**GeneWatch UK response to the Nuffield Council on Bioethics' Call for Evidence on Genome Editing and Farmed Animals**

September 2019

## Summary


(i) Genome editing techniques open up many more opportunities for researchers to alter the genomes of animals in fundamental ways. However, they do not overcome many of the concerns regarding genetic modification of animals, or the problems in delivering viable commercial products. Therefore, the potential harm to genome edited farm animals needs to be considered in a context where many research projects will cause suffering to animals without delivering the claimed benefits.

(ii) Exaggerating the likely delivery and effectiveness of potential future technological applications can lead to opportunity costs when alternative solutions are neglected, and can close down public debate about the best ways of developing knowledge collectively in order to tackle societal problems. Alternative approaches to tackling challenges must be a key part of public engagement with the scientific, regulatory and science policy debates: including questions about what kinds of research should be funded.

(iii) GeneWatch UK considers that genetic modification of animals is an assault on the integrity of living beings. It should not be undertaken without extremely compelling reasons and the presumption in every case should be against such interventions. Regarding regulation, GeneWatch UK supports the opinion of the Trans Atlantic Consumer Dialogue, which states that new genetic engineering techniques will create genetically modified organisms (GMOs) that require, *inter alia*, risk assessments and labelling (TACD, 2016).


## Current research

**1. What current or planned projects of research into the use of genome editing in farmed animals do you think we ought to take into account in our inquiry?**

1.1 Current research on genome editing in farmed animals includes traits such as enhanced muscle growth, hornlessness in cows, disease resistance and medical applications, such as developing pig organs for transplantation into humans (xenotransplantation). The farm animals used in this research include mammals, birds and fish.

**2. What kinds of innovation does genome editing make possible (or practical) that selective breeding or transgenic modification techniques do not?**

2.1 GeneWatch UK is concerned that hype about the future benefits of genome editing in animals is leading to misleading claims about what will be achievable. The social and economic causes of this hype are discussed further later in this submission. Here, we begin by noting that we have heard many of these claims before.

2.2 For example, in 1992, the US National Agricultural Biotechnology Council (NABC) reported that transgenic cattle, sheep, swine and rabbits had been made by microinjection of DNA into a pronucleus of a one cell zygote, transgenic fish by

injection of DNA into oocytes and chickens by infection of genes into eggs (First, 1992). The main traits under development were enhanced growth, using growth hormone transgenes, and disease resistance. The report states, "*When appropriate disease resistance genes are identified, it should be possible to engineer high-producing animals for survival in high-disease environments*" and speculates that "*It is likely that some cows will be designed to produce milk for speciality dairy products whilst most cows may be engineered to produce little or no fat in their milk*". The report also notes that transgenic mice, sheep goats and pigs had already been created which could make pharmaceutical products such as clotting factors and growth hormones, for potential use in human medicine.

2.3 In 2002, GeneWatch UK published a report about the genetic modification and cloning of animals (Rutowitz & Mayer, 2002). The report warned prophetically that "*The profit driven manner in which the technology is being applied has led to sustained overstatement of the achievements of genetic modification in order to maintain investor confidence*". Since that date, many more animals have been genetically modified with no tangible public benefit, whilst repeated claims are made that major breakthroughs are just around the corner. In 2011, an expert report to the European Food Safety Authority (EFSA) found that around 50 species/trait combinations of GM mammals and birds had been catalogued (Henry et al., 2011). Species included mice, rats, sheep, goats, pigs, horses, donkeys, rabbits, cattle, yaks, water buffalo, ferrets, cats, dogs, golden hamsters, rhesus monkeys, and marmosets; but success rates remained low (meaning much suffering for the animals, as described below) and there was little potential for commercialisation. In addition, over 50 species of fish had been the subject of genetic modification, with over 400 fish/trait combinations produced by 2010, although only the fluorescent GloFish (for the aquarium market) was available commercially (in the USA) (Cows et al., 2010). Despite the enormous investment in this type of research, there are still no GM meat, fish or dairy products available on the global market (although a small quantity of GM salmon produced in an experimental facility has been sold, unlabelled, on the Canadian market).

2.4 Although the development of gene editing can speed up part of the process of making GM animals, it suffers from its own technical difficulties and moreover does not address many of the problems associated with producing GM animals using transgenesis.

2.5 For example, most applications of gene editing in mammals still rely on the use of cloning (somatic cell nuclear transfer, SCNT) to reproduce gene edited animals, resulting in serious welfare impacts, including effects on health, on a significant proportion of the clones and surrogate dams involved in the cloning process (Kirkden and Broom, 2012). This includes large numbers of failed pregnancies, still births, deformities and early deaths. Alternatives such as cytoplasmic injection (CPI) have somewhat lower failure rates, but still raise animal welfare issues regarding failure rates, and cause additional problems because the desired trait is not successfully expressed in all offspring or all cells of offspring (known as mosaicism). More than 300 gene edited pigs, cattle, sheep and goats were produced between 2010 and 2015, with a single gene edited animal typically requiring hundreds or thousands of embryo transfers (with CPI or SCNT respectively) (Tan et al., 2016). Alternative methods which seek to reduce these negative impacts are under development, but so far have been largely unsuccessful (Proudfoot & Burkard, 2017). Thus, there is no reason to expect an imminent breakthrough in the delivery of claimed benefits, or in reduction of the harms to the animals involved in such experiments.

2.6 In theory, the genome editing of farm animals is intended to lead to the creation of one or more founder animals, that can then be bred with each other, or with other non-edited animals, to create a large enough group of animals with the desired trait to be used commercially (e.g. a herd of cattle). However, there are many problems with achieving successful germline gene editing in practice and with scaling production up to spread the desired trait into a group of animals. Firstly, it is unclear whether germline gene editing is actually successful enough to create even a small group of animals without repeated use of cloning to transfer the trait to future generations. For example, Hauschild et al. (2011) and Reyes et al. (2014) report the need for multiple re-clonings of foetuses during their experiments with gene editing in pigs. Mosaicism is an additional challenge if cytoplasmic injection is used, as this means that the eggs and sperm of the gene edited animal may not all carry the desired genetic change. Secondly, if a genetic trait is recessive (requiring the inheritance of two copies of a gene) or complex (requiring the inheritance of multiple genetic changes), it is impossible to spread it rapidly though a population through sexual reproduction, as each descendant will inherit only half its genome from each parent. Finally, even simple dominant traits (which require only a single copy of a gene to be inherited) must be bred into an animal population in a way which does not lose the other genetic characteristics of the breed which are regarded as important. In practice, this is likely to require the repeated use of cloning over multiple generations, as illustrated by the example of hornless cattle below.

2.7 A recent paper which uses modelling to argue that gene editing could be used to rapidly decrease the frequency of horned cattle in US dairy cattle populations, assumes that the top 1% of bulls would be gene edited and cloned in each generation (Mueller et al., 2019). In this model, which assumes that germline transmission of the genetic trait works perfectly as intended, further gene editing eventually became unnecessary after 15 years because all of the highest-ranking bulls were already homozygous polled (i.e. had two copies of the genetic mutation required for hornlessness) based on inheritance from their parents. The base population assumed for both breeds in this 20-year simulation was 35,000 cows distributed over 200 herds and 350 bulls. According to this paper, the US dairy cow population is approximately 90 times larger than the modelled population. The paper concludes that, "*Irrespective, for any of the gene editing scenarios, multiple unrelated high-merit bulls would need to be edited for each dairy breed to prevent an unacceptable increase in inbreeding*". Thus, even if the germline transmission of the intended trait works perfectly (as assumed in this computer model), and the trait is dominant (requiring a single copy of the required genetic change to be passed to offspring), cloning is expected to be used repeatedly were such gene edited animals ever to be commercialised, with numerous adverse effects on animals, such as stillbirths and miscarriages (discussed further below). These difficulties scaling up applications of genome editing in farm animals are likely to have economic as well as ethical implications.

2.8 The University of Minnesota and Recombinetics have used genome editing and reproductive cloning to create hornless dairy cows (Carlson et al., 2016). Although this is a naturally occurring trait, the researchers claim it would take over 20 years to reach a frequency of 50% polled dairy cattle by natural mating and that this would lead to loss of productivity. However, different breeders today focus on naturally polled dairy cows, reporting high frequencies and competitive breeding values (Norwegian Red, 2018; O'Keefe, 2016). In 2015, the highest Red Holstein Sire in Germany was a polled bull and his high testing offspring have topped many sales. According to GGI-SPERMEX this proves again "that the quality of polled bulls is at the same level as their horned contemporaries" (GGI-SPERMEX, 2015).

Since Carlson et al. (2016) did not identify and report any off target effects in the gene edited animals, Carroll et al. (2016) argued that *"…genome editing can be used to produce precise analogs of the naturally occurring mutations…".* In 2019 however, the US Food and Drug Administration (FDA) found that apart from the intended edit, the whole plasmid, including a second copy of the repair template and the plasmid backbone, were integrated into the target location of both calves (Norris et al., 2019). The FDA scientists detected this error accidentally by running publicly available whole genome sequencing data from Carlson et al. (2016) through new DNA screening software. The FDA study further shows that plasmid integration is known to happen at the intended target site as well as at off-target sites. They assume that such errors are "…under reported or overlooked". The findings of the FDA scientists also raised biosafety issues, since the plasmid backbone that was unintentionally integrated into the calves' genome also included genes conferring antibiotic resistance. Concerns were expressed that these genes could be taken up by bacteria present in the gastrointestinal tract or the body of the calves (Regalado, 2019).

2.9 The 2002 GeneWatch UK report on GM and cloned animals identified research on GM animals for agricultural applications (in cattle, pigs, rabbits, sheep and goats); pharmaceutical production (in the same animals, plus chickens; and xenotransplantation (in pigs) (Rutowitz & Mayer, 2002). This expensive and controversial research has not led to any viable commercial products, with the exception of three very expensive human proteins produced in GM animals for the treatment of rare diseases. According to Sheridan (2016), production systems based on mammalian cell culture continue to set the standard for production of human proteins used in medicine, and alternative systems have failed to keep pace with them, although few niche products are on the market. It is clear that any future production systems using genome edited animals will have to compete with well-developed alternative cell-based systems, which will work better and be more cost-effective for most applications. Today, research on gene edited farm animals has largely shifted its focus away from producing pharmaceuticals in GM animals, however these many failed attempts will have caused unnecessary harm to animals.

2.10 One example of past hype is the case of Dolly the sheep, the first mammal to be cloned from adult cells using a technique known as nuclear transfer; born on 5th July 1996 (Fransman, 2001). A further sheep, Polly, and other transgenic lambs were able to produce the blood clotting Factor IX in their milk, considered to be of major economic potential. PPL Therapeutics was floated on the London Stock Exchange in 1996, with an initial commercial value of £110 million. However, in June 2003 PPL's German pharmaceutical partner Bayer AG decided to put on hold plans to develop a lung drug from the milk of genetically modified sheep. The firm had to slaughter up to 3,000 transgenic sheep, sack three-quarters of its workforce and sell its intellectual property to other companies (Ward, 2003; Foley, 2003). By December 2003, PPL was up for sale, having made a loss of £13.6 million. It is important to note that the inefficiency of cloning or mass-producing GM mammals is still a major issue which will also apply to GM mammals produced using genome editing techniques.

2.11 Production of industrial materials in GM animals has also been a failure, despite much past investment in research. Montreal-based company Nexia Biotechnologies, which genetically engineered goats to produce 'Biosteel', went bankrupt in 2009. A researcher at Utah State University USU) still maintains a herd of the GM goats, although 'Biosteel' has never been commercialised (Hirsch, 2013). In 2018, USU received a major grant from the US navy to further develop synthetic spider silk (USU, 2018). However, its focus was on synthetic spider silk made from transgenic bacteria and silkworms (not from the herd of GM goats) and on how to

scale-up production of (non-GM) hagfish-derived proteins to manufacture commercial-level quantities of these materials. Any similar products produced by gene edited animal in future will therefore also have to compete with non-GM alternatives (and potentially with less controversial 'contained use' applications using GM microbes).

2.12 By 2002, pigs had also been genetically modified to seek to 'humanise' their organs for use in transplantation (known as 'xenotransplantation') and thousands of other animals had been used in xenotransplantation research (Rutowitz & Mayer, 2002). For example, kidneys had been transferred between sheep, tiger, pig, cat, lion, wolf, fox and dingo to dog; dog to wolf; cat, hare and pig to rabbit; rabbit to cat; pig to dog, baboon, monkey, goat and rabbit; sheep and pig to goat; and guinea pig and mouse to rat (Langley & D'Silva, 1998). Many of the recipient animals will not only have endured surgery but will also have suffered the effects of organ failure and the side effects of immunosuppressive drug regimes. Because genetic modification techniques are variable in their effectiveness, many animal 'failures' will also have been destroyed. For example, when the $\alpha$-gal gene (thought to be important in organ rejection) was removed from mice, all the mice developed cataracts and became blind (Tearle et al., 1996).

2.13 At that time, the main barrier to using pig organs in humans was organ rejection, in which the transplanted organ is detected as 'foreign' by the immune system and attacked (Rutowitz & Mayer, 2002). Further, there were significant uncertainties about whether pig organs could ever be compatible with humans, due to important differences in physiology and biochemical functions. In addition, a major concern was the potential transfer of pig viruses to humans, particularly 'porcine endogenous retroviruses' (PERV), which can infect human cells in laboratory tests. Retroviruses may cause tumours, alter gene expression, or combine with other retroviruses to produce novel viruses with unexpected properties. In 2000, the Roslin Institute pulled out of xenotransplantation research because of the risks from retroviruses, focusing instead on tissue regeneration from stem cells through its alliance with the US biotech company, Geron (Clark, 2000). Whilst genome editing researchers are working on the challenges both of PERVs and of organ rejection, these are extremely complex processes and success is far from guaranteed. Mass production of such organs for use in transplantation would also be extremely costly and suffer from the same difficulties of scaling up (e.g. via cloning) as existing GM animals.

2.14 Attempts to use genetic modification to create disease resistant animals are also not new. By 2002, concerns raised in scientific papers included: if an animal or bird does not display symptoms but continues to carry a disease, this could create a hidden reservoir of disease; making animals resistant to disease could lead to pathogens evolving to become more virulent; or if mutated pathogen genes are introduced into an animal to make them resistant to the pathogen, these might recombine with wild pathogens to create resistant pathogens (Rutowitz & Mayer, 2002). For example, pigs resistant to African Swine fever (see below) still get infected by the virus (Devlin, 2015). More recently, a participant from a company at a COGEM workshop (COGEM, 2017) voiced reservations about investing in gene edited virus-resistant chickens because the single genetic mutation in the virus-resistant chicken could allow the virus to quickly adapt so the chicken is no longer resistant. This problem has been seen with past attempts to develop GM virus-resistant papaya, where the virus has adapted itself to the mutation, leading to reduced efficacy in China (Hamim et al., 2019; Wu et al., 2018). For the same reason, another participant also argued for a focus on 'polygenetic robustness and tolerance' instead of virus resistance based on mutations in a receptor gene

(COGEM, 2017). Thus, attempts to genetically engineer pathogen resistance into animals, raises additional concerns which are not addressed merely by using gene editing rather than transgenesis. These concerns are particularly relevant to situations where diverse strains exist and novel strains of virus can arise with high mutation rates, as has been observed for example with porcine reproductive and respiratory syndrome (PRRS) in China (Chen et al., 2019; Zhang et al., 2019).

2.15 Broader concerns include that animals genetically engineered to be resistant to disease could permit them to be kept in crowded conditions, which could facilitate the spread of the same or additional diseases. Thus, genetically engineered animals could perpetuate the factory farming model, which many criticise on animal welfare and other grounds. In fact, most envisaged agricultural applications of genome editing in farm animals aim to produce more meat, faster, using less space and thus support a highly industrial form of agriculture that causes a lot of animal suffering (instead of changing/adapting the system so that there is less animal suffering). This includes applications that are advertised as "animal welfare applications" such as hornless cows, or disease resistant animals, which allow less space per animal and poor sanitary conditions.

2.16 Other examples of genome edited mammals explicitly intended for industrial agriculture include so-called 'super-muscly' animals. Genome editing techniques including TALEN, ZNF and CRISPR/Cas have been used to create animals with increased muscle growth. These include pigs (Bi et al., 2016; Kang et al., 2017; Qian et al., 2015; Rao et al., 2016; Wang et al., 2015a; Wang et al., 2017), cows (Proudfoot et al., 2015), sheep (Crispo et al., 2015; Proudfoot et al., 2015; Wang et al., 2016; Zhang et al., 2014), goats (Ni et al., 2014; Guo et al., 2016; Wang et al., 2015b) and rabbits (Guo et al., 2016). In these animals, genome editing is used to knock out the myostatin gene (MSTN) that inhibits the growth of muscle cells. Problems observed include birthing difficulties due to large offspring size, enlarged tongues and severe health problems.

2.17 The GeneWatch UK 2002 report also highlights the hype surrounding the genetic modification of chickens, referring as an example to reports in December 2000 in which a chicken called 'Britney' was characterised as the transgenic chicken helping to fight cancer even though it had merely been announced that scientists intended to *try and produce* a transgenic chicken which could produce cancer drugs (Rutowitz & Mayer, 2002; Highfield, 2000). A similar story (without the name 'Britney') resurfaced recently, in January 2019, in relation to GM chickens produced at the Roslin research centre in Scotland, which the researchers again claimed would be commercially viable at some point in the future (Ghosh, 2019).

2.18 There is a specific problem with the use of gene editing in birds because egg cells are difficult to access while still inside the hen. This means CRISPR elements cannot be injected directly into the egg cells itself. To get around this, the researchers are developing the use of Primordial Germ Cells (PGCs). These are immature cells that eventually turn into sperm or egg cells. Researchers 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. However, using PGCs is a complex, multi-stage process which is still under development, although a small number of live gene edited chickens have been born (Tizzard, 2019). The main areas of current research are attempts to develop virus-resistant poultry, sex-selection for layer hens (and the removal of males), removal of allergens from egg white and attempts to produce vaccines in hen's eggs (Tizzard, 2019).

2.19 Again, past failures to commercialise GM chickens highlight some of the complexities. The company Viragen was granted a worldwide exclusive license to commercialize avian transgenic technology by the Roslin Institute in Scotland, which led to AviGenics suing Viragen in March 2001: this dispute was settled in 2002, with both companies and the Roslin Institute continuing to work on GM chickens (Fakler, 2002). Although two companies (GeneWorks and Avigenics) had already claimed in 1999 that the production of pharmaceuticals in transgenic poultry was close to commercialisation, details had not yet appeared in peer reviewed papers (EurekAlert,1999). In reality, the first such product was approved for use by the US Food and Drug Administration (FDA) in 2016 (Sheridan, 2016). This drug, marketed under the trade name Kanuma, was developed by Avigenics, which changed its name to Synageva and was then bought by Alexion Pharmaceuticals in 2015, for $8.4 billion in cash and shares (Sheridan, 2016). Kanuma is produced using chickens which are genetically modified to produce the recombinant form of the enzyme lysosomal acid lipase (LAL) in their egg white. It is now approved for use in the US and EU for the treatment of people with the rare genetic disease lysosomal acid lipase deficiency (LAL-D). However, in 2017 the UK National Institute of Clinical Excellence (NICE) rejected the use of Kanuma in the NHS, stating that the high cost of the drug - nearly £500,000 per patient per year - could not be justified by its long-term treatment benefits (McKee, 2017). Severe hypersensitivity reactions occurred in 21 of 106 patients who received Kanuma during clinical trials, three of whom developed anaphylaxis: this necessitates strict medical supervision during administration of the drug (Sheridan, 2016).

2.20 Attempts to produce virus-resistant GM chickens also bring concerns about the real-world impacts of using this approach. In its guidance on environmental risk assessment for GM animals, EFSA (2013) notes that, although disease-resistant GM animals may not become infected, "*one can also imagine a situation where colonisation still could take place, virulence of the pathogen remains unchanged, but the GM animals with enhanced disease tolerance could serve as a reservoir/carrier for that pathogen and may thereby in the long term increase the exposure of other, more susceptible, animals (their non-GM comparators or other susceptible species)*". This report cites the example of avian influenza-resistant chicken which can still be infected experimentally and replicate the virus. Another potential hazard cited by EFSA (2013) is that a population of GM animals with increased resistance, compared with populations of its non-GM comparators, may select for pathogen strains with increased virulence, causing more severe disease in non-GM animals than did the previously circulating strains.

2.21 Gene editing research is also being undertaken in fish and other aquaculture species. Gratacap et al. (2019) note that, "*The high fecundity and external fertilization of most aquaculture species can facilitate genome editing for research and application at a scale that is not possible in farmed terrestrial animals*." Studies to date have focused on proof-of-principle of gene editing techniques in a number of different species (Atlantic salmon; rainbow trout; Rohu, grass, and common carp; channel and southern catfish; Pacific oyster; Nile tilapia and gilthead sea bream). Studied traits include sterility, enhanced growth, and disease resistance. However, many gene edited fish exhibit mosaicism and not all traits are successfully passed to the next generation. There are also practical limitations to gene editing in shrimp. As with mammals, there are concerns regarding unintended off-target effects and further challenges include the successful identification of causative variation underpinning the desired polygenic traits; and the need to edit multiple alleles simultaneously in the same broodstock animal(s) (Gratacap et al., 2019). Disease resistant traits will raise similar concerns to those in birds and mammals, discussed above, namely the development of resistance (more likely in gene edited animal reliant on a single

modification than in animals bred for polygenic disease resistance), the risk of the pathogen evolving to become more virulent, and the potential to create a 'silent reservoir' of disease.

2.22 Another problem is that changing genes related to one aspect of metabolism, can have multiple effects (known as 'pleiotropy'). Dunham (2003) includes a number of examples of transgenic GM fish where changing complex biological pathways leads to unintended effects on other traits: for example, enhanced growth hormones can lead to developmental abnormalities and changes in the nutritional composition of the fish. Such problems are well-known from many experiments on GM crops and are fundamental problems related to the complexity of biology, rather than the specific genetic engineering technique applied. Pleiotropy is widespread in humans and animals and has been well-studied in mice: however, we did not find any recent discussion of this problem in relation to GM farm animals, apart from fish.

2.23 The standard methodology to create gene edited fish is injection of the CRISPR/Cas9 complex into newly fertilized eggs as close as possible to the one-cell stage of development. Aquaculture species allow ease of access to many thousands of externally fertilized embryos, and the large size of those embryos facilitate microinjection by hand (Gratacap et al., 2019). However, microinjection has been used to create transgenic fish since the mid-1980s, with research on numerous farmed species focusing on growth, cold tolerance and disease resistance (Dunham, 2003). Despite decades of investment in research on GM fish, the main products so far are genetically modified (GM) fluorescent aquarium fish being marketed as pets in the USA (tradename GloFish). In addition, some GM salmon from experimental production of GM salmon by US company AquaBounty has been sold (unlabelled) as food in Canada (CBAN, 2017). The GM salmon includes a growth hormone gene from Chinook salmon (native to the North Pacific) and an anti-freeze protein gene from the ocean pout (native to the North West Atlantic) (Yaskowiak et al., 2006). As a result, the transgenic fish produces growth hormone all year (rather than just during the warmer months) and reaches maturity faster than non-GM salmon. AquaBounty has received approval from the US Food and Drug Administration (FDA) to market GM Atlantic salmon in the US. However, many retailers have said they will not stock GM salmon and environmental groups have also warned about risks to wild salmon populations should the fish escape (FoE USA, 2019). Originally, the company proposed shipping GM fish eggs from its Canadian facility to Panama, for production in an on-land facility: however, this facility never received approval for commercial production of GM fish from the Panamanian authorities. Therefore, in future, AquaBounty plans produce its GM salmon commercially on-land in Canada and the USA (CBAN, 2019; Smith, 2019). It may also seek to sell eggs for production of GM salmon elsewhere in the world.

2.24 In December 2018, AquaBounty announced that a gene-edited tilapia, also with enhanced growth rates, would be exempted from GM regulation in Argentina (Anon, 2018). However, no detail is available about the stage of development of this gene edited fish. Production without regulation would raise serious concerns about the lack of health and environmental risk assessments, and the absence of labelling for consumers. Further, this is a GM fish under EU regulations (as discussed further below) and therefore could not be exported to the EU without fulfilling EU regulatory requirements.

2.25 Genome editing techniques open up many more opportunities for researchers to alter the genomes of animals in fundamental ways. However, they do not appear to overcome many of the concerns regarding genetic modification of animals, or the problems in delivering viable commercial products. Therefore, the potential harm to

genome edited farm animals needs to be considered in a context where many research projects will cause suffering without delivering the claimed benefits.

**3. Are there biological reasons why particular (kinds of) applications in farmed animals are more or less likely to be developed and used than others?**

3.1 As noted above, applications in fish may be more likely due to the larger numbers of eggs that can be obtained. However, there remain many technical difficulties, as well as environmental and ethical concerns.

**4. Are there any technical constraints or bottlenecks holding up genome editing research in this field?**

4.1 There is a poor likelihood of successful, sustainable applications, due to numerous technical difficulties and the complexity of biology: these range from technical problems with the use of genome editing itself (on- and off-target effects), to the complexity of biological pathways and the existence of pleiotropic effects, which make it hard to achieve intended outcomes without other unintended effects. Further, there are significant costs and practical difficulties, including adverse impacts on animal welfare, particularly in relation to the use of cytoplasmic injection and cloning to produce such animals and to attempt to scale up to commercial production. For some applications (such as in fish) there may be significant environmental risks. Finally, depending on the application, there are often many alternative approaches which may produce the desired outcomes with fewer practical difficulties and ethical concerns.

**5. What are the expected timescales within which we might expect to see particular genome editing applications being used on farms?**

5.1 As outlined above, GeneWatch UK is sceptical that many of the claimed applications will ever be delivered as commercial products.

5.2 Nevertheless, numerous animals may suffer at the research and development phase.

**The socioeconomic context**

**6. What are the societal, production, environmental and policy challenges to which genome editing applications in farmed animals might offer a response?**

6.1 Genome editing applications are primarily a response to the system of incentives embedded in the science funding system, rather than a response to specific societal, production, environmental and policy challenges.

6.2 Inventions made with public funding in the USA originally belonged to the federal government: however, the adoption of the Bayh-Dole Act in 1980 made it possible for universities to own and commercialise publicly-funded, in-house inventions, and to license their intellectual property to private firms. This policy change was subsequently adopted in the UK and elsewhere. A watershed moment in biotechnology was when venture capitalists learned that intellectual property (IP) could be bought and sold independently of the final product (Pisano 2006). This has allowed hype around new technologies to influence both public and private R&D

investments, and allowed money to be made from promises about future products, even when useful final products are often not delivered and when there is no net benefit to society or the economy. A related problem is scientists 'over-promising' in order to secure research funding, "*by sending messages of being close to their goals, even if this is not true*" (Gannon 2007).

6.3 The privatisation of agricultural research and development is related to economic policies and to reductionism in science, i.e. to "*the promises associated with the biotechnology revolution, and specifically the 'molecularisation' of life sciences, which prompted major changes in research and development (from the experimental field to the research laboratory, increasingly disciplinary and reductionist research and development, concentration of research in a small number of institutions), and the patentability of life forms*…" (Joly 2005). As a result, (claimed) biotechnological 'solutions' (which can be patented) become the main focus of R&D investments.

6.4 This trend can be exacerbated by the patenting of research tools (such as CRISPR) which are then widely promoted by 'star' scientists, leading to financial benefits for their institutions and a gravy train for researchers working in the field, as whole new areas of research funding become available. To facilitate the growth of research funding in these areas, policy makers must be convinced that breakthroughs are just around the corner and that major public benefits (particularly to the economy) are likely to flow from such research.

6.5 Whilst some new knowledge is likely to be generated, exaggerating the likely delivery and effectiveness of potential future technological applications can lead to opportunity costs when alternative solutions are neglected, and can close down public debate about the best ways of developing knowledge collectively in order to tackle societal problems.

6.6 In GeneWatch UK's view, alternative approaches to tackling challenges must be a key part of public engagement with the scientific, regulatory and science policy debates: including questions about what kinds of research should be funded.

**7. How might genome editing technologies help to address these challenges, and what practical benefits and drawbacks would genome editing applications have over existing or envisaged alternative approaches?**

7.1 Significant drawbacks to attempting to use genome editing techniques to address these challenges include:

- Poor likelihood of successful, sustainable solutions, due to numerous technical difficulties and the complexity of biology;
- Significant adverse impacts on animal welfare, particularly in relation to the use of cytoplasmic injection and cloning to produce such animals and to attempt to scale up to commercial production.

**8. What groups or organisations are likely to benefit most from the use of genome editing in farmed animals and what groups or organisations might be disadvantaged?**

8.1 The principle benefits are to scientists, universities and research institutions which receive significant public and private investments in R&D, as well as managers and directors of biotech firms. Those institutions with patents on research tools such as CRISPR also stand to benefit significantly. Venture capital investors in early stage R&D also stand to benefit significantly provided they can pull off an exit strategy before the long-term failure of many such investments is exposed.

8.2 This type of innovation bubble is likely to lead to a net loss to the economy overall (Pisano, 2006) and to cause significant suffering for the many animals involved in this type of research. There are also significant opportunity costs as alternative research strategies and potential solutions are neglected and underfunded.

**9. What do you think are the broader social, economic and political drivers that will facilitate, impede or otherwise shape the development and use of genome editing applications in farmed animals, and what effect do you think these will have?**

9.1 The biggest impediment to the development of useful genome editing applications is not over-regulation (as some advocates claim) but the complexity of biology and the many technical challenges which are unlikely to be overcome, along with the significant negative impacts on the welfare of the animals involved and the difficulties of scaling up to commercial production. Further, if any commercial products are ultimately developed in farm animals, these are likely to face significant barriers in terms of ethical, environmental and consumer concerns. The economic viability of such products is also in significant doubt, given the many difficulties of mass production, and the likely competition from alternatives.

**10. How might differing regional social, economic and political drivers influence the likely development and adoption of genome editing applications in the UK, the EU and the rest of the world?**

10.1 There is a risk that significant sums of money are wasted on this type of R&D, based on misleading claims of what it can deliver. A major concern would be the promotion of such technologies in developing countries in ways that could undermine more locally-developed solutions to problems such as animal diseases. Lack of regulation in some countries could also lead to difficulties in preventing animal suffering, protecting consumers, and tracing and recalling products when there is a problem.

**11. What effect do you think public attitudes will have on innovation in this field (in the UK, the EU and internationally) and how should researchers and policy makers take account of these?**

11.1 Civil society organisations have highlighted major concerns about proposals from vested interests to avoid proper regulation of genome edited products (e.g. TACD, 2016).

11.2 For credible public engagement to take place, uncertainty about what can be delivered needs to be openly acknowledged and unrealistic promises must be avoided. In addition, public concerns about food safety, environmental impacts and animal welfare must be taken seriously.

11.3 GeneWatch UK believes that public engagement should never begin with the promotion of a claimed technological 'solution'. Public engagement has to take place at the very beginning of the process, when funders, innovation stakeholders and researchers define what a problem is and set R&D priorities. A broader approach would begin with different definitions of the problem that is being investigated (such as the challenge of animal diseases), and a serious consideration of all the alternatives that could be used or developed in order to tackle it, including social and economic measures such as discouraging intensive factory farming methods.

**Ethics**

**12. Are there any categorical ethical objections to genome editing farmed animals and if so on what grounds are they based?**

12.1 In 2002, GeneWatch UK published a report which took the view that existing applications of the genetic modification and cloning of animals, with the possible exception of medical uses, were not justified and that genetic modification or cloning of farm animals should not be allowed. This report argued that genetic modification other than for direct medical benefit should be stopped and any applications for medical uses should undergo the most rigorous scrutiny (Rutowitz & Mayer, 2002).

12.2 Ethical issues discussed in this report include species integrity and the human-animal relationship. The conclusion of the chapter on ethical issues states: "*GeneWatch UK considers that genetic modification of animals is an assault on the integrity of living beings and rejects a utilitarian approach to its assessment. Genetic modification should not be undertaken without extremely compelling reasons and the presumption in every case should be against such interventions. Genetic modification of animals changes our relationship with the natural world and contributes to the commoditisation of animals. Our treatment of other species in this way reflects on human dignity and diminishes human society*."

12.3 GeneWatch UK has not changed its view in the light of the availability of new genetic engineering techniques such as genome editing.

12.4 Cloning remains an integral part of many of the proposed applications of genome editing in animals. The European Group on Ethics (EGE, 2008) notes that: "*The Group is aware that there are differing viewpoints on the moral acceptability of using animals in modern farming and is aware that there are some very strongly held views against the instrumental use of animals for human purposes regardless of positive consequences this might have for humans. The Group therefore recognises that, for some people, animal cloning for food supply is an ethically unacceptable practice, whatever conditions are required*."

12.5 This report continues: "*The EGE wishes to emphasise that embarking on cloning for food supply means opening up a new dimension in the general context of breeding that is not merely technical, and which for some people may create a moral unease that cannot be simply dismissed*".

12.6 In addition, as noted above, many envisaged applications would lead to a continuation and increase of farm animal suffering because they support intensive industrial animal farming and the associated negative impacts on animal welfare.

**13. What, if any, are the ethical differences between using genome editing and deliberately altering an animal's physiology in other ways, for example, by using hormones, surgical procedures or drugs?**

13.1 Many ways in which animals are treated may be seen as assaults on their integrity. This does not, however, justify other infringements. Germ line genetic modification is a fundamental alteration of the genome, one of the most basic attributes of both individual and species. It continues beyond the individual lifetime, reaching into future generations of animals. Production of gene edited animals also requires the extensive use of hormones and surgery (discussed further below), where these procedures are not applied in the interests of the animal that is being treated. In humans, such uses would automatically be regarded as unethical.

**14. What, if any, are the ethical differences between using genome editing and using alternative methods such as traditional selective breeding methods, or marker assisted selection to alter the characteristics of a breed of farmed animals?**

14.1 The poor efficiency of gene editing and cloning and cytoplasmic injection techniques in farm animals means that many gene edited eggs need to be implanted to create one successfully gene edited mammal. Tan et al. (2016) provides figures in Table 1, which include columns for the 'total embryos transferred/total recipients/total pregnancies' and 'live/total born' and 'F0 edited/live'. A single gene edited animal typically requires hundreds (or sometimes thousands) of embryo transfers.

14.2 For the animals, the impacts of the following should be considered (Kirkden & Broom, 2012; Tan et al., 2016):

- Egg harvesting procedures, including superovulation, typically involving hormones, and/or surgery, in female egg donors (except where these eggs can be obtained from slaughterhouse carcasses);
- Pregnancy in surrogate mothers, typically also involving hormone injections and surgery;
- Miscarriages, stillbirths, deformities and deaths associated with the numerous unsuccessful pregnancies;
- Slaughter of live animals which do not carry the required genome edits;
- Any adverse effects in the surviving ('successfully edited') animals.

14.3 Only the animals generated by the initial cloning rather than re-cloning are listed in Table 1 of Tan et al. (2016): many more clonings would be needed to establish a substantial number of animals. For example, a report of a COGEM workshop states that, according to the researchers, it would take ten years to set up a population of hornless cows that would be big enough to produce for the market (COGEM, 2017): the journal paper cited above includes a timescale of 20 years, with repeated re-editing and cloning (Mueller, 2019).

14.4 The problem of 'inefficiency' (and associated animal suffering) is not significantly different in terms of its ethical implications that applications of GM animals and cloning considered in the past (e.g. EGE, 2008). This concern applies to all applications of genome editing in mammals. For example:

- The University of Minnesota and Recombinetics have edited the genomes of dairy cows to make them hornless, using CRISPR-Cas9: according to a journal paper published by the research group, 295 nuclear transfers resulted in 26 implanted embryos and only 5 live births (from 14 pregnancies at day 40). Of these, three were non-viable and only two calves survived to 90 days (Carlson et al., 2016).
- The Roslin Institute at the University of Edinburgh has used genome-editing tools to make European domestic pigs resistant to a deadly viral disease called African swine fever. The pigs have their immune gene RELA replaced with a version carried by African pigs, such as warthogs, by means of molecular scissors (Lillico et al., 2013). The researchers injected both TALEN and ZNF directly into pig zygotes to produce live genome edited pigs. 502 embryos transferred to 14 recipients led to 8 pregnancies (one subsequent abortion at 15 weeks) and finally 55 piglets being born. Of those, 9 piglets (16%) were edited, 5 (9%) were biallelic and 2 (3.6%) were homozygous (both were accidentally killed by their mother within 24 hours after birth). Of the 9 edited piglets, two were stillborn.
- The Institute of Animal Reproduction Uruguay is developing gene edited 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,

so they can be used to test therapies. According to COGEM (2017), 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 genetically engineered CRISPR lambs.

14.5 The animal suffering caused by the high failure rates of gene editing, cytoplasmic injection and cloning in mammals is not an issue with conventional breeding or marker assisted selection. Further, for many applications conventional breeding or marker assisted selection (MAS) is likely to produce the desired traits in a manner which is more robust. For example, polygenic disease resistance developed through breeding or MAS is less likely to be quickly overcome by the evolution of pathogen resistance.

14.6 As well as concerns regarding the in large numbers of failed pregnancies, still births, deformities and early deaths associated with cloning and cytoplasmic injection, issues of concern include the impacts on animal welfare of unintended on-target and off-target effects (unintended genetic changes), as well as the effects of the intended genetic edit. The latter can include trait-specific concerns (such as the animal welfare implications of creating higher-yielding animals). Some of these concerns may also apply to animals bred using conventional breeding or MAS. However, this does not mean that these issues should be ignored.

14.7 Broader implications of the use of gene editing techniques, include:

- potential inadvertent effects on animal welfare resulting from interference with complex biological pathways (e.g. through pleiotropy);
- trait-specific issues (e.g. the potential for gene edited disease resistance to create a 'silent reservoir' for disease; adverse impacts on animals of enhanced production traits);
- loss of genetic diversity.

**15. What, if any, are the ethical differences between using genome editing, which relies on the cell's own repair mechanisms, and using genetic modification techniques that insert transgenes into organisms?**

15.1 Genetic modification techniques that insert transgenes into organisms involve a random process of insertion of the gene, which may lead to unintended effects, some of which may be harmful to the animal. However, different types of 'on-target' or 'off-target' effects may be caused by the process of gene editing (Kosicki et al., 2018; Chakraborty, 2018). New unintended effects that derive from the use of such nucleases to cut animal DNA are mainly related to uncertainties regarding target specificity (i.e. whether the nuclease cuts only the intended target site or also other sites in the DNA) and double-stranded break repair (i.e. whether the repair mechanism works as intended or introduces errors). In addition, because gene editing allows the introduction of new traits into plants or animals, the effects of these traits and their impact on health and the environment need to be considered, as they do for GM animals produced using transgenesis.

15.2 From an ethical point of view, some people may have greater concerns about assaults on the integrity of animals using a genetic modification process that may cross species barriers and that involves a haphazard process of gene insertion. Nevertheless, as argued above, all GM animals (including gene edited animals) are produced using processes which alter the germ line and may therefore be regarded as an assault on the integrity of the animal. A single genome edit may still lead to an animal that is radically different from the parent line. Further, many unnecessary operations and hormones are involved in the process of producing genome edited

animals (without benefit to the animals involved) and there are numerous failed pregnancies, miscarriages and early deaths.

15.3 Further, one aim of gene editing in some cases is "introgression-by-editing", which involves attempts to edit the unfavourable allele in the target strain and/or species to correspond to the sequence of the favourable allele found in another strain or species (Gratacap et al., 2019). This process could in theory be used to create gene edited animals with similar genomes to those intended to be created by cross-species transgenesis. As Lillico (2019) notes: "*The recent advent of genome editors allows us to re-write the genetic code of all major farmed species. [*…] *Furthermore, not only can we import alleles from diverse populations where cross-breeding was previously impractical, but also from other species where cross-breeding would be impossible*".

**16. Are some but not other applications of genome editing in farmed animals acceptable and, if so, on what does their acceptability depend (for example, improving animal welfare, meeting objectives of importance for animals or humans, etc.)?**

16.1 Early attempts at genetically engineering animals were already subject to criticism by those concerned about the welfare of animals. For example, Fox (1992) describes the adverse effects of growth hormone transgenes in lambs, which were diabetic and had such severe health problems that they died before reaching puberty. According to Rutowitz & Mayer (2002), the majority of GM animals at that time had been modified to attempt to develop the production of pharmaceutical proteins. The expression of growth hormones and growth factors was another major application being developed, with disease resistance also being researched. Increased wool production, altering the protein content of milk and producing 'BioSteel' were other aims of genetically modifying farm animals.

16.2 Gene editing which focused on production traits (such as enhanced growth) may be less publicly acceptable than some other traits, since enhanced production is an instrumental end, largely driven by the economics of large-scale factory farming, with no potential benefit to the animal itself. Further, such traits often result in phenotypes which cause the animal distress. However, traits which may appear more beneficial, such as disease-resistance or hornlessness, in practice introduce more potential problems (e.g. the evolution of the pathogen) and may encourage poor animal welfare standards (such as high-intensity production). Further, complex traits are less likely to be deliverable due to technical problems and the complexity of biology (e.g. pleitropy).

**Law, regulation and policy**

**17. Are there reasons to think that genome editing approaches are inherently more likely than alternative approaches to result in adverse outcomes, or to result in outcomes that are potentially more harmful; what are the major risks or uncertainties that regulation should seek to manage?**

17.1 Organisms developed using genome editing techniques are regulated as genetically modified organisms (GMOs) in the European Union (EU), which requires risk assessments relating to food safety and environmental impacts, as well as traceability and labelling of food and feed containing or consisting of GMOs. The applicability of these regulations has been confirmed by a ruling by the European Court of Justice (ECJ, 2018).

17.2 EFSA (2013) has published detailed guidance for the environmental risk assessment of GM animals. This guidance highlights numerous risks and uncertainties that need to be addressed before GM animals are placed on the market in the European Union (EU). For example, as discussed above, these include whether a disease-resistant GM animal could act as a silent reservoir of disease or facilitate the evolution of a pathogen so it becomes more virulent. Similarly, EFSA (2012) provides guidance on the risk assessment of food and feed from genetically modified animals (such as toxicological assessment, allergenicity assessment and nutritional assessment); and on animal health and welfare aspects. These are important issues that need be assessed, regardless of whether the GM animal has been produced using transgenesis or gene editing techniques.

17.3 The case of the hornless cattle described above also shows that regulation is required as researchers may overlook relevant on- and off-target effects.

17.4 Further, as noted above, the European Group on Ethics' opinion on cloning, remains highly relevant (EGE, 2008).

17.5 The recent example of antibiotic resistance genes being identified in gene edited hornless cows, as described above, also highlights the continued need for strict regulation.

17.6 Regulation should also facilitate consumer choice, as it does in the EU, by requiring foods produced from GM animals to be traceable and labelled for consumers.

**18. What are the roles of policy and markets in shaping livestock farming practices and what should be the key policy objectives in this area?**

18.1 New policies should focus on improving livestock welfare conditions. Policies should promote livestock farming practices that allow the animals enough (indoor and outdoor) space to feel comfortable and engage in social behaviour with other animals. For example, horns are important for cattle social behaviour and communication, grooming and comfort, Policies should thus rather focus on livestock farming systems that, for example, allow cattle to keep their horns, rather than to genetically modify animals to fit an even more intensified system.

**19. Do you think that the existing EU regulatory framework for the production and sale of GMOs is appropriate for genome editing applications in farmed animals and, if not, what alternatives might be considered?**

19.1 Yes, gene edited animals clearly fall within the scope of EU regulations on GMOs. The existing framework is appropriate and necessary, as described above. However, there should also be a presumption against the gene editing of animals for the ethical reasons described above.

19.2 GeneWatch UK supports the opinion of the Trans Atlantic Consumer Dialogue, which states that new genetic engineering techniques will create genetically modified organisms (GMOs) that require risk assessments and labelling (TACD, 2016). In its opinion, the TACD urges the EU and US governments to:

- Regulate products of new genetic engineering techniques as genetically modified organisms (GMOs);

- Strengthen regulatory systems to include mandatory pre-market human health evaluation that will screen all foods produced using new genetic engineering techniques for potential hazards;
- Develop strong systems of pre-market environmental safety evaluation and post-market monitoring;
- Fully consider the welfare of animals altered using new genetic engineering techniques prior to approval;
- Adopt mandatory labelling rules for all food produced using new genetic engineering techniques;
- Adopt and enforce strict rules for corporate liability and mandatory insurance for companies that want to release organisms altered using new genetic engineering techniques into the environment;
- Establish and maintain systems to ensure that identity-preserved supplies of non-genetically-engineered ingredients remain available.

19.3 If the UK Government were to consider weakening or abandoning EU regulations, it would risk exposing members of the public to unassessed risks, and could potentially face a backlash regarding consumer choice and the need to protect the welfare of animals. In GeneWatch's view, this might lead to increased R&D involving genome edited animals, with associated suffering for such animals: however, in practice useful real-world applications have a low chance of delivery. Were any commercial products ever to be delivered, these are likely to bring their own concerns and controversies (such as gene edited, growth-enhanced fish, which might escape and harm wild species) and would likely struggle to find acceptance in the marketplace. Further, international markets could be affected if such products cannot be sold elsewhere. Within the UK, devolved governments might take different views and this could lead to additional complexities regarding traceability, labelling and regulation.

**20. How might national or regional differences in policy or regulation influence the development and diffusion of genome editing applications in farmed animals internationally?**

20.1 Different countries may take different views based on commercial and economic considerations, policy context (for example, the role of the precautionary principle in decisions about environmental releases) and cultural and societal considerations (for example, regarding people's relationships with animals). However, protecting the environment and human health should be important requirements for all regulatory regimes, as should clear labelling to enable consumer choice.

**Finally**

**21. Is there any important question that you think we should have asked or an area that we ought to have covered, or any other information that you would like to bring to our attention in order to help us with this inquiry?**

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