not last longer than a week. In severe cases (5% of infected people) the disease may develop into dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). In addition to the mild symptoms, this can cause bleeding, either subcutaneous or internal, which can lead to organ failure and hypovolemic shock (reduction of the volume of circulating blood). There are no specific treatments for dengue fever. Dengue fever occurs in more than 100 countries in America, South East Asia, the eastern Mediterranean and the western part of the Pacific Ocean. Worldwide, an average of 500,000 people are admitted with severe symptoms each year and 2.5% of these people die of the disease.[164] Vaccines are currently being developed and one vaccine has been approved for use in endemic countries.[163] However, the approved vaccine has been shown to increase the likelihood of severe dengue fever developing in vaccinated individuals who have not previously been exposed to the dengue virus. The vaccine is effective in people who are seropositive for dengue.[165]

A British company has developed a mosquito containing a 'self-limiting' gene, which prevents the mosquito from reaching the adult stage. The gene encodes a protein that inhibits the expression of other genes critical for the survival of the mosquito, which dies as a result. The working of the self-limiting gene itself is inhibited only when the insects grow in the presence of a specific antibiotic. The mosquitoes can therefore be kept in a controlled environment in which they develop and reproduce, but in the wild, in the absence of the antibiotic, the offspring of the mosquitoes will die before they become adults (**see Figure 8**).[166] In 2014 the company was given permission to release the mosquito in Brazil, Panama and the Caiman Islands. In Panama the mosquito was introduced into a 10 hectare area and the company claimed that within six months it had reduced the population of wild mosquitoes by 93%. The release of the mosquito in Brazil and the Caiman Islands is claimed to have reduced the population of wild mosquito larvae by 82%. The company also intends to hold field trials in Florida. In 2016 the American Food and Drug Administration (FDA) announced that these experiments presented no risk to human health and the environment.[167] In 2017 the Netherlands National Institute for Public Health and the Environment (RIVM) published a report on a technical evaluation of this GM mosquito on the island of Saba and concluded that the risks to human health and the environment are negligible.[168]

Until now the development of GM insects to control infectious diseases has mainly been for the self-limiting strategy, in which large numbers of sterile insects are released into the environment, temporarily suppressing the natural population. Because the sterility is not passed onto the next generation, the effect decreases as the insects released into the environment die. However, it is possible to exterminate isolated populations (on islands, for example) by releasing sterile males.

z The sterile insect technique (SIT) is a technique in which male insects are made sterile by irradiation and then released into the environment. Because fertilisation by the sterile males does not lead to the production of offspring, the population size is reduced and thus also the occurrence of the disease (or infestation). The advantage of SIT is that no chemical pesticides are released into the environment.

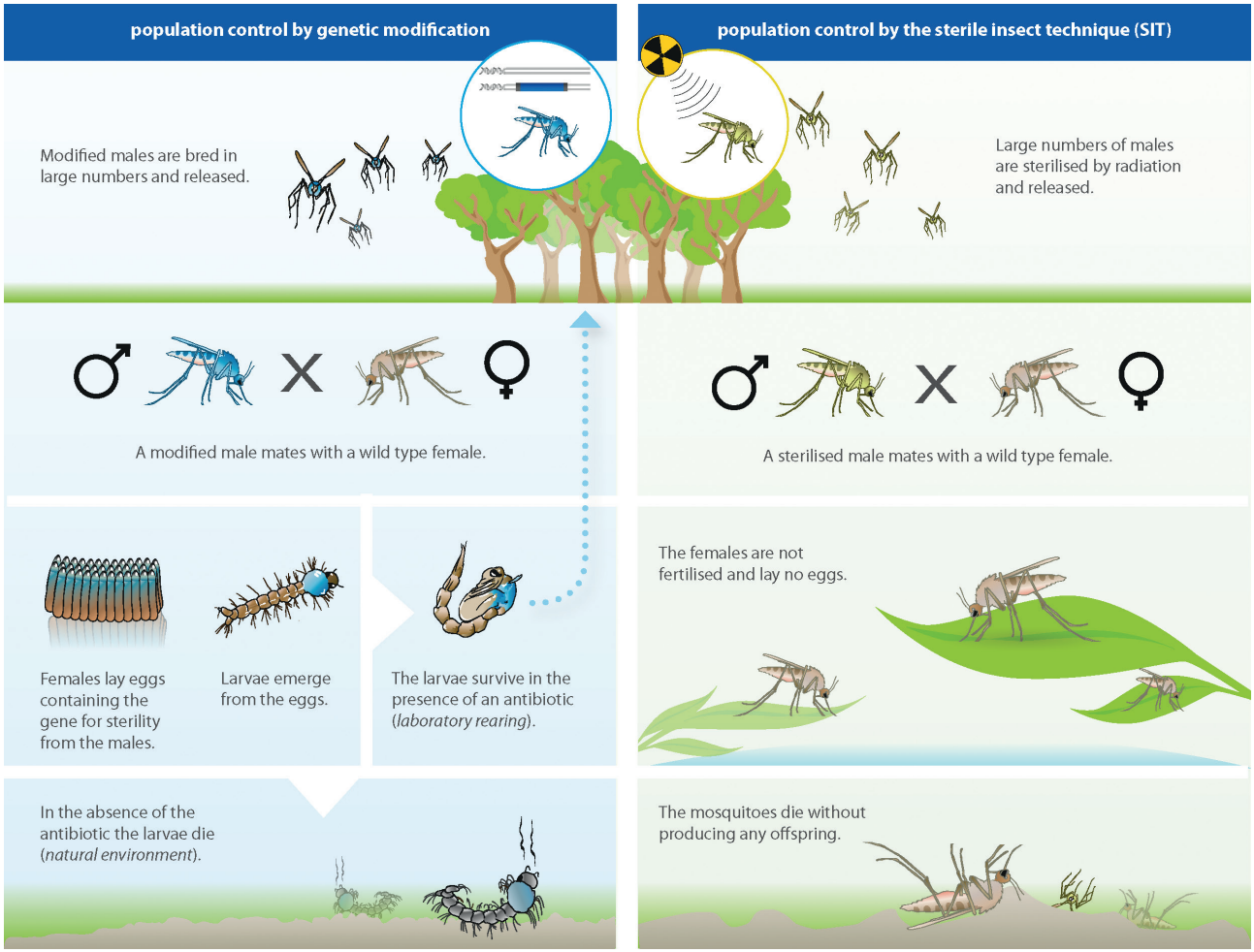

Figure 8: Methods for controlling pests and diseases that use sterile insects.

A specific application of CRISPR-Cas can make the effect of releasing the GM insects more efficient. A technique known as gene drive increases the inheritance of certain traits so that, in principle, all offspring will possess the introduced trait. In theory, releasing even just a few insects could result in the spread of the introduced gene or trait throughout the population within a short time.

Gene drives are not new and are also found in nature. Examples of gene drives are Medea, homing based drives and sex-linked meiotic drives.[169,170] Over the years much research has been done on using gene drives to control populations, but without much success. Some researchers expect that by using CRISPR-Cas it will be possible to develop efficient and work-

able gene drives. By inserting CRISPR-Cas complexes into the genome of an organism in a gene that has to be switched off or in another specific target location in the genome, in combination with the transgene, the desired modification will be made in each chromosome in every copy of the gene or genome location in question. Each gamete will therefore contain the modification and after reproduction all gene copies, including in the gametes in the offspring, will also contain the modification (**see Figure 9**). In theory, this leads to the rapid spread of the trait throughout the population.[171]

Gene drives can only be used in organisms that reproduce sexually and the rate at which the gene drive mechanism can propagate a trait through a population depends on the number of offspring and the generation time. For these reasons, work on gene drives is mainly limited to controlling insects and rodents. However, gene drives are only effective if sexual reproduction takes place between all the animals in a population. If mating is restricted to within certain groups, such as colonies or families, as in some rodents, spread of the gene drive will be restricted to that group. The introduced trait is also an important factor. A trait that has an adverse effect on the individual will spread through a population less efficiently because these individuals will have lower reproductive success. In fact, model calculations show that genes that have adverse traits will eventually disappear from the population, despite the gene drive.[172] Only neutral traits spread efficiently through the population. Research into population control therefore focuses mainly on changing the sex of the offspring. In theory, it is possible to control or even exterminate a population by ensuring that only male or only female offspring are born.[173,174,175]

The successful use of gene drives in insects may also be constrained by the development of resistance. Experiments on mosquitoes in contained areas have shown that after an initial rapid spread, the development of resistance leads to a progressive reduction in the presence of the gene drive in the population in each succeeding generation, starting as early as the sixth generation.[176] To overcome the development of resistance, researchers are now working on the insertion of multiple gene drives, but the success of such an approach has yet to be demonstrated.

Research into gene drives for controlling diseases is largely restricted to malaria. Extermination of the mosquito species that transmits this disease would also result in the eradication of the disease. Another possible approach is to block the transmission of the malaria parasite from the mosquito to humans.[177,178] Gene drives may also offer solutions for other diseases transmitted by mosquitoes, such as Zika or dengue fever.[171,179] In addition, it may be possible to use genome editing or gene drive techniques to control infectious diseases transmitted by other organisms, such as ticks (Lyme disease) and aquatic snails (schistosomiasis, also known as bilharzia).[180]

Several questions still remain concerning the consequences of gene drives, such as whether and how the malaria parasite would develop following the introduction of gene drives, and the possibility of other parasites filling the niche vacated by malaria. Eradicating a species

from an ecosystem, whether or not the species is invasive or the eradication is achieved through the use of biotechnology or other means, can have consequences for other spe-

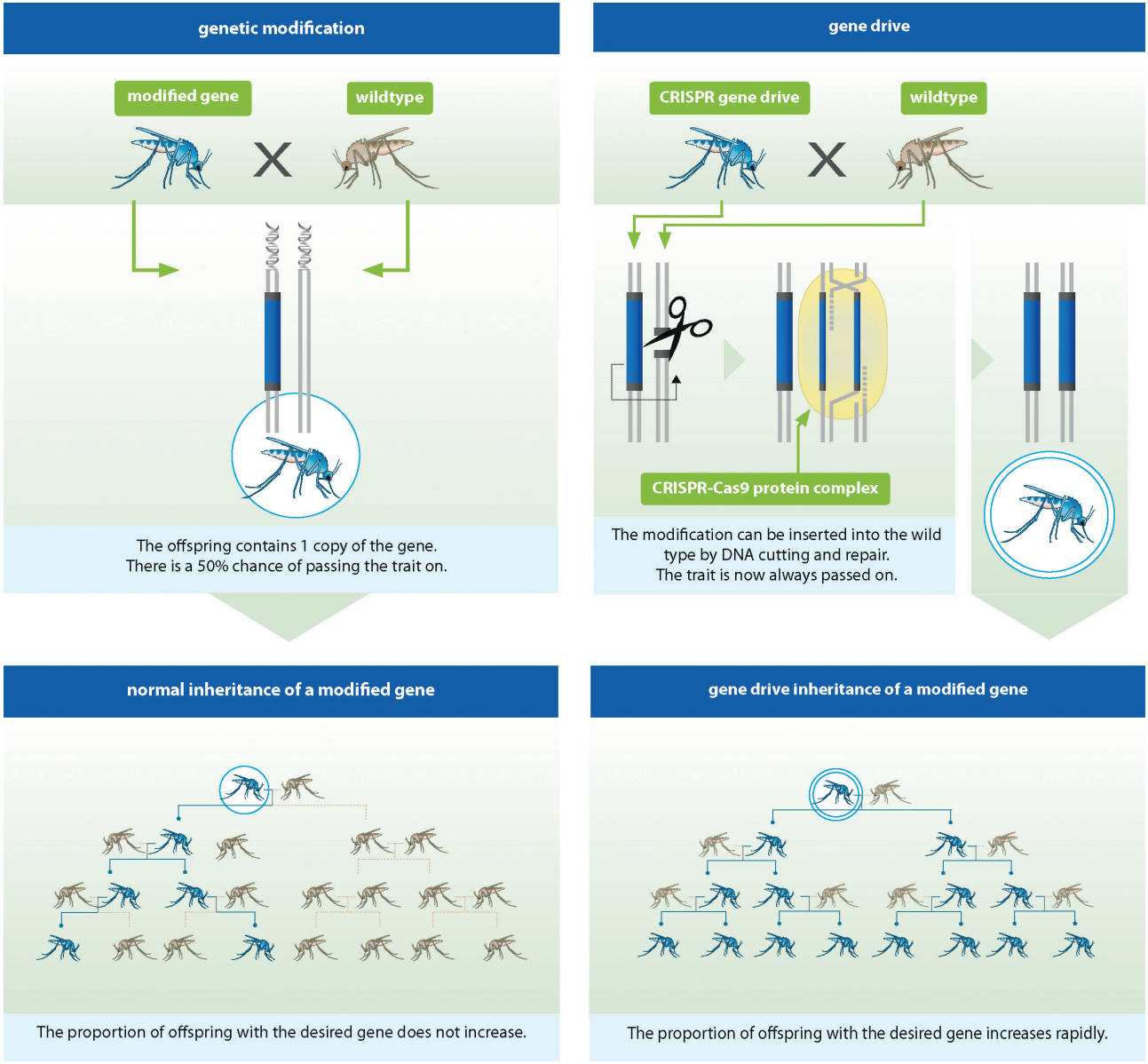

Figure 9: Gene drive mechanism in mosquitoes: genetic modifications ensure that a certain trait is passed on very efficiently to successive generations.

cies. For example, the extermination of a rat species from Palmyra Atoll by poisoning had the unintended, but positive, side effect of also eradicating the tiger mosquito.[181] And on a Caribbean island where invasive rats and goats have been eradicated, several salamander species are now going through a more rapid phase of evolution.[182]

3.4.2 Controlling invasive species

Gene drives can also be used as biocontrol mechanisms for invasive exotics.[aa] Introducing non-native organisms (plants, animals), also called exotics, can have a negative impact on native species. In such cases the organisms are called 'invasive exotics'. Well-known exotics that have caused problems include rabbits in Australia, the cane toad (native in Central and South America) in Asia and Australia, the red lionfish (native to the Indo-Pacific) in the Caribbean, the python (native to Africa, Asia and Australia) in Florida, the coypu (native to South America) in the Netherlands, and the tiger mosquito (native to Asia) which is regularly found in Europe.

In extreme cases, exotics can be a factor in driving native species to extinction. Invasive species may also cause economic loss (such as the damage to Dutch dikes by the tunnelling of the coypu[183] and the costs of controlling them) or pose a threat to human health (e.g. the transmission of diseases by the tiger mosquito).[ab] Besides the above questions about the real effectiveness of gene drives as a mechanism for controlling invasive species and diseases vectors, these applications also raise societal issues (**see text box D**).

D: Societal issues

Releasing gene drives into the environment is controversial. On the one hand, this technique has the potential to control infectious diseases and pests far more effectively than existing alternatives. On the other hand, there are doubts and uncertainties about the consequences of using it in complex ecosystems and our ability to manage or reverse the effects. Researchers have been critical of the idea of using gene drives to control invasive animals. They argue that a successful gene drive is itself not much different from an invasive species and can therefore pose an unacceptable risk.[184,185] Escape or deliberate unauthorised transport or introduction could lead to the uncontrollable spread of animals with gene drives. Based on model calculations, the researchers say that releasing even a handful of organisms with gene drives could have far-reaching and even global consequences.[186] Other research indicates that resistance to gene drives develops quickly and can prevent their further distribution.[187,188] In any case, research is being carried out into ways of managing or 'reversing' gene drives, such as the reverse gene drive and the daisy drive.[171] The daisy drive works in stages to gradually eliminate the gene drive from the population and so prevent a gene drive having an unintentional and possibly global impact on a population.[184]

aa Invasive exotics are animal or plant species that do or did not naturally occur in the Netherlands and which have been introduced intentionally or unintentionally by humans, or which are likely to become established in the near future (Nature Conservation Act).

ab Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union 4.11.2014, L317/35.

3.5 Ecological engineering: endangered and extinct species

Another ecological engineering application is the revival, reintroduction or restoration of endangered or extinct species. These are niche applications which have been worked on for many years. Genome editing techniques present new opportunities for this area of research.

3.5.1 Restoration of endangered animal species

All over the world animals are being threatened with extinction. The causes usually involve a human element, such as hunting or loss of habitat, but can also be the spread of disease through a population. Populations threatened with extinction also often suffer from the effects of inbreeding. As genetic diversity diminishes, the population becomes vulnerable to disease and genetic disorders start to emerge. Biotechnology can contribute to the restoration and conservation of endangered species in various ways: biobanking (storing biological material and genetic information), genomics (mapping genetic information and variation within species), biosynthesis (as a possible alternative to producing materials harvested from endangered species), assisted reproductive technologies (ART) and genome editing or gene drives.[189] Eliminating genetic causes of diseases or introducing more genetic diversity, with or without the help of gene drives, are considered to be possibilities for the conservation or restoration of endangered species.[175,190]

3.5.2 De-extinction

It may be possible with new techniques such as CRISPR-Cas to 'resurrect' extinct animal species, such as the mammoth and the passenger pigeon. This is referred to as 'de-extinction'. There are three main de-extinction methods: artificial selection and back-crossing, somatic cell nuclear transfer (SCNT) and genetic modification (**see Figure 10**).

Artificial selection is a breeding technique in which species that are related to the extinct species are crossed to create an animal that resembles the extinct species. Back-crossing is a form of artificial selection in which the breeder selects for traits within a species that resemble the traits of the extinct species. Continuing to select for these traits will lead to a species with phenotypic traits that closely resemble those of the extinct species. Artificial selection and back-crossing were used as early as 1930 in the Netherlands to reconstruct the auroch.[191] The method is now being used in South Africa to revive and reintroduce the quagga, a subspecies of zebra that became extinct around 1883.[192] A new programme to breed auroch type cattle, extinct in Europe since 1627, was launched in 2009.[193] Researchers hope that by back-crossing with various breeds of cattle they will be able to create an auroch type of cattle that will be able to become established in the wild.[194]

Previous de-extinction research focused on obtaining and cloning DNA from extinct animal species (e.g. from fossils, resin or glacier ice) by SCNT. An egg from a related non-extinct animal species is harvested, the DNA is removed from the nucleus and replaced with the DNA from the extinct animal. However, it is rarely possible to recover a complete and un-

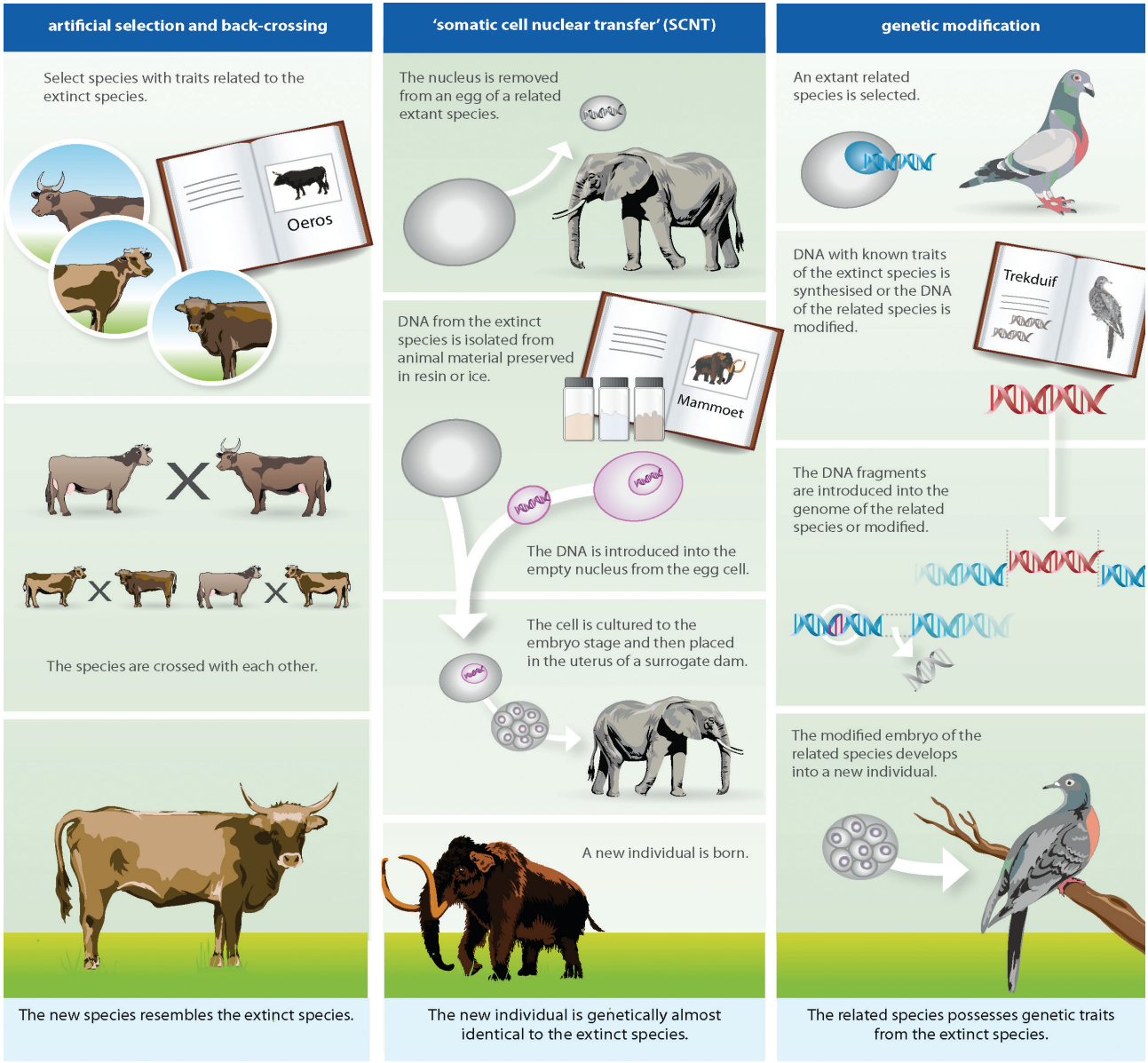

Figure 10: Different strategies for reviving extinct animal species: artificial selection and back-crossing, SCNT and genetic modification.

damaged complement of the extinct animal's DNA. In such cases genetic modification or genome editing can be used to insert fragments of DNA from the extinct species into the

genome of an existing species. Or, genome editing techniques can be used to make targeted alterations to the genome of the related species so that the sequence is the same as in the extinct species. These techniques are designed to 'reverse evolve' existing related species to recreate an extinct species.

Several research groups in America are using genome editing techniques to de-extinct the passenger pigeon, the mammoth and the Tasmanian tiger.[195,196] The woolly mammoth lived about 600,000 years ago and was about as big as the present-day Asian elephant. It died out probably because of climate change and the loss of habitat. The research group has succeeded in modifying the DNA in cell cultures of modern elephants using CRISPR-Cas so that the cells express various traits that distinguish mammoths from elephants: producing more haemoglobin (the protein that carries oxygen through the body), more fat cells and longer hairs to protect the animal from low temperatures. The eventual aim is to 'reverse evolve' the elephant.

The passenger pigeon used to be numerous in forested areas of North America, but became extinct in the early 1900s as a result of hunting for human consumption and loss of habitat.[197] Although a number of stuffed specimens are available in museums and institutes, the DNA is mostly contaminated or damaged. Researchers are now looking to combine DNA fragments from various related species to create a pigeon with as many genetic traits of the passenger pigeon as possible.[198] Actually reviving extinct species or reintroducing endangered species is still a long way off. Moreover, this is not universally considered to be desirable (**see text box E**).

E: Societal issues

Some people fear that bringing back the passenger pigeon could have adverse effects on the ecosystem. The idea of bringing back the mammoth is also not viewed in a positive light by everyone. Moreover, some people think that it is not right to think about de-extincting the mammoth while the elephant populations in large parts of Africa are in sharp decline and conservationists are fighting hard to protect them.[199] Also, where would the mammoth be reintroduced, given that climate change has eliminated its ecological niche and habitat? Besides, there are other more general questions about the consequences and potential risks to ecosystems of reintroducing extinct species. Restoring populations of endangered species, with or without genetic modifications to make them more resilient, would in some cases seem to be a possible answer for animal species threatened with extinction. However, not everyone thinks conserving endangered animals is such an obvious thing to do.[200] Also, some people are concerned that the need to protect and conserve nature may seem less urgent if there are technical fixes available to bring back species that become extinct.

3.6 Sub-conclusions

Domesticated animals

- The aim of most genome editing applications in farm animals (cows, pigs, goats, sheep and chickens) is essentially the same as in current selective breeding practices: increasing yields, conferring disease resistance and improving product quality. The difference is that genome editing can speed up the breeding process where one or just a few spe-

cific genes are involved. Traditional selective breeding is mostly directed at the whole genome and involves selecting for genes distributed across the whole genome that are involved in certain characteristics of production.
- Some applications of genome editing can lead to an improvement in animal welfare. Within existing farming practices, the introduction of disease resistance in animals can be seen as a relative improvement in animal welfare. However, modifying traits with the aim of increasing yields (such as muscle mass) may also lead to physical complications, irrespective of the method used to modify those traits.
- The use of genome editing in farm animals to increase yields, confer disease resistance and improve product quality puts the issue of the desirability of current farming practices back on the agenda.
- Some genome editing applications are so subtle that they are almost impossible to distinguish from naturally occurring mutations. This presents challenges both to policy (enforcing regulations) and to society (consumer choice).
- In theory, several applications are also relevant to Dutch livestock farming (such as those for increasing yields and improving product quality) or are even urgently needed (such as those for disease resistance). A number of these applications are almost ready for market. However, introducing a new trait into existing populations (while necessarily retaining variability and other carefully selected traits) is expected to take at least five to ten years from the first introduction.
- The authorisation for placing on the market of genome editing in animals is itself an unpredictable process. No market authorisations have been given for GM animals in Europe and in the US the authorisation process for the first GM animal for food production purposes (the GM salmon) took almost 20 years.

Laboratory animals
- Genome editing techniques make it easier, quicker and cheaper to produce specific and more complex disease models in laboratory animals for research purposes. This can make a major contribution to advancing research and improving our knowledge of these issues.
- But this may or may not lead to one or more of the following consequences:
  - an increase in the absolute number of GM laboratory animals;
  - an increase in the number of GM laboratory animals in combination with a decrease in the number of non-GM laboratory animals;
  - a shift towards the use of other animal species as test animals.
- In China, genome editing appears to be rekindling interest in the use of primates in experiments, whereas in Europe the aim is to phase out the use of primates. Opinions are divided about the need to use primates as laboratory models.
- The Netherlands has strict legislation on the application of biotechnology in animals held in the Netherlands, but GM animals are imported for use as laboratory animals and some products from GM animals are imported (e.g. medicines). International differences in legislation can encourage 'moral freeriding' in which use is made in the Netherlands of products, applications or research results for which the research or production processes are not permitted in the Netherlands.

Xenotransplantation / chimeric animals for organ transplantation
- Genome editing for xenotransplantation opens up new possibilities for resolving some technical problems surrounding immune reactions and the possible transmission of animal diseases. In addition, progress is being made with the production of human organs in chimeric animals.
- These developments are still at an early experimental stage and clinical or commercial applications are still a long way off. In fact, there is a chance they may never materialise.

- Clinical xenotransplantation is prohibited by law as long as the safety of this application is in doubt. Should these applications become available elsewhere and the prohibition on their use in the Netherlands remains in force, medical tourism will be a distinct possibility.

Ecological engineering: population control

- Gene drive techniques would seem to offer new possibilities for controlling infectious diseases, pests and invasive exotics.
- In some cases, such as the control of invasive exotics, these techniques may offer efficiency and animal welfare advantages over existing alternatives (poison, traps). On the other hand, there are concerns about possible negative, unforeseen or irreversible consequences at the population and ecosystem levels. In Europe, gene drives fall under the GMO legislation and must be assessed for possible risks to human health and the environment.
- Any environmental effects could have consequences beyond national borders, which can be problematic when controlling invasive exotics that are not undesirable in other countries, such as their country of origin. National policy is therefore of limited effectiveness and international cooperation will be required.

Ecological engineering: de-extinction

- Genome editing opens up possibilities (although still theoretical) for reintroducing extinct animal species. Using these techniques to revive extinct animal species is a niche application at an early stage of research.
- What is more likely as a practical application is the reintroduction or restoration of endangered species.
- These applications are controversial and some people consider that other areas of research should be given higher priority for funding. The ability to reverse the extinction of animal species could also undermine the perceived urgency of nature conservation efforts.

4. Legislation relevant to GM animals

This chapter gives an overview of the legislation relating to the genetic modification of animals, covering not only the specific regulations on genetic modification but also more general legislation on the use of animals for various purposes. After a brief review of EU law, we focus on the Dutch legislation.

4.1 EU legislation

The Dutch legislation is largely derived from EU law. EU law on genetic modification consists of several directives and regulations. The implementation of EU directives can vary between member states because they are permitted to take specific national preferences and laws into account. EU regulations are directly applicable in all member states.
EU law relating to genetic modification is primarily geared to safeguarding human and environmental safety. In addition, there are various laws and rules on animal welfare, housing, transport and permitted medical interventions that apply to the use of animals in general, as well as for animals kept for breeding and improvement, and for the trade in gametes (sperm and egg cells) and embryos. The global trade in gametes is considerable. Major players in the trade in genetic material for cattle breeding are the United States and Canada, followed by the Netherlands. Lastly, various EU and national laws and regulations apply to wild, protected and endangered animal species.

4.1.1 Genetically modified organisms (GMOs)

In the EU, applications involving the use of genetic modification are subject to an authorisation procedure in which they are assessed for potential risks to human health and the environment. EU law[ac] defines a GMO as 'an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.' Techniques such as cloning, embryo splitting and cell nucleus transplantation do not fall under the GMO legislation. Organisms obtained by mutagenesis (causing mutations in genetic material, for example by irradiation or chemicals) are exempt from the GMO legislation. In Europe there is a debate about whether or not site-directed mutagenesis techniques, such as genome editing, should be subject to the GMO legislation or exempt from it.[201,202,203,204] The European Court of Justice is expected to provide more clarity on this issue in the course of 2018.[ad]

ac Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

ad This report was compiled before the judgement of the European Court of Justice was published on 25 July 2018. The Court ruled that organisms obtained by new mutagenesis techniques (such as genome editing) are subject to the obligations laid down by the GMO Directive and that these organisms are not exempted from those obligations because there is no proven safety record. This is in contrast to organisms obtained by traditional mutagenesis techniques (radiation, chemicals) which are exempted from the GMO directive. URL: http://curia.europa.eu/juris/documents.jsf?num=C-528/16

EU directives have been adopted on the contained use of GMOs (Directive 2009/41/EC) and on their deliberate release into the environment (Directive 2001/18/EC). In addition, GM food (e.g. meat and GM animals) and GM feed are subject to Regulation 1829/2003/EC, while Regulation 1830/2003/EC concerns the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs (**see text box 'Traceability of GM animals and products derived from them'**).

Traceability of GM animals and products derived from them

Regulation 1830/2003/EC concerns the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs. This regulation applies to both plant and animal material and to food and feed as well as living organisms. GM animals and products derived from them that have been authorised for placing on the market in Europe, either through production or importation, must be traceable and labelled. Applicants are required to provide sequencing information and a method for detecting their product. This method is then validated by the European Joint Research Centre (JRC). The detection method is important for international trade and inspection by customs.

The current inspection methods used by customs and inspection agencies are based on mapping the DNA of a sample from the plant or animal material and looking for general signs of genetic modification or for a specific modification. These methods can be broken down into three levels of specificity:[205]

- Screening methods: these look for DNA sequences that are often used to assist the process of genetic modification, such as promoters, terminators and certain resistance genes. These methods can be used to establish whether or not a modification has been made, but cannot identify the modification itself.
- Construct-specific methods: these look for combinations of elements in a transgene construct. These methods can establish the presence or absence of a specific construct, but cannot distinguish between different events or introduced traits with the same or similar constructs.
- Event-specific methods: these look for sequences unique to a certain GMO at the junction between the DNA of the host and a specific transgene from the donor. This method is highly specific and detects one specific event only.

The possibilities for detection depend on the type of genetic modification made in the GM animal and the information that is available on the modification. If there is no information on what to look for, detection can be very difficult, if not impossible. The emergence of techniques like CRISPR-Cas makes detection even more difficult. A general distinction can be made between insertions (addition of DNA), deletions (removing a gene or part of a gene) and substitutions (replacing DNA nucleotides).

Insertions are the simplest to detect because of the presence of donor sequences. In many cases event-specific detection is possible because when DNA is added to the host genome two unique junction sequences are created at the insertion location. Detection is more difficult when genes from the same species are inserted, because it is harder to detect the difference between a natural variation and an introduced mutation. The same applies to deletions. Although, like insertions, deletions create a new unique junction in the DNA, it still has to be demonstrated that this is not the result of a natural deletion. The smaller the deletion, the harder it is to detect. Nucleotide substitutes (such as point mutations) are harder still to detect and differentiate from natural variations. Finally, there must be a suitable reference for comparison. For animals, this will, ideally, be an animal of the same species, but without the genetic modification. In practice, this is not easy.

In Europe there are no specific regulations for GM animals. The GMO legislation applies to microorganisms (fungi, viruses, bacteria, parasites), plants and animals (vertebrates and invertebrates[ae]). With a view to possible future applications for GM animals, in 2012 the European Food Safety Authority (EFSA) prepared a guidance document on the risk assessment of food and feed derived from GM animals[206] and in 2013 a guidance document on the environmental risk assessment of GM animals,[206] which also covers animal health and welfare. The environment within which GM animals are commercialised depends on the individual species and can vary considerably. For this reason, GM fish, insects, mammals and birds are treated separately in the guidance document. The document identifies the following risks: persistence and invasiveness, including vertical gene transfer; horizontal gene transfer; interactions with target organisms; interactions with non-target organisms; environmental impacts resulting from the use of GM animals; impacts of the animal on biogeochemical processes; and the impact of the GM animals on human and animal health. In addition, several general issues relevant to the environmental risk assessment are treated in depth, such as which animals should be used for comparison[af] and how the environment within which the animal is used can be characterised.

The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the US Food and Drug Administration have published similar documents on the assessment of GM animals. Pharmaceutical applications, such as medicinal proteins produced in animals, are tested for safety by the European Medicines Agency (EMA).

4.1.2 Breeding, keeping and trading animals and animal products

EU legislation on breeding and keeping animals is mainly concerned with animal health issues and environmental risks. The EFSA plays a key role in advising on and assessing risks to animal welfare.[207] A number of EU directives and regulations go into more detail on keeping, housing, caring for, trading, transporting and slaughtering animals, the identification and registration of animals, and the prevention and control of animal diseases and zoonoses (infectious diseases that can be transmitted from animals to humans).[208] Besides general directives and regulations, there are more specific rules for individual species, for example on the trade in gametes (sperm and egg cells) and embryos and on breeding.[209]

4.1.3 Soft law and other initiatives (EU)

Besides the EU directives and regulations, several international and national agreements and codes of conduct have been drawn up on animal breeding practices. These are all concerned with the ethical and social aspects of breeding objectives. An example is the Code

ae Genetic modification in humans is prohibited. See also COGEM & The Health Council of the Netherlands (2017). Editing Human DNA: Moral and social implications of germline genetic modification (CGM/170328-01).

af In the environmental risk assessment it is usual to compare a new application with the existing situation, the baseline. In animal biotechnology, for example, the baseline for a GM farm animal is the most comparable farm animal without the modification. This is simpler to do for plants.

of Good Practice for European Farm Animal Breeding (Code-EFABAR),[210] a joint initiative by industry, animal welfare organisations, ethicists and economists. The code applies specifically to cattle, pigs, poultry and aquatic organisms. For the pig farming industry there is also an international partnership organisation that promotes best practices on health, production, animal welfare and product quality.[211]

4.2 Dutch legislation

EU law is reflected in Dutch legislation on GMOs and animals. The relevant parts of these laws are discussed in this chapter. The Netherlands has a number of committees and councils that advise on the use of animals in different sectors, such as the Committee on Animal Biotechnology (*Commissie Biotechnologie bij Dieren, CBD*) (**see 4.2.2**), the Council on Animal Affairs (*Raad voor Dierenaangelegenheden, RDA*) (**see 4.2.2**) and the Central Authority for Scientific Procedures on Animals *(Centrale Commissie Dierproeven, CCD*) (**see 4.2.3**).

4.2.1 Genetically Modified Organisms Decree

The EU GMO legislation is implemented in the Netherlands in the Genetically Modified Organisms Decree[ag] and the Ministerial Regulation on Genetically Modified Organisms. Applications for laboratory research (contained use) and field trials (deliberate release into the environment) are assessed nationally and licences are issued by the Ministry of Infrastructure and Water Management; applications for placing on the market are assessed at EU level. Activities involving GM laboratory animals, such as mice and rats, that take place in laboratories and animal houses fall under the contained use category. The deliberate release into the environment category includes activities such as field trials with GM mosquitoes. Marketing authorisation is needed for the commercial production of and trade in GM animals and products derived from them. In the Netherlands, applications of animal biotechnology are also subject to a compulsory ethical review (**see 4.2.2**).

4.2.2 Animals Act

The Animals Act covers activities that involve the use of animals and products of animal origin.[ah] It contains rules on animal welfare, animal health, animal veterinary medicines and products of animal origin. The Act applies to all kept animals, unless stated otherwise. The principle underlying this law is that 'the intrinsic value of animals, being sentient beings, is recognised and that any infringement of the integrity or welfare of animals beyond what is reasonable and necessary, must be prevented.' The Act does not explain what exactly is

ag Genetically Modified Organisms Decree of 1 April 2014, containing rules on the contained use and deliberate release into the environment of genetically modified organisms [*Besluit van 1 april 2014, houdende regels met betrekking tot het ingeperkt gebruik en de doelbewuste introductie in het milieu van genetisch gemodificeerde organismen (Besluit genetisch gemodificeerde organismen milieubeheer 2013*)].

ah Animals Act of 19 May 2011, containing a comprehensive framework for rules on kept animals and related topics [*Wet van 19 mei 2011, houdende een integraal kader voor regels over gehouden dieren en daaraan gerelateerde onderwerpen (Wet dieren*)].

meant by the 'intrinsic value of animals' or interventions that are 'reasonable and necessary'.

This law is the basis for various more extensive regulations concerning animals, including the Keepers of Animals Decree (*Besluit houders van dieren*), the Keepers of Animals Regulation (*Regeling houders van dieren*) and the Breeding Decree (*Fokkerijbesluit*). The Keepers of Animals Decree[ai] includes a description of what is meant by the prevention of health and welfare problems when breeding pets.[212] The Breeding Decree[aj] includes provisions on stud books and the identification and registration of breeding animals.

The Animals Act also defines the remit of the Council on Animal Affairs.[ak] This council discusses issues of national and international policy on the health and welfare of animals (**see text box 'Council on Animal Affairs'**).

Council on Animal Affairs (RDA)

The RDA considers issues across the full range of policies concerning animals: kept and not kept ('living in the wild') animals, hobby animals, pets, farm animals (for production) and laboratory animals. The RDA is an independent council of experts that advises the minister of agriculture, nature and food quality, either on request or on its own initiative, on multidisciplinary issues in animal welfare, including animal health and ethical questions. Over the years the Council has published various opinions on keeping animals and on breeding and reproductive techniques, including genome editing. It has also developed an assessment framework and set up an initiative for testing it in 'trial polders'.

Animal biotechnology

The Netherlands has adopted a 'no, unless' policy on genetic modification in animals (both vertebrates and invertebrates). This means that applications for genetic modification are subject to a compulsory ethical review, the outcome of which must be satisfactory before the minister of agriculture, nature and food quality, can issue a licence.[al] Licences for animal biotechnology applications[am] are granted if the activities have no unacceptable consequences for the health or welfare of animals and there are no ethical objections to the activities. There must also be no realistic alternatives available for the proposed research or the proposed practical applications.[213] The law prohibits biotechnological procedures on animals for the purposes of improving sporting performance or for entertainment. This review does not apply to importing GM animals, which falls under EU law.

ai Keeper of Animals Decree of 5 June 2014, containing rules for keepers of animals [*Besluit van 5 juni 2014, houdende regels met betrekking tot houders van dieren (Besluit houders van dieren)*].

aj Breeding Decree of 8 August 1994, containing rules on breeding livestock and other animals [*Besluit van 8 augustus 1994, houdende regelen betreffende het fokken van vee en overige diersoorten (Fokkerijbesluit)*].

ak Article 10.8 of the Animals Act.

al Article 2.23 Biotechnology Licence, Animal Act.

am Animal biotechnology is a broader field than genetic modification and includes, for example, the cloning of animals.

A committee of independent experts advises the state secretary for agriculture, nature and food quality on animal biotechnology. Until 2010 this was the CBD.[214] For many years this committee assessed licence applications and held hearings with stakeholders. During the course of its work, which is documented in its annual reports and advisory reports, it refined and developed the concept of animal integrity in relation to genetic modification.215 However, from 1 January 2010, biotechnological procedures in animals for biomedical research have been exempt from the ethical review required under the Animals Act, and because since then no other types of applications have been made in the Netherlands, the CBD is no longer active. Biomedical research with GM animals still falls under the Experiments on Animals Act and a licence has to be obtained from the CCD.[214]

4.2.3 Experiments on Animals Act

Experiments on animals, whether or not they are GM animals, must first be approved under the Experiments on Animals Act.[an] Two licences are required, one for the institute responsible for the experiment and a separate project licence for each procedure. Licences are issued by the CCD (**see text box 'Central Authority for Scientific Procedures on Animals'**).

Central Authority for Scientific Procedures on Animals (CCD)

In the Netherlands the CCD is the agency responsible for issuing licences for animal experiments. In this capacity it is advised by the Animal Experiments Committees at the individual institutes. As a rule, licences are given if the conditions set out in the Act are complied with. It is prohibited to conduct animal experiments for a purpose that can be achieved by other means or whose importance does not outweigh the distress caused to the animal. In addition, each breeder, supplier and user of laboratory animals must appoint an animal welfare body (*Instantie voor Dierenwelzijn*). Small licence holders are exempt from this requirement. The Netherlands Food and Consumer Product Safety Authority (NVWA) checks compliance with the rules.

The Experiments on Animals Act applies to all living vertebrates and invertebrates[ao] designated by order in council.[216,217,217] Whether or not certain animal species are included depends on how likely it is that the experimental activities will cause distress to the animals or will result in the birth (intended or unintended) of an animal which will suffer distress. Distress is understood to include pain, suffering, discomfort or permanent injury or harm. The presence of a central nervous system is a key factor in making this distinction. Growing, breeding or using most invertebrates is therefore not subject to authorisation under the Act.

Experiments on primates are subject to additional restrictions.[130] EU law states that tests on great apes are only permissible for research that is essential for the preservation of the spe-

an Experiments on Animals Act of 12 January 1977, containing rules on carrying out experiments on animals [*Wet van 12 januari 1977, houdende regelen met betrekking tot het verrichten van proeven op dieren (Wet op de dierproeven)].*

ao From 2010 the scope of Directive 2010/63/EU was widened to include the cephalopods (squid, octopus, cuttlefish and nautilus) and independently feeding larval and foetal stages of mammals from the last (third) stage of their normal development.

cies or in relation to life-threatening or debilitating clinical conditions, and only if the purpose of the procedure cannot be achieved by the use of other species or alternative methods.[ap] Member states that want to permit such research must submit the information that is needed to come to a decision to the European Commission.

However, when transposing this directive into the Experiments on Animals Act the Netherlands introduced a total ban[aq] on the use of most great ape species[ar] (chimpanzees, bonobos, orangutans and gorillas) for experiments on animals and therefore takes a stricter line than the EU directive.[as] A number of other member states have also adopted stricter rules on research involving great apes than required by the EU directives. In the Netherlands, other non-hominid primates (such as macaques, marmosets and rhesus monkeys) may be used, under strict conditions, in fundamental and applied research, for statutory assessments of the safety of chemicals and the efficacy and safety of medicines, for research into infectious diseases, and in transplant medicine. The Netherlands has one of the largest publicly funded primate research centres in Europe.[at]

4.2.4 Special Medical Procedures Act

In Chapter 3 we looked at the new possibilities for xenotransplantation created by the development of genome editing techniques. The legal definition of xenotransplantation is 'the introduction or insertion of living components of an animal or of an animal's fetus or embryo, or of a human component that has been deliberately brought into contact with it, into or onto the body of a human being.' In the Netherlands clinical xenotransplantation is prohibited by law[au] as long as its use is considered by experts not to be sufficiently safe.[av] However, in the second paragraph of this prohibition it is stated that an exception can be made if current medical understanding indicates that unacceptable risks to the patient and to public health can be ruled out.

Scientific research into xenotransplantation is permitted in the Netherlands and as long as it falls within the scope of medical research it is not subject to an ethical review as described in article 2.23 of the Animals Act (animal biotechnology licence). However, this type of research also falls under the Experiments on Animals Act and must be licensed by the CCD. If the procedure involves any genetic modification, an assessment of the environmental risks under the GMO legislation is also required.

ap Article 8(3) in conjunction with Article 55(2) of Directive 2010/63/EU.

aq Article 10e of the Animal Act.

ar Gibbons are also usually included among the great apes.

as Explanatory Memorandum to the Animals Act.

at Biomedical Primate Research Centre in Rijswijk.

au Article 6a, Special Medical Procedures Act.

av Special Medical Procedures Act of 24 October 1997, containing rules for special procedures in the field of healthcare [*Wet van 24 oktober 1997, houdende regels betreffende bijzondere verrichtingen op het gebied van de gezondheidszorg (Wet op bijzondere medische verrichtingen)*].

4.2.5 Nature Conservation Act

Chapter 3 not only described applications in domesticated animals and laboratory animals, but also ecological engineering applications of genome editing. These genetic modifications are made not in kept animals, but in wild animals such as insects, invasive exotics and extinct or endangered species. To what extent these fall under the GMO legislation, the Animals Act or the Experiments on Animals Act, depends, among other things, on the type of animal, how the genetic modification is brought about, and the purpose of the modification (the application area). Genetic modification always falls under the GMO legislation irrespective of the species of animal in question, but insects, for example, are not covered by the Experiments on Animals Act (because they are invertebrates). The Animals Act concerns only 'kept animals' and would therefore appear not to apply, for example, to insects or rodents in the wild (when invasive exotics).

Ecological engineering applications may in some cases also fall under the Nature Conservation Act.[aw] This law is based on the government vision on nature, which sets out the broad lines of government policy for 'the conservation and where possible strengthening of biological diversity, the sustainable use of the components of biodiversity and the protection of valuable landscapes, in both the national and international contexts, as well as the conservation and where possible strengthening of the recreational, educational and amenity values of nature and the landscape, in conjunction with policies for a more sustainable economy.'[ax] This law also contains the 'red lists' of endangered species and the definition and identification of invasive and other exotics.

The legislation that applies to the reintroduction of extinct animal species depends on the method used to revive or reconstruct the species and the purpose for which it is done. The use of ecological engineering techniques to reintroduce extinct animal species raises several (so far theoretical) legal, technical and ethical questions.[218,219,220]

4.2.6 Soft law and other initiatives (Netherlands)

Besides the legislation, in the Netherlands there are various initiatives and groups exploring the direction that agriculture, and livestock farming in particular, should take. For example, the initiative group on sustainable breeding has investigated how the breeding industry can make livestock farming more sustainable and in 2012 produced a sustainable breeding action plan.[221] In 2016, as part of this initiative, the participants examined the role of genome editing in livestock farming.[222] Similar initiatives are ongoing in the dog breeding sector. In 2014 the Dutch kennel club (*Raad van Beheer op Kynologisch Gebied*) introduced the *Fairfok* (fair breeding) programme, which aims to breed a healthy and social dog, starting with pedigree dogs.[223]

aw In 2017 this Act consolidated three laws: the Nature Conservancy Act 1998, the Forestry Act and the Flora and Fauna Act.

ax Article 1.5(2), Nature Conservation Act.

In addition, various bodies in the livestock farming sector have initiated an ethical review process. For example, the Dutch/Belgian CRV, an international organisation for improving cattle, has instituted an ethical review committee to draw up proposals for sustainable livestock farming in Europe.[224] A similar initiative has been launched by international pig breeding company Topigs.[225] An ethical review of animal welfare is not compulsory in the livestock breeding industry.

4.3 Sub-conclusions

- The law relevant to genome editing in animals includes general legislation on the use of animals for various purposes as well as specific regulations on genetic modification. For specific applications, regulations on medical procedures or nature conservation may also apply.
- The Dutch legislation is largely derived from EU law. Genetic modification is subject to authorisation and in the EU these activities must first be assessed for their potential risks to human health and the environment. Authorisation is required for both laboratory research (contained use) and deliberate release into the environment (field trials and marketing authorisation).
- In the Netherlands the laws applying to animals can be characterised roughly by type of application and type of organism.
  - The GMO legislation applies to all organisms – microorganisms, plants and animals – and focuses on environmental risks.
  - The Animals Act applies to all kept animals and would therefore appear not to apply to animals in the wild or free to roam in protected areas and nature reserves. The focus is on animal health and welfare.
  - The Experiments on Animals Act applies to all vertebrates and invertebrates that are designated by order in council. The main aim of this law is to facilitate important scientific research and as far as possible prevent or minimise any distress to laboratory animals. Experiments on animals are subject to authorisation and fall under the responsibility of the Central Authority for Scientific Procedures on Animals (CCD).
  - The Nature Conservation Act applies to both plants and animals. Its aim is the conservation, protection and restoration of wild and cultivated nature. It contains lists of endangered species and invasive exotics. It is not clear whether or how reintroduced extinct animals would fall under the legislation pertaining to these lists.
- In the Netherlands, animal biotechnology applications are subject to an ethical review, with the exception of biotechnological procedures for medical research purposes. The latter are now the only activities involving biotechnology in animals in the Netherlands and consequently the Committee on Animal Biotechnology, which is responsible for the ethical review of proposals for other types of applications, is no longer active. This review does not apply to imported GM animals or products derived from them. These fall under EU legislation, which does not include such a review.
- Besides the formal assessment frameworks for GMOs and laboratory animals, there are other practical arrangements for monitoring and assessing the use of animals, for example in the livestock farming industry.
- There is debate about which genome editing applications should remain or come under the GMO legislation. In 2018 the European Court of Justice is expected to come to a decision on the legal status of site-directed mutagenesis, which could include certain genome editing applications.dd
- Outside the Netherlands, developments are moving fast and it is expected that the results will soon arrive at the European and Dutch borders in the form of imported laboratory or other animals, their gametes and products de-

rived from such animals (food, medicines and other products such as leather and wool). These must be labelled, but will not always be detectable and will therefore be hard to regulate. The same applies to the international trade in gametes (sperm and egg cells) and embryos.

5. Dialogue and governance: why and about what?

This policy report focuses on the emergence and development of genome editing techniques for modifying animals, the political and policy implications of these techniques and their consequences for professional groups, scientists and society. Opinions on the use of animals and the use of genetic modification differ so widely that it is unlikely that all groups will be able to unite behind a clear and unequivocal position. Nevertheless, decisions will have to be taken on the regulation of genome editing in animals. This chapter summarises how genome editing techniques can influence the public dialogue on the use of animals. The essential aim of a dialogue is to explore possible perspectives and to jointly formulate solutions.[226] Then, in Chapter 6, we examine how this dialogue might be organised, and by whom.

5.1 Speed: dialogue on GM animals acquires urgency

Genome editing techniques such as CRISPR-Cas have recently become a standard practice in scientific research and product development. These developments are international in scope and the Netherlands, despite its strict policy on animal biotechnology, will be faced with the consequences, for example via the importation of GM laboratory animals or GM animal products or, indirectly, via the results of international research in which use is made of genome editing in animals. A complicating factor is that in many cases it is difficult if not impossible to detect that genome editing has been used. Given the speed at which these developments are progressing, a linear process from public and expert opinion to policymaking does not seem to be feasible. A parallel, more anticipatory procedure would appear to be more practical.

Genetically modified animals are not new and are a recurring theme on the scientific, political and public agendas, while media reports of new scientific developments periodically reignite public interest in the topic. So far almost no GM animals have been introduced into the public domain and just one GM animal in the world has been put on the market for the production of food. The emergence of genome editing techniques and the new possibilities they offer has again attracted renewed attention to the subject – but this time it is different. Various factors indicate that the debate about these animals has now moved out of the academic sphere and is no longer merely theoretical. Genome editing techniques such as CRISPR-Cas are efficient, effective, broadly applicable and quick to develop and put into practice, overcoming some of the obstacles which in the past have stood in the way of the commercialisation of GM animals.

The international scope of biotechnological research can lead to 'geographical pressure' when GM animals are developed and commercialised elsewhere and then put on the mar-

ket and traded internationally. For example, there are differences in the public acceptance of such animals and in the legislation governing them between North and South America, Asia and Europe, and even within Europe itself. The Netherlands is one of the few countries in Europe that requires an additional ethical review of applications to create a GM animal, but this requirement does not apply to imported animals because they are subject to EU law, which does not require such a review.

Differences in opinion on GM animals will probably remain, but the science continues to develop and it is likely that genome-edited animals and products from these animals will one day appear on the market, and possibly in a form that cannot be detected. Policymakers, the relevant professional groups and bodies (such as the livestock farming sector) and consumers will all have to decide whether or not they accept these developments as they arise.

5.2 Enforceability: regulations unable to cope with international developments

In countries that have ratified the Cartagena Protocol on Biosafety, any transboundary movements of genetically modified organisms must be notified to the competent authority. Moreover, in the EU, all GMOs and products produced from GMOs must be labelled. This requirement also applies to imported products.[ay]

It is theoretically possible, at the molecular level, to detect genetic modifications in animals made by genome editing if it is known which DNA sequences to look for. However, if genome editing is used to introduce genes from the same species or to make small, targeted mutations or deletions, this will in practice be virtually impossible, because it is very difficult or impossible to distinguish these changes from natural mutations. The effectiveness of any detection test will depend on the information provided by the producer. By law this information must be supplied when marketing authorisation is given (either for production or import and for both animals and products of animal origin (**see Chapter 4**)), but not for animals used for research purposes only. Cases have been confirmed of laboratory animals entering the food supply chain, which shows that this type of incident cannot be ruled out.[227]

Genome-edited animals, their gametes, body tissue or products derived from them could enter the Netherlands, intentionally or unintentionally, as a result of differences in the legislation between jurisdictions (when applications fall under the GMO legislation in the Netherlands and the EU, but not in other countries) or as a result of illegal importation or production. Besides being illegal, this can infringe the freedom of choice of the producer

ay Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

and the consumer and, in cases of mixing or unintentional use of products of animal origin made by genome editing, may lead to considerable economic losses in the organic food or other sectors through loss of certification.

5.3 Complexity: transdisciplinary research needed

Some genome editing techniques, such as gene drives, aim to bring about ecological changes. The use of GM animals to make changes in the environment used to be restricted almost entirely to modifying mosquitoes, for example to control infectious diseases or agricultural pests, and the strategies used were self-limiting. Now, gene drive technology is being used in other animals as well, such as rodents, to control infectious diseases or pests in complex ecosystems. This raises questions about the irreversibility and controllability of the consequences. Applications such as these will probably not be geographically restricted and so the spread of gene drive organisms may not stop at national borders. This means that any Dutch policy initiatives will have little effect and international consultation and cooperation will be needed when such applications become available in neighbouring countries.

The accessibility and practicality of genome editing techniques may well broaden the scientific and commercial arena within which applications are developed and affect a wider range of activities, organisations and businesses, from livestock breeding and farming to environmental protection and nature conservation. All this is influencing the further development of the technique, as well as the allocation of rights, responsibilities and powers. It is not only the scientific possibilities that are changing, but the commercial playing field, professional boundaries and implications for society as well.[228] Evaluating these applications is made more complicated by the larger variety of applications and number of stakeholders involved. Bringing transdisciplinary expertise and perspectives to bear can help with getting to grips with this complexity.

5.4 Mobilisation potential: ecological applications fuel debate

Applications that involve a combination of GMOs, animals and environmental effects have a greater social mobilisation potential and may further stoke existing public discussion. This also applies to niche applications such as reviving and reintroducing extinct or endangered animal species. Much of the debate on these applications, as for genome editing for artistic purposes, has to do with questions about the need for them and their usefulness, certainly if they involve uncertainty and risks (**see text box 'Mobilisation potential of alarming studies and art with animals'**).

Mobilisation potential of alarming studies and art with animals

In 2003, COGEM reported on the response to alarming studies about the safety of GMOs, pointing out that too much emphasis on scientific facts and objectivity hampers the underlying and unavoidable debate about values,[229] and stressing the importance of exploring these context-related arguments and articulating them in the policymaking process. In addition, in 2014, at the request of the Ministry of Infrastructure and the Environment, COGEM reported on the use

of genetic modification and animals in art projects and observed that the need and utility discussion about these types of applications cannot be hedged by legal framing.[10] There are different views on the intrinsic value of animals which do not always align with their legal or lawful right to protection. Moreover, no strict division can be made between vertebrates and invertebrates, between animals and products of animal origin, or between applications in educational art and scientific settings. Acknowledging and explicitly stating these perspectives can create space for a mutual learning process and a possible willingness to come to a decision.

In Chapter 2 we discussed the general themes which play a role in the debate on the use of animals for human purposes. We saw that opinions can vary considerably and depend on both the animal concerned and the context in question; the use of an animal may be considered acceptable in one context but not in another. Moreover, the degree of acceptance depends on the purpose or the interest being served. In general, the greater the importance or advantage to humans, the greater the acceptable costs to animal welfare. Dutch law is based on this assumption. Also, many people have an ambiguous attitude towards the use of animals[230] and the new possibilities for modifying animals provided by genome editing will probably do nothing to dispel this ambiguity, and may even heighten it. It is hard to predict what might spark off a heated public debate and how it will progress; licence applications, imports (legal or illegal) of GM animals or products derived from them, and media attention are all possibilities.

5.5 Naturalness: existing terminology of limited use

Naturalness is a recurring term in the debate about biotechnology and genetic modification and has a whole range of possible meanings. It is used in legal (definition of a GMO), scientific (biological) and social contexts, where it has different meanings and connotations. Some genome editing applications are so subtle that they can be almost impossible to distinguish from spontaneously occurring mutations. These modifications are therefore no longer by definition associated with transgressing species boundaries or the use of foreign DNA, which is one of the most frequent objections to genetic modification in the Netherlands. In the genome editing debate this is sometimes associated with the term 'natural' and the argument is made that such modifications ought not to meet with objections (s**ee text box 'Naturally occurring mutations not necessarily acceptable'**). On the other hand, the term 'natural' is generally used by opponents of genetic modification, who posit it as the opposite of genetic modification. This illustrates how terms can have different meanings to different groups of stakeholders.

Naturally occurring mutations not necessarily acceptable

In discussions about the limits in breeding or modifying animals it is sometimes argued that a naturally occurring mutation will meet with little objection and that if such a mutation is brought about by a genome editing techniques it should be exempt from the legislation. This argument is based on an assumption about the nature of these mutations (positive or acceptable) which does not necessarily reflect public opinion.

Hornlessness in cattle is a natural mutation which at first sight has few consequences for the welfare of the animals themselves, but is of benefit to cattle farmers. It also avoids the suffering or distress caused by the physical removal of

horns, which is normal practice. But naturally occurring mutations can also lead to health problems, an example being the mutation that affects the growth of muscle tissue in the Belgian Blue breed (**see Chapter 3**). There are also examples in pet breeding of selecting for 'natural' genetic traits that are not always beneficial to animal health and welfare. These examples show that in different contexts or within different practical settings, the same mutations can be valued differently and that 'naturalness' is not by definition a good criterion for assessing the desirability of a certain modification, irrespective of how it occurred (naturally, artificially maintained or introduced).

The terms 'natural' and 'not natural' and 'genetically modified' and 'not genetically modified' no longer accurately reflect the subtleties of the technical possibilities, which sometimes build on natural occurrences or biological processes, although this does not automatically mean they are not problematic or do not meet with public resistance. This is because the size or impact of the genetic modification does not have to be proportional to its degree of acceptance. Research indicates that the metaphors and terminology of genome editing influence the framing of public opinion.[231] Terms such as 'editing' and comparisons with computer code suggest a precision and accuracy that is not justified by the uncertainties of scientific practice.

This strips the term 'naturalness' of some of its value and utility, particularly in legal and public debate. In 2010 the Council on Animal Affairs (RDA) observed that in the debate it can be useful to state in concrete terms what the underlying values are.[230] The question then is what the important values are regarding the use of animals and do we want to protect or conserve, and under what conditions? This may provide a better basis for the substantive dialogue between stakeholders (**see Chapter 6**).

5.6 Proportionality: shift in public perception

In the past, genetic modification of animals was used mainly in the service of human interests, mostly in the form of GM laboratory animals for both fundamental and applied (mainly medical) research. The few commercial applications also have a strong human interest: the fast growing GM salmon, a pig that excretes less phosphate in its manure, and animals with increased muscle growth (for meat production).

Genome editing offers possibilities for conferring disease resistance in farm animals and controlling pests that are less animal unfriendly than current practices (disease resistance instead of vaccination[az] or culling infected animals; gene drives for invasive exotics instead of poisoning and setting traps). Such applications not only serve the interests of humans (such as increasing yields) but can also improve animal welfare (within the existing livestock farming and food production system). However, it must be said that these applica-

az In many cases vaccination means excluding the animal from the production chain because it is impossible to distinguish between vaccinated animals and animals that have been infected. In practice, this means that animals are more likely to be destroyed than vaccinated. Vaccination is therefore not necessarily a desirable alternative to current practice.

tions probably do not match up to the standards required by those who subscribe to an absolute interpretation of integrity or consider the existing context within which animals are used to be unacceptable. They simply aim to minimise or prevent distress to animals held within the current agricultural and scientific systems. Existing systems have a long history of functional use of animals for the production of meat and milk (livestock farming) or for research purposes (laboratory animals) in which concessions are made to animal welfare to greater or lesser degrees.[28] Specific applications of genome editing in animals do not in themselves necessarily have an adverse effect on animal welfare, but they do take place in a system which has an inbuilt tendency towards increasing instrumentalisation of animals and a restriction of animal welfare. Moreover, developing applications of genome editing in animals may involve the need for more experiments, which in turn may lead to an increase in the number and type of modified laboratory animals. Genome editing can also facilitate a more progressive instrumentalisation of animals than is now the case, for example for xenotransplantation and the generation of chimeric animals.

The driving forces behind applications of genetic modification in animals have been mainly scientific, medical or therapeutic, economic and the interests of human convenience. The introduction of genome editing techniques adds to these motives the relative interest of the animal itself, which can alter the tenor of the debate and shift the balance of interests and degree of support for applications. Whether this will happen, and if so, to what degree, will depend on the context of each specific application.[61] When assessing the relation between the purpose of an application and the use of genome editing to produce it, the principle of proportionality therefore deserves to be given greater attention.

Sub-conclusions

Based on the content of the previous chapters, COGEM has identified six themes of substantive or procedural importance for the political, policy, professional, scientific and public debate on genome editing in animals:

1. Speed: The pace of developments urgently requires international coordination, the informing of public opinion and the development of policy in anticipation of changing public perception of genome editing in animals.
2. Enforceability: Detecting applications of genome editing in animals is in many cases only possible if it is known which DNA sequences to look for – and even then it is hard to tell the difference between introduced mutations and naturally occurring mutations. Enforcement is therefore seen as increasingly problematic, both in terms of practical implementation and international coordination.
3. Complexity: Applications with potential cross-border environmental and ecosystem effects require a multidisciplinary approach to risk assessment and international consultation procedures.
4. Mobilisation potential: The use of genome editing in animals may fuel an intensification of existing public debates. It is crucial that in these situations the government not only draws on scientific facts, but also acknowledges contextual and value-related arguments and articulates these in its policymaking processes.
5. Naturalness: The nature of the applications makes the concept of naturalness less useful, particularly in the legal context. It is therefore important to clarify the arguments underlying the interpretation and understanding of this concept.

6. Proportionality: Some new applications not only provide benefits to people, but also provide relative benefits to animal welfare, while other applications facilitate further instrumentalisation of animals, which can harm animal health and welfare. This can alter the balance of risks and benefits.

6. Dialogue and governance: how?

The Dutch government wants biotechnology and the relevant government policies to meet society's needs and address its problems. Engagement with stakeholders is one way it tries to achieve this, for example in stakeholder meetings organised by the Ministry of Infrastructure and Water Management as input to the modernisation of its biotechnology policy.[232,233] International initiatives have also been developed for public input to biotechnology research agenda-setting ('upstream participation') and the development and use of the results of research and applications.[234,235,236] However, the outcomes of these initiatives also present challenges, as described in the following sections.

6.1 Stakeholder engagement achieves little

Meetings regularly take place with stakeholders and interested members of the public to discuss developments in biotechnology and obtain input to policy and decision-making on biotechnology. However, there is an impression that these meetings are seldom successful. Proponents and opponents rarely seem to move any closer to agreement and discussions tend to degenerate into an exchange of well-worn positions, which makes it extremely difficult to incorporate the outcomes of such meetings into the policymaking process to everyone's satisfaction. Also, participants at stakeholder meetings sometimes feel that developments are overtaking them, that it is too late for any meaningful input and that little can be done to influence the further course of developments.[237] The importance of normative aspects in the debate about genetic modification is acknowledged, but these normative considerations are sometimes excluded from legal decision-making,[238] which can lead to frustration among stakeholders.

There is a continuing interest in and a need for clarity about the purpose of the dialogue with civil society – and about stakeholder engagement in particular – and its role in shaping the future of biotechnology. This is also an issue in the Netherlands and it has COGEM's full attention.[ba]

6.2 Available methods not at fault

Engaging stakeholders in decision-making about the development of technologies is known as adaptive governance for responsible research and innovation. Core values in such processes include anticipation, inclusion, reflexivity and responsiveness.[239] There is a considera-

ba In 2017 the COGEM Subcommittee on Ethics and Societal Aspects established a working group to explore the role of stakeholder engagement and the conditions necessary for it to be effective. The findings of this working group have been incorporated into an internal memorandum and in this chapter applied to the identified issues relating to genome editing in animals.

ble body of qualitative literature on how to put this approach into practice.[240,241,242,243,244,245] Besides analyses of the effectiveness of certain methods, guidance is offered on which methods to use and when.[246,247,248,249] COGEM has commissioned several research projects on stakeholder engagement and genetic modification.[249,250] In addition, COGEM has reported on the failings and stumbling blocks in processes that involve stakeholder participation, mentioning in particular transparency about the goal of the process, limitations of the input, and the inclusion and continuity of stakeholder groups.[251] Several methods and assessment frameworks can be used to help identify the societal implications of technology or to weigh up a broad range of factors.[252,253] COGEM observes that the disappointing results of stakeholder engagement may not be down to the available methods, but to the stage preceding the choice of a particular method. Often there is confusion about the goal of stakeholder participation (to refine the substance or improve the process), the facts of the matter at hand, the nature or form of engagement (participation, consultation or representation) and who the stakeholders are. In summary, we can identify three problems with stakeholder engagement:

1. disagreement about the definition of the problem;
2. disagreement about the goal and methods to be used to solve the problem;
3. tackling unstructured problems[bb] with participation methods for structured or semi-structured problems.

If there is confusion or disagreement (either deliberate or unintentional) about the nature of the problem, whether it be structured or unstructured, it will not be possible to set an unambiguous goal. And if there is no clear goal, opinions on whether or not the stakeholder engagement has been successful will be divided.

6.3 Problem structuring: an essential starting point

To determine whether or not stakeholder engagement has been a success, the goal of that process has to be clear from the start. Success can be measured through different prisms, based on internal criteria (determined by the participants) or external criteria (determined by the initiators of the participation process), and on substantive criteria or process-related criteria. Participants may have different (intrinsic and extrinsic) objectives and may also come to a different evaluation of the success or otherwise of the process. In some cases, stakeholder engagement may simply be a goal in itself as part of a democratic legitimation of policy or decisions.

In attempting to deal with the three problems listed above, we can make a distinction between two phases of stakeholder engagement in the debate about genome editing in ani-

bb In the literature, biotechnology and genetic modification are often identified as 'unstructured problems' (Hisschemoller & Hoppe, 1995), which are characterised by disagreement about the scientific facts and the social values at stake.

mals: 1) the problem structuring phase and 2) the identification and evaluation of possible solution strategies.

6.3.1 Phase 1: Problem structuring

Phase 1 is about exploring and structuring the problem. This phase of stakeholder engagement has a largely normative character.[254] The discourse is predominantly normative (to identify opportunities and risks in the societal context) and reflective (via argumentation, confrontation and adjustment).[248] The starting point is not a problem already defined by the initiators of the stakeholder activity, but the subject about which there is disagreement or dissensus.[255] For the problem structuring phase, Castle & Culver (2013) developed the 'contested exchange' method in which instead of looking for shared values, the aim is to uncover exactly what there is disagreement about.[256] This helps to structure and clarify the preparatory stage of stakeholder engagement. The underlying idea is that a frequent mistake is to look for consensus too soon, under the misapprehension that the problem is already clear, with the danger of overlooking alternative solution strategies. Poort (2012) breaks down a joint learning and opinion-forming process for problem structuring into two steps:

1. identifying and documenting different perspectives on the theme/problem (articulation);
2. systematic exchange of insights from these perspectives, focusing on the contentious points (confrontation).[257]

Structuring and clarifying the problem makes it easier to identify policy objectives and find suitable methods for achieving these objectives (phase 2). However, the aim of problem structuring is not to make policy; it is a method that can be used in the preparatory stages to reveal difficulties and obstacles and to facilitate opinion forming.

The societal implications of a technology are often not known beforehand, even to those developing the technology. The applications of a new technology do not just elicit public approval or rejection, they also change our social and cultural structures, which is why it is important to be flexible during this preparatory phase, with the ultimate aim of obtaining a common problem definition.[245] Different levels of understanding, polarised positions and attitudes, and the withholding of interests all make it difficult to draw up a broad agenda that encompasses both technical and social issues. It is therefore advisable to uphold a participation ethic in which reciprocity is a core value: participants have obligations towards each other, but may also have expectations of each other (**see 6.4.3**).

New questions about the development of a technology or issues which raise new perspectives on existing issues require a phase of problem structuring before a start can be made with looking for solution strategies. Phase 1 can help to articulate the nature of those new perspectives.

6.3.2 Phase 2: Identification and evaluation of solution strategies

Phase 2 is about identifying solution strategies, but this only makes sense once a common problem definition has been agreed. This form of stakeholder engagement is pragmatic in na-

ture and therefore often involves a more restricted and specific group of participants.[248] The discourse may be more cognitive (problem-oriented, solutions within a professional or user context) and/or regulative (testing and reflecting on procedures and regulations).[254] Phases 1 and 2 do not necessarily have to take place consecutively and may be conducted separately.

Phase 2 is not just about policy options and regulation (hard law), but also 'soft law' options. The term soft law refers to semi-formal instruments with limited legal force in which agreements are made between stakeholders and other interested parties. It includes things like codes of conduct, covenants, quality marks and certifications. By its nature, soft law provides low threshold options for organising new and uncertain developments, with the possibility in future of turning working agreements into actual regulations (hard law).

Issues relating to technology development that are not new but which clearly stretch existing policy or legislation to the limit, and which will necessitate revision to policy or legislation, can be taken on board during phase 2. Issues which have been identified in sufficient detail in phase 1 and have been given a clear problem definition, can also be included in this phase.

6.4 Conditions

Successful stakeholder engagement depends on meeting at least three conditions, which can be considered as constituting a professional ethic for stakeholder engagement: a transparent goal, a transparent process and the principle of reciprocity.

6.4.1 A transparent goal

Organisers of stakeholder engagement operate within a specific context and with a certain goal in mind. However, the goal of stakeholder participation is not always made explicit by the initiators of the process. The goal and method of stakeholder engagement must be appropriate to the topic under discussion and a precise definition of the goal is a prerequisite for successful stakeholder engagement. A key factor in evaluating the success or otherwise of stakeholder participation is the degree of participation. It is imperative, therefore that the goal, and who has set it, is clearly stated at the outset.

6.4.2 A transparent process

Stakeholder engagement can take many forms, although the terms used to describe different methods are often not used consistently. First of all, a distinction can be made between stakeholder engagement and public dialogue[bc] And within stakeholder engagement, different forms of involvement can be identified. Stakeholder consultation is about gathering information and different perspectives. Stakeholder participation is when the stakeholders

bc Stakeholder engagement is primarily used for input to policymaking and professional and political decision-making processes. Public dialogue can have direct or indirect consequences for policymaking or decision-making, but not necessarily. It can also help to raise awareness of technological developments and facilitate opinion forming.

take an active part in a specific process with a particular goal. Different degrees of participation are possible: for example, what mandate do the stakeholders have in the decision-making process? Do they have a formal vote or are they a sounding board or consultative group? In the interests of managing expectations, this must be made clear at the start of the process.

6.4.3 Principle of reciprocity

In addition to carefully determining the goal of the stakeholder engagement and clearly communicating the nature of the process itself, the relation between the participants should be one of reciprocity, whatever their positions and competencies may be. All participants then become 'owners' of the process, each with their own rights and obligations (own responsibilities). Initiators have a duty to make it clear exactly what their goal is, what their own agenda is and what the level of participation is (how much influence stakeholders have on the decision-making process). They should also clearly state which problem (or aspect of a problem) is being addressed. These obligations can also be seen as rights of the participants. At the same time, participants have a duty to reveal their own agendas, interests and expectations, because only then will initiators be able to respond to these expectations – or not. If neither side is clear about what their goals are, what expectations they have of participation, or which problems are being addressed, there will be a risk of participants going off in different directions without being aware of it or without taking responsibility. Some participants may take part in stakeholder activities for strategic reasons or with a hidden agenda. A reciprocal process brings this into the open. Although participants may still have goals that deviate from that of the process being followed, putting these on the table at the start gives the initiators the clarity they need to decide on an appropriate course to follow.

6.5 Dialogue – with whom?

Reports on emerging technologies often call for a public dialogue to determine the direction a policy on the emerging technology should take and build broad support for it. First, this suggests that a consensus can and should be found. Second, it raises the question of who should take part in this public dialogue and who should organise it. These are difficult questions which have no clear-cut answers.
Strictly speaking, stakeholders are people or representatives from organisations with an interest. In practice, the term stakeholders sometimes refers to a much wider group of people who are potentially affected by, or involved with, or have an interest in the issue or problem. In this section we identify several main groups of stakeholders and interested parties and give an indication of the types of contributions they could make to the public debate and stakeholder engagement on genome editing in animals. For other technologies or applications, the relevant groups may be different.

This is by no means an inclusive or exclusive list. It is important that the three main groups of stakeholders (society, technology and policy) can be involved interactively in both phase 1 and phase 2. While normative discussions (phase 1) require the broadest possible input,

for the more pragmatic discussions about concrete problem definitions (phase 2) it may be sufficient to involve selected representatives from the main groups.

6.5.1 Society: consumers, patients and the public

Stakeholders: consumers, patients, the public and the umbrella organisations that represent the interests of these groups.

This group of stakeholders indirectly determines the acceptance or level of support for the application of a new technology by deciding to use or not to use the end products. Their decisions to use certain products and services or not to use them may or may not be a conscious choice, depending on the availability and accessibility of the relevant information. It also assumes that consumers form an opinion and consider the subject to be important. However, dialogue can also be designed to facilitate opinion formation.

Regarding genome editing in animals and products derived from these animals, stakeholders hold a wide range of preferences (some are meat eaters, vegetarians, vegans, etc.), priorities (price, quality, amount) and convictions (animal welfare, sustainability, etc.), which are sometimes contradictory or inconsistent. This does not have to be problematic, because Dutch consumers also have a wide choice of products of animal origin, which allows them to put their wishes and priorities into practice as consumers. However, public acceptance of the way certain products are produced can change over time. For some biotechnologies, such as xenotransplantation and growing human organs in chimeric animals, the level of acceptance may be as yet entirely unknown. Now that the emergence of genome editing promises to remove the technical obstacles to the development of such technologies, it makes sense for technology developers and users as well as policymakers to assess the level of public support for these applications.

Incorporating public preferences and wishes into practice takes specialist technical expertise and knowledge about specific animal species. In its report on breeding and reproductive techniques, the Council on Animal Affairs (RDA) concluded that buyers of animal products know too little about breeding practices to make informed decisions about what the objectives of this sector should be.[258] However, social stakeholders play a crucial role in the problem identification and structuring phase (phase 1), while the feasibility of putting public preferences and wishes into practice is only addressed in phase 2. To generate acceptance or build public support for new technologies, social stakeholders must remain involved in phase 2 by including interest groups such as patients associations, consumer organisations and NGOs from the organic sector and environmental organisations in meetings with technology users and regulators of genome editing.

6.5.2 Technology: developers and users

Stakeholders: scientists, professional groups such as breeders, veterinarians and breeding support services (inseminators, breeding extension and pet supply shops) and umbrella organisations (breeding associations).

These stakeholders not only have expertise in genome editing technology, but also know the professional practices in which it can be applied, and as such they form a bridge between civil society and government policymakers. However, it should be pointed out that this expertise needs to be maintained, which means that professional groups such as breeders and vets should keep themselves informed about the latest technical and scientific advances. An important part of this is coordination and dialogue between these groups on technical possibilities and future developments. The RDA has also pointed out that educational and training courses, for professional and support groups (users) as well as scientists and technologies, should include scientific advances in reproductive techniques as a permanent element in the curriculum.[258]

Besides increasing yields and improving quality, animal breeding also has to take account of environmental and biodiversity interests, which go beyond the scope of the individual breeder's competence. Breeding objectives are therefore formulated at a higher level, such as that of the stud or herd book and breeding societies. To bring about the desired changes, choices are then made at the level of the individual animal. The RDA has concluded that the breeding industry and other parties in the animal value chain have a responsibility to identify ethical issues in animal breeding and to tackle them jointly. One element in this process could be proactive engagement in the genome editing debate, which in turn will involve exchanging information with the scientific community.

The breeding industry also has its own interests, preferences, priorities and wishes, such as the maintenance and improvement of certain animal species, the maintenance and improvement of production and quality levels, and the maintenance and improvement of the level of sustainability of animal production. The stakeholders in the technology developers and users group should have an input not only to the problem structuring (phase 1), but also to the identification of possible solution strategies (phase 2).

The legal possibilities for putting these preferences into practice are in turn determined by the next group of stakeholders: policymakers and policy implementers. Policymaking requires specialist knowledge of the legislation and the international context.

6.5.3 Policy: professional experts and implementing organisations

Stakeholders: multidisciplinary experts and advisory committees, policymakers, legal experts.

The government is responsible for making policy on the minimum standards for animal health and welfare in the Netherlands, including supporting instruments such as identification and registration. In doing so, the government is expected to take account of the wishes and preferences of the social stakeholders as well as those of the technology developers and users. The government not only promotes dialogue between the various stakeholder groups to facilitate opinion formation, but also stimulates innovation (such as the development of alternatives to laboratory animals and stimulating sustainable livestock farming).

The government's role is therefore primarily as a process manager with a responsibility for problem structuring, conflict management and balancing all relevant viewpoints, while disregarding the dominance or power of a certain group in an economic sector or field of application.[248] This is an important point that also touches upon the issue of who should represent each group and thus demand the right to be spokesperson. Sometimes it is hard to assess how representative individual players are, but much can be learned from investigative studies or surveys held before the start of the policymaking process. The form of stakeholder engagement must also be appropriate to the institutional and legal context, which is sometimes referred to as 'scalar fit'. The scalar fit of the dialogue is the degree to which it is aligned with existing jurisdictions and institutions, such as the existing legislation (Dutch and EU) and the existing advisory and regulatory bodies on biotechnology. The government, as process manager, also has the responsibility for overseeing this aspect.

The government and policymakers must have access to a broad range of information and expert opinion in order to properly assess and weigh up the various interests at stake. They can draw upon the expertise in advisory bodies (such as COGEM and the RDA), but can also maintain direct contacts with stakeholders in the field.

As an exercise, in **Appendix A** to this report the societal and ethical aspects of genome editing in animals are associated with the relevant stakeholder groups and the goal of the dialogue to produce an outline of an indicative dialogue agenda.

Sub-conclusions

Stakeholder engagement is seen as an important instrument in the development of policies for technological applications and for gaining acceptance and support for those policies. At the same time, holding stakeholder activities and interpreting and implementing the results present a considerable challenge, and in many cases those involved are dissatisfied with the outcome. This chapter looked at the causes of these problems with stakeholder engagement and the possible improvements that can be made in the procedures.

- COGEM observes that the dissatisfaction with stakeholder engagement is not so much to do with the available methods for stakeholder engagement itself, but with the process that precedes it: the problem structuring (phase 1). It is only when this is resolved that stakeholder engagement can focus on the identification and evaluation of possible solution strategies (phase 2).
- Successful stakeholder engagement depends on meeting at least three conditions, which can be seen as constituting a professional ethic for stakeholder engagement: a transparent goal, a transparent process and recognition of the principle of reciprocity.
- In the debate and dialogue about genome editing in animals, three main groups of stakeholders can be identified: social stakeholders (such as consumers, patients and the public), technology developers and users (such as scientists, professional groups and umbrella organisations) and policy-oriented stakeholders (such as advisory bodies, policymakers and legal experts).
- The three main groups of stakeholders (society, technology and policy) should be involved interactively in both phase 1 and phase 2. Whereas normative discussions (phase 1) require the broadest possible input, for the more pragmatic

discussions about concrete problem definitions (phase 2) it may be sufficient to involve selected representatives from the main groups.

7. Conclusions and observations

Genetically modified animals are not new and the topic is a recurring theme on the scientific, political and public agenda. However, almost no animals of this type have yet been introduced into the public domain (outside medical research). The newest techniques, referred to as genome editing, are expected to change this situation. Techniques such as CRISPR-Cas are efficient, effective, broadly applicable and can be developed very quickly. This removes many technical and economic barriers which in the past have hampered the commercialisation of GM animals. At the same time, it means that detecting these animals, and therefore enforcing the relevant legislation, will in practice be virtually impossible. The debate about the genetic modification of animals is therefore no longer purely academic or theoretical; stakeholders and policymakers now need to take a proactive approach.

7.1 National legislation inadequate to deal with international developments

Genome editing techniques are already widely used in research on animals, including laboratory animals, farm animals, pets and wild animals, including insects. The applications are highly diverse and each will present its own technical challenges and pose a broader range of problems should they be used in any future commercial applications. What they have in common is that in some cases the use of genome editing techniques will be all but impossible to detect.

The emergence of genome editing will highlight existing international differences in GMO legislation if applications of the technique are regulated in some countries but not in others. Moreover, with genome editing it is no longer always possible to distinguish between genetic modification and natural variation or naturally occurring mutations. It is theoretically possible to detect applications, but only when it is known where in the genome to look, which in practice means that detection and therefore enforcement of existing regulations will be problematic, particularly in relation to the international movement of animals and products.

The breeding and improvement of farm animals is a very international business. Gametes (sperm and egg cells) and embryos are traded internationally to achieve breeding objectives. The United States and Canada are the biggest players in the global trade in genetic material for cattle breeding, followed by the Netherlands. Differences between national legislation and regulations make it almost inevitable that applications of genome editing will, intentionally or unintentionally, find their way into the Netherlands. It is therefore important that professional groups such as veterinary surgeons and breeders are informed about these developments and are given any necessary training. They must also be consulted periodically to remain alert to the situation on the ground and ascertain whether or not policy is sufficiently in line with practice.

7.2 Public debate: same topics, different implications

Opinions and preferences on the use of animals and the use of genetic modification reflect a diversity of cultural and individual preferences. It is therefore unlikely that it will be possible to come to a single clear vision on the place of animals in society and the types of applications that are acceptable or desirable. Moreover, people's attitudes towards animals are highly ambiguous. Issues concerning the relationship between people and animals vary depending on the context and can vary for the same animal in different contexts.

Nevertheless, decision will have to be taken on the use and regulation of genome editing in animals. Genome editing in animals is expected to cause shifts in the public debate. Some new applications not only provide benefits to people, but also (relative) health and welfare benefits to animals, while other applications harm animal health and welfare. In both cases, these applications will also call existing practices into question.

Telos, integrity, naturalness, instrumentalisation, animal welfare and current practice are recurring topics in the debate about the use of animals. The nature of some genome editing applications (e.g. very small point mutations that can also occur naturally) will change the way we think about some of the fundamental concepts in the debate about genetic modification. For example, genome editing makes the concept of naturalness less meaningful in legal contexts and as a general concept in the debate, and it forces a fundamental rethink and re-evaluation of underlying values. Genome editing applications in animals will present consumers, patients and the public with new choices about which they will have to make their own decisions. To make these decisions people will need information about how products have been made. For applications which have never before been available, such as xenotransplantation, it is important to establish what people think about them and to facilitate informed opinion formation.

7.3 Stakeholder consultation and dialogue

The pace of change in genome editing in animals requires international coordination on potential cross-border issues, a process of opinion forming and preparedness in anticipation of a shift in the substance of the public debate on animals. The Netherlands is one of the few countries that has a statutory ethical review for animal biotechnology applications. The commission responsible for this review is no longer active, however, because such application have so far not come forward in the Netherlands. If the Netherlands wants to maintain this review, it will be essential to re-evaluate the purpose and remit of any commission involved in this review in the light of developments in genome editing in animals.

This review does not apply to the importation of GM animals or products derived from them, which are subject to EU law and this – as yet – does not require an ethical review. In preparation for these developments, the government and relevant stakeholders should move quickly to adopt a position on the possible importation of genome-edited animals (for

example via the trade in sperm, egg cells and embryos). As part of this process, they can consult with various existing initiatives in the breeding industry that are examining the direction being taken in the selective breeding of farm animals and pets and the desirability of these developments. The joint exploration of perspectives with different stakeholders is an important first step towards forming opinions and standpoints, which can then be taken further in an international setting.

Other applications, such as environmental and ecosystem applications (e.g. gene drives for combating infectious diseases or invasive exotics) require a multidisciplinary approach to risk assessment. From a policy perspective, applications such as these will probably not be geographically restricted and so the spread of gene drive organisms may not stop at national borders. This means that any Dutch policy initiatives will have little effect and international consultation and cooperation will be needed when such applications are considered for authorisation in neighbouring countries.

For this reason, the government and policymakers must not delay in gathering information from different stakeholder groups in various settings, and where necessary work with these groups to look for solution strategies for identified problems or improvement areas. The success of any stakeholder engagement will depend crucially on having a transparent goal, on the form of engagement and on acknowledging the reciprocity of the process. This applies not only to the initiators, but also to the participants.

Appendix A: Outline of public dialogue: who, what, where, why and how

In the table below the societal and ethical aspects of genome editing in animals are linked to relevant stakeholder groups and the goal of the dialogue, with an indication of the dialogue agenda. This is not a strict or exhaustive breakdown indicating which groups should or should not decide about which issues. It simply gives an initial indication of the phase of normative and pragmatic issues surrounding genome editing in animals and the stakeholders who could be engaged in the dialogue.

Public dialogue on genome editing in animals: what, who, why and how

Theme	Engagement	Goal	Agenda
Naturalness	As broad as possible: Consumers Animal welfare, environmental and nature conservation interest groups, professional groups ...	1*	The nature of applications makes the concept of naturalness less useful in legal and social contexts. What are the values underlying the use of this term and the associated problem areas?
Mobilisation potential	As broad as possible: Consumers Animal welfare, environmental and nature conservation interest groups, government ...	1	The use of genome editing in animals can in combination with ecosystem applications potentially lead to fierce public debate. What are the sore points and controversies?
Proportionality	As broad as possible: Consumers Animal welfare, environmental and nature conservation interest groups ...	1	Some new applications not only provide benefits to people, but also (relative) benefits to animal welfare, while other applications facilitate further instrumentalisation of animals. Exploring the acceptability and possibilities of genome editing in animals by holding a need and purpose debate on the use of animals. What are the limits and have the boundaries been shifted?
	Professional groups	2**	Determining the direction and goals of animal breeding and improvement.

Speed	Government Professional groups Scientific community ...	2	The pace of technological change and increase in geographical pressure; Dealing with international differences; Importation issues.
Complexity	Government Transdisciplinary science ...	2	Applications with potential cross-border environmental and ecosystem effects require a transdisciplinary approach to risk assessment and international consultation.
Inevitability	Government Professional groups Retail / consumer organisations Organic sector ...	2	Detection of applications is theoretically possible, but problematic in practice and in international contexts. What are the problems and possible solution strategies?

*Phase 1: Problem structuring: opinion formation & learning process

**Phase 2: Identification of solution strategies: soft law & policymaking

Appendix B: Report on the symposium 'Gene editing in animals'

19 AND 20 OCTOBER 2017 IN AMSTERDAM

Why this symposium?

New gene-editing tools such as CRISPR/Cas9 make it easier to modify the genomes of animals. Since the first transgenic mouse was created in 1980, however, there has been an ongoing debate about the genetic modification of animals. During the symposium 'Gene editing in animals' on 19 and 20 October in Amsterdam, 160 animals scientists, regulators and other professionals gathered to discuss the current developments and the ethical and societal aspects linked to gene-edited animals. One of the main questions was: Should these animals still be considered GMOs?

Biotechnologists are working on animals such as cows without horns, virus resistant chickens, lambs with more wool and pigs for xenotransplantation. To obtain these animals they have been using gene editing technologies including CRISPR/Cas, that make it possible to precisely 'edit' one or a few DNA base pairs. CRISPR/Cas is seen as easier, cheaper and more precise than older techniques.

But gene-editing also raises questions. Important questions that came up during the meeting were to what extent do gene-edited animals need the same kind of regulation as genetic modified animals required 25 years ago? Does gene editing (always) result in genetic modification as defined in the law? And should gene editing in animals be regulated differently from gene editing in plants? 'In the Netherlands, genetic modification of animals is seen as a violation of their integrity,' said Sybe Schaap, chair of COGEM, at the opening of the symposium. Genetically modified animals are therefore only allowed for specific purposes where no alternatives exist. They may be used for biomedical research purposes, but are prohibited for recreation and sport.

Schaap emphasized that the new gene-editing technologies bring these premises into question. The modifications made to the genome with gene editing can be extremely small compared with previously used modification techniques. Besides, changes made by using CRISPR/Cas are also ones that could arise spontaneously in nature. Schaap: 'So should these animals still be considered GMOs?' No answers were provided during this symposium, but the discussion has clearly started. Schaap: 'Gene editing puts questions about the relationship between humans and animals back on the agenda.'

Breeding farm animals

Cows without horns

Genome-editing technologies have the potential to become the next 'game changer' in cow breeding, said animal scientist Han Mulder from Wageningen University & Research. His study, which he carried out together with animal breeding companies, found that genome editing can accelerate the introduction of the monogenic trait of 'hornlessness' in a population of dairy cows. Companies are eager to introduce this trait, which is rare in dairy breeds. At present farmers have to remove the horns to protect themselves and other cattle from injury. But this practice, which is painful for the cows, has come under increasing scrutiny from animal rights activists. To solve this problem, several laboratories have now edited the genomes of dairy cows to make them hornless. Mulder and his group calculated the breeding time and cost of using genetic selection only with the time and cost of using a smart combination of genetic selection and gene editing. 'Gene editing results in an enormous increase in speed,' Mulder said. Scientists who use gene-editing, are able to fix the allele for hornless in an elite population of Holstein cows in 2-5 generations, which is four times faster than it would take using only selection and results in less loss of useful polygenic traits. But there are challenges. The break even costs depend on the value of the desired phenotype and the target population size. Furthermore, detecting off-target edits is still quite time-consuming. Mulder also stressed that public dialogue is 'very much needed'. Companies will probably be unwilling to use CRISPR-Cas9 as long as the cows are seen to be genetically modified cows.

Chickens producing eggs with more influenza vaccine

Tim Doran, a molecular biologist at CSIRO in Geelong, Australia, also concluded that gene-editing technologies can speed up the introduction of mutations that already exist in a species: the technology is precise and does not introduce deleterious or unwanted traits that can arise when traditional selective breeding is used. His group is working on chickens that produce eggs for enhanced influenza vaccine production. Since 1970, pharmaceutical companies are using chicken eggs to produce influenza vaccine. The yield of the vaccine, however, is still very low. To achieve eggs with higher vaccine yields, the group deleted a small part of a specific chicken gene to remove inhibitors of vaccine growth.

But, before being able to do this editing, the group had to solve a problem that is specific to birds. Because egg cells are difficult to access while still inside the hen, CRISPR elements cannot be injected directly into the egg cells itself. To get around this, the researchers now use Primordial Germ Cells (PGCs) – immature cells that eventually turn into sperm or egg cells. They remove these cells from the blood, edit the genome and then put them back into the developing chicken in an early stage. They can also edit cells by injecting the CRISPR elements directly into the blood. Doran is also using these germ cells to create chickens that produce eggs without the proteins that cause egg-allergies (2.5 % of Australians have this allergy) and chickens that are resistant to a virus.

Tan sheep with higher body weight

'The future Tan sheep will grow faster, have a short tail and be hornless', animal scientist Xiaolong Wang predicted. His group, of Northwest A&F University Yangling in Chi, has created sheep and goats with higher body weight, higher meat quality and increased hair length to help improve the growing livestock husbandry sector in China. The group has edited genes from the indigenous Tan sheep by injecting one-cell-stage embryos with Cas9 mRNA and sgRNAs. These CRISPR elements, called nucleases, disrupted one or several genes with known functions: e.g. MSTN for muscle growth, FGF5 for fibre growth and BMPR1B and GDF9 for fecundity.

However, public debate is important in China too, Wang said. Despite the fact that hundreds of Chinese laboratories are working on GM animals and plants, there are still only two GM crops on the market: a cotton cultivar (1997) and a papaya cultivar (2006). 'It will take a long time before the public will accept these new GM sheep,' Xiaolong said. Public attitude can be influenced by ethical issues or by uncertainties about safety. One of the safety concerns is off-target mutations. So to provide a foundation to this safety question, his group studied the off-target mutations from 50 CRISPR sheep and goats including single nucleotide polymorphisms (SNPs) and deletions in the whole genomes. They found no detectable off-target modifications that could be attributable to the injected CRISPR elements.

Virus resistant sheep and sheep as a model for human diseases

Alejo Menchaca, a biotechnologist at the Institute of Animal Reproduction Uruguay, also works on sheep. His laboratory has created sheep that combine traits from the Australian Merino sheep (superfine wool) and the Dutch Texel sheep (good meat quality). Menchaca confirmed that the CRISPR/Cas system has become a relevant tool for sheep breeding. His laboratory is also breeding sheep that are resistant to Jaagsiekte sheep retrovirus, a virus that causes lung cancer in sheep. The disease have been called Jaagsiekte, after the Afrikaans words for "chase" (*jaag*) and "sickness" (siekte), to describe the respiratory distress observed in an animal out of breath from being chased, indicating the breathing difficulty experienced by infected sheep. To make them resistant, elements from the CRISPR-Cas system were injected into zygotes to obtain a mutation in the gene that is responsible for the receptor protein that recognizes the Jaagsiekte sheep retrovirus. Without this receptor, virus infection is not possible. Fifteen lambs with this mutation were born this summer, the group is now testing their resistance.

Menchaca is also working on another application: sheep as models for human diseases. In October 2017, 35 deaf GM lambs were born with a mutation that causes a specific genetic type of deafness that also occurs in humans. These sheep may be used to test therapies. 'Our models will have practical implications in the field of livestock production, animal health and medicine,' Menchaca concluded. But technical challenges still lie ahead. The success rate of the transformation and pregnancies (from the transferred embryos) is still quite low: the laboratory needed more than 300 embryos and 86 recipient sheep to produce the 35 deaf GE CRISPR lambs.

CRISPR/Cas: not so easy to communicate

'So if you say that the CRISPR technology is more precise,' a science journalist commented during the discussion, 'then you have to be specific about what exactly is more precise.' Menchaca agreed, and said that he wished he could speak more often with science journalists. 'Help me to communicate better.' Another question from the audience was how long it is likely to take from the birth of a first gene edited animal to an animal reaching the market. According to the researchers, it would take 10 years to set up a population of hornless cows that would be big enough to produce for the market. For gene edited chickens this would only take two years. But a participant from a company voiced reservations: companies are not so eager to invest in this technology. This is not only because the authorization process can take years (gene edited animals are still regarded as GMOs), but also because the monogenetic mutation that Doran's first virus-resistant chicken had could be less sustainable. 'We have seen this also in plants: you do a massive amount of work and then the virus adapts itself to the mutation.' This is the reason another participant gave for preferring to focus on 'polygenetic robustness and tolerance' instead of virus resistance based on mutations in a receptor gene.

Eradicating pests

Further global trade and climate change will lead to more invasive species including pest species. So new methods to combat pest species are very welcome. During this symposium, it became clear that laboratories can use CRISPR/Cas not only to accelerate farm-animal breeding, but also for the eradication of pest species. Governments could use this method to eradicate a pest in a particular area, such as mice on a tropical island or malaria mosquitos in East Africa. The pest animals are given a 'gene drive': a construction based on CRISPR/Cas9 that accelerates the spread of a specific trait among a population, for instance disease resistance or male or female sterility.

Malaria mosquitos with a *gene drive*

The international 'Target Malaria' programme, financed by the Gates Foundation, is using this gene-drive technology to obtain sterile male mosquitos. 'The current methods won't enable us to eliminate the malaria mosquito' said Samantha O'Loughlin from Imperial College London. Gene drive might be a method to realize this goal. The teams are testing mosquitos with a gene drive in laboratories (England and Burkina Faso) and in large cages (Italy). And Mali and Uganda want to start with laboratory experiments.

Gene drives, however, are raising concerns that the technology is too powerful, as it would theoretically enable researchers to eradicate a species on a global level. Scientists, NGOs, regulatory authorities and the media have all been warning about this danger since the idea for this technology arose in 2013. As a result, Target Malaria is employing a step-wise, multi-disciplinary approach for the transition from lab to field. The interdisciplinary teams in the different countries are not only involved in technical and safety issues, but also in regulatory issues, science communication and community engagement on all levels – from

international to national and local levels. 'We want to ensure that all concerns from stakeholders can be addressed in a transparent and systematic way,' O'Loughlin said.

Jianghong Min from MIT Media Lab (US) described how his lab has developed DNA technology to control spread of a gene drive on a global level. 'We are in need of technologies that can limit the geographical spread of gene-drive organisms, as deployment of global drive systems ought to be reserved for only the most extreme of circumstances,' he said. The team has therefore developed locally confined drive systems: by adapting the design of a specific CRISPR construct, a laboratory can 'build in' the number of generations for which the gene drive will spread the trait. The group is now testing their constructs in nematodes.

Eradicating house mice on islands

Some nature conservation organizations are against the use of CRISPR-based gene-drive constructions because of the perceived risks; others want to find out whether the technology can be used to eradicate exotic invasive animals. Now exotic animals are often poisoned, or shot off, but these methods have severe disadvantages.

A huge problem for nature conservation are rodents on tropical islands. 'Mice on islands attack young birds such as albatrosses, and are therefore partly responsible for the extinction of birds,' explained John Godwin, a scientist at North Carolina State University in the US. His organization, Genetic Biocontrol of Invasive Rodents, is an international partnership made up of six research centres and a nature conservation organization, Island Conservation. The partnership is trying to solve the problem using CRISPR-based gene drive technology. The researchers are now producing house mice that spread the trait of sterility. The goal is to reduce reproduction and potentially eliminate invasive rodent populations on islands.

'Carefully assessing this potential, however, is important,' said Godwin. 'You can test the mice in small cages, but this doesn't provide a complete picture for the field situation.' In the field, there are many more variables that are of influence on a population. So the researchers first want to test the mice in simulated natural environments measuring 30 x 30 feet (9 x 9 metres). The partnership is committed to being overseen by an external ethics advisory committee and to early and sustained engagement with stakeholders, island communities and regulatory authorities.

Could local communities decide?

Philosopher Jeantine Lunshof, of the University of Groningen and Harvard Medical School, raised the question of who should make decisions about gene drives? On a global or even national level, stakeholders are unlikely to agree about the (possible) advantages and the risks. So should local communities decide, and if so, how do you organize such a process? Lunshof is an advisor on an MIT-project that is trying to combat Lyme disease on two islands in the US: Martha's Vineyard and Nantucket. Lyme disease is spread by ticks that are carried by mice, and now the biotechnologists have bred mice that are immune to the ticks.

Releasing these mice might help solve the problem of Lyme disease. The biotechnologists have made immune GM mice with and without a gene drive (the latter will spread the new trait quicker, so this intervention might be cheaper and easier). The island residents are now deciding whether they want these GM mice, and if so, do they want mice with or without a gene drive? The laboratory will design a mouse in accordance with their (ethical) preferences.

'We have developed a model for Responsive Science,' Lunshof explained. 'This is a way of conducting research that invites community involvement from the earliest stages of each project.' The continuous interaction between scientists and citizens allows concerns to be identified before experiments are performed. In addition, it encourages new technologies to be redesigned in response to societal feedback. Developing DNA constructs that limit the geological spread of gene-drive organisms, as explained by Min, is also an example of adapting a technology in response to ethical concerns.

Interaction with citizens full of pitfalls

During the discussion, several participants stressed the need for better interaction between scientists and citizens. Philosopher John Dupré from the University of Exeter, however, foresaw problems concerning communication about CRISPR-based technologies. When explaining what the technologies could mean for society, scientists and science journalists tend to use metaphors for the genome such as 'blueprint', 'recipe' and 'book of life'. 'But these terms suggest a genetic programme for the different traits,' Dupré said. 'As if, in a magical thinking, you change a gene and the new trait appears. But that's quite misleading: a genome is not a static entity, but constantly changing in a dynamic interaction with the environment.' So we need a more sophisticated understanding, and communication, of what genome editing is.

Dupré also stressed the importance of discussing the bigger picture when talking about the advantages and risks of the new CRISPR technology. For instance, when it comes to hornless cows, we can say that gene editing is good for animal welfare because the farmer doesn't need to remove the horns anymore, which is quite painful. But are hornless cows really needed for sustainable food production? We should also discuss the fact that the same intensive cattle rearing industry that is pushing for hornless cows has many disadvantages for the environment and animal welfare. So wouldn't it be better to search for alternative methods of food production?

Large animals for xenotransplantation and disease models

A solution for the current shortage of kidney and heart transplants in hospitals could be xenotransplantation, the transplantation of organs from animals. Decades of scientific setbacks has kept clinical trials of this approach on the horizon. Gene editing, however, increases the possibilities for xenotransplantation. This is because the animal in which the (human or pig) organ has to be grown, can easier be made immune compatible with hu-

mans. Improved technologies to modify pluripotent germ cells are also bringing xenotransplantation closer to clinical practice.

Pig-human chimeras

Scientists can use CRISPR/Cas, and other new technologies, to develop chimeras – animals that can produce human organs for transplants. American laboratories have already made a few mouse-rat chimeras and the oldest one is now 24 months. Juan Carlos Izpisua Belmonte, a biotechnologist at the Salk Institute for Biological Studies in the US, showed a picture of his two-year-old chimeric 'rat-mouse' or 'mouse-rat'. 'In the lab I say mouse, but I really don't know what it is,' he added. Belmonte explained how his group has created mice embryos that have rat organs. Using the gene-editing tool CRISPR/Cas9, the researchers disabled several genes involved in heart and eye development so that the mice would not grow functioning mice versions of those organs. Rat stem cells introduced into mouse embryos filled in for these, growing functional organs, including one the researchers didn't expect. Rats don't have gallbladders, but rat stem cells introduced into mice embryos are able to form gallbladders.

Belmonte has also succeeded in producing pig embryos that have incorporated a small number of human cells. However no functional organs were formed, possibly due to the shorter development time of the pig embryo compared to human embryos. The group is also trying to introduce human cells into horse embryos. The Salk researchers aim to use CRISPR/Cas9 to engineer farm animals that lack certain organs just as they did with rodents. Human cells able to supply the missing organs might then have a growth advantage and survive better. For ethical reasons, the group is developing genetic tools to prevent human cells from integrating in the brain of the recipient animal.

But how can laboratories monitor the welfare of chimeras, one of the participants asked. 'There are no classifications or protocols for these new animals.' Belmonte agreed that his laboratory has to develop welfare guidelines for chimeras, together with regulators. 'At the moment, how-ever, these first chimeras are perhaps the best treated animals in the world.'

Pigs as disease models

Angelika Schnieke, from the Technical University of Munich, stressed that gene editing improves the possibilities for xenotransplantation, but it can also be used to create farm animals as disease models. 'Large animal models of human diseases can bridge the gap between basic biomedical research in rodents and the translation of new knowledge into clinical practice to improve diagnosis and treatment.' Pigs, horses and cows are anatomically and physiologically more similar to humans than rodents in a number of ways. Their immune systems are more similar and they have a longer lifespan than rodents. Schnieke showed how laboratories throughout the world have already made twenty porcine models including models for various types of cancers, cardiovascular diseases, diabetes mellitus and neurodegenerative diseases such as Alzheimer's diseases. Gene editing has enormously accelerated the speed of this process. 'We can have a new animal model in a couple of

weeks or months.' For the pancreatic cancer model, her group mutated three genes that were known to be involved in pancreatic cancer in humans. They applied the modifications in both pigs and mice. Whereas the CRISPR pig developed this cancer as predicted, mice with these three mutations did not. This strengthens the argument that animals such as pigs are better disease models for humans.

The Technical University of Munich is doing work on xenotranplantation as well: they transplant pig organs to other species. Schnieke's laboratory has successfully transplanted a pig heart from a gene-edited pig into a baboon. In September, the researchers announced that they had nearly doubled the previous survival record for a life-saving pig heart transplant in a baboon to 90 days – at that age the baboon was still healthy. Three months might be long enough for regulators to permit clinical trials in humans, so the group is therefore working to repeat the results in more baboons. In order to increase the likelihood of the pig organs being accepted by the body of the patient, tens of pig genes have to be edited. The German researchers modify genes that are involved in hyper-acute rejection, vascular rejection and cellular rejection.

More mice models than ever

Michael Wiles from the Jackson Laboratory in the US stressed the many advantages this technology offers for creating disease models in mice. To test a gene-disease hypothesis in mice now costs 90 per cent less and takes less than a quarter of the time that it previously took. Besides, laboratories can now modify any species, they can regulate the expression of genes (epigenetics), and they can refine or adapt the genetics of a mouse model in response to new insights into the disease. 'It is much quicker and more efficient to use CRISPR technology to alter a fertilized egg and thus engineer a mouse than the traditional route, which starts by modifying an embryo cell.' One of the results, however, is that pioneers in garages, with less money and expertise than certified laboratories, can also change the genetics of species. According to Wiles, CRISPR and similar technology will change society. 'Perhaps having our genetic destiny within our hands is a next enabling evolutionary step.' One of the participants asked what role Wiles sees for himself as a biotechnologist in addressing ethical issues. In response Wiles described a project in a small country that he declined to participate in because gene editing did not seem to be the best solution for the problem (diminished fertility due to inbreeding).

Monkeys for disease models

Primates are closely related to humans and known to be probably the most representative disease model for research. In Europe however, the possibilities for using monkeys to develop disease models are limited because of ethical concerns. Weizhi Ji, from the Yunnan Key Laboratory of Primate Biomedial Research in Kunming (China), however, emphasized the importance of using monkeys. He believes Non Human Primates (NHPs) are an ideal model for complex human diseases. 95% of our genome sequence is similar to that of monkeys, whereas we share only 84% of our genome with rodents or pigs. In addition, our minds are more similar to those of primates than to those of rodents, which could help advances in

the neurosciences. It is much easier to bring about changes in monkeys by using CRISPR than it is with older genetic technologies. In the five years between 2001 and 2016, only six conventional GM-monkey disease models were published, while 11 gene-edited monkey models have been published in the last three years, including a monkey with autism.

Weizhi's institute has made a monkey with Rett Syndrome (RTT) by mutating one gene (called Mecp2). Children with this severe monogenetic disease show impaired development and have a lot of pain; they are prone to infections and they lose language and motor skills. Weizhi showed that the phenotype of the rodent RTT model is different from that of human patients, but the phenotype of the monkey RTT model is comparable to that of humans. For example, most girls and most female monkeys survived, and most boys and male monkeys with the mutation resulted in abortion during pregnancy (in humans RTT affects mainly girls). Rodents showed no gender differences. And compared with humans, rodents displayed completely different social behaviour, whereas monkeys evolved more similar social behaviour. Weizhi: 'Monkey models are therefore very helpful for understanding diseases as well for drug screening and clinical therapy.'

In Europe, however, it is likely to be difficult to obtain permission for performing behaviour experiments with (genetically modified or gene edited) monkeys. Other participants stressed that in all cases laboratories will first need rodents to find out which technologies or therapies are the best.

Bringing back extinct animals

Many conservation programmes have saved iconic birds and other species from extinction through interventions such as captive breeding and the reintroduction of endangered species in an area. Nature conservationists in the Netherlands, for example, introduced primitive 'Heckrunderen' in the Oostvaardersplassen thirty years ago. These Heckrunderen were created around 1930 by the German breeder Lutz Heck. He crossed indigenous cattle with specific 'primitive' traits in breeds from Hungary, Corsica, Scotland and Spain. CRISPR/Cas could also enable re-introduction of extinct animals.

Reintroducing a pigeon in the eastern US

Ben Novak is a scientist at Revive & Restore, an international organization for the genetic rescue of endangered and extinct species, based in California. He explained how his organization is trying to bring back the North American passenger pigeon and heath hen, using CRISPR and other biotechnologies developed for chickens. The passenger pigeon is an iconic species in the eastern part of the US. Before they were shot down in large numbers in the nineteenth century, the passenger pigeon population numbered billions. Their disappearance from the skies was a sign that even the most abundant of natural resources could be exhausted by unchecked human consumption, heralding a new age of conservation regulation and game management. Reintroduction of the passenger pigeon may also help restore forest biodiversity in the eastern US.

To 'revive' the extinct bird, the organization plans to edit the germ-line of living band-tailed pigeons according to genes for ancestral traits that have been found in the genome of a passenger pigeon from a museum. Next steps will include breeding the edited band-tailed pigeon/passenger pigeon in captivity, reintroducing these new 'hybrids' into the wild after proper conditioning, and monitoring. Novak stresses that 'de-extinct' species will not be transgenic or genetically modified. 'They most closely resemble selectively bred hybrids.' According to Novak, many species and environments would benefit from such restoration. But who decides what kind of landscape or ecosystem we want, one of the participants asked. Passenger pigeons were once part of a specific natural landscape in the eastern US, but farmers today might regard passenger pigeons as a pest. Moreover, the disappearance of the passenger pigeon might have stimulated the rise of other species which flourish in another natural landscape. 'Our view of nature and what is natural will keep changing,' this participant remarked. Revive & Restore, however, sees the advantage of bringing back the passenger pigeons not in restoring a specific form of ancestral nature, but in providing measurable ecological services such as enlarging the biomass in forests.

How should we regulate gene-edited animals?

Definition of GMO

One of the major questions that arose during the symposium was how: should we define a GMO? 'It is really tough to come up with a definition,' one of the participants declared. 'Using CRISPR you can change just one or two base pairs, but also tens, hundreds or thousands of base pairs. The mutations may be similar to what could happen in nature, but they can also result in brand new organisms.'

According to Alison Van Eenennaam from the University of California, regulators should restrict themselves to the risks of a product, regardless of the way they are made. But that is not the way it works in the United States, she explained. In January, the FDA announced a draft guideline that classifies all gene-edited animals that have had their genomes intentionally altered as drugs. This means that the authorization process can take years. That was the case with the AquaBounty's fast growing GM-salmon, so far the only GM-animal that has made it to the agricultural world market. It took almost 30 years from the initial production of this salmon at the University of Toronto to obtaining Canadian Government approval for its sale in Canada. The company has already spent 16 million dollars on the regulation processes. Because it will often be impossible to distinguish between natural mutations and intentionally altered base pairs, the new FDA guidelines will be unworkable, Van Eenennaam predicted. Her own group has also created CRISPR cows without horns. The mutations in these cows are similar to the mutations that produce hornless cows when traditional breeding techniques are used. In the Netherlands regulation on animal biotechnology is also based on ethical and social issues. Van Eenennaam, however, sees no advantage to including these issues. 'That would open up a lot of uncertainty. Ethical and social issues should be part of broader discussions about food production, and companies can use labels to let the public choose.'

Regulation that stimulates innovation

Ann Bruce, an innovation researcher at the University of Edinburgh, proposed another idea for regulation: 'adaptive governance'. 'CRISPR/Cas9 is a first step in a new direction', she explained. 'It is a novel technology, and, as scientists and companies are unlikely to have exhausted all ideas for its use, we might underestimate the impact in the long term.' Regulatory authorities could therefore go for stepwise regulation including 1) pre-regulatory standards; 2) pre-regulatory guidelines (these are already more formal); 3) regulation, and 4) post-regulatory standards and guidelines to support compliance with regulation. According to Bruce, a regulatory system for a 'new' technology should take into account four aspects: the innovation strategies of companies, the extent to which innovations could respond to currently unmet social needs, innovativeness of particular sectors of industry, and geographic location and scale of operations. In this way, the regulation process could encourage positive changes in industry and distinguish between products on the basis of social and economically relevant criteria.

Bruce also mentioned some disadvantages of not regulating CRISPR animals. 'Companies could then be open to accusations of 'hiding' the new technology, and if you are hiding something, it must be bad.' From the debates on GM plants she learned that the first products to reach the market are likely to frame the CRISPR technology. Unfortunately, the first products are not necessarily the most socially desirable applications, the first GM plant being a good example: Monsanto's herbicide soy. What might make it even more difficult to introduce CRISPR animals onto the market is that conventional livestock farming already has a bad image, at least in the UK. This can lead to unexpected reactions: biotechnologists show the advantages of hornless cows, and the public is shocked to learn that horn-cutting is a practice and starts criticizing current, industrialized farming. Bruce: 'So my question is also: how might gene editing help deliver alternative systems, with an emphasis on local production and breeds adapted to local conditions, and with an emphasis on animal welfare and reduced environmental impact? Are there alternative, innovative pathways for the current pathways?'

GM animals back on the agenda

'I don't dare to summarize what we have discussed,' concluded Frans Brom, chair for the day and member of the Dutch Scientific Council for Government Policy at the end of the symposium. He therefore came up with three remarks: The first one was that gene editing could be a game changer: it can lead to new laboratories, new companies, new people and new discussions in animal breeding, medicine and nature conservation. The second one? The relationship between animals and humans is back on the agenda. 'Some of the ethical issues are new', Brom said, 'and they are being discussed on a global level.'

His third remark, finally, was that regulation is back on the agenda too. Rules and practices developed in the early phase of genetic modification are no longer suitable for the current DNA-technology in its mature state: in the early phase, genetic modification was only feasible for a few prosperous laboratories; now CRISPR can be used much more widely and

by anyone. Brom: ‘So the question we have to answer again is how should we organize the regulation of this kind of technology?’

Text: Marianne Heselmans, science writer

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