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Genome editing in food and feed production – implications for assessing risk

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Humans have always influenced the genetic composition of other species through selective hunting and harvesting, breeding and cultivation. Today... feeding the world population relies heavily on a few domesticated animals and eight selectively bred crop species. This improvement (for human ends) of domestic organisms has persisted for some 10 000 years, and still is important. Yet, for the past few decades, radically new methods of breeding by direct, genomic intervention have been implemented.

The use of gene technology in breeding allows transfer of genes among organisms and among species, and can design genotypes with novel traits. Until recently, gene technology has been technically challenging and untargeted, resulting in insertions of recombinant DNA at random sites in the genome. A new paradigm started in the early 2000's with the development of genome-editing tools. The new techniques are mainly based on the use of engineered site-directed nucleases (SDNs) for targeted editing of genes or targeted insertion of DNA

sequences (Friedrichs et al., 2019; Grohmann et al., 2019). Genome-editing techniques can efficiently induce specific changes in the genome of the target organism and include the utilization of DNA cutting enzymes, such as Meganucleases (MN), Zinc Finger Nuclease (ZFN), Transcription activator-like effector nucleases (TALENs), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) system, and Oligonucleotide-directed mutagenesis (ODM). The CRISPR/Cas9 system is currently the predominant genome editing technique across organisms. This system is developed to also facilitate base-editing of single nucleotides, as well as to alter sequences of DNA at specific sites without involving double-strand breaks (DSBs).

The CRISPR system normally serves as a natural protection against viral attacks in bacteria. The CRISPR system has been repurposed to facilitate targeted engineering of the genome in a wide variety of organisms. This discovery of the applied potential of CRISPR/Cas9 (Doudna and Charpentier, 2014) was awarded the Nobel Prize in chemistry in 2020.The Royal Swedish Academy of Sciences called it the discovery of "one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors" which can be used "to change the DNA of animals, plants and microorganisms with extremely high precision".

The SDN based techniques (CRISPR, TALENs and Zinc Finger) and ODM now represent the pinnacle of targeted genome editing approaches (Figure 1 and 2, and Box 1).

These methods combine the precise DNA-cutting abilities of different enzymes and the intrinsic DNA repair system of all cells to perform tailored alterations in the genome. The significantly reduced time, effort, and costs associated with these methods has changed genome editing from being a niche technology to a mainstream method used in basic and applied life science research (Pramanik et al., 2021).

The breakthrough of CRISPR/Cas9 in genetic engineering in 2012 came almost 50 years after discovering that genetic material from one bacterium could be cut and spliced into another. This bacterium was the first genetically modified organism (GMO), and soon after the same technique was adopted for plants and animals. The use of genetic modification has been particularly successful in plant breeding, enabling the introduction of novel traits considered impossible to achieve through conventional breeding.

Unlike genetic modification techniques that have relied on the insertion of foreign DNA fragments, the new genome-editing techniques are primarily used to change the phenotype through a few single nucleotide edits or short insertions/deletions in an organism's genome. However, new phenotypes may also be produced through introducing targeted deletions or even without nucleotide changes to the genome at all, through epigenetic changes. The repertoire of SDN techniques also allows for larger DNA insertions that resemble the outcome of genetic modification (Pramanik

et al., 2021). The boundaries between genetic modification and genome editing are thus becoming increasingly hard to define, thereby excluding a straightforward and concise definition of new organisms produced by genome editing (Pramanik et al., 2021).

The opportunities offered by the new approaches and the ease with which they can be applied to various systems come with some potential risks to humans and the environment. This concerns the potential for an imbalance between rapid technological developments versus the health and environmental implications of novel genome edited organisms. Understanding the risks and developing a consensus for assessing risks are thus a key to ensure biodiversity, food and feed safety, and sustainable agriculture and aquaculture.

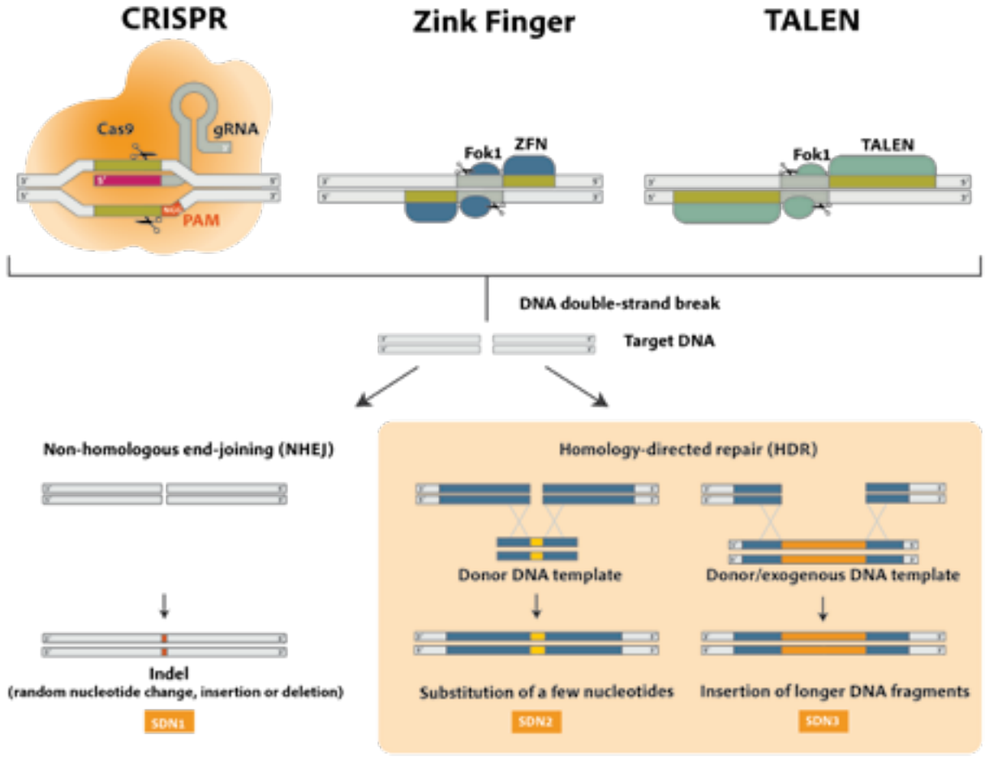


Figure 1. The outcome of genome editing with engineered site-directed nucleases (CRISPR, ZFNs and TALENs) divided into three categories, SDN1-3. The starting point for each genome edit is that SDN "molecular scissors" cuts DNA at specific sites directed by their DNA-binding moiety, introducing a double-strand break (DSB) which triggers cellular DNA repair mechanisms. If no template (donor DNA) is added, the induced break is repaired by NHEJ (Non-Homologous End Joining) pathway and the outcome is defined as a SDN1 category. If a homologous repair template containing one or several single nucleotide variants is added, the break is repaired by HDR (Homology Directed Repair) pathway and the the outcome is defined as a SDN2 category. If the added template contains DNA insertions flanked by sequences homologous to the target DNA site, the construct is inserted by either HDR or NHEJ. This outcome is defined as a SDN3 category. Base editing and prime editing techniques (not shown in the schematic figure) use modified Cas9-protein (nCas9-nickase) and they edit DNA bases without inducing DSBs or without donor DNA templates.

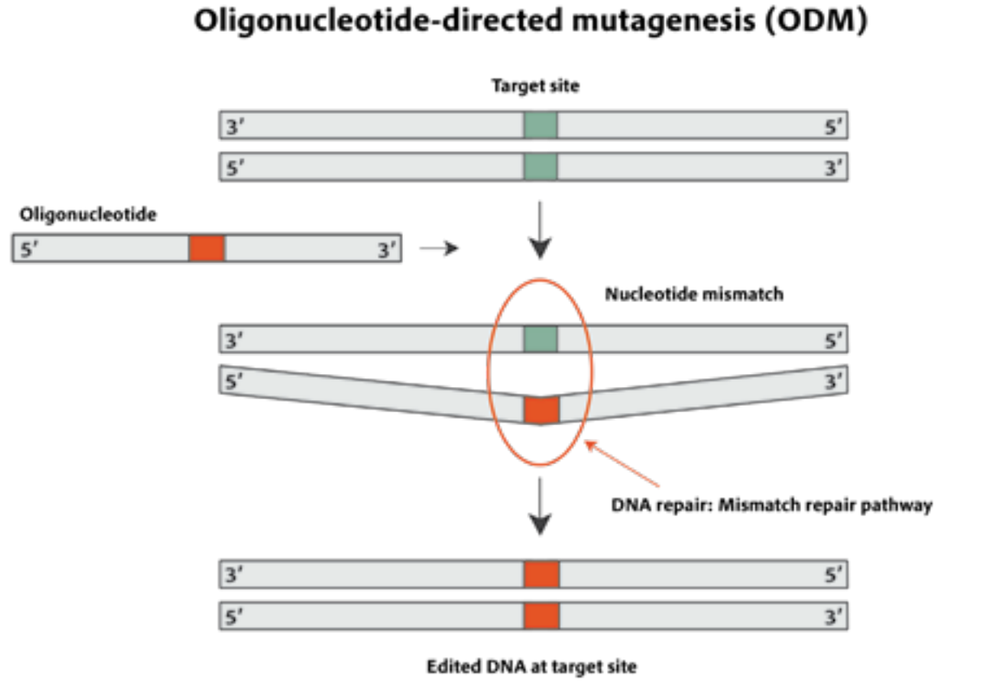


Figure 2. Genome-editing with the use of oligonucleotide-directed mutagenesis (ODM). A short DNA fragment (oligonucleotide; <200 nucleotides long) homologous to the target sequence with exception of a few nucleotides (1-5 nucleotides) is temporarily exposed to the cells. The oligonucleotide containing the desired modification targets binds to the corresponding homologous DNA sequence. Once bound, the cell's natural repair machinery recognises the small mismatch between its own DNA and that of the repair template. DNA binding triggers cellular DNA mismatch repair mechanisms. ODM can change, insert or delete one or a few base pairs of DNA.

Background and introduction

Box 1
Key concepts used in the report

Genetic modification

The process of inserting novel DNA/genes from the same or foreign species or deleting genes. Common to all is the use of recombinant DNA technology.

Genome editing

The process of editing DNA with techniques such as CRISPR, ZNF and TALEN to target genetic changes to a specific location in a genome. Most often with the aim to change single nucleotides or produce short insertions/deletions (indels).

Site-directed nucleases (SDN)

Group of enzymes that are capable of targeted cleavage of a double-stranded DNA molecule/genome, based on recognition of a defined nucleotide sequence. The main site-directed nucleases are ZFNs, TALENs and Cas of the CRISPR system (Figure 1). They are usually engineered forms of enzymes found in bacteria. The outcome of their use has been categorised in 3 groups (EFSA, 2012a).

SDN1; Category of genome-edited organism where the edited genome contains a single or a few base-pair changes after random repair of targeted double-strand breaks in the genome.

SDN2; Category of genome-edited organism where the edited genome contains single or a few defined base-pair changes after template-based repair of targeted double-strand breaks in the genome.

SDN3; Category of genome-edited organism where the edited genome contains longer DNA fragments inserted after template-based homologous repair of targeted double-strand breaks in the genome. This edit may resemble classic transgene-based modification but avoids issues with random DNA insertions, vector sequences and unintended foreign DNA.

Oligonucleotide-Directed mutagenesis (ODM)

Oligonucleotide-directed mutagenesis can be used to insert minor edits into the nucleotide sequence (Figure 2). Various versions of ODM have been developed. In the field of agriculture, it is often referred to as Rapid Trait Development System (RTDS) technology.

Base editing (BE)

The process of producing single nucleotide changes without introducing double-strand breaks in the genome. The technique can also be used to make changes in the epigenetic pattern (e.g. methylation) at targeted genome sites.

Off-target activity

The use of site-directed nucleases may in some cases cause DNA cleavage at sites in the genome not intentionally targeted. Such unintended effects are called off-target effects. The occurrence of such effects mainly depends on the enzymatic characteristics and cellular context of the SDN technology used. Causes of off-target activity include the presence of similar nucleotide motifs elsewhere in the genome, lack of 100% specificity of the SDN used, as well as mechanistic aspects of the nuclease delivery technology used and how it will control nuclease concentration, etc. Double-strand breaks occurring off-target may be repaired through normal cell repair mechanisms and can result in nucleotide changes, rearrangements or indels at those sites (Modrzejewski et al., 2020).

Cisgenesis, intragenesis and transgenesis

Cisgenesis is the genetic modification of a recipient organism with a gene from a crossable, sexually compatible organism (same species or closely related species). This gene includes its introns and is flanked by its native promoter and terminator in the normal sense orientation (EFSA 2012b).

Intragenesis is a genetic modification of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of the same or a sexually compatible species as the recipient. These may be arranged in a sense or antisense orientation compared to their orientation in the donor organism.

Transgenesis is a genetic modification introducing an exogenous or modified gene (transgene) into a recipient organism of a different species than the species from which the gene is derived.

The word "guidance" in this report

There are several EFSA guidance documents available for risk assessment of GMOs. These guidance documents are developed by the EFSA GMO Panel and provide a set of both requirements and recommendations of experimental data needed for a comprehensive risk assessment. The areas covered include molecular characterisation, toxicity, allergenicity, nutrition and environmental risk assessment. In this report, the five main EFSA guidance documents for GMOs have been considered; 1) Guidance on risk assessment of food and feed from genetically modified plants, 2) Guidance for risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects, 3) Guidance for environmental risk assessment of genetically modified plants, 4) Guidance on the environmental risk assessment of genetically modified animals, and finally 5) Guidance for risk assessment of genetically modified microorganisms and their products intended for food and feed use. These are the core set of EFSA guidance documents referred to in the report, outlining the main areas of concern and principles behind the assessments. It is acknowledged that the approaches developed in these Guidance documents are continually refined/amended through subsequent Opinions and Technical notes published by EFSA. As of October 2021, more than 20 documents are available and applicable (EFSA, 2021b).

The case-by-case approach in risk assessment

One of the fundamental concepts in the EFSA guidance documents is the case-by-case approach. This approach allows case-specific assessments to be made and for data requirements to depend on the context. In the case of risk assessment of genetically modified or genome-edited organisms, the organism, derived product and intended uses can vary substantially. It is not realistic to develop detailed guidance that can cover all aspects of the assessment for all possible uses. Hence, the guidance will necessarily have to be generic. The various areas of concern presented in the guidance may then be considered for their relevance on a case-by-case basis. The case-specific assessments relate to all aspects regarding the organism, e.g. species, modification/edit, trait, and uses, etc.

The regulation of gene technology in food and feed production

The broad opportunities for various forms of genome engineering and editing offered by site-directed nucleases have raised questions about how they fit into the regulation of GMOs, and whether some uses warrant a different regulatory approach. In 2018, the European Court of Justice decided to include genome-edited organisms in the GMO definition, and within the EU regulatory system for GMO (and the obligations laid down by the EU legal framework). This sparked an international debate about the suitability and continued use of the regulatory system for GMOs (Van der Meer et al., 2021).

In the EU, all new GMO products for import and processing, food, feed and cultivation are assessed by the European Food Safety Authority (EFSA). The EFSA GMO Panel provides scientific opinions on the health and environmental safety of GMOs on a case-by-case basis to the European Commission. EFSA has developed several guidance documents on risk assessment of GMOs.

In Norway, the Norwegian Scientific Committee for Food and Environment (VKM) carries out risk assessments of GMOs for the Norwegian Food Safety Authority and the Norwegian Environment Agency. As a response to the rapidly developing field of genome editing and the new challenges that emerge for risk assessors, VKM initiated a project to address these challenges. The purpose of the project is defined by the terms of reference given by VKM. This is an abbreviated version of the resulting full report (VKM, 2021).

Terms of reference (ToR)

- **Describe the various methods that constitute the genome-editing technologies.** Different methods and their technologies, including the variation within these and the genomic alterations they result in, should be described.
- **Describe the use of genome-editing technologies today, including future perspectives.** The main applications of new genome-editing technologies within plant breeding, animal breeding (including farmed fish), and microorganisms should be described, and examples relevant for Norway should be highlighted.
- **Discuss implications for risk assessment regarding genome-edited organisms.** Potential challenges for risk assessment of genome-edited organisms (and products thereof) with the EFSA guidance for genetically modified organisms should be investigated and described.
- **Discuss possible implications for biodiversity in Norway.** Potential effects stemming from the spread and establishment following the use of or production of genome-edited organisms should be discussed.

In considering the ToR, VKM decided not to include assessment of insects for food and feed production. Insects for food and feed production are not expected to have any substantial impact on the Norwegian market within the next ten years. There are a few examples of market-ready genome-edited insects for food and feed uses (Xu et al., 2019).

Background and introduction

Box 2
The regulation of GMOs in the EU and Norway

The European and Norwegian regulatory frameworks regulate the production, import and market placement of food and feed containing, consisting of, or produced from GMOs, as well as the release of GMOs into the environment. The legal frameworks for GMOs ensure that no genetically modified organism or product from GMOs, can be placed on the market before it has been granted an authorisation. The frameworks are interdependent and are all process-oriented. The use of certain gene technologies to develop a product will trigger the regulatory framework and the regulated status, inter alia that authorisation is required before placing on the market. The Court of Justice of the EU decided in 2018 that organisms obtained by genome-editing techniques are also defined as GMOs (EU, 2018). Hence, organisms developed by new genome-editing techniques are also subject to the obligations laid down by the EU legal framework.

The European Food Safety Authority (EFSA) evaluates the safety of GMOs on a case-by-case basis before they can be authorised for use as food or feed and/or for import and processing, or cultivation in the EU. EFSA performs a scientific risk assessment, in cooperation with the scientific bodies of the Member States. Authorisations are granted for a ten-year period by the European Commission through a centralised procedure under Regulation No. 1829/2003 (EC, 2003a) concerning genetically modified food and feed, or Directive 2001/18/EC (EC, 2001) on deliberate release into the environment of genetically modified organisms. The frameworks regulate genetically modified plants, microorganisms, and animals. GMOs are assigned a unique identifier, and food or feed consisting of, containing, or produced from GMOs must be labelled to ensure traceability and to enable consumers to make informed choices in accordance with Regulation (EC) 1830/2003.

In Norway, the use of GMOs and derived food and feed are regulated under the Gene Technology Act (Government.no, 1993) and the Food Act (Government.no, 2003). The purpose of the Gene Technology Act is to ensure that the production and use of GMOs and the production of cloned animals takes place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development, and without adverse effects on health and the environment. The provisions of the Act also apply to substances and products that consist of or contain GMOs. Additionally, there are requirements for labelling and traceability of GMOs.

The purpose of the Food Act is to ensure safe and wholesome food, to promote health, quality, and consumer concerns along the whole production chain, and to provide for sustainable production. Processed and derived genetically modified products for food and feed are regulated by different provisions founded on the Food Act. The provisions lay down authorisation and labelling requirements, were the labelling requirements concern both derived and living GMOs for food and feed.

The EU Directive 2001/18/EC is implemented in the EEA Agreement (European Economic Area Agreement) and transposed into the Norwegian Gene Technology Act. Norway is therefore affiliated with the GMO authorisation process in the EU for applications submitted under the directive (mainly products other than food and feed). The Regulation 1829/2003/EC is currently not a part of the EEA Agreement. However, in preparation for a legal implementation of the Regulation in Norwegian law, Norway adheres to the EU proceedings for GMO applications.

Current debate on regulation of GMOs in the EU and Norway

The European Court of Justice decision in 2018 (Van der Meer et al., 2021), that included genome-edited organisms in the GMO definition and hence the regulatory system, sparked a debate about the suitability and continued use of the regulatory system for GMOs. The debate often emerges from various perceptions on the suitability of process- versus product-based approaches to safety assessments. Further, the current lack of international harmonization has resulted in national decision making with various assessment provisions in e.g. the US, Japan, Argentina, Australia, and others (Menz et al., 2020; Thygesen, 2019; Van der Meer et al., 2021). This heterogeneous landscape of regulatory approaches taken at the national level, combined with a rapidly developing technology, new commercial opportunities, and lack of standardised terminology for new product categories, currently represents a substantial uncertainty for developers, producers and consumers. At the core, international trade requires transparency and consistent regulations. In this context, the Norwegian Biotechnology Advisory Board, on its own initiative, presented in 2018 its proposal for how GMOs could be regulated (Bioteknologirådet, 2018a). The board suggested that the requirements for risk assessment and approval could be differentiated in a tiered system based on the genetic change made. However, details enabling a regulatory categorisation, as well as other topics, including the relationship to EU legislation, definitions and terms, and risk assessment, were not fully addressed in the board's proposal.

The principles for regulation of GMOs in both EU and Norway were developed in the 1990s (Van der Meer et al., 2021). Now in 2021, there are ongoing processes for considering possible regulatory amendments to the GMO frameworks, both in the EU and in Norway. In December 2020, the Norwegian Ministry of Climate and Environment assigned a Public Committee to assess questions related to gene technology. The mandate of the Committee is to prepare an updated knowledge base in the field of gene technology, and to consider amendments to the legal national framework. The report is expected in June 2022 (Government.no, 2020). In late April 2021, the European Commission informed the public that processes for discussing a new legal framework for new genomic techniques will be put in motion (EC, 2021d).

The risk assessment process of GMOs in EFSA and VKM

EFSA is funded by the European Union to provide independent scientific advice and communication on risks associated with the food chain. It is working through its Scientific Committee and various standing Scientific Panels. The GMO Panel provides advice through its opinions on the health and environmental safety of specific genetically modified organisms for placing on the market.

EFSA, in collaboration with member states, assesses possible risks from GMOs to human and animal health, and to the environment. EFSA's risk assessment of a GMO is based on the documentation presented by the applicant and other relevant scientific information. EFSA has prepared several guidance documents for the risk assessment of GMOs (EFSA, 2021b).

EFSA applies the criteria laid down in the EU regulatory framework as decided by the European Commission when evaluating the safety of a GMO. The GMO risk assessments consider the following aspects: molecular characterisation, comparative analysis, evaluation of potential toxicity and allergenicity and evaluation of potential environmental impact. Under EU legislation, applications for import and processing, cultivation or breeding of GMOs must contain a plan for detailed post-market environmental monitoring (PMEM). This plan should describe how the GMO will be monitored for possible adverse effects on the environment. Taken together, environmental risk assessment and PMEM are important parts of the measures in place to protect the environment. In addition, a validated protocol for detection is needed, and reference

material must be provided to the EU reference laboratory for GM food and feed (EC, 2021b).

In Norway, VKM carries out health and environmental risk assessments of GMOs and products containing or consisting of GMOs applied for approval in the EU under Directive 2001/18/EC or Regulation 1829/2003/EC. The risk assessments are performed on behalf of the Norwegian Food Safety Authority and the Norwegian Environment Agency (VKM's assignment, 2020). The VKM assessments form a key part of the documentation supporting the national GMO approval process (approval process only for GMOs applied for under the Directive, as Regulation 1829/2003 is not yet implemented). The Norwegian Biotechnology Advisory Board evaluates ethics, societal benefit, and sustainability, according to the Norwegian Gene Technology Act.

The VKM GMO Panel evaluates GMOs with reference to their intended use in the EEA, and according to the principles described in relevant national and EU frameworks. VKM also takes into account the appropriate principles described in the EFSA guidance documents for risk assessment of GMOs and derived food and feed, and the environmental

risk assessment of GMOs, as well as other supporting documents developed by EFSA. (2021b)

In this report, five of the main EFSA guidance documents have been considered;

1. Guidance on risk assessment of food and feed from genetically modified plants (EFSA, 2011a)
2. Guidance for environmental risk assessment of genetically modified plants (EFSA, 2010)
3. Guidance for risk assessment of food and feed from genetically modified animals, and on animal health and welfare aspects (EFSA, 2012a)
4. Guidance on the environmental risk assessment of genetically modified animals (EFSA, 2013)
5. Guidance for risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA, 2011d)

These guidance documents outline the baseline for risk assessment, including the areas of concerns, and the various steps of the risk assessment process. It is emphasised that the assessment process that is structured and guided by these documents also draws strong support from subsequent opinions and technical notes developed by EFSA. It is the total collection of these documents that form the basis for assessment practices. The document base is continually updated to take into account developments in technology and the evolving knowledge base.

VKM has used the five core guidance documents and case examples (Box 3) relevant for Norway to describe the methodology and assess whether the EFSA guidance is adequate for risk assessments of genome-edited organisms in general.

Is the EFSA guidance for GMO adequate for risk assessment of genome-edited organisms?

The present discourse on how new genome-editing techniques will be regulated lacks an analysis of whether current risk assessment methods can be applied to organisms arising from these new techniques. Therefore, VKM's report aims to provide an overview of the new techniques and to examine whether current risk assessment methodologies are adequate to evaluate potential risks from organisms developed by targeted genome editing.

Specifically, the report addresses the question whether EFSA guidance documents for GMOs are sufficient to evaluate the risks to health and environment posed by genome-edited plants, animals, and microorganisms. The report also provides some perspectives on topics that may need to be addressed as part of the further scientific and regulatory approach to genome-edited organisms. Possible implications for biodiversity in Norway are also discussed.

Use of genome editing in food and feed production

Use of genome editing in food and feed production

The new genome-editing techniques can be applied to most types of organisms, including those of commercial interest. The report gives attention to the genome editing of plants, animals, and microorganisms intended for food and feed production.

Genome-editing techniques have been used for research purposes for several years already, especially for knocking out genes to study gene function. More recently, the advances made in genome editing, combined with increased biological understanding, present new opportunities in development of food and feed crops. Most of the market-oriented traits under development are point mutations or indels (SDN1) that knock out gene function to improve nutritional value or stress tolerance, while a much smaller fraction are plants containing insertions of whole genes or gene fragments (SDN3) (Menz et al., 2020). In Norway, researchers are now developing potatoes and strawberries resistant to certain pathogens of fungi, through genome editing with CRISPR.

Genome editing also has a broad range of potential applications in production animals, including making livestock more adapted to farming or environmental conditions, increasing disease resistance, or improving growth, fertility, and animal welfare. Genome editing has been used to alter targeted genes to be either active or inactive, both for research purposes and for direct applications (Van Eenennaan, 2017).

Fish are marine sources of PUFA (polyunsaturated fatty acids) in human diets. By using genome-editing technology, it is possible to increase the endogenous synthesis of PUFA in farmed salmon (Box 3). Escaped farmed salmon are a substantial challenge in fish farming as they may enter rivers, interbreed with wild salmon, and introduce maladapted traits to wild populations (Bolstad et al. 2017). Therefore, CRISPR/Cas9 technology is being used to target genes for gonad development to obtain sterility in farmed fish (Box 3). Producing fish that are sterile also opens opportunities for genome editing of other traits.

Genome editing can also be used to increase food safety by targeting the production of specific proteins, such as the bovine prion protein, resulting in resistance towards Bovine Spongiform Encephalopathy (BSE) in cattle (Bevacqua et al., 2016). Also, the improvement of milk quality, free from major allergens, has been the focus of many genome engineering projects (e.g., Sun et al., 2018). The chicken egg is a widely consumed protein source and the genome editing could be used for production of allergen-free or allergen-reduced chicken eggs by disrupting ovalbumin and ovomucoid genes in parent generations (Oishi et al., 2016).

The use of bacteria and yeasts in fermented foods is common worldwide, and in this respect genome-editing technologies offer possibilities for more rapid development

Genome editing in plants

of probiotics and starter strains/cultures for the food and feed industry. Genome-editing techniques have been used for research purposes in the model plant *Arabidopsis thaliana* (thale cress) for several years and many genes have been targeted to generate mutant plants. Scientists and commercial companies have used the technology for faster, cheaper, and more precise development of new crop varieties in plant breeding. Studies on more than 20 crop species developed with CRISPR genome editing have been published (Ricroch et al., 2017) and several new projects on other crops are underway. Most edited plant species in the pipeline are relevant for Norway, either for import, like soybean used in fish feed production, or for agricultural production, like potatoes. Plants where CRISPR has been used to knock-out a gene to improve traits, e.g., stress tolerance and improved nutritional value, are closest to the market today. Knock-in mutants harbouring a gene or part of a gene from a related plant or another species are expected to be introduced in the future.

Successful genome editing depends on efficient genetic transformation and regeneration of plants from edited plant cells (Altpeter et al., 2016). There are mainly two methods that are used for transferring the components of the genome editing apparatus to the plant cell nucleus: *Agrobacterium*-mediated transformation and particle bombardment or "gene gun". For both particle bombardment and *Agrobacterium*-mediated transfer, plants are regenerated from cells where the gene expression

constructs are integrated into the plant cell genome (Altpeter et al., 2016). Genome editing is even useful in polyploid plants, since it is possible to knock-out or modify several redundant gene copies on all the homologous chromosomes in a single transformation event, if the targeting region is conserved among the alleles (van de Wiel et al., 2017; Wang et al., 2018).

Many emerging genome-edited crop varieties are not relevant for cultivation in Norway. This is due to growth requirements of the crop itself, e.g., soybean, rice or maize, none of which are well adapted to the Norwegian climate, and/or they may have redundant traits, e.g., resistance to a disease or pest not found in Norway. Genome-edited plants not suitable for cultivation in Norway may still be relevant for import if the new traits confer other benefits, e.g., improved taste or nutritional value.

The use of genome-editing techniques have led to development of new varieties within a broader diversity of agricultural crops, including crops commonly cultivated in Norway, e.g., potatoes, oilseed rape, tomatoes, and camelina. Case examples discussed in the report are presented in Box 3.

Genome editing in animals

Genetic improvements through selective breeding have significantly boosted livestock and aquaculture production. Selective breeding has resulted in farmed animals that are more resistant to diseases, grow faster and have a higher meat quality, or produce more offspring. Genetic improvement in animal husbandry traditionally relies on observation and characterisation of given traits in a limited number of elite individuals and their progeny (progeny testing). In aquaculture breeding, information is collected from siblings of the animal and also from other close relatives in the pedigree. The process of generating production animals from this elite population is limited by several factors, such as ability to accurately identify high merit individuals for further expansion, selection intensity, generation time of the species, maintaining existing genetic diversity and conversion of genetic variation into genetic gain (Gonen et al., 2017; Lillico, 2019).

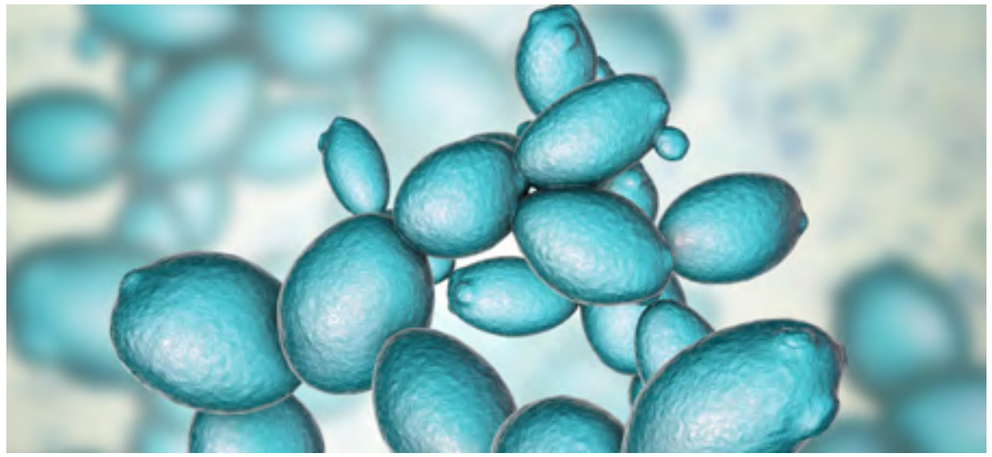
Genome editing could be used to correct heritable diseases or substitute alleles of a given gene into more desirable alleles, without the need for repeated backcrossing or outcrossing with an animal carrying the desired allele (Van Eenennaan, 2017).

Delivering nuclease-mediated genetic changes to livestock will likely occur in synergy with conventional breeding programs. Most of the economically interesting traits in animal breeding are typically polygenic traits, for which a high number of small effect genes together control the trait. The majority of these small effect genes remains to be identified and are therefore not available for being edited. However, some single genes with strong effects on certain traits are known and are typical candidates for genome editing.

Several genome-edited animals with relevance to Norway, e.g., Atlantic salmon, cattle, domestic pig, chicken and sheep, have been developed using genome-editing techniques. Case examples discussed in the report are presented in Box 3.

Use of genome editing in food and feed production

Genome editing in microorganisms



The use of bacteria and yeasts in fermented foods is common. Bread, dairy products, fermented meats and fermented beverages, such as beer and wine, have been consumed by many cultures for thousands of years. In addition to their traditional uses, bacteria and yeasts are currently utilised by the industry to synthesise diverse value-added compounds that have applications in pharmaceutical-, cosmetic-, food- and feed products. However, the choice of a particular strain or species for a specific industrial application is often based on historical, rather than scientific grounds. Genome-editing tools, such as CRISPR technology, have now become available for use in many different species of bacteria and yeasts (Stout et al., 2017).

Techniques for targeted genome editing have, for many species of microorganisms, been available long before CRISPR technology. Hence, the practical implications of new CRISPR based tools may not be as important as for higher organisms. On the other hand, more species of microorganisms may be targeted and possibly more efficiently, i.e. a broader set of yeast species and other eukaryotic microorganisms including phytoplankton (Cai et al., 2019).

CRISPR/Cas-based technologies have been used to develop probiotics and starter strains/cultures for the food and feed industry. Microorganisms intended for contained use are often extensively genetically altered to optimise particular production goals. These organisms can be considered production scaffolds and fall within the concept of synthetic biology (EFSA, 2020).

The larger field of synthetic biology is growing rapidly, especially in model - host systems, such as Saccharomyces cerevisiae, Escherichia coli, and Bacillus subtilis. The CRISPR/Cas-based technologies have also been shown to be adaptable to an increasing number of non-conventional species, hence, their limitations as synthetic biology platforms no longer represent a major obstacle. This will likely enable industrial biotechnology to use a broader set of non-conventional microorganisms for the economical production of small molecules and proteins.

Genome-edited microorganisms are not expected to be entering the Norwegian food chain in the near future.

Use of genome editing in food and feed production

Box 3

Genome-edited plants and animals used as case examples in the report

Genome-edited plants

Case 1 is a genome-edited potato (*Solanum tuberosum* L.) developed with the CRISPR/Cas9 technique and categorised as a SDN1 (Andersson et al., 2017). Improved starch quality was achieved after the introduction of a few nucleotide changes (1-10 bp indels) into all four alleles of the potato granule-bound starch synthase (GBSS) gene. The resulting loss of function of the GBSS enzyme elimnates the synthesis of amylose, thereby increasing the amylopectin content of the potato. This gene target and phenotype is the same as for the genetically modified Amflora potato developed by BASF (https://basf.com/).

Case 2 is a genome-edited soybean (*Glycine max* (L.) Merr.) developed with the CRISPR/Cas 9 technique and categorised as a SDN2 (Li et al., 2015). Tolerance to the herbicide chlorsulfuron was introduced by editing the acetolactate synthase 1 (ALS1) gene. The edit causes an alteration in the ALS enzyme making it less sensitive to the herbicide. ALS is a key metabolic enzyme in biosynthesis of branched-chain amino acids like valine and isoleucine, targeted by many herbicides. In conventional soybeans, chlorsulfuron would block branched-chain amino acid biosynthesis by disrupting the enzyme, killing the plants.

Case 3 is a genome-edited maize (*Zea mays* L.) developed with the CRISPR/Cas9 technique and categorised as a SDN3 (Shi et al., 2017). The drought-tolerant maize variety was developed by overexpressing the negative ethylene regulator ARGOS8. This was achieved by inserting the constitutively expressed native maize promotor GOS2 in the promotor region of the ARGOS8 gene. Ethylene is a phytohormone known to play an important role in regulating plant response to abiotic stress, including water deficits and high temperature. A higher yield can be achieved by decreasing the sensitivity of maize to ethylene.

Case 4 is a genome-edited oilseed rape (*Brassica napus* L.) developed with the oligonucleotide-directed mutagenesis (ODM) technique (Songstad et al., 2017). Tolerance to sulfonylurea and imidazolinone herbicides was achieved by single nucleotide changes in two genes encoding subunits of the AHAS (acetohydroxyacid synthase, also known as ALS enzyme (as in case 2 and 5)). The changes result in a single amino acid substitution in each protein subunit, which induces conformational alterations in AHAS conferring tolerance to the herbicides. In conventional rapeseeds, sulfonylurea and imidazolinone would block branched-chain amino acid biosynthesis, killing the plants.

Case 5 represents both a genome-edited tomato (*Solanum lycopersicum* L.) and a genome-edited potato (*Solanum tuberosauma* L.) developed with base editing (BE) techniques (Veillet et al., 2019). Tolerance to the herbicide chlorsulfuron was achieved in both plants with cytidine base editors to direct a C-to-T base conversion, editing the acetolactate synthase (ALS) gene. The edit causes an alteration in the ALS enzyme making it less sensitive to the herbicide. ALS is a key metabolic enzyme in biosynthesis of branched-chain amino acids like valine and isoleucine, targeted by many herbicides. In conventional tomatoes and potatoes, chlorsulfuron would block the enzyme ALS, killing the plants.

Case 6 is a genome-edited apple tree (*Malus ×domestica* (Suckow) Borkh.) developed with the CRISPR/Cas9 technique and categorised as a SDN1 (Pompili et al., 2019). Reduced susceptibility for fire blight infection was achieved by knockout of the gene MdDIPM4. Fire blight is a contagious disease affecting apples and pears. The bacterium *Erwinia amylovora* is the causal agent of fire blight disease in apple.

Case 2, case 4 and case 5 represent genome-edited plants (soybean, oilseed rape, tomato and potato) obtained through the use of the editing CRISPR/Cas9, ODM and base editing, respectively. Despite differences in the systems, all three cases lead to base-changed variants of the endogenous enzyme ALS conferring tolerance to sulfonylurea herbicides, and other related herbicides that target ALS.

Potato, rapeseed, tomato and apples (cases 1, and 4-6) were chosen based on their relevance for cultivation in Norway, whereas soybean and maise (cases 2-3) were chosen because of their significance as imported food and feed.

Genome-edited animals

Case 1 represents two examples of genome-edited farmed Atlantic salmon (*Salmo salar* L.) developed with theCRISPR/Cas9 technique and categorised as SDN1 (Datsomor et al., 2019a; Datsomor et al., 2019b). In both cases, genes encoding enzymes involved in the production of polyunsaturated fatty acids (PUFA) were edited, resulting in altered fatty acid composition.

Case 2 is a genome-edited farmed Atlantic salmon developed with the CRISPR/Cas9 technique and categorised as SDN1 (Wargelius et al., 2016). Introduced edits in the dead end (dnd) gene leading to knockout of this gene resulted in a sterile fish without germ cells. The dnd gene is a factor required for germ cell survival in vertebrates.

Case 3 is a genome-edited channel catfish (*Ictalurus punctatus)* developed with CRISPR/Cas9 technique and categorised as SDN1 (Khalil et al., 2017). Knockout of the MSTN gene encoding the protein myostatin which normally suppresses muscle growth, resulting in enhanced growth of the fish.

Case 4* is a genome-edited cattle (*Bos taurus*) developed with the TALEN technique and categorised as a SDN3 (Carlson et al., 2016). Insert of a 212 bp duplication (homology-directed) into bovine embryo fibroblasts leads to alteration of the gene responsible for development of horns. The altered gene resembles a gene variant found naturally in cattle of Celtic origin (Polled Celtic, PC POLLED) that does not produce horns.

**This example also illustrates the occurrence of unintended effects of the engineering approach. Independent analyses of sequencing data made available by the developers revealed that vector sequences remained in the final cow genome. FDA discovered a stretch of bacterial plasmid DNA including several genes conferring antibiotic resistance. The unintended integration of the DNA fragment is likely to have occurred during the genome-editing process (Norris et al., 2020).*

Use of genome editing in food and feed production

Case 5 is a genome-edited pig (*Sus scrofa domesticus*) developed with the CRIPSR/Cas9 technique and categorised as SDN1 (Burkard et al., 2017; Burkard et al., 2018). Resistance towards porcine reproductive and respiratory syndrome (PRRS) was achieved by a deletion in the CD163 gene. The virus causing the disease enters immune cells via the CD163-receptor to establish an infection. Animals carrying the modified CD163 receptors seem to be fully resistant to PRRS virus infection.

Farmed Atlantic salmon, cattle, and the domestic pig (case 1-2 and 4-5) were chosen based on their relevance for breeding and production in Norway. Channel catfish (case 3) was chosen because the gene edit targets muscular growth, which is relevant for most domesticated animals, and because it is an alien fish species in Norway.

The cases of genome-edited animals listed above represent animals intended for confined or semi-confined conditions. There are currently few examples of genetically modified or genome-edited animals intended for open environmental release. A notable exception of environmental release is the genetically modified male sterile mosquitoes developed by Oxitec (www.oxitec.com), which have been field-released in various parts of the world for the purpose of population control of disease-carrying mosquito populations. Gene drive traits facilitated by the use of CRISPR technology has been proposed as a tool to further develop measures for insect population control (www.targetmalaria.org). Gene drive as case examples of traits enabled by CRISPR are not covered in this report. A recent advisory on the risk assessment of engineered gene drives was published by EFSA (EFSA, 2020).

It is noted that several of the cases above have been developed through a two-step approach during which, in the initial development step, the organism was genetically transformed through chromosomal insertion of the CRISPR machinery. In the second step, the CRISPR machinery was removed through excision mechanisms or negative segregation. The extent of genome editing present in the final product constitutes the basis for the SDN class assignments suggested above. The regulatory aspects of negative segregants (EFSA et al., 2011) are not considered in further detail in this report.

Risk assessment of genome-edited organisms

Applicability of the EFSA guidance

Six genome-edited plants and five genome-edited animals were selected as case examples to evaluate the applicability of the EFSA guidance (Box 3). These cases were chosen based on the editing techniques used, types of edits introduced, and their potential relevance in Norwegian food and feed production. The types of edits are categorised based on the extent of changes introduced (Figures 1 and 2 and Box 1). The evaluation of microorganisms was performed with a generalised approach.

Potato, rapeseed, tomato, and apples (cases 1 and 4-6) were chosen based on their relevance for cultivation in Norway, whereas soybean and maize (cases 2-3) were chosen because of their significance as imported food and feed. Farmed Atlantic salmon, cattle, and the domestic pig (case 1-2 and 4-5) were chosen based on their relevance for breeding and production in Norway. Channel catfish (case 3) was chosen because the gene edit targets muscular growth, which is relevant for most domesticated animals, and because it is an alien fish species in Norway.

According to the EFSA guidance, health and environmental risk assessments of genetically modified plants and animals should be based on scientific information compiled from several aspects within key areas of concern (Figures 3 and 4). For each main section of the EFSA guidance an evaluation of the applicability to genome-edited plants and animals are provided with the case examples (Box 3).

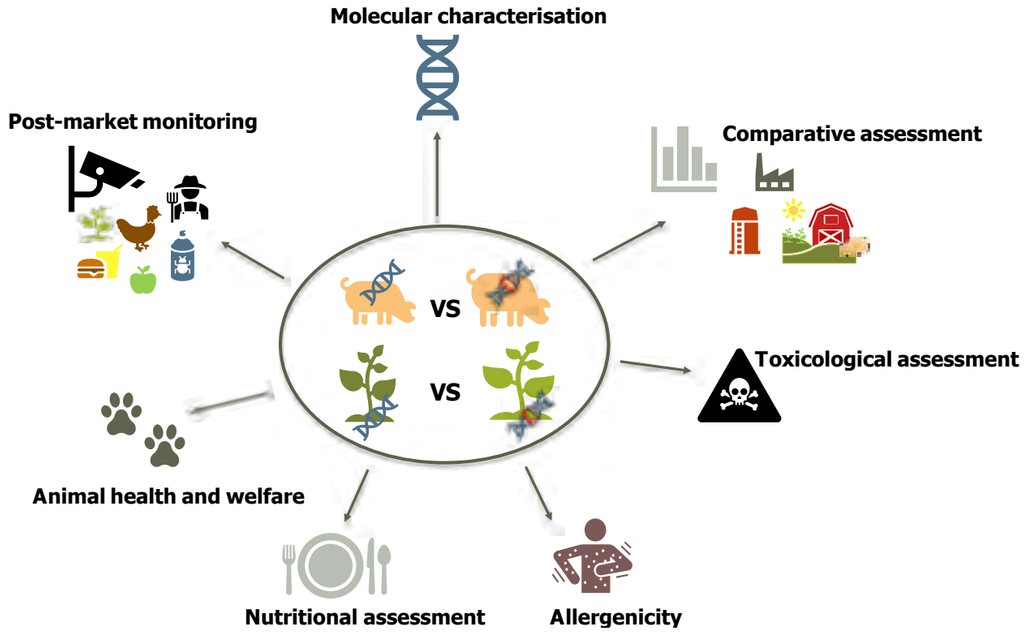
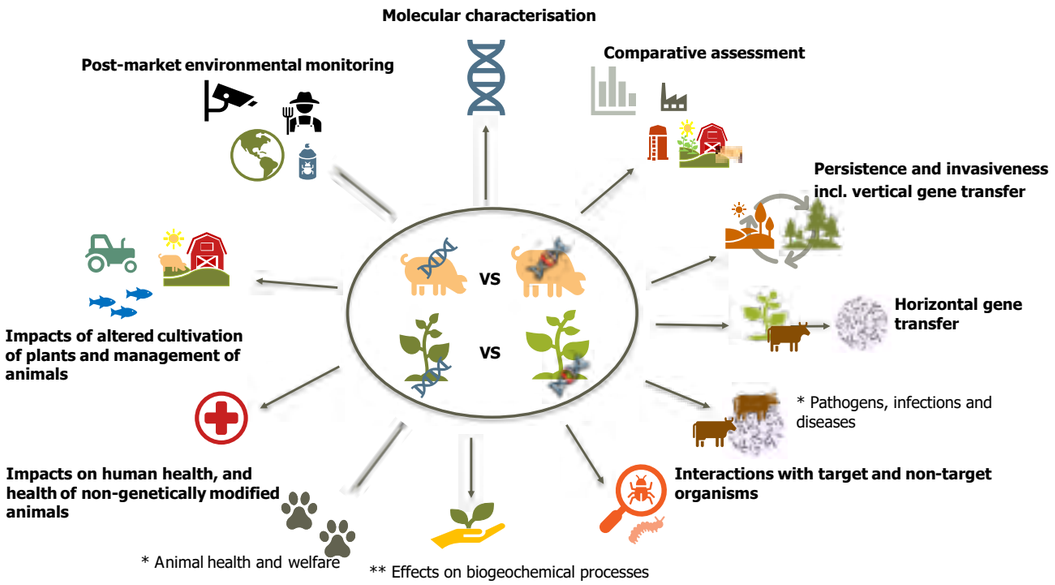


Figure 3. Key areas of concern in health risk assessment of GMOs. A simplified depiction of key areas of concern in the EFSA guidance that need to be addressed and weighted on a case-by-case basis in health risk assessments of genetically modified plants and animals intended for food and feed production. Depending on the organism, traits introduced and intended uses, various parts of the guidance may vary in importance for a risk assessment. For detailed information on the stepwise procedure in the guidance, see the VKM full report (VKM, 2021).



*** Risk assessment of genetically modified animals, ** Risk assessment of genetically modified plants.**

Figure 4. Key areas of concern in environmental risk assessment of GMOs. A simplified depiction of key areas of concern in the EFSA guidance that need to be addressed and weighted on a case-by-case basis in environmental risk assessments of genetically modified plants and animals intended for food and feed production. Depending on the organism, traits introduced and intended uses, different parts of the guidance may vary in importance for a risk assessment. For detailed information on the stepwise procedure in the guidance, see the VKM full report (VKM, 2021).

Risk assessment of genome-edited plants

Genome-editing techniques provide a new continuum of products ranging from those containing minor genetic changes similar to products selected in conventional breeding to those currently generated through genetically modified organisms. The suitability of the EFSA guidance documents has been evaluated using selected cases of genome-edited soybean, potato, maize, oilseed rape, tomato, and apple (Box 3).

Risk assessment of genome-edited plants should include information on the modification methods used, potential identity of new proteins in the plant to known toxins or allergens, effects on phenotypic and agronomic traits, effects of processing and storage of plant-derived products, effects on the content of chemical components including nutrients and anti-nutrients, estimation of exposure, and, lastly, a monitoring plan on potential effects on human dietary patterns and/or side-effects. The environmental risk assessment is concerned with potential risks associated with the introduced trait(s) and whether it may have an effect on survival, fitness, fecundity, and potential spread of the organism, with implications for the ecosystem and biodiversity.

Molecular characterisation and comparative assessment of genome-edited plants

The requirements for the molecular characterisation can be fully or partially applied for plants developed with genome-editing techniques, as exemplified in the six cases presented in Box 3. The requirements for the molecular characterisation are fully applicable for the genome-edited plant in case 3 (SDN3), in which an exogenous DNA fragment was inserted into the maize genome. The sections of the guidance focusing on the molecular characterisation of the exogenous DNA intended for introduction in the plant genome is not relevant for plants in case 1 (SDN1), 2 (SDN2), 4 (ODM) and 5 (BE), since no exogenous DNA fragment is present in the final product. A characterisation of the introduced changes at the edited loci would still be required. Depending on the technique used to generate the edits, the absence of vector sequences needs to be demonstrated.

Through the evaluation of the examples of genome-edited plants, it is generally concluded that the guidance is fully applicable and all analyses equally relevant for the comparative assessment. VKM finds that the high resolution offered by "omics"-based approaches (transcriptomics, proteomics and metabolomics) may in some cases improve the comparative and molecular approach and hence risk assessment of both genetically modified and genome-edited plants.

Risk assessment of food and feed from genome-edited plants

Depending on the genetic changes and traits introduced, as well as the species, a health risk assessment of a genome-edited plant will require variable amounts of data, a flexibility already embedded in the guidance.

The guidance on toxicological assessment can be applied to genome-edited plants. However, some analyses described in the guidance may not be applicable for genome-edited plants without DNA insertions. Contrary to case 3 (SDN3 category), the remaining case examples represent the SDN1-2 category, or are obtained by ODM or BE. The latter cases may therefore trigger fewer toxicological analyses than case 3.

Depending on the modifications introduced, all elements of the toxicological assessment may be performed for plants developed in the SDN3 category. If the assessment reveals compositional differences to the comparator, whole food feeding studies should be performed for genome-edited plants on a case-by-case basis.

The guidance can be applied for assessment of allergenic potential of genome-edited plants. Allergenicity testing is indicated in the guidance for the newly introduced proteins, epitopes or other constituents, and is of special importance if the edited plant is a known allergen (e.g., wheat or soy). For genome-edited plants, this would mostly apply to plants in the SDN3 category, especially if exogenous DNA fragments with a lack of history of safe use have been introduced.

The guidance is suitable for nutritional assessment of genome-edited plants. According to the guidance document, a nutritional feeding study should be performed on a case-by-case basis in plants with traits affecting nutritional composition, or if unintended effects are documented, in the molecular characterisation or comparative assessment. The guidance specifies that genetically modified plants carrying specific traits, e.g., herbicide tolerance and insect resistance, require appropriate treatment comparisons to evaluate safety. This can also be relevant for genome-edited plants carrying herbicide tolerance (cases 2, 4 and 5) or carrying insect resistance.

Environmental risk assessment of genome-edited plants

Depending on the genetic changes and traits introduced, as well as the species, an ERA of a genome-edited plant will require variable amounts of data. Equally important and applicable are the fundamental aspects described under the cross-cutting considerations. The cross-cutting considerations constitute key information that would also be required to complete risk assessments of genome-edited plants.

The guidance for assessment of specific areas of concern to be addressed in the ERA is applicable also for genome-edited plants. The information required is case-dependent, with more and thematically wider information needed when the plant has the potential to spread into natural ecosystems or hybridise with native species. For instance, soybean and maize (cases 2 and 3) are not considered environmental threats under the present environmental conditions in Norway, while oilseed rape and apple (cases 4 and 6) have wild relatives and, therefore, have the potential to hybridise and/or spread in the environment. More information would therefore be required for the environmental risk assessment of the two latter plants. Information on risk to threatened or key-stone species and threatened nature types should be considered in a Norwegian context.

Concerning impacts of specific cultivation, management, and harvesting techniques, the guidance can also be used for risk assessment of genome-edited plants. As for genetically modified plants, the introduction of genome-edited plants for cultivation may lead to changes in management and production systems. The guidance can be used to assess the effects on biogeochemical processes of genome-edited plants. Assessment of effects on human and animal health will also cover genome-edited plants, in accordance with the risk assessment for food and feed products.

The guidance for post-market environmental monitoring can also be applied to genome-edited plants. The need for a case-specific monitoring will vary among cases depending on the outcome of the environmental risk

assessment. Technical issues may be present in monitoring efforts, depending on the extent and type of genome edits introduced and the scope of the environmental release. For instance, case-specific monitoring of species, where risks of hybridisation or long-distance dispersal have been identified in the environmental risk assessment, requires careful design of monitoring programmes. For soybean, maize, potatoes, and tomatoes (cases 1, 2, 3 and 5), the monitoring would presumably be limited to general surveillance for unanticipated effects, whereas for oilseed rape and apple (cases 4 and 6) inclusion of a case-specific monitoring, in line with the potential environmental effects identified in the environmental risk assessment, might be needed. It is noted that, depending on the extent of genomic changes introduced (as categorised as SDN1 to SDN3), monitoring of some genome-edited plants may be technically challenging, i.e., to distinguish on a molecular level between conventional, naturally occurring mutants and genome-edited plants.

The guidance is considered suitable for all steps of the risk assessment of genome-edited plants. However, the extent of data and emphasis will depend on the genome-edited plant and intended use. Not all data requirements in the guidance are considered relevant for risk assessment of genome-edited plants when no foreign DNA has been added, and data requirements should therefore be determined on a case-by-case basis.

Risk assessment of genome-edited animals

Genome editing has a broad range of potential applications in livestock and aquaculture production, including making livestock and farmed fish more adapted to farming or environmental conditions, and improving disease resistance, growth and fertility, and animal welfare.

The suitability of the EFSA guidance has been evaluated using case examples of genome-edited animals, such as Atlantic salmon with altered fatty acid composition or sterility, channel catfish with increased muscle growth, hornless cattle and disease-resistant pigs, to exemplify the use and adequacy of the guidance documents.

Molecular characterisation and comparative assessment of genome-edited animals

The molecular characterisation requirements in the guidance can be fully or partially applied for genome-edited animals. The application of genome-editing techniques will in some cases only result in minor genetic alterations of the organisms. If no exogenous DNA has been inserted, the parts of the guidance focusing on molecular characterisation of the insert may not be relevant. This is the case for animals presented in cases 1, 2, 3 and 5 (SDN1) which have no inserted exogenous DNA. The data requirements linked to the intentional introduction of exogenous DNA fragments, i.e., the presence of foreign DNA in the final product, would therefore not be fully applicable for animals in the SDN1 category. Absence of unintended insertions of DNA in the target organism must be demonstrated for animals in the SDN2-3 category, which includes introduced DNA fragments and homologous recombination approaches. In all cases where vector DNA has been used in the genome-editing process, its absence must be shown in the edited organism.

The requirements in the guidance on the molecular characterisation are fully applicable for the genome-edited animal in case 4 (SDN3) where an exogenous DNA fragment was inserted with homologous recombination.

The guidance specifies that a comparative analysis of the genetically modified animal with the traditionally bred animal, comparing the phenotypic characteristics including health and physiological parameters, is an important component in the risk assessment. Through the examination of the different cases (1-5) of genome-edited animals, it is concluded that the comparative analysis in the guidance can also be used for genome-edited animals.

Risk assessment of food and feed from genome-edited animals and assessment of animal health and welfare aspects

Depending on the genetic changes and traits introduced, as well as the species, a health risk assessment of a genome-edited animal will require variable amounts of data, a flexibility already embedded in the guidance.

It is concluded that the guidance for toxicological risk assessment can be applied for genome-edited animals. When no new protein(s) is expressed and molecular, compositional, and nutritional assessments, as well as animal health and welfare considerations, show no difference from the comparator, the guidance states that animal testing would not be required. This follows the case-by-case approach.

The guidance for assessment of allergenicity can also be applied to genome-edited animals. When the modification is associated with alterations in allergenicity of the whole food, the allergenic potential of the genome-edited food should be further investigated. The case-by-case approach will determine the extent of allergenicity investigations needed.

The guidance is also applicable to nutritional assessments of genome-edited animals. Unless the assessment indicates significant differences in composition between the animal and its comparator, or the introduced trait affects nutritional properties directly, no further nutritional analyses are required.

However, if nutritional equivalence has not been established, a nutritional feeding study can be performed on a case-by-case basis.

The guidance also addresses the scientific requirements for the assessment of health and welfare of genetically modified animals, bred to be used for food and feed. The assessment is made in terms of the effective functioning of their body systems in a given environment and is also applicable to genome-edited animals.

Environmental risk assessment of genome-edited animals

Depending on the genetic changes and traits introduced, as well as the species, an ERA of a genome-edited animal will require variable amounts of data. Equally important and applicable are the fundamental aspects described under the cross-cutting considerations. The cross-cutting considerations constitute key information required to perform appropriate risk assessments of genome-edited animals. The risk assessment may use a staged approach, which suggests that the different end points of the ERA may be the target population, wild-type relatives of the target organism, related species or the entire ecosystem. The staged (step-by-step) procedure as well as a case-by-case approach will define the specific types of information and considerations needed for the ERA.

As net pen-based aquaculture is unconfined, the interactions between the edited fish and the biotic components and processes in the environment are among the most complex interactions that should be contemplated in an ERA. They include genetic effects on wild populations of the same fish species, interspecific hybridisation with closely related species, ecological effects on other fish species, and ecological effects on other trophic levels, biodiversity, and ecosystem services (IPBES, 2019).

The guidance is applicable for ERA of fish pathogens, infections, and diseases. Transmission of disease agents between farmed and wild fish populations is an issue of central importance for risk assessment. The guidance is also adequate with respect to assessing aspects of interactions with the abiotic environment.

The management techniques associated with both conventional aquaculture and genetically modified fish have been subject to considerable debate. This is reflected in the EFSA guidance that considers a variety of topics and procedures for generation and containment of farmed fish. The guidance for risk assessment of effects on human and animal health will also cover genome-edited fish, in accordance with the risk assessment for food and feed products.

The guidance addresses the potential persistence or invasiveness of genetically modified mammals and birds, and their potential to hybridise with non-modified relatives. Mammals and birds with differing confinement levels will require separate considerations. These considerations will also be applicable on a case-by-case basis to genome-edited mammals and birds.

The guidance for assessing the risk of disease transmission is also applicable to genome-edited mammals and birds. This is also the case for aspects concerning interactions with their target organisms (TOs). The EFSA guidance on interactions between genetically modified mammals and birds and non-target organisms (NTOs) covers many aspects. These will also be relevant for genome-edited mammals and birds. Nevertheless, a complete assessment will not be achievable due to the inherent complexity of ecosystems. Thus, as emphasised by the guidance, attention should be given to description of data gaps, uncertainties and mitigation measures. When it comes to interactions of genetically modified mammals and birds with the abiotic environment, the guidance will also be adequate for genome-edited mammals or birds.

The guidance for assessing the environmental impacts of genetically modified animals, for which the management practices are changed, will also apply for genome-edited mammals and birds. As for genetically modified animals, a case-by-case conclusion for the overall risk on animal health and

welfare should also be required for genome-edited mammals and birds. The guidance for risk assessment of effects on human and animal health will also cover genome-edited mammals and birds, in accordance with the risk assessment for food and feed products.

The principles laid down in the guidance are adequate for the monitoring of potential adverse effects of genome-edited animals including fishes, mammals, and birds. The need for case-specific monitoring will vary among cases depending on the outcome of the ERA. Technical issues may be present in monitoring efforts, depending on the extent and type of genome edits introduced, and the scope of the environmental release. The five cases above are quite different with respect to monitoring environmental effects. Data and monitoring plans following the guidelines for case-specific monitoring or general surveillance should be provided for all cases. Cases 1 and 2 represent Atlantic salmon, which is a valuable species for Norway, both as a domesticated farmed fish and as a native, wild species, and would require case-specific monitoring plans. Further, any data gaps and uncertainties concerning the environmental impact (including risks for human and animal health) of genome-edited cows and pigs compared to non-edited conspecifics should be surveyed. Depending on the extent of genomic changes (SDN1 versus SDN3) introduced, the monitoring of some genome-edited animals may be technically challenging, i.e., to distinguish on a molecular level between conventional, naturally occurring mutants and genome-edited animals.

The guidance is considered suitable for all steps of the risk assessment of genome-edited animals. These steps include hazard identification, hazard characterization, as well as exposure assessment and risk characterization. The extent of data and emphasis will depend on the genome-edited animal and intended uses.

Risk assessment of genome-edited microorganisms

Genome editing has a broad range of potential applications in microorganisms, particularly those in contained fermentation systems for the production of various fine chemicals, including drugs. Fewer products are developed with the purpose of including them in the food chain. The suitability of the EFSA guidance was evaluated for genome-edited microorganisms. It is noted that the regulatory landscape of microorganisms is not straightforward. Due to the heterogenous uses of microorganisms/products, their regulatory landscape can be considered complex, falling under both an EU directive, different EU regulations, as well as various guidance documents developed by several of the EFSA panels. The product categorization presented in the guidance allows for differentiation in the amount of data needed for the assessment. In contrast to animals and plants, the core concept of qualified presumption of safety (QPS) provides a clear baseline for the comparative approach. This, combined with a case-by-case approach, provides both structure and flexibility to the risk assessment process. The same flexibility is offered to genome-edited organisms within this regulatory framework.

The EFSA guidance for the risk assessment of genetically modified microorganisms and their products intended for food and feed use is also applicable to genome-edited microorganisms.

Conclusions

The Norwegian Scientific Committee for Food and Environment (VKM) initiated this work to examine the extent to which organisms developed by genome-editing technologies pose new challenges in terms of risk assessment. In 2018, the European Court of Justice decided to include genome-edited organisms in the GMO definition. Therefore, organisms developed by new genome-editing techniques for the production of food and feed are also subject to the obligations laid down by the EU legal framework. In the EU, all new GMO products for food, feed and cultivation are assessed by the European Food Safety Authority (EFSA). This report considers whether the risk assessment guidance on genetically modified organisms, developed by EFSA, is applicable for genome-edited organisms.

- The inherent flexibility of the EFSA guidance makes it suitable to cover health and environmental risk assessments of a wide range of organisms with various traits and intended uses. Combined with the embedded case-by-case approach including the initial hazard identification step, that determines the type and extent of information needed for the assessment, the guidance is applicable to genome-edited organisms. VKM's evaluation has not identified new hazards specific to genome-edited organisms that fall outside the areas of concern established in the guidance.

- The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with novel traits (i.e. the phenotype of the organism) may be fully applied to all categories of genome-edited organisms. The guidance for environmental risk assessment, largely concerned with novel traits and assessment of potential effects on biodiversity (e.g. in Norway), stemming from the spread and establishment of genome-edited organisms, is fully applicable.

- The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with genetic modification (i.e. the genotype of the organism) may be fully applied to genome-edited organisms having inserted genes or long fragments of DNA, i.e. edits categorised as Site-Directed Nuclease type 3 (SDN3).

  However, these parts are not fully applicable for genome-edited organisms with minor insertions, deletions or single mutations, i.e. edits categorised as Site-Directed Nuclease type 1-2 (SDN1-2), edits obtained by oligonucleotide directed mutagenesis (ODM), or base editing (BE).

In summary, VKM finds that the EFSA guidance on risk assessment of genetically modified organisms provides a functional framework for risk assessment of genome-edited organisms. However, inclusion of specific considerations in the guidance regarding different properties of genome-edited organisms would be beneficial to ensure a common understanding between product developers and risk assessors regarding the type and extent of data needed to perform a risk assessment.

Further considerations

New genome-editing techniques provide a continuum of organisms ranging from those containing very minor genetic changes, to those currently generated through genetic modification. It will be challenging to fit such a heterogenous set of outcomes from genome-editing techniques into the regulatory system developed for genetically modified organisms. Moreover, many of the definitions, terminology and concepts used in the EFSA guidance documents were developed at a time when genetically modified organisms were nearly synonymous with the use of species-foreign transgenes inserted at random locations into the recipient genome. The applicability of such descriptors may or may not be valid for organisms developed through genome-editing techniques. VKM identified several topics that would benefit from further attention. These included the development of a common understanding of the following issues.

i) The dynamic nature of EFSA guidance. EFSA is continually refining and updating its guidance for risk assessment of genetically modified organisms as new products and processes emerge. Today there are more than 20 applicable documents. Thus, collectively, the guidance with technical notes also covers new technological developments such as the potential use of omics and next-generation sequencing technologies, as well as new genome-editing approaches.

ii) The importance of the case-by-case approach. As mentioned above, the guidance documents are developed to cover a broad set of organisms, environments and intended uses. Hence, not all sections of the guidance will be equally important or relevant for single cases. The case-based approach is commonplace in today´s risk assessment of genetically modified organisms, and should not differ for genome-edited organisms in the same regulatory framework.

iii) The substantiation of claims of naturalness. The use of genome-editing techniques has shifted focus from producing novel traits by adding foreign DNA (transgenes) to obtaining new traits by editing existing combinations of nucleotide sequences in the genome. The current focus on the techniques to edit single nucleotides in a genome has led to statements that such organisms also could have occurred naturally. With the implicit claim, they should therefore be exempt from GMO status and regulation. The types of edits and organisms warranting such claims may be further clarified.

iv) The need for precise definitions and harmonised use of terminology. Gene editing draws on specific methods and produces different types of outcomes that require unambiguous and harmonised descriptors. For instance, the words “new” or “novel” or “newly expressed” in relation to the altered trait/protein may benefit from further clarification. Until now, a “new protein” or “newly expressed trait” is typically understood as a protein derived from a newly inserted gene obtained by transgene-based engineering. Today, however, in some cases, the same “new” trait could be obtained through either expression of transgenes, cisgenes or intragenes, or through genome editing. Thus, the meaning of “new” would have different nuances depending on the methods used. Moreover, genome editing allows minor nucleotide changes to be introduced at desired places in the genome, for example in regions of the genome controlling expression of a gene of interest, in regulatory sequences and at multiple sites in the same or different genes. These different approaches will certainly create phenotypes with variable degrees of perceived “novelty” and may require developments in terminology beyond what has been established so far for genetic modifications.

v) The key function of the risk assessment is to reduce uncertainty and increase understanding of the evidence base. It is noted that a key consideration behind EU regulations and EFSA guidance is focused on excluding unintended effects and hence on reducing uncertainty. The assessment thus extends beyond considering the producer´s data needed to document the intended effects of the introduced change(s). The risk assessment seeks to understand and reduce concerns of unintended effects. To exclude unintended effects, phenotypically based nutritional, whole food toxicological testing, and allergenicity considerations have usually been expected in applications for food and feed use. These aspects extend beyond reporting on the genotype and the molecular characterisation of the intended genetic change. It is important to have the broader scope of risk assessment in mind, when considering its relevance and applicability to organisms with targeted genome edits.

vi) The lack of consistency of organisms in the SDN1-2 categories. The SDN1-2 categories are named outcomes of some uses of genome-editing techniques. In many example cases, the approach has targeted one or a few protein coding genes with the aim to alter the phenotype based on changed protein characteristics/expression patterns, including loss of function. Such examples with well characterised phenotypes may show some consistency. Some, but not all, could be obtainable through mutation-based breeding as well. However, another example of edits in the SDN1 category, e.g. targeting three genes simultaneously, is unlikely to occur through classic breeding in a relevant time frame (Sanchez-Leon et al., 2018). Moreover, introducing a few single nucleotide changes in regulatory genes can cause large changes in the phenotype, proteome and nutritional profile etc. Thus, a minor edit in a genome (compared to nucleotide alterations of previous transgene-based insertions) may not translate linearly to a minor edit in the phenotype. The risk assessment of phenotypes in the SDN1 category may be vastly different. This aspect of the broad opportunities inherent in the SDN1-2 categories must be considered when categorisation and alternatives to the current case-by-case approach is considered.

vii) The need to determine the absence of vectors and the regulatory status of negative segregants. Negative (null) segregants arise when genetically modified organisms lose the transgene insertion through segregation/outcrossing, for instance when the CRISPR machinery encoding locus/vector is removed from a plant genome after having obtained the desired edit elsewhere in the same genome. It is noted that many of the organisms produced by genome-editing techniques will, at an early developmental stage, contain DNA-based CRISPR vectors in the cytoplasm or have the CRISPR locus inserted as a transgene into the genome of the edited organism. At that stage, the organism resembles a genetically modified organism carrying a novel trait/transgene. However, subsequent breeding is done to ensure segregation of traits and that the final product will not be carrying the CRISPR locus, but only the intended genome edit. The practice of genomic integration of the SDN encoding locus early in the developmental phase of genome-edited organisms may vary. This heterogeneity in the early developmental stages of introducing genome edits is likely to have implications for risk assessment and may complicate the regulatory approaches.

viii) The requirements for validated detection protocols. EU regulation specifies the need for validated detection protocols for genetically modified organisms placed on the market to ensure clear labelling and traceability. This requirement can be met by genetically modified organisms because all current commercialised ones contain DNA sequences inserted stably at unique genomic sites. For some uses of genome editing (e.g., SDN1-2, ODM and BE), it is not fully clear whether validated detection protocols can be achieved. Such protocols will also be difficult to apply for organisms containing several edits that are not genetically linked. Regulatory aspects of detection should be further clarified. VKM, however, notes that the opportunity to complete a risk assessment of a genome-edited organism based on EFSA guidance is usually not dependent on validated detection protocols. This is because the assessment is on the product and the process of production.

ix) The emergence of new approaches to data collection. The collection of data relevant to risk assessment has followed a conventional path in which the applicant has collected the main part of the data submitted with the application. Scientific and technical developments now allow broader approaches to data collection, sharing and analyses, including artificial intelligence-based approaches. The further build-up of (eco)system approaches, combined with strong computing capacity including vast amounts of data, are likely to be increasingly valuable in environmental risk assessment. For instance, the recent EFSA report on ERA of bees discusses modelling and advocates a more holistic environmental approach to risk assessment (EFSA, 2021).

x) The urgent need for harmonised regulatory frameworks. Genome-edited organisms and products thereof intended for the market have reached various developmental stages. Several field trials have been conducted under national legislation, and some products have reached a commercialised stage (Menz et al., 2020). National authorities have taken different approaches to assessment and regulation in the absence of a harmonised international regulatory framework and established consensus of their regulatory status (Schmidt et al., 2020). This heterogenic landscape has created uncertainty for product developers, both in terms of regulatory status and opportunities for export/international markets. This also applies to the European market, where national authorities have approached applications for field releases differently. SDN-based products are not yet authorised for the EU market. There is therefore limited harmonisation and experience available to risk assessors, applicants and other stakeholders.

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