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SCIENTIFIC OPINION

New developments in biotechnology applied to animals: An assessment of the adequacy and sufficiency of current EFSA guidance for animal risk assessment

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

EFSA was requested by the European Commission to provide a scientific opinion on new developments in biotechnology, including new genomic techniques, as applied to animals for food, feed and other agricultural uses. A horizon-scanning exercise identified a variety of animals obtained with new genomic techniques, with the potential to reach the EU market in the short, medium and long term. No novel hazards have been identified that are linked to either the modification process or the newly introduced trait, when SDN-1, SDN-2 and comparable techniques (e.g. base editing or prime editing) were compared to established genomic techniques (EGTs) or conventional breeding. Hazards posed by SDN-3 are of the same nature as those posed by EGTs and the targeted insertion may reduce the potential hazards associated with the disruption of endogenous genes and/or regulatory elements in the recipient genome. Hazards posed by the new trait resulting from the introduced transgenic or intragenic DNA sequence are of the same nature as those posed by EGTs. Hazards posed by the new trait resulting from the introduced cisgenic DNA sequence are of the same nature as those posed by conventional breeding. Off-target mutations from genome editing are similar in nature to those from conventional breeding and do not pose novel hazards. Consequently, based on the currently available data, no new potential hazards, and thus, no new risks to humans, animals or the environment have been identified. A thorough evaluation of existing EFSA guidance documents for the risk assessment of GM animals revealed that their principles and recommendations provide the basis for assessing the risks of new genomic technique (NGT) animals for food, feed and other agricultural uses; however, the current texts cover only partially some areas (e.g. animal health and welfare) and may need updates, adaptations or enhancements on a case-by-case basis to fully address NGT-related risks.

KEYWORDS

animal health and welfare, conventional breeding, established genomic techniques, genetically modified animals, hazard, new genomic techniques, risk, risk assessment, synthetic biology

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SUMMARY

EFSA received a request by the European Commission, in accordance with Article 29 of Regulation (EC) No 178/2002, to provide a scientific opinion on new developments in biotechnology, including synthetic biology (SynBio) and new genomic techniques (NGTs), as applied to current or near-market animals for food, feed and other agricultural uses (hereinafter referred to as NGT animals), with implications for risk assessment methodologies and adequacy of the current EFSA risk assessment guidance documents, covering all aspects of molecular characterisation, food and feed safety, animal health and welfare, and environmental impact.

This mandate considered farmed animals – mammals, birds, fish and invertebrates – bred to obtain food, feed and other agricultural products falling within the remit of EFSA, as identified by Parisi and Rodríguez-Cerezo (2021) and Van Eenennaam (2023). Animals bred only for medical purposes or gene drive applications did not fall within the remit of this mandate. The NGT animals identified in a knowledge gathering report were grouped according to their stage of (pre) market development.

In particular, EFSA was requested to provide (1) a knowledge gathering on known cases of animals, and their food and feed products, which were obtained by new developments in biotechnology and (2) an EFSA opinion on potential novel hazards/risks from new developments in biotechnology applied to current and near-market animals and adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food and feed safety, animal health and welfare, and environmental impact (details in Section 1.1).

A horizon-scanning exercise informed the GMO Panel about NGT animals and confirmed that several have been authorised for market placement. In some instances outside the EU, NGT animals were judged to be 'non-GMO' if no exogenous DNA was added to the breed (SDN-1 and SDN-2), meaning that in some countries (e.g. Argentina and Brazil) such animals will be considered equivalent to conventionally-bred animals (Van Eenennaam, 2023). Projects in the research and development phase are also reported in the scientific literature, including mammals, birds, fish and invertebrate (e.g. insects, crustaceans and shellfish) species which may be intended for food, feed and other agricultural uses. Available knowledge suggests that the highest number of NGT animals reported in the scientific literature is represented by mammals, followed by fish, then birds and invertebrates. The majority of NGT applications involved genetic changes achieved by means of SDN-1 techniques, followed by SDN-3 and SDN-2. Among the various NGTs reported, the most frequently used were CRISPR/Cas, followed by TALENS and ZFNs. The most common trait category targeted was increased meat and fibre yield, followed by reproduction, biotic stress, production of hypoallergenic products, combinations of traits, colour, product quality, abiotic stress and a few others.

No novel hazards have been identified that are linked to either the modification process or the newly introduced trait, when SDN-1, SDN-2 and comparable techniques (e.g. base editing or prime editing) were compared to established genomic techniques (EGTs) or conventional breeding. Hazards posed by SDN-3 are of the same nature as those posed by EGTs and the targeted insertion may reduce the potential hazards associated with the disruption of endogenous genes and/or regulatory elements in the recipient genome. Hazards posed by the new trait resulting from the introduced transgenic or intragenic DNA sequence are of the same nature as those posed by EGTs. Hazards posed by the new trait resulting from the introduced cisgenic DNA sequence are of the same nature as those posed by conventional breeding. Off-target mutations from genome editing are similar in nature to those from conventional breeding and do not pose novel hazards. Consequently, based on the currently available data, no new potential hazards, and thus, no new risks to humans, animals or the environment have been identified.

Animal health and welfare hazards in EGT and NGT animals related to pre-commercial phases should be addressed through case-by-case evaluations when informative to the risk assessment, as earliest procedural steps may result in unintended phenotypes that are sometimes associated with advanced breeding technologies.

A thorough evaluation of existing EFSA guidance documents (EFSA GMO and AHAW Panel, 2012; EFSA GMO Panel, 2013) for the risk assessment of GM animals revealed that their principles and recommendations provide the basis for assessing the risks of NGT animals for food, feed and other agricultural uses; however, the current text only partially addresses the topics in several areas (e.g. animal health and welfare) and might require further updates, adaptations or enhancements on an ad hoc basis, to address the risk assessment of NGT animals, as outlined in this opinion.

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1 | INTRODUCTION

On 25 June 2018, the European Food Safety authority (EFSA) received from the European Commission (EC) a mandate referring to the 'Request for an EFSA opinion on genetically modified organisms developed through SynBio and their implications for risk assessment methodologies' submitted under Article 29 of Regulation (EC) No 178/2002.[1]

Within the scope of this mandate, limited to animals for food, feed and other agricultural uses (hereafter referred to as 'NGT animals') modified by new biotechnologies, including SynBio and NGTs, EFSA was asked:

- to consider whether and which newer sectors or advances should be considered among SynBio developments, in addition to the six[2] identified by the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Consumer Safety (SCCS)[3]
- to identify, where possible, potential risks in terms of impact on humans, animals and the environment that current and near-future SynBio developments could pose; in this respect, EFSA was also asked to identify potential novel hazards compared to established techniques of genetic modification
- to determine whether the existing guidelines for risk assessment are applicable and sufficient for current and near-future SynBio developments or whether there is a need for updated guidance, and, if so, to identify the specific areas where such updated guidance is needed
- to provide technical and scientific expertise on risk assessment of GMOs obtained through SynBio to support the EU in the work under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety

The mandate received from the EC (M-2018-0205) was organised into six work packages (WP) by EFSA to be reflected in six scientific opinions, covering the adequacy of the current EFSA guidance for:

- WP1: GM microorganisms – molecular characterisation and environmental risk assessment
- WP2: GM plants – molecular characterisation and environmental risk assessment
- WP3: GM microorganisms – food and feed risk assessment
- WP4: GM plants – food and feed risk assessment
- WP5: GM animals – food and feed risk assessment
- WP6: GM animals – environmental risk assessment

At the time of publication of this opinion, EFSA has finalised four opinions (EFSA GMO Panel, 2021, 2022a; EFSA Scientific Committee, 2020, 2022) that cover microorganisms and plants developed through SynBio for the environment and food feed aspects (WPs 1–4).

The current opinion addresses WP5 and WP6, with some adaptation from the original mandate. On 20 December 2022, EFSA received from EC the request to continue the work on animals developed through SynBio, expanding the scope of the mandate to cover new developments in biotechnology applied to animals, including SynBio as well as NGTs, given also that NGTs can be used for SynBio applications. EC provided the background and expanded Terms of Reference for this request (Sections 1.1.1, 1.1.2 and 1.2), with the request to have the final opinion adopted by 30 June 2025.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

The Commission's study[4] on NGTs published in April 2021 covered the use of NGTs in plants, animals and microorganisms, and in a broad variety of potential applications, including in the agricultural, food, medicinal and industrial sectors. The study was supported by expert work, including a report of the Commission Joint Research Centre (Broothaerts et al., 2021). Referring to the JRC definition, the Commission did include NGTs inducing epigenomic changes or acting specifically on RNA, which is not within the scope of this mandate.

The study noted that, in comparison to plants, less information is available on application of NGTs to animals. In particular, safety data are mainly available for genome editing in plants, making it difficult to draw relevant conclusions in other areas. It therefore recognised the importance of generating relevant information in the application of NGTs to animals.

[1]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[2](i) genetic part libraries and methods; (ii) minimal cells and designer chassis; (iii) protocells and artificial cells; iv. Xenobiology; (v) DNA synthesis and genome editing; (vi) citizen science (Do-It-Yourself biology).

[3]SCENIHR, SCCS and SCHER, 2014. Synthetic Biology I Definition, Opinion, September 2014. Available online: https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf; SCENIHR, SCCS and SCHER, 2015. Synthetic Biology II – Risk assessment methodologies and safety aspects, Opinion, May 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf; SCENIHR, SCCS and SCHER, 2015. Synthetic Biology III – Research priorities, Opinion, December 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_050.pdf.

[4]https://food.ec.europa.eu/document/download/5135278b-3098-4011-a286-a316209c01cd_en?filename=gmo_mod-bio_ngt_eu-study.pdf. Accessed: 23 October 2024.

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Based and building on the above, the Commission intended to initiate work to map new developments in biotechnology applied to animals and to get an updated scientific understanding of aspects related to the relevant safety and risk assessments.

These new developments in biotechnology applied to animals include SynBio applications. Therefore, this mandate encompasses the Request for an EFSA opinion on genetically modified organisms developed through SynBio and their implications for risk assessment methodologies,[5] received on 25 June 2018 under Article 29 of Regulation (EC) No 178/2002.

1.1.2 | Terms of Reference as provided by the requestor

The European Commission requested EFSA to produce an opinion, in accordance with Article 29 of Regulation (EC) No 178/2002, on new developments in biotechnology applied to animals. For the purpose of this opinion, EFSA was requested to deliver the following two-step work on new developments in biotechnology, including SynBio and NGTs applied to animals and their implications for risk assessment methodologies. The scope of the present mandate is limited to agri-food uses.[6]

First deliverable: Knowledge gathering on known cases of animals (and their food and feed products) obtained by new developments in biotechnology (ToR 1).

For the purpose of this task, the JRC report mapping "Current and future market applications of NGTs"[7] was indicated to EFSA as a basis to complement and update. The expected outcomes of this activity were:

- identify animals and their products obtained by new development in biotechnology described since 2001 including their traits and uses.
- list the techniques and modifications used, including explanation of relevant terminology.
- identify animals and their products developed since 2001 that are subject to authorisation procedures by international authorities, and the corresponding available risk assessments (e.g. opinions, guidances, authorisation) that exist.
- collect per case the data and information relevant for risk assessment, and structure it according to the EFSA guidances.

Second deliverable: EFSA opinion on the potential novel hazards and risks from new developments in biotechnology applied to current and near-market animals and adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety and welfare, and environmental impact (ToRs 2, 3 and 4).

Based on the data collection report, EFSA was requested to produce an opinion on new developments in biotechnology applied to animals. The expected outcome of this activity is the current opinion which:

- ToR 2: identifies, where possible, novel potential hazards and risks which new developments in biotechnology as applied to current or near-market animals could pose for humans, animals and the environment compared to conventional breeding or established techniques of genetic modification.[8]
- ToR 3: determines whether the existing guidelines for risk assessment of genetically modified animals are applicable, fully or partially, adequate and sufficient to risk assess new developments in biotechnology applied to animals.
- ToR 4: identifies on which specific areas and aspects the existing guidelines should be updated, adapted or complemented in the case that the existing guidelines for risk assessment are considered not applicable, partially applicable, not adequate or not sufficient.

1.2 | Interpretation of the Terms of Reference

An interpretation of the Terms of References (ToRs) was necessary to complete the mandate, and a detailed explanation is provided below, with reference to specific sections.

a. 'New developments in biotechnology, including SynBio and NGTs, applied to current or near-market animals for agri/food/feed products falling within the remit of EFSA'.

   a.1 'New developments in biotechnology, including SynBio and NGTs' – The new biotechnologies applied to animal breeding represent a broader field than the use of genomic techniques alone (e.g. artificial insemination, cell cultures, embryo transfer, in vitro fertilisation, somatic cell nuclear transfer); however, given the European Commission's interest in identifying possible new genetically modified animals that could enter the EU market in the near future, and the lack of animal developments obtained through SynBio and the growing interest in the NGTs applied to animals, the attention of the

[5] Ares(2018)3356496 of 25/06/2018.

[6] For the purpose of this mandate agri-food uses means agri/food/feed products falling within the remit of EFSA.

[7] https://publications.jrc.ec.europa.eu/repository/handle/JRC123830.

[8] For the purpose of this mandate the terms "established techniques of genetic modification" refers to various genetic engineering techniques which have been significantly used over the last 30 years to produce genetically modified organisms, such as those which have been authorised under Directive 2001/18/EC and Regulation (EU) No 1829/2003.

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working group was mainly focused on these latest technologies developed after the publication of Directive 2001/18/EC.[9] It is worth noting that there is currently no indication of SynBio approaches being applied to animals for food, feed and other agricultural uses at commercial or pre-commercial stages, nor are such developments expected in the near future, making it difficult to predict whether animals developed through SynBio, or their products, will reach the EU market in the coming years. This is supported by knowledge gathering reports (Parisi & Rodríguez-Cerezo, 2021; Van Eenennaam, 2023), which did not identify any examples of animals developed through SynBio at commercial, pre-commercial, or research and development stages, and verified by expert insights from the working group. The SynBio and NGTs taken into consideration in the context of this mandate are further defined in Section 1.3.1.

a.2 'Current or near-market' – For the purpose of this mandate, this stage is interpreted as the potential to reach the EU market based on the current stage of an NGT product application, as proposed in Van Eenennaam (2023) (also reflected in Section 2.3 when selecting case studies):

- Commercial stage: NGTs applied to animals farmed for food, feed and other agricultural uses, currently marketed in at least one country worldwide.
- Pre-commercial stage: NGTs applied to animals being developed for food, feed and other agricultural uses, for which an application has been made for regulatory assessment (in at least one country) but for which a product is not yet on the market.
- Research and Development stage: NGTs applied to animals being investigated for food, feed and other agricultural uses, at proof-of-concept stage (e.g. testing gene targets for the enhancement of traits of commercial interest).

a.3 'Animals for agri/food/feed products falling within the remit of EFSA' – For the purpose of this mandate, farmed animals were considered, i.e. those mammals, birds, fish and invertebrate species bred for food, feed and other agricultural uses falling within the remit of EFSA, as identified in Parisi and Rodríguez-Cerezo (2021) and Van Eenennaam (2023). Pollination and natural pest and disease control, facilitated by insects and animals, are just two examples of ecosystem services that could be considered part of 'agricultural uses'. Therefore, NGT applications aimed at these purposes would fall within the mandate. Animals bred only for medical purposes and gene drive applications were not considered to fall within the remit of this mandate. Companion animals, which by definition are not intended for food, feed and other agricultural uses, fall outside the scope of this mandate. However, it is well known that some species serve dual purposes, such as horses or rabbits, which can be kept either as a pet or for food, feed and other agricultural uses.

b. 'Identify animals and their products developed since 2001 that are subject to authorisation procedures by international authorities, and the corresponding available risk assessments (e.g. opinions, guidances, authorisations) that exist'.

b.1 'International authorities' – A clarification was needed on the wording international authorities. The knowledge gathering report on known cases of animals obtained by NGTs (Van Eenennaam, 2023) identified animals and their products developed since 2001 that are subject to authorisation procedures by 'non-EU authorities, both national (e.g. FDA) and international (e.g. FSANZ)', and the corresponding available risk assessments (e.g. opinions, guidances, authorisations) that exist'.

c. 'Identifies, where possible, novel potential hazards and risks which new developments in biotechnology applied to current or near-market animals could pose for humans, animals and the environment compared to conventional breeding or established techniques of genetic modification'.

c.1 'Conventional breeding' – With the term conventional breeding, the WG refers to techniques applied to animals aimed at selecting preferred phenotypes, as further defined in Section 1.3.1.

c.2 'Established genomic techniques (EGTs)' – With the term EGTs, the WG refers to the molecular techniques developed prior to the publication of Directive 2001/18/EC, as further defined in Section 1.3.1.

d. 'Determines whether the existing guidelines for risk assessment of genetically modified animals are applicable, fully or partially, adequate and sufficient to risk assess new developments in biotechnology applied to animals'.

d.1 'Applicable, fully or partially, adequate and sufficient' – Further clarifications were needed on footnote 11 of the mandate stating: 'In the context of this mandate, applicable means that can be used for the purpose, fully applicable means that can be used in full, partially applicable means that can be used only in part and sufficient means that does not need to be complemented'. These terms were interpreted as:

- Fully applicable: all the principles and recommendations included in the selected EFSA Guidance documents provide a basis for conducting the risk assessment of NGT animals for food, feed and other agricultural uses.

[9]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

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- Partially applicable: only some of the principles and recommendations included in the selected EFSA Guidance documents provide a basis for conducting the risk assessment of NGT animals for food, feed and other agricultural uses, while others are not applicable.
- Sufficient: there is no need for further additions to, or modifications of, the existing text/guidance of the selected EFSA Guidance to conduct the risk assessment of NGT animals for food, feed and other agricultural uses.
- Adequate: this term is considered a synonym of 'sufficient' by the EFSA GMO Panel.

e. 'EFSA opinion on potential novel hazards or risks from new developments in biotechnology applied to current and near-market animals and on the adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety and welfare, and environmental impact'.

e.1 As already defined by the guidance on risk assessment for animal welfare (EFSA AHAW Panel, 2012a), the term animal welfare includes animal health. This is also underlined by the methodological guidance for the development of animal welfare mandates in the context of the Farm to Fork strategy (EFSA AHAW Panel, 2022a). 'The welfare of an individual is defined as its state as regards its attempts to cope with its environment' (EFSA AHAW Panel, 2012a). In this understanding, the welfare of an animal is good if, as indicated by scientific evidence, the animal is healthy, comfortable, well-nourished, safe, able to express key aspects of behaviour and is not experiencing unpleasant states such as pain, fear and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter or killing. Animal welfare refers to the state of the animal, whereas the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, management and humane treatment. Within the remit of this mandate, consideration on the welfare assessment has been contextualised on NGT animals potentially ready for the market, but pre-commercial stages need to be considered when they provide important information for the risk assessment. Ethics and socio-economic aspects related to animal welfare associated with gene editing technology are out of the scope of EFSA and therefore are not considered.

f. Cell culture-derived products

NGTs applied to the production of cell culture-derived products are out of the scope of this mandate.

g. Cloning

Cloning is an advanced breeding technique that can be used in conventional breeding as well as in the production of NGT and EGT animals. At the time of writing this opinion, no EU legislation specifically concerning cloned animals and/or food and feed derived from them has been enacted. No applications for such authorisation have been submitted and no cloned animal products are authorised for sale in the EU. Therefore, this technique is not included when referring to conventional breeding.

1.3 | Additional information to set the scene for the execution of the EC Mandate

1.3.1 | Definitions and concepts applicable to the scope of the mandate

Given the lack of EFSA guidance on the application of genomic techniques in animals for food, feed and other agricultural uses, the working group believes it is helpful to define relevant concepts to aid the reader's understanding of this document. The definitions reported below are aligned with those given in previous EFSA output (EFSA GMO Panel, 2020a, 2021, 2022a, 2022b, 2022c, 2024; EFSA Scientific Committee, 2020, 2022), and adapted to be fit for the purpose of genetic modifications in animals, or alternatively, they arise from the scientific literature on the topic.

Chimerism and mosaicism

Chimeric and mosaic animals are organisms that have two or more cell types with different chromosomal constitutions. Chimerism involves cells from genetically distinct zygotes, whereas mosaicism involves genetically distinct cells, but from the same zygote. A common form of chimerism occurs when two fertilised eggs (zygotes) fuse early in development, resulting in a single organism that has two sets of cells with different genetic material. In mosaicism, these cells arise from a single fertilised egg (zygote), due to mutations or errors during cell division early in development.

Conventional (or classical) breeding

Crossing animals, by natural mating or through assisted reproduction techniques (e.g. artificial insemination, superovulation of females, in vitro embryo culture and embryo transfer/surrogacy), with desired characteristics to naturally recombine genomes, and selection of offspring with improved combinations of traits inherited from both parents, potentially resulting in animals with new phenotypic characteristics. To refine this process, genomic selection may be applied, whereby single-nucleotide

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polymorphisms in linkage disequilibrium with quantitative trait loci are used to assign breeding values to individuals based on the predicted frequencies of both desirable and undesirable traits. In some species genetic modification techniques may be used, such as the application of physical or chemical mutagens or polyploidy induction, which are excluded from the requirements of Directive 2001/18/EC (see Annex Ia, Part 2 and Annex IB for details) and may similarly produce animals with new phenotypic characteristics. For the purpose of this mandate, the term 'conventional breeding' is used.

Established genomic techniques (EGTs)

Techniques developed prior to the publication of Directive 2001/18/EC, used to intentionally alter the genetic material of an animal in a way that cannot occur naturally by mating (e.g. adding a new exogenous gene to the genome). The use of EGTs may end up in the production of animals with new phenotypic characteristics. EGTs consist in random integration (via retroviruses, transposons or microinjection of a zygote/laid egg with DNA) of DNA cassette(s) encoding a transgene, intragene or cisgene of interest.

Genetic alteration

Any modification made to specific DNA sequences within an animal's genome, to introduce, delete or modify specific traits. This can include (i) insertion of an additional genetic sequence or (ii) alteration of an endogenous genetic sequence.

Classification of genetic alterations produced by site-directed nucleases (SDNs)

Site-directed nucleases are enzymes that recognise a specific sequence and cleave the DNA, usually creating a double-strand break (DSB). Three types of SDN approaches have been defined:

- SDN-1 (targeted mutagenesis): no sequence template provided, the repair of the DSB occurs randomly, introducing random mutations (substitutions, insertions, deletions or inversions) at the target site(s).
- SDN-2 (targeted mutagenesis): a template is provided for homology-directed repair, resulting in small, precise alterations at the SDN target site(s).
- SDN-3 (targeted insertion of a cisgenic, intragenic or transgenic sequence): a template is provided for insertion of large sequences at the SDN target site(s).

New approaches have been developed that do not produce DSBs. In this scientific opinion, the GMO Panel considers that the genetic alteration obtained using base editing and changes induced by prime editing are comparable to those created by SDN(s) technology.

Various definitions of SDN-2 and SDN-3 are used by different sources. The definitions provided above are consistent with those provided in previous EFSA documents on NGTs in plants as well as with the JRC report (Broothaerts et al., 2021), with further adaptation to take into account the emerging nature of the field.

Types of genetic alteration of a recipient animal:

- Genome editing: genetic modification that induces mutation(s), through NGTs, in one or more selected target locations of the genome. Mutations can be substitutions, insertions, deletions or inversions.
- Cisgenesis: is a genetic modification involving the transfer of an exact copy of a DNA sequence already present in the species or in a sexually-compatible species to the host, using various delivery strategies, e.g. EGT or SDN-3. The transferred genetic material can replace an endogenous DNA sequence of the host, in the same genomic context, resulting in a modification that could be obtained by either NGTs or conventional breeding. On the other hand, the transferred genetic material can be introduced in a different genomic context of the host, resulting in a modification that could be obtained by NGTs and EGTs, but that would be unlikely to arise by conventional breeding.
- Intragenesis: a genetic modification consisting of the insertion of two or more endogenous DNA sequences (e.g. promoter, intron, terminator, coding regions), each of which can be found in a member of the same species, or a sexually-compatible species, but in a combination that does not exist in nature. A new combination of promoters, regulatory elements and coding sequence can thus be created. The location of the insertion may be random through EGTs, or targeted, through NGTs.
- Transgenesis: a genetic modification consisting of the insertion of one or more DNA elements (e.g. promoter, intron, terminator, coding regions) that cannot be found in the same species, or a sexually-compatible species. DNA elements may be from another species, or they may be synthetic. A new combination of promoters, regulatory elements and coding sequence can thus be created. The location of the insertion may be random through EGTs, or targeted, through NGTs.

Integration of genetic elements

- Random integration: introduces a DNA element [gene or one or more element(s) of a gene (e.g. promoter, intron, terminator, coding regions)] into an unspecified location of the host genome through EGTs or as an unintended consequence of NGTs.

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- Targeted integration: introduces a DNA element [gene or one or more element(s) of a gene (e.g. promoter, intron, terminator, coding regions)] into a target location of the host genome through NGTs.
- Safe harbour: A safe harbour is a neutral genomic site into which new genetic material can be integrated and that ensures that the inserted elements function in a predictable manner. At this time there is no known example of the use of a safe harbour in animals intended as food or feed. However, examples of targeted DNA integration into such sites (e.g. the *Rosa26* locus, extensively for targeted transgene expression) have been reported primarily in mice, showing that targeted integration is technically possible.
- Multiple copy number: number of copies of a particular gene or segments of DNA that are present in the genome of an animal, due to processes like gene duplication (more than one copy of a gene in one animal), or copy number variation (different numbers of copies of a particular gene or DNA segment across animals of the same species). In animals bred for food, feed and other agricultural uses there are currently no examples of NGT applications in which the copy number of endogenous genes has been increased. However, it is possible that integration of additional copies of the same gene (same promoter, gene, terminator) could be of interest.

New Genomic Techniques (NGTs)

Techniques developed after the publication of Directive 2001/18/EC, used to intentionally alter the genetic material of an animal in a targeted way. They include (i) targeted mutagenesis (e.g. SDN-1 or SDN-2 to induce nucleotide modification, insertion, deletion or inversion) and (ii) targeted insertion of a DNA cassette encoding a transgene, intragene or cisgene of interest. (e.g.: SDN-3).

Sexual compatibility across animal species

- Sexually-compatible animal species: sexually-compatible species are those which are able to mate, either naturally or through assisted reproduction, such as embryo transfer or in vitro fertilisation, producing fertile offspring.
- Sexually incompatible animal species: sexually incompatible species are those which are able to mate, either naturally or through assisted reproduction, such as embryo transfer or in vitro fertilisation, often producing non-fertile offspring. Sexual incompatibility between closely related animal species is usually due to differing chromosome organisation and/or number. Where breeding between two species produces viable offspring, these are often infertile. However, there are exceptions among animals used in agriculture and aquaculture. The following non-exhaustive list are examples of such crosses:

  - Beefalo – a cross between male cattle (*Bos taurus*) and female American bison (*Bison bison*). Female beefalo resulting from such a breeding are usually fertile, while males are usually not fertile.
  - Dzomo and Dzo – a cross between cattle (*Bos taurus*) and yak (*Bos grunniens*), the offspring demonstrate hybrid vigour. Dzomo (females) are fertile, but Dzo (males) are sterile. Dzomo can be further crossed with either yak or cattle.
  - Mule – a cross between a female horse (*Equus caballus*) and a male donkey (*Equus asinus*). As the horse has 64 chromosomes and the donkey has 62, mules are usually (but not always) infertile.
  - Some commonly used farmed fish families including, salmonid (*Salmonidae*), tilapia (*Oreochromis*) and carp (*Cyprinidae*) species can hybridise and produce fertile offspring (e.g. the splake, which is a hybrid between Brook trout (*Salvelinus namaycush*) × Lake trout (*Salvelinus fontinalis*).
  - Domestic chickens (*Gallus* domesticus) can be crossed, and produce fertile offspring, with silver pheasants (*Lophura nycthemera*), red junglefowl (*Gallus gallus*) and green junglefowl (*Gallus varius*). They have also produced hybrids with peafowl (*Pavo cristatus*), chachalacas *Ortalis vetula, Ortalis ruficauda*), capercaillie (*Tetrao urogallus*), grouse (*Tetrao tetrix, Lagopus lagopus, Canachites canadensis*), quail (*Coturnix coturnix*), curassows (*Crax rubra*), pheasants (*Phasianus colchicus, Chrysolophus amherstiae*) and guans (*Penelope purpurascens*).
  - Mulard duck – a cross between a Muscovy duck (*Cairina moschata domestica*) and a domestic duck (*Anas platyrhynchos domesticus*). The progeny are sterile.

Synthetic biology

SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms (EFSA Scientific Committee, 2020). It is possible to create novel genetic information, biological networks (including complete genomes) and/or novel biological functions, either completely de novo or by combining and/or modifying existing naturally occurring DNA modules.

Unintended genetic changes

Application of NGTs may induce unintended genetic changes. These can include unintended changes at the target site (unintended on-target mutations), or changes to the genome at a site other than the intended target site (off-target mutations).

- Unintended on-target mutations: an unintended/unexpected mutation (e.g. insertions, deletions, substitutions or inversions) that occurs proximal to the target sequence for which the NGT is designed.

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– Off-target mutations: an unintended mutation that can occur when the target sequence shares sequence similarity with other regions in the genome. These mutations happen at sites distant from the intended target. Sometimes, off-target activity can result in chromosomal changes due to large insertions or deletions. These chromosomal changes also occur spontaneously, for example during meiosis of germ cells, often as a result of poor gamete quality; whether they arise spontaneously or are introduced by NGTs, such deleterious aberrations rarely survive to the next generation.

1.3.2 | Genome editing in livestock breeding programmes

Genetic changes occur in individuals as a consequence of natural processes, giving rise to variation in farmed animals. Conventional breeding relies on this genetic variation in a population of bred animals, allowing selection for desirable traits.

The rate of genetic gain depends on the four components of the breeders' equation (Box 1) – intensity, accuracy, genetic diversity and generation length – applied in a systematic approach based on objective phenotyping, genomic information, statistical methodologies and advanced reproductive techniques (Bishop & Van Eenennaam, 2020).

BOX 1 Breeders' equation

$$\Delta G = \frac{i \times r \times \sigma_A}{L}$$

Intensity (i):	The extent to which individual animals in the breeding nucleus at the top of the pyramid breeding structure can be used as parents of the next generation.
Accuracy (r):	How certain we are of the true genetic merit of selection candidates.
Genetic diversity (σA):	The additive genetic standard deviation of the population.
Generation length (L):	The interval calculated as the average age of parents when progeny are born.

Considerable productivity gains have been made using conventional breeding to date, due to the development of new knowledge and technologies in this field,[10] but the fact remains that genomic selection is mainly limited to the variation present within a given animal population or, in some cases, to variation introduced from closely related species. Indeed, although spontaneous mutation rates represent a theoretically infinite resource of genetic variation within a given population, it is, nevertheless, important to assume that the appearance of a desired mutation is based on chance and will occur without a defined time scale (Whitelaw, 2019).

Genome editing is the most important NGT developed since 2001. This technology facilitates the introduction of defined genetic variants into a population under selection. These may be variants that are difficult to access using conventional breeding schemes (e.g. from a different breed or a related species, or a genetic variant which has not been observed previously).

The use of genome editing has several potential applications in conventional animal breeding programs, such as: (i) functional genomics to elucidate gene function and identify causal variants underlying monogenic traits; (ii) introduction of targeted genetic variations considered useful in structured livestock breeding programs (e.g. repair of genetic defects, inactivation of unwanted genes, movement of alleles and haplotypes between breeds in the absence of linkage drag); (iii) acceleration of the rate of genetic change by wider dissemination of the germ cell lineage of genetically changed lines through commercial breeding.

1.3.2.1 | *NGTs in farmed mammals*

In farmed mammals, the goal of genetic modification by means of NGTs is to genetically alter single cells which will subsequently contribute to the germline. There are currently three alternative routes by which this may be achieved, but more may be developed as the technology matures. Reagents used are typically mRNA (ZFN, TALEN or CRISPR) or protein + guide RNA (CRISPR) +/– a DNA template for repair.

– Direct manipulation of the single-cell zygote. Access to this cell may be achieved by collection of oocytes followed by in vitro fertilisation or by mating (natural mating or artificial insemination) and zygote recovery from the oviduct. Once collected, genome editors are typically delivered by either microinjection or electroporation. The mode of delivery may change as the technology matures. Manipulated zygotes are either transferred directly to a synchronised surrogate (in

[10]E.g. Artificial insemination, embryo transfer, in vitro breeding, in vitro gametogenesis and fertilisation, cell culture development (Spermatagonial stem cells (SSCs), Embryonic stem cells (ESCs), Primordial germ cell-like cells (PGCLCs), Mesenchymal stromal cells (MSCs), induced pluripotent stem cells (iPSC)) (Bishop & Eenennaam, 2020); sophisticated numerical tools relying on complex probability-based algorithms to select the desired outcome within a given population; whole genome sequence information which, combined with imputation based on haplotype data and analysis of large numbers of animals (hundreds of thousands to millions), enables low-coverage (e.g. 0.1× genome) sequence data to be informative (Whitelaw, 2019).

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pigs 30–40 zygotes are typically transferred into a single recipient), or they are cultured in vitro for several days until the blastocyst stage, at which point they are transferred to a synchronised surrogate (in sheep two to four blastocysts are typically transferred, while in cattle this is typically one to two). At parturition, a small tissue sample is collected and genotype assessed. While the goal of direct zygote manipulation is to alter the genome of the single cell (and hence the whole organism derived from it), it is common to observe editing events which have occurred later in development. As such, it is common for founder animals (F0) to be mosaic, i.e. they may contain cells which contain different editing events. This is resolved by breeding to the F1 generation, although generation intervals for some species make this approach problematic.

- Manipulation of a primary somatic cell, clonal expansion and characterisation of manipulated cells in culture, then somatic cell nuclear transfer into an enucleated oocyte to reconstitute a zygote. Manipulation is typically by transfection (e.g. lipofection or electroporation). As with approach (1), reconstituted zygotes may be transferred directly to recipients (in pigs this involves typically 100–300 putative zygotes) or cultured in vitro to the blastocyst stage, at which point they are transferred to a synchronous recipient. Due to issues associated with incomplete reprogramming there is typically a lower pregnancy rate than with approach (1), with subsequent losses throughout pregnancy duration and occasional neonatal health problems. Founder offspring may also have unexpected phenotypic characteristics. The benefit of this approach, however, is that the F0 offspring have the same genetics as the manipulated somatic cell from which they were derived, and do not display mosaicism.
- Manipulation of a spermatagonial stem cell, with subsequent engraftment into the testis of a sterile surrogate. As with approach (2), this benefits from the ability to clonally expand and subsequently characterise the outcome of the manipulation prior to engraftment. This is a relatively new approach and will require further optimisation of culture conditions before it becomes widely used. It is also currently limited to the male germline.

Examples of gene edited mammals at commercial stage are (refer also to Van Eenennaam, 2023):

- Pigs (*Sus scrofa*) with removal of an exon of the *CD163* gene, making them resistant to porcine reproductive and respiratory syndrome virus (Burkard et al., 2017)
- Cattle (*Bos taurus*) with introduction of a premature stop codon in the prolactin receptor gene, making them better able to cope with hot/humid conditions (Rodriguez-Villamil et al., 2021)
- Cattle (*Bos taurus*) with duplication of a short region of non-coding sequence, introducing the well characterised Celtic polled (hornless) allele (Carlson et al., 2016)
- Cattle (*Bos indicus*) with a knockout of the *MSTN* gene, increasing muscle mass (Proudfoot et al., 2015)

1.3.2.2 | *NGTs in farmed birds*

New genomic techniques applied to birds mainly concern certain species such as chickens, ducks and quail (Van Eenennaam, 2023).

In farmed birds, the goal with NGTs is to genetically modify the germ cells that will form the sperm and eggs of the adult animal. The offspring then inherit the genetic edit as a heterozygous allele. Breeding will produce offspring with the genetic edit on both chromosomal alleles. The genetic modification is almost always performed at embryonic stages in a laid egg, as an avian egg can be windowed (a hole is cut in the shell), sealed and then hatched and raised to sexual maturity. The hatching rate of the chicks is reduced after windowing.

The reagents used are typically plasmids, adenoviral vectors, lentiviral vectors or mRNAs encoding ZFNs, TALENs or CRISPR/Cas genome editors +/– a DNA template for repair. The vector system used typically depends on the stage and cells being targeted.

- Gene editing of in vitro cultured chicken embryonic germ cells.

  The embryonic germ cells are removed from a fertilised egg and cultured in vitro for a period of several weeks to several months. During this time, the genome editors are introduced into the germ cells. DNA or RNA vectors, usually expressing CRISPR/Cas9 modules, are most commonly used.
  The edited germ cells can be analysed and grown clonally. The edited germ cells are injected into a surrogate host egg which is hatched and raised to sexual maturity. The surrogate host chicken is mated naturally or using artificial insemination and the genome edit is inherited by some of the offspring. The surrogate host chicken may be genetically or chemically modified to be rendered sterile, e.g. by way of an introduced transgene.

- Gene editing of in vitro cultured embryonic germ cells, with subsequent engraftment into the testis of a sterile surrogate.

  This approach benefits from the ability to clonally expand and subsequently characterise the outcome of the genome edit prior to engraftment. In this case, the edited germ cells are injected into the testes of an adult sterile surrogate host cockerel. Surrogate cockerels may have been treated by irradiation with chemotherapeutic agents to kill the native population of dividing cells in the gonads prior to engraftment. Some of the offspring will inherit the genetic change.

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- Direct modification of embryonic germ cells in the chicken embryo.

  DNA-based genome editing tools are injected directly into the vascular system of the embryo within the fertilised egg. The injected egg is hatched and raised to sexual maturity. The chicken is mated using standard husbandry practices and the genome edit is inherited by some of the offspring. Some of the offspring, usually ~1% of the chicks, will inherit a genetic change (usually an insertion or deletion of a small region of genetic information – indel) in the target genetic locus of a germ cell. Few somatic cells of the injected chicken will be modified.

- Direct injection of adenovirus into a laid egg.

  Adenovirus-containing expression cassettes for the genome editing tools are injected into the laid chicken egg. A percentage of the somatic cells and germ cells of the injected embryo will contain genetic changes in the target genetic locus. DNA vectors can also be used to transfect the somatic and germ cells at embryonic stages. The injected egg is hatched, raised to sexual maturity and bred. Some of the offspring, usually ~1% of the chicks, will inherit an indel in the target genetic locus of a germ cell. The somatic cells of this chicken will also be modified with different numbers of indels.

Examples of gene edited poultry at pre-commercial stage are (refer also to Van Eenennaam, 2023):

- Chickens (*Gallus gallus*) with a single amino acid change in the NEPH transporter gene, making them resistant to infection by the avian leukosis-J virus (Koslova et al., 2020)
- Quail (*Coturnix japonica*) with a small deletion introduced into the myostatin gene, resulting in a 10% increase in skeletal musculature (Lee et al., 2020)
- Chicken (*Gallus gallus*) with resistance to influenza obtained by a CAS9/guide plasmid and a short oligonucleotide transfected into culture chicken primordial germ cells (Idoko-Akoh et al., 2023)

1.3.2.3 | *NGTs in farmed fish*

Fish have a different reproductive strategy to birds and mammals. Distinctively, in most fish species, both fertilisation and embryonic development occur outside the mother's body (oviparous). Furthermore, fish typically possess a high reproductive capacity, which allows them to significantly increase population sizes from one generation to the next. Fish also have genome characteristics that differ from birds and mammals, since whole genome duplication(s) have occurred in the evolutionary history of fish. Due to this, fish species may have up to several paralogs (i.e. gene copies within the genome) of each gene while other genes come in one copy due to rediploidisation. This is relevant when editing in fish species, since other gene copies than the targeted one need to be considered in the evaluation of gene function. Duplicated genes may diverge to perform different functions, exhibit additive function where both copies contribute to a single function, or experience loss of function where one of the duplicated genes becomes non-functional.

The reagents used are typically mRNAs encoding CRISPR/Cas genome editors, ZFNs, TALENs. Cas proteins may also be injected instead or the corresponding mRNA. For some applications a DNA template can be used to guide the change towards a desired change.

- Gene editing in fish embryos

In most fish species, the eggs are fertilised and develop outside the mother, simplifying the gene editing process significantly compared to mammals. Manipulation involves direct injection of reagents, such as CRISPR-Cas9 components (gRNA and mRNA encoding the Cas9 protein or Cas9 protein), into the cytoplasm or nucleus of the one to four cell stage embryo. This typically results in the creation of a mosaic first-generation gene-edited fish (F0), with various mutations at the target site, including potential off-target mutations and chromosomal deletions. Since fish may have several similar gene copies within their genome, multiple genes with potentially similar functions often need to be targeted and evaluated in fish. F0 broodstock can be crossed with wild type (wt) fish or another F0, to produce a second generation (F1). In the F1 generation, individuals will carry only two alleles, one from the mother and one from the father.

- Gene editing of in vitro cultured embryonic germ cells, with subsequent injection into sterile fish embryos (surrogates) or into sterile gonads of adult fish

Surrogacy in farmed fish is a technique where germ cells are transplanted into a sterile host (e.g. into gonads, peritoneal cavity, bloodstream, intramuscular), from another fish (within species or between species), including the edited germ cells. Within the host, these germ cells will mature and ultimately produce either sperm or oocytes. This method is used in farmed fish species for various purposes, including the production of mono-sex populations for research or commercial use. The applications of surrogacy in farmed fish are numerous: surrogacy allows the application of editing to introduce traits considered desirable into embryos, ensuring the passage of these traits to the next generation, thus helping maintain specific genetic lines. It can also help bypass mosaicism, resulting in offspring with more consistent genetic modifications. Surrogacy expedites selective breeding programs by allowing embryos with high genetic merit (including those altered by

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NGTs) to be carried by surrogate fish with superior reproductive capabilities or survival rates. Furthermore, this technique can be used for conservation purposes, enabling the storage, preservation and propagation of valuable genetic material, upon demand in a sterile host. Additionally, surrogacy technology often leads to distinct genetic differences between the host and implanted germ cells, which can facilitate large-scale production of sterile offspring. This approach has proven effective in the model species medaka.

Examples of gene edited fish at (pre)-commercial stage are (refer also to Van Eenennaam, 2023):

- Tilapia *(Oreochromis niloticus)* with a knockout of the *mstn* gene, generating fish with double the muscle mass, thicker fillets and faster growth (Wu et al., 2023)
- Red Sea Bream *(Pagrus major)* with knockout of the *mstn* gene, generating fish with double the muscle mass, thicker fillets and faster growth (Kishimoto et al., 2018)
- Pufferfish *(Takifugu rubipes)* with knockout of four leptin receptor genes, generating fish with an increased appetite that results in faster growth (Kishimoto et al., 2019)
- Olive flounder *(Paralichthys olivaceus)* with disruption in *mstn* gene, generating fish with an increased appetite that results in faster growth (Kim et al., 2024)

1.3.2.4 | *NGTs in invertebrates*

Gene editing in invertebrates for food, feed and other agricultural uses (excluding gene drive), has limited application to date and no cases at the pre-commercial stage have been identified so far (e.g. Van Eenennaam, 2023 and literature referred below). However, this approach may become a significant field in the future.

Insects

Existing methods for insect gene editing typically involve the microinjection of RNAs or RNA and protein into early embryos. However, this approach significantly limits the potential for gene editing in many insect species, particularly those with reproductive systems that prevent access to early embryos for injection. Recently, it was shown that injecting CRISPR/Cas9 ribonucleoproteins (RNPs) into the haemocoel of adult females of the German cockroach *Blattella germanica* effectively introduces heritable mutations in developing oocytes (Shirai et al., 2022). This technique, known as 'direct parental' CRISPR (DIPA-CRISPR) can utilise readily available standard Cas9 protein, and so this method may prove highly practical and achievable for many species (Van Eenennaam, 2023). According to the scientific literature, research on gene editing on different insect orders is moving forward, including the modification of species of agricultural importance (*Apis mellifera, Bombyx mori, Plutella xyllostella, Tribolium castaneum*) and species that can be used for food and feed (*Gryllus bimaculatus, Hermetia illucens*) (reviewed in Sun et al., 2017; Nie et al., 2021; Kou et al., 2024; Sui et al., 2024).

Other invertebrates

There are research groups working on gene editing in shrimp (Gui et al., 2016; Song et al., 2024; Sun et al., 2017; Zhang et al., 2018) and oysters (Yu et al., 2019). Gene editing in these animal groups is still at the early research stages, and no applications at the pre-commercial stage were identified in these invertebrate groups.

1.3.3 | EFSA risk assessment of animal health and welfare

The risks for animal health and welfare have been considered in EFSA's scientific opinions since 2004, providing a science-based, transparent and reproducible framework to address specific welfare concerns within a limited timeframe and with available scientific data. As explained in Section 1.2, the term animal welfare includes animal health. Recently, a revision of the methodologies for animal health and welfare risk assessment was accomplished by the AHAW Panel of EFSA in response to mandates received from the European Commission (EFSA AHAW Panel, 2022a). From 2020 the EC has started a comprehensive evaluation of the animal welfare legislation, and mandated EFSA to develop scientific opinions on welfare aspects of various farm animal species and categories. Under this framework, EFSA has delivered scientific opinions on the welfare of animals during transport (EFSA AHAW Panel, 2022c, 2022d, 2022e, 2022f), and on farm (EFSA AHAW Panel, 2022b, 2023a, 2023b, 2023c, 2023d).

One of the aims of EU legislation on animal protection has been to ensure that animals are not exposed to avoidable negative affective states such as pain and distress, therefore focusing on preventing the negative welfare consequences. In addition, the EFSA guidance on animal health and welfare of GM animals (EFSA GMO and AHAW Panel, 2012), is predominantly focused on ensuring that the animals are able to function, which today does not cover the full range of animal welfare (e.g. Fraser, 2009; Mellor & Beausoleil, 2015). Since the publication of that guidance, knowledge about animal welfare and the ability of animals to experience negative as well as positive affective states has grown considerably, leading to more recent animal welfare assessment tools focusing on a combination of animal needs and motivations, affective states, cognitive states and ability to function in a given environment (e.g. Mellor, 2017). This knowledge allows a more comprehensive assessment of animal welfare, and is reflected in the more recent EFSA AHAW guidance document on animal welfare mentioned above,

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published in the context of the Farm to Fork Strategy (EFSA AHAW Panel, 2022a), where focus is directed at a list of potential welfare consequences and their associated hazards and animal-based-measures (ABMs).

The new methodology for risk assessment in animal welfare included the identification of relevant welfare consequences (i.e. the result of factors that can worsen the welfare of an animal) as included in the latter guidance (EFSA AHAW Panel, 2022a), consisting of a list of 33 potential welfare consequences along with their descriptions. Each welfare consequence refers to one or more negative affective states (e.g. pain, fear, fatigue). These high-level states are the direct results of the occurrence of one or more welfare consequences and can lead to animal suffering.

Besides welfare consequences, animal welfare risk assessment also involves the identification of animal-based measures (ABMs), defined as the quantifiable response of an animal or an effect on an animal that can be used as indicators of welfare consequences (EFSA AHAW Panel, 2012a, 2012b; EFSA AHAW Panel, 2022a). These measures can be taken directly or indirectly from the animal and include the use of animal records along the production chain from early experiments to commercial release. For the purpose of this opinion, the focus is on the post-commercial phases. However, pre-commercial stages are to be taken into account when informative of risk assessment on animal health and welfare (see Section 1.3.4). An ABM may be used, for example, to assess impaired functioning due to injury, disease or malnutrition, provide information on animal needs and affective states like hunger, pain and fear by measuring preferences, motivations and aversions, or assess physiological, behavioural and immunological responses to a certain factor (EFSA AHAW Panel, 2012a, 2012b). According to the same guidance the identification of endpoints is used to quantify the impact of a factor (or hazard) in terms of its welfare consequence. Examples of the link between welfare consequences and ABMs are reported in Table 1, while the full list of welfare consequences included in EFSA AHAW Panel guidance (2022a) is showed in Appendix A.

TABLE 1 Example of welfare consequences, with related animal-based measures in broilers (chickens produced for meat) (EFSA AHAW Panel, 2023b).

Welfare consequence	Definition	Animal-based measure
Bone lesions	The animal experiences negative affective states such as pain, discomfort and/or distress due to fractures or dislocations of the bones (excluding those fractures leading to locomotor disorders).	Keel bone fractures
Cold stress	The animal experiences stress and/or negative affective states such as discomfort and/or distress when exposed to low effective temperature	Huddling Cloacal temperature Surface temperature
Locomotory disorders	The animal experiences negative affective states such as pain, discomfort and/or due to impaired locomotion induced by, e.g. bone, joint, skin or muscle damage	Flock activity Leg deformation
Prolonged hunger	The animal experiences craving or urgent need for food or a specific nutrient, accompanied by a negative affective state and eventually leading to a weakened condition, as metabolic requirements are not met.	Impaired growth rate Polydipsia

Note: Experiencing one or more of these welfare consequences results in negative affective states in the animals, such as pain, fear, fatigue and distress (EFSA AHAW Panel, 2022a).

Once ABMs indicative of the relevant welfare consequences have been identified, the assessment will continue identifying and describing risk factors (i.e. hazards) and the preventive, corrective or mitigating measures to correct the identified hazards or welfare consequences (EFSA AHAW Panel, 2022a). Preventive, corrective and mitigating measures are expressed quantitatively when possible or qualitatively. Review of literature (including grey literature when needed), data analysis and expert knowledge elicitation are used to address the request of the mandate in terms of risk assessment.

It is important to highlight that, so far, EFSA guidance for animal welfare (EFSA AHAW Panel, 2012a, 2022a) has been applied to mammals and birds, but not to fishes or other aquatic animals, nor to invertebrates such as insects.

1.3.4 | Risk assessment of gene-edited animals through the different stages of commercial development

As described in Section 1.3.2, the breeding process of NGT animals bearing the intended genetic modifications involves several stages and generations of animals to obtain the final NGT animal that is intended to be placed on the market for food, feed or released in the environment. These steps are similar to those required to produce the final EGT animals and encompass both ex vivo and in vivo phases. The ex vivo phase includes selecting and using primary materials (e.g. oocytes, eggs, somatic cells) for gene editing. The in vivo phase involves a variable number of steps (depending on the specific trait, animal species and technology used), as simplified in Figure 1.

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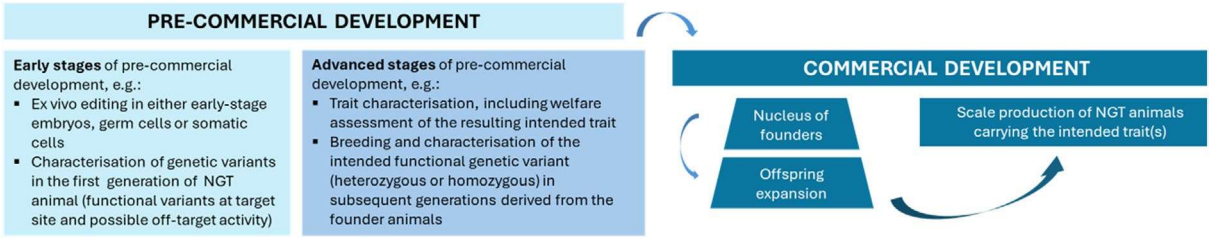


FIGURE 1 Simplified description of pre-commercial and commercial development of gene-edited animals.
Development of NGT animals involves ex vivo modification of either somatic cells, germ cells or early-stage embryos from genetically elite donor animals through the introduction of NGT reagents using various methodologies. The production of founder offspring at the pre-commercial stages, uses species-specific methods. For example, in mammals, edited embryos are implanted into a uterus, while in fish, they are incubated in water. Advanced pre-commercial development will include breeding and trait characterisation. Once the optimised NGT trait(s) has been successfully established in elite breeding animals (i.e. the nucleus or founder population), the process transitions from pre-commercial development to commercial development. Commercial development will involve expansion and dissemination through a pipeline appropriate to that species, often involving phenotypic characterisation of each generation for both the trait associated with the NGT intervention and other relevant production traits.

EFSA's mandate for this opinion (see Sections 1.1.2 and 1.2) focuses on exploring novel potential hazards and risks which NGTs as applied to animals could pose for humans, animals and the environment compared to conventional breeding or established techniques of genetic modification and on assessing the applicability and sufficiency of existing EFSA risk assessment guidelines, covering aspects such as molecular characterisation, food and feed safety, animal welfare and environmental impact.

It is acknowledged that certain elements, such as data on molecular and phenotypic characterisation obtained during the pre-commercial development stages are relevant to inform the risk assessment of the EGT and NGT animals and derived products intended to be placed on the market. Certain phenotypic aspects also involve animal health and welfare.

Welfare considerations are acknowledged as crucial to the risk assessment of GMOs involving animals. However, further dialogue with risk managers is needed to determine how these aspects should be integrated into the overall risk analysis framework.

2 | DATA AND METHODOLOGIES

2.1 | Knowledge gathering on known cases of animals and their food and feed products obtained by new developments in biotechnology

ToR 1 was supported by a specific contract (PO/EFSA/NIF/2023/01) to procure a knowledge gathering report on NGTs in farmed animals and their food, feed and other agricultural products. This report (Van Eenennaam, 2023) was used as a starting point for addressing ToRs 2, 3 and 4. The data collection on marketed or near-market applications of NGTs in the different animal species was carried out through a literature review to compile a comprehensive list of the types of gene-edited animals that are being developed for food, feed and agricultural applications, to provide an overview of the types of applications that are forthcoming. Furthermore, a survey was also developed and sent to public and private technology developers working in the field, to broaden data collection beyond published literature. A total of 195 publications reporting the production of NGT animals were compiled. Several applications have been authorised for placement in various markets around the world. In some instances, NGT animals were judged to be 'non-GMO' if no exogenous DNA was added to the breed (SDN-1 and SDN-2), meaning that in some countries such animals will be equivalent to conventionally-bred animals. Many projects in the research and development phase are also reported in the scientific literature, including studies on mammals, poultry, fish and invertebrate species which may be intended for food, feed and other agricultural uses. Further details on the methodology used by the author to collect data and the outcome of results are detailed in the scientific report. Available knowledge suggests that the highest number of NGT animals reported in the scientific literature is represented by mammals (livestock), followed by fish, then birds (poultry) and then invertebrates. The majority of NGTs applications involved genetic changes achieved by means of SDN-1 techniques, followed by SDN-3 and SDN-2. Among the various NGTs reported, the most frequently used were CRISPR/Cas, followed by TALENS and ZFNs. The most common trait category targeted was increased meat and fibre yield, followed by reproduction, biotic stress, production of hypoallergenic products, combinations of traits, colour, product quality, abiotic stress and a few others.

2.2 | Ad hoc expert working group and its methodology, and existing guidelines

For ToRs 2, 3 and 4, EFSA established an ad hoc expert Working Group of the GMO Panel that met regularly to address the mandate of the European Commission for the development of a scientific opinion according to the mandate.[11]

[11]EFSA web page: https://www.efsa.europa.eu/en/science/scientific-committee-and-panels/gmo#working-groups.

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The EFSA GMO and AHAW Panel (2012) guidance was the result of a joint adoption by both the GMO and AHAW Panels. Regarding aspects related to animal health and welfare risk assessment, selected sections of this scientific opinion were also discussed by the AHAW Panel and endorsed.

In delivering its scientific opinion, the GMO Panel, together with the ad hoc expert Working Group, considered the EFSA guidance documents relevant for molecular characterisation, food feed safety and animal welfare, and environmental impact of GM animals. Besides the two EFSA guidance documents identified in the Terms of Reference of the EC mandate (EFSA GMO and AHAW Panel, 2012; EFSA GMO Panel, 2013), the GMO Panel notes that EFSA has published also two guidances targeted on the welfare of animals (EFSA AHAW Panel, 2012a, 2012b, 2012c, 2012d, 2022a), relevant for the welfare assessment also of GM and NGT animals, presented in Table 2, which were also considered.

TABLE 2 Existing guidelines relevant for molecular characterisation, food feed safety and animal welfare and environmental impact.

EFSA GMO and AHAW Panel (2012)	Guidance on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects
EFSA GMO Panel (2013)	Guidance on the environmental risk assessment of genetically modified animals
EFSA AHAW Panel (2012a)	Guidance on risk assessment for animal welfare
EFSA AHAW Panel (2022a)	Scientific Opinion on the methodological guidance for the development of animal welfare mandates in the context of the Farm to Fork Strategy

The GMO Panel has also drawn from previous assessments of EFSA guidance documents that focused on the adequacy of molecular characterisation, food feed safety and environmental risk assessment for plants and microorganisms developed through SynBio, NGTs and Gene Drives. These evaluations pertain to the respective mandates of the European Commission (EFSA-Q-2012-00241, EFSA-Q-2018-01000, EFSA-Q-2018-00619, EFSA-Q-2019-00297, EFSA-Q-2021-00052, EFSA-Q-2021-00361, EFSA-Q-2022-00508), in a series of scientific opinions published in the last decade (EFSA GMO Panel, 2012, 2020a, 2020b, 2021, 2022a, 2022b, 2024).

The WG adopted a methodology based on a four-phase approach, outlined in Table 3.

TABLE 3 Methodology of the EFSA GMO Panel for addressing the mandate.

Phase 1	
Aim	To identify novel potential hazards and risks which NGTs applied to current or near-market animals could pose for humans, animals and the environment compared to conventional breeding or established techniques of genetic modification.
Approach	Expert knowledge was the basis to address the aim. The ad hoc working group was assembled to ensure a multi-disciplinary approach, aligning with the requirements of the mandate.
Outcome	Refer to Section 3.1
Phase 2	
Aim	To assess the applicability and sufficiency of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) for the risk assessment of NGT animals for food, feed and other agricultural uses, with regards to humans and animal safety, animal health and welfare and environmental risk assessment.
Approach	A thorough section-by-section evaluation of the two guidance documents was performed, based on expert knowledge.
Outcome	Refer to Sections 3.2 and 3.3
Phase 3	
Aim	To test the applicability and sufficiency of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) for the risk assessment of NGT animals for food, feed and other agricultural uses, with regards to humans and animal safety, animal health and welfare and environmental risk assessment.
Approach	A thorough section-by-section evaluation of the two guidance documents was performed, based on selected case studies. This exercise served also as the foundation for specific recommendations.
Outcome	Refer to tables 5–29 in Sections 3.2 and 3.3 and their subsection
Phase 4	
Aim	To identify specific areas and aspects of the existing guidelines which should be updated, adapted or complemented, in those cases in which existing guidelines for risk assessment are considered not applicable, partially applicable or insufficient.
Approach	Expert knowledge was the basis to address the aim.
Outcome	Refer to Sections 4.1 and 4.2

The evaluation of the applicability and sufficiency of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) guidances for the risk assessment of NGT animals highlights several challenges. The two guidances, developed over a decade ago for GM animals, do not fully address the complexities introduced by the emerging and rapid evolution of NGT applications in animals. The lack of submitted applications, to date, requiring the risk assessment of either GM or NGT animals for food, feed and other agricultural uses has limited practical experience in applying these guidances to food and feed safety, animal welfare and environmental risks. Additionally, significant recent updates to animal welfare risk assessment paradigms, reflecting revised scientific understanding (discussed in Section 1.3.3), further contributes to this gap; as a

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result, the two guidances no longer align with current standards or the latest scientific methodologies. Moreover, the EFSA GMO and AHAW Panel (2012) guidance focused primarily on the risk assessment for GM animals and their derived food and feed products, without addressing agricultural uses. There is now a need to adapt risk assessments to account for the emerging of NGTs, incorporate contemporary approaches to animal welfare, and address specific requirements for food and feed safety as well as environmental impacts. These issues are further explored in Sections 3.2 and 3.3, which refer to the EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) guidances, respectively.

2.3 | Selection and rationale of case studies

In order to test the applicability and sufficiency of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) for the risk assessment of NGT animals for food, feed and other agricultural uses, with regards to humans and animal safety, animal health and welfare and environmental risk assessment, eight case studies (Table 4) were chosen. These were either drawn from the knowledge gathering report (Van Eenennaam, 2023), or identified by the EFSA GMO Panel as relevant, based on expert knowledge on the state of the art.

The information available in these studies was used to assess whether the existing guidance would be applicable and sufficient for the risk assessment of NGT animals, as further reported in Sections 3.2 and 3.3 and respective subsections.

The case studies outlined in Table 4, while not exhaustive, were carefully chosen as examples to represent a diverse array of animal's class (mammals, birds, fish, insects), intended traits (hypoallergenic food and feed products, polled cattle, hypermuscularity, disease resistance, sterility),[12] NGT tools (TALEN, CRISPR) and techniques (SDN-1, –2 and –3), as well as potential environmental exposure based on intended confinement regimens (confined, semi-confined and open release) and to encompass diverse scenarios in welfare assessment. These case studies were deemed suitable for testing the existing guidance documents within the context of this scientific opinion in relation to molecular characterisation, food and feed safety, animal welfare and environmental risk. It is worth noting that the GMO Panel has not done a formal assessment of the case studies. While these case studies helped highlight the current status of NGT developments, the field is rapidly evolving and the selected cases may not fully represent future applications.

Each of the case studies was used to test the applicability and sufficiency of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) for the risk assessment of NGT animals for food, feed and other agricultural uses, with regards to humans and animal safety, animal health and welfare and environmental risk assessment. Refer to tables 5–29 in Sections 3.2 and 3.3 and their subsection for a thorough section-by-section evaluation of the two guidances.[13][14]

TABLE 4 Description of the selected case studies.

Study number	Case study reference	Species	Short description of the intended trait modification(s), method and scope	Site-directed nuclease (SDN) approach
1	Wei et al. (2018)	Cattle (*Bos taurus*)	Production of hypoallergenic milk through TALEN-mediated knockout of Beta-Lactoglobulin (*BLG*)	SDN-2

Abstract: We applied precise zygote-mediated genome editing to eliminate beta-lactoglobulin (BLG), a major allergen in cows' milk. To efficiently generate LGB knockout cows, biopsied embryos were screened to transfer only appropriately modified embryos. Transfer of 13 pre-selected embryos into surrogate cows resulted in the birth of three calves, one dying shortly after birth. Deep sequencing results confirmed conversion of the genotype from wild type to the edited nine bp deletion by more than 97% in the two male calves. The third calf, a healthy female, had in addition to the expected nine bp deletion (81%), alleles with an in frame 21 bp deletion (< 17%) at the target site. While her milk was free of any mature BLG, we detected low levels of a BLG variant derived from the minor deletion allele. This confirmed that the nine bp deletion genotype completely knocks out production of BLG. In addition, we showed that the LGB knockout animals are free of any TALEN-mediated off-target mutations or vector integration events using an unbiased whole genome analysis. Our study demonstrates the feasibility of generating precisely biallelically edited cattle by zygote-mediated editing for the safe production of hypoallergenic milk.

Reference: Wei, J., Wagner, S., Maclean, P., Brophy, B., Cole, S., Smolenski, G., Carlson, D. F., Fahrenkrug, S. C., Wells, D. N. and Laible, G. (2018). Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin. *Scientific Reports*, 8, 7661.

Selection criteria:

- Animal's class: mammal
- Intended trait: hypoallergenic food and feed products
- NGT tools: TALEN
- Techniques: SDN-2
- Potential environmental exposure: confined or semi-confined

[12]Intended traits are classified in Van Eenennaam (2023) as: abiotic stress, biotic stress, quality, yield, colour, hypoallergenic, reproduction, welfare.

[13]For the case study the following references were also considered: Tan et al. (2013) (a), Carlson et al. (2016), Norris et al. (2020).

[14]Only the *dnd* knock out animals were taken in consideration.

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TABLE 4 (Continued)

Study number	Case study reference	Species	Short description of the intended trait modification(s), method and scope	Site-directed nuclease (SDN) approach
2	Ezaki et al. (2023)	Chicken (*Gallus domesticus*)	Production of hypoallergenic eggs through TALEN-mediated knockout of Ovomucoid (*OVM*)	SDN-1
	Abstract: Among the major egg allergens, ovomucoid (OVM) is very stable against heat and digestive enzymes, making it difficult to remove physiochemically and inactivate allergens. However, recent genome editing technology has made it possible to generate OVM-knockout chicken eggs. To use this OVM-knockout chicken egg as food, it is important to evaluate its safety as food. Therefore, in this study, we examined the presence or absence of mutant protein expression, vector sequence insertion and off-target effects in chickens knocked out with OVM by platinum TALENs. The eggs laid by homozygous OVM-knockout hens showed no evident abnormalities, and immunoblotting showed that the albumen contained neither the mature OVM nor the OVM truncated variant. Whole genome sequencing (WGS) revealed that the potential TALEN-induced off-target effects in OVM-knockout chickens were localised in the intergenic and intron regions. The WGS information confirmed that plasmid vectors used for genome editing were only transiently present and did not integrate into the genome of edited chickens. These results indicate the importance of safety evaluation and reveal that the eggs laid by this OVM-knockout chicken solve the allergy problem in food and vaccines. **Reference:** Ezaki, R., Sakuma, T., Kodama, D., Sasahara, R., Shiraogawa, T., Ichikawa, K., Matsuzaki, M., Handa, A., Yamamoto, T. and Horiuchi, H. (2023). Transcription activator-like effector nuclease-mediated deletion safely eliminates the major egg allergen ovomucoid in chickens. *Food and Chemical Toxicology*, 175, 113703. **Selection criteria:** – Animal's class: bird – Intended trait: hypoallergenic food and feed products – NGT tools: TALEN – Techniques: SDN-1 – Potential environmental exposure: confined or semi-confined			
3	Kishimoto et al. (2018)	Red sea bream (*Pagrus major*)	Increase of skeletal muscle mass through CRISPR/Cas9 knockout of Myostatin (*mstn* and *mstnb*)	SDN-1
	Abstract: Genome editing is a powerful tool as a new breeding technology including for aquaculture because of the high efficiency of gene targeting without the requirement for exogenous gene integration. CRISPR/Cas9 system, a genome editing tool, has been widely used in various species due to its efficiency and flexibility. We demonstrate the establishment of a new breed of myostatin (Pm-mstn) complete knockout red sea bream (Pagrus major) using CRISPR/Cas9. This is the first report of the establishment of a new breed in aquaculture marine fish using genome editing. The mutations were formed by deletions in the first exon of the Pm-mstn, which cause disruption of the C-terminal active domain of MSTN. The breed exhibited a 16% increase of skeletal muscle, that is, an increase of edible parts. The breed showed the phenotype of short body length and small centrum, which is not observed in mice and other teleost fish. We established the homozygous gene disrupted breed in 2 years, which is far shorter than the conventional breeding method. Our study indicates that genome editing can accelerate the speed of aquaculture fish breeding. **Reference:** Kishimoto, K., Washio, Y., Yoshiura, Y., Toyoda, A., Ueno, T., Fukuyama, H., Kato, K. and Kinoshita, M. (2018). Production of a breed of red sea bream Pagrus major with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. *Aquaculture*, *495*, 415–427. **Selection criteria:** – Animal's class: fish – Intended trait: yield – NGT tools: CRISPR – Techniques: SDN-1 – Potential environmental exposure: confined and semi-confined			
4	Whitworth et al. (2019)	Domestic pig (*Sus domesticus*)	Increased resistance to gastrointestinal coronavirus infection through CRISPR/Cas9 knockout of Amino peptidase N receptor (*ANPEP*)	SDN-1
	Abstract: The alphacoronaviruses, transmissible gastroenteritis virus (TGEV) and Porcine epidemic diarrhoea virus (PEDV) are sources of high morbidity and mortality in neonatal pigs, a consequence of dehydration caused by the infection and necrosis of enterocytes. The biological relevance of amino peptidase N (ANPEP) as a putative receptor for TGEV and PEDV in pigs was evaluated by using CRISPR/ Cas9 to edit exon 2 of ANPEP resulting in a premature stop codon. Knockout pigs possessing the null ANPEP phenotype and age matched wild type pigs were challenged with either PEDV or TGEV. Faecal swabs were collected daily from each animal beginning 1 day prior to challenge with PEDV until the termination of the study. The presence of virus nucleic acid was determined by PCR. ANPEP null pigs did not support infection with TGEV, but retained susceptibility to infection with PEDV. Immunohistochemistry confirmed the presence of PEDV reactivity and absence of TGEV reactivity in the enterocytes lining the ileum in ANPEP null pigs. The different receptor requirements for TGEV and PEDV have important implications in the development of new genetic tools for the control of enteric disease in pigs. **Reference:** Whitworth, K. M., Rowland, R. R. R., Petrovan, V., Sheahan, M., Cino-Ozuna, A. G., Fang, Y., Hesse, R., Mileham, A., Samuel, M. S., Wells, K. D. and Prather, R. S. (2019). Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Research*, *28*, 21–32. **Selection criteria:** – Animal's class: mammal – Intended trait: biotic stress – NGT tools: CRISPR – Techniques: SDN-1 – Potential environmental exposure: confined or semi-confined			

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TABLE 4 (Continued)

Study number	Case study reference	Species	Short description of the intended trait modification(s), method and scope	Site-directed nuclease (SDN) approach
5	Song et al. (2022)	Domestic pig (*Sus domesticus*)	Improved growth performance and infectious disease resistance through Cytidine base editor mediated single-nucleotide replacement at multiple sites of Myostatin *(MSTN)*, Insulin-like growth factor-2 (*IGF2*) and Cluster of Differentiation 163 (*CD163*)	SDN-1

Abstract: The precise and simultaneous acquisition of multiple beneficial alleles in the genome is in great demand for the development of elite pig breeders. Cytidine base editors (CBEs) that convert C:G to T:A have emerged as powerful tools for single-nucleotide replacement. Whether CBEs can effectively mediate C-to-T substitution at multiple sites/loci for trait improvement by direct zygote injection has not been verified in large animals. Here, we determined the editing efficiency of four CBE variants in porcine embryonic fibroblast cells and embryos. The findings showed that hA3A-BE3-Y130F and hA3A-eBE-Y130F consistently resulted in increased base-editing efficiency and low toxic effects in embryonic development. Further, we verified that using a one-step approach, direct zygote microinjection of the CBE system can generate pigs harbouring multiple point mutations. Our process resulted in a stop codon in CD163 and myostatin (MSTN) and introduced a beneficial allele in insulin-like growth factor-2 (IGF2). The pigs showed disrupted expression of CD163 and MSTN and increased expression of IGF2, which significantly improved growth performance and infectious disease resistance. Our approach allows immediate introduction of multiple mutations in transgene-free animals to comprehensively improve economic traits through direct embryo microinjection, providing a potential new route to produce elite pig breeders.
Reference: Song, R., Wang, Y., Zheng, Q., Yao, J., Cao, C., Wang, Y. and Zhao, J. (2022). One-step base editing in multiple genes by direct embryo injection for pig trait improvement. *Scientific China Life Sciences*, 65, 739–752.
Selection criteria:
- Animal's class: mammal
- Intended trait: biotic stress and yield
- NGT tools: CRISPR
- Techniques: SDN-1
- Potential environmental exposure: confined or semi-confined

6	Young et al. (2020)	Cattle (*Bos taurus*)	Production of hornless dairy cattle through TALEN-mediated knock-in of the Celtic POLLED allele	SDN-3

Abstract (a): We have expanded the livestock gene editing toolbox to include transcription activator-like (TAL) effector nuclease (TALEN)- and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9- stimulated homology-directed repair (HDR) using plasmid, rAAV and oligonucleotide templates. Towards the genetic dehorning of dairy cattle, we introgressed a bovine POLLED allele into horned bull fibroblasts. Single-nucleotide alterations or small indels were introduced into 14 additional genes in pig, goat and cattle fibroblasts using TALEN mRNA and oligonucleotide transfection with efficiencies of 10%–50% in populations. Several of the chosen edits mimic naturally occurring performance-enhancing or disease-resistance alleles, including alteration of single base pairs. Up to 70% of the fibroblast colonies propagated without selection harboured the intended edits, of which more than one half were homozygous. Edited fibroblasts were used to generate pigs with knockout alleles in the DAZL and APC genes to model infertility and colon cancer. Our methods enable unprecedented meiosis-free intraspecific and interspecific introgression of select alleles in livestock for agricultural and biomedical applications.
Reference: Tan, W., Carlson, D. F., Lancto, C. A., Garbe, J. R., Webster, D. A., Hackett, P. B., and Fahrenkrug, S. C. (2013). Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proceedings of the National Academy of Sciences of the USA*, *110*, 16526–16531.
Abstract (b): Genome editing followed by reproductive cloning was previously used to produce two hornless dairy bulls. We crossed one genome-edited dairy bull, homozygous for the dominant PC Celtic POLLED allele, with horned cows (pp) and obtained six heterozygous (PCp) polled calves. The calves had no horns and were otherwise healthy and phenotypically unremarkable. We conducted whole genome sequencing of all animals using an Illumina HiSeq4000 to achieve ~20× coverage. Bioinformatics analyses revealed the bull was a compound heterozygote, carrying one naturally occurring PC Celtic POLLED allele and an allele containing an additional introgression of the homology-directed repair donor plasmid along with the PC Celtic allele. These alleles segregated in the offspring of this bull, and inheritance of either allele produced polled calves. No other unintended genomic alterations were observed. These data can be used to inform conversations in the scientific community, with regulatory authorities and with the public around 'intentional genomic alterations' and future regulatory actions regarding genome-edited animals.
Reference: Young, A. E., Mansour, T. A., McNabb, B. R., Owen J. R., Trott, J. F., Brown, C. T., and Van Eenennaam, A. L. (2020). Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. *Nature Biotechnology*, *38*, 225–232.
Selection criteria:
- Animal's class: mammal
- Intended trait: welfare
- NGT tools: TALEN
- Techniques: SDN-3
- Potential environmental exposure: confined or semi-confined

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TABLE 4 (Continued)

Study number	Case study reference	Species	Short description of the intended trait modification(s), method and scope	Site-directed nuclease (SDN) approach
7	Fujihara et al. (2022)	Rainbow trout (*Oncorhynchus mykiss*)	Production of sterile fish through CRISPR/Cas9 mediated knockout of Dead end (*dnd*)	SDN-1
	Abstract: In germ cell transplantation experiments, the use of sterile recipients that do not produce their own gametes is an important prerequisite. Triploidisation and dnd gene knockdown (KD) methods have been widely used to produce sterile fish. However, triploidisation does not produce complete sterility in some fish species, and gene KD is labor and time intensive since it requires microinjection into individual fertilised eggs. To overcome these problems, in this study, we generated homozygous mutants of the dead end (dnd) gene in rainbow trout (Oncorhynchus mykiss) using the clustered regulatory interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system, analysed their reproductive capacity and evaluated their suitability as recipients for germ cell transplantation. By crossing F1 heterozygous mutants produced from founders subjected to genome editing, an F2 generation consisting of approximately 1/4 homozygous knockout mutants (dnd KO) was obtained. The dnd KO hatchlings retained the same number of primordial germ cells (PGCs) as the wild type (WT) individuals, after which the number gradually decreased. At 1 year of age, germ cells were completely absent in all analysed individuals. To evaluate the dnd KO individuals as recipients for germ cell transplantation, germ cells prepared from donor individuals were transplanted into the abdominal cavity of dnd KO hatchlings. These cells migrated to the recipient gonads, where they initiated gametogenesis. The mature recipient individuals produced only donor-derived sperm and eggs in equivalent numbers to WT rainbow trout. These results indicate that dnd KO rainbow trout are suitable recipient candidates possessing a high capacity to nurse donor-derived germ cells. **Reference:** Fujihara, R., Katayama, N., Sadaie, S., Miwa, M., Sanchez Matias, G.A., Ichida, K., Fujii, W., Naito, K., Hayashi, M. and Yoshizaki, G. (2022). Production of germ cell-less rainbow trout by dead end gene knockout and their use as recipients for germ cell transplantation. *Marine Biotechnology*, *24*, 417–429. **Selection criteria:** – Animal's class: fish – Intended trait: reproduction – NGT tools: CRISPR – Techniques: SDN-1 – Potential environmental exposure: confined or semi-confined			
8	Kandul et al. (2022)	Spotted wing sdn drosophila (*Drosophila suzukii*)	Pest population control by means of female-specific lethality and male sterility through Cas9/gRNA mediated knockout of Sex lethal (*Sxl*), Transformer (*Tra*), Doublesex (*Dsx*) and bTubulin 85D *(bTub)*	SDN-1
	Abstract: The Drosophila suzukii invasion of western countries has created an immense agricultural and economic threat to crop production. Despite many attempts to suppress its population, D. suzukii continues to destroy soft-flesh fruits. Precision guided sterile insect technique (pgSIT) utilises the accuracy of programmable CRISPR gene targeting to generate sterilised males that can be deployed to suppress populations. Here, we generate pgSIT in D. suzukii and empirically and mathematically demonstrate that sterilised males are fit, competitive and can eliminate populations of D. *suzukii*. Altogether, we describe an efficient way to generate sterile D. *suzukii* for release and safe effective population suppression. **Reference:** Kandul, N. P., Liu, J., Buchman, A., Shriner, I. C., Corder, R. M., Warsinger-Pepe, N., Yang, T., Yadav, A. K., Scott, M. J., Marshall, J. M., and Akbari. O. S. (2022). Precision Guided Sterile Males Suppress Populations of an Invasive Crop Pest. *GEN Biotechnology*, 1(4), 2022. https://doi.org/10.1089/genbio.2022.0019 **Selection criteria** – Animal's class: insect – Intended trait: reproduction – NGT tools: CRISPR – Techniques: SDN-1 – Potential environmental exposure: open release **Rationale:** as an example of a possible application of NGTs in animals for food, feed and other agricultural uses, considered representative for assessing whether the existing guidance would be adequate and sufficient to carry out the risk assessment from the perspective of molecular characterisation, animal health and welfare, and environmental risk assessment. This case study was selected primarily for the relevance of the trait for environmental risk assessment. Two transgenic parental strains (one expressing Cas9, the other expressing gRNAs targeting genes essential for both female viability and male fertility) were crossed to obtain male sterile F1, making this case primarily suitable for the impact of the phenotype and not for the genetic modification.			

2.4 | Consultations

In line with its policy on openness and transparency, EFSA consulted EU Member States and its stakeholders via an online public consultation. Between January and March 2025, interested persons were invited to submit their comments on the draft GMO Panel Scientific Opinion. Following this consultation process, the document was revised by the EFSA GMO Panel and comments received have been incorporated whenever appropriate. The outcome of the online public consultation is published on the EFSA's website as supporting document together with this scientific opinion as adopted by the EFSA GMO Panel (Annex A).

A webinar meeting was organised on February 2025 to support the launch of public consultations.

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3 | ASSESSMENT

3.1 | ToR 2 – Novel potential hazard and risk identification

The GMO Panel compared the potential hazards and risks associated with the application of NGTs in animals for food, feed and other agricultural uses to those derived from EGTs and conventional breeding. The comparison focused on two primary areas: hazards and risks (intended and unintended) related to the modification process, and those associated with the nature of the resulting new trait.

Hazards and risks related to the modification process considered the origin of any new genetic material and potential risks associated with changes to the host genome at both the intended target site and elsewhere in the genome (i.e. alterations to the host genome), including the possible incorporation of unintended sequences.

Hazards and risks related to the nature of the resulting new trait considered the gene products (i.e. source of DNA and safety of gene products), with broader implications associated with the consumption of animal-derived food and feed, animals' management and their deliberate release into the environment taken into consideration compared to those associated with EGTs and conventional breeding.

These comparisons aimed to determine how the hazards and risks associated with NGTs align with those from EGTs and conventional breeding at the commercial stage, providing a comprehensive risk assessment framework for their use in food, feed and other agricultural productions.

(i) SDN-1, SDN-2 and comparable techniques (e.g. base editing or prime editing) can be used to modify an endogenous DNA sequence without the intended introduction of any additional genetic material (SDN-3 or EGTs). Furthermore, the changes induced are similar to spontaneous mutations which can be introduced using conventional breeding.

- As a result, no novel hazards have been identified that are linked to either the modification process or the newly introduced trait, when compared to EGTs or conventional breeding.

(ii) SDN-3 represents a significant refinement of EGTs allowing insertion of a transgene, intragene or cisgene to a specific target site in the animal genome, instead of random introduction.

- While hazards posed by SDN-3 are of the same nature as those posed by EGTs, the targeted insertion may reduce the potential hazards associated with the disruption of endogenous genes and/or regulatory elements in the recipient genome.
- Hazards posed by the new trait resulting from the introduced transgenic or intragenic DNA sequence are of the same nature as those posed by EGTs.
- Hazards posed by the new trait resulting from the introduced cisgenic DNA sequence are of the same nature as those posed by conventional breeding.

(iii) Application of NGTs may result in off-target alterations to the genome.

- Where off-target mutations are found, they are of the same nature as those that arise in conventional breeding and therefore do not pose a novel hazard (Atkins et al., 2021; Burger et al., 2024; Dong et al., 2019; Hennig et al., 2020; Hruscha et al., 2013; Peterson et al., 2023; Seruggia et al., 2015; Wang et al., 2018; Zhang et al., 2015).

Although NGTs can introduce traits at a rate that exceeds that of conventional breeding, considering the literature and the case studies analysed, the GMO Panel did not identify new traits that are different from those that can be introduced by EGTs or conventional breeding.

The assessment of risk always takes into consideration the hazard and the exposure. Given that no novel potential hazards related to animals obtained by NGTs were identified at the commercial stage, no novel potential risks to humans, animals and the environment are expected even when there is exposure.

It is worth noting that pre-commercial development of EGT and NGT animals may involve procedural steps that are not commonly used in current conventional breeding processes. The earliest procedural steps may result in unintended phenotypes (e.g. still birth, deformity) that are sometimes associated with advanced breeding technologies (e.g. cloning, embryo transfer) resulting in hazards impacting animal health and welfare. These hazards are case-specific (see Sections 3.2.3.1.1 and 3.3.4.10).

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3.2 | ToR 3 – Evaluation of the applicability and sufficiency of the guidance on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects (EFSA GMO and AHAW Panel, 2012)

EFSA GMO and AHAW Panel (2012) provide guidance for the risk assessment of food and feed containing, consisting of or produced from GM animals within the framework of Regulation (EC) No 1829/2003,[15] as well as for the health and welfare assessment of these animals. The evaluation of the applicability and sufficiency focused on the four parts of this guidance consisting of:

(i) General principles governing the comparative approach for the risk assessment of food/feed from GM animals, including animal health and welfare aspects (see Section 3.2.1)
(ii) Food and feed risk assessment, including molecular characterisation, toxicology, allergenicity and nutrition (see Section 3.2.2)
(iii) Assessment of animal health and welfare (see Section 3.2.3)
(iv) Post-market monitoring (see Section 3.2.4)

3.2.1 | EFSA GMO and AHAW Panel (2012), Part B – General principles governing the comparative approach for the risk assessment of food/feed from GM animals, including animal health and welfare aspects

The EFSA GMO and AHAW Panel (2012) outlined the general principles governing the comparative approach for the risk assessment of food and feed from GM animals, including their health and welfare. The principles seek to deploy appropriate approaches to compare GM animals and derived food and feed products with their respective comparators. However, they do not apply just to agricultural uses.

In this opinion, the GMO Panel considers that the principles are applicable for the risk assessment of NGTs applied to animals for food, feed and also for other agricultural uses, but insufficient, requiring further additions/modifications of some parts of the existing text.

The underlying assumption of the comparative assessment approach, that conventionally-bred animals have a history of consumption as food and feed and can serve as a baseline for the health and welfare assessment of the NGT animals and for the risk assessment of NGT animal-derived food and feed, is applicable.

The implementation of the comparative approach, including the health and welfare status of a food and feed producing NGT animal, is applicable for the safety assessment of derived foods and feed, and for the safety assessment of NGT animals themselves.

The principle that a comparative safety assessment of the phenotypic and compositional characteristics should be performed on a routine basis (as described in the guidance for GM animals), is partially applicable but insufficient because it requires further additions to and/or modifications of the existing guidance as follows. Molecular characterisation should be the starting point for risk assessment. The risk assessment approach in NGT animals should be primarily driven by the function of the trait and the knowledge derived from a comprehensive characterisation of the trait obtained by NGTs, in support of a well-founded risk hypothesis. A thorough characterisation of the genetic modification, along with the identification of intended and unintended effects, should be the starting point for the risk assessment of NGT animals and their derived food and feed products.

In the absence of identification of an appropriate comparator, the current guidance recommends a comprehensive safety and nutritional assessment. However, this approach is insufficiently described, and requires further additions to and /or modifications of the existing guidance to integrate and clarify details which would enable an applicant to gain a clearer understanding of how the risk assessment should be conducted (for details see below Section 3.2.2).

3.2.2 | EFSA GMO and AHAW Panel (2012), Part C – Food and feed risk assessment

The EFSA GMO and AHAW Panel (2012) summarised the objectives of the various steps involved in the risk assessment of GM animals and their derived food and feed products (i.e. hazard identification and characterisation, exposure assessment and risk characterisation), but not focusing on agricultural uses, providing detailed information on the specific requirements for the key elements of the assessment: molecular characterisation, comparative analysis, toxicological assessment, allergenicity assessment and nutritional assessment.

[15]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

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3.2.2.1 | *EFSA GMO and AHAW Panel (2012), Section 1 – The objectives of the different steps of the risk assessment procedure for GM animals and derived food/feed and issues to be considered*

3.2.2.1.1 | *EFSA GMO and AHAW Panel (2012), Section 1.1 – Objectives of the different steps of the risk assessment*

The objectives of the different steps of the risk assessment (i.e. hazard identification and characterisation, exposure assessment and risk characterisation) described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for NGT animals, although mainly focused on those intended for food and feed uses. This section, however, is insufficient to address recommendations for NGT animals intended for agricultural uses, particularly concerning exposure assessment beyond intake estimation related to food and feed-derived products.

3.2.2.1.2 | *EFSA GMO and AHAW Panel (2012), Section 1.2 – Elements to be considered for the risk assessment of GM animal and derived food and feed products*

The elements (list a–h, Box 2) to be considered for the risk assessment of GM animals and their derived food and feed products described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses.

For some traits obtained by NGTs (e.g. polled cattle described in case 6 and sterile fish in case 7, Table 4), not all of the elements proposed in EFSA GMO and AHAW Panel (2012) may be necessary to draw a robust conclusion on safety (e.g. the potential for nutritional impact in traits not intended to modify edible tissues). The elements to be considered should, therefore, be guided by an understanding of the function of the trait, the results of comparisons with conventionally-bred animals with a history of safe use, and the findings from molecular characterisation that support a science-based hypothesis regarding potential hazards to be further evaluated in the risk assessment.

BOX 2 Text from EFSA GMO and AHAW Panel (2012), Section 1.2 – Elements to be considered for the risk assessment of GM animal and derived food and feed products

The following elements should be considered for the risk assessment of GM animals and derived products:

a. The characteristics of the donor and recipient organisms;
b. The genetic modification and its functional consequences in the GM animal, intended as well as unintended;
c. The phenotypic characteristics of the GM animal, including health and physiology;
d. The analysis of key components of relevant animal tissues, organs, fluids and/or derived products;
e. The influence of processing on the characteristics of the GM animal-derived food and feed;
f. The potential toxicity, bioactivity and allergenicity of gene products, metabolites and the derived GM animal-derived food and feed;
g. The potential for nutritional impact of GM animal-derived food and feed;
h. The potential for changes in dietary intake as a result of the introduction of the GM animal-derived food or feed.

3.2.2.2 | *EFSA GMO and AHAW Panel (2012), Section 2 – Information required for risk assessment of GM animal-derived food and feed*

3.2.2.2.1 | *EFSA GMO and AHAW Panel (2012), Section 2.1 – Hazard identification and characterisation*

3.2.2.2.1.1 | EFSA GMO and AHAW Panel (*2012*) – *Section 2.1.1 – Information relating to the recipient or (where appropriate) parental animals*

The information (list a-e, Box 3) relating to the recipient or (where appropriate) parental animals described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products are applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed.

Regarding point b, the information required on the 'geographical distribution and husbandry of the animal(s), including its distribution and/or husbandry in Europe and information on natural predators, parasites, competitors and symbionts', is open to interpretation and lacks details relevant to the NGT animal risk assessment.

Regarding point c, the 'information on recipient or parent animals relevant to their safety assessment, including information on any known toxicity and allergenicity of constituents and susceptibility to pathogens', should focus more explicitly on known toxicity and allergenicity, specifically addressing their potential impacts on humans and animals, avoiding to present an overly broad or generalised discussion, ensuring relevance and clarity in the content.

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BOX 3 Text from EFSA GMO and AHAW Panel (2012) – Section 2.1.1 – Information relating to the recipient or (where appropriate) parental animals

The applicant should provide the following information.

a. Complete names: (i) family, (ii) genus, (iii) species, (iv) subspecies, (v) breeding line or strain, (vi) common name;
b. Geographical distribution and husbandry of the animal(s), including its distribution and/or husbandry in Europe, and information on natural predators, parasites, competitors and symbionts, where appropriate;
c. Information on the recipient or parental animals relevant to their safety assessment, including information on any known toxicity and allergenicity of constituents and susceptibility to pathogens;
d. Data on the past and present use of the recipient organism. This information should include the history of consumption as food or feed, information on how the animal is typically bred, reared, transported and housed, and whether special processing is required for safe consumption of derived food or feed. In addition, the normal role of the animal-derived product in the diet should be described (e.g. which part of the animal is used as a food/feed source, whether its consumption is important in particular subgroups of the population, which macro- and/or micronutrients in the product make nutritionally significant contributions to the diet);
e. Information on the ploidy of animal(s).

3.2.2.2.1.2 | *EFSA GMO and AHAW Panel (2012) – Section 2.1.2 – Molecular characterisation*

a. Information relating to the genetic modification.

a.1 Description of the methods and vectors used for the genetic modification.

The information (list a–e, Box 4) relating to description of the methods and vectors used for the genetic modification described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products are partially applicable and insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed.

This section provides information required for the risk assessment of GM animals, based on the methods applicable for transgenesis (i.e. vector used for DNA delivery). In the case of NGTs applied to animals for food, feed and other agricultural uses, a vector is not always used (e.g. Ribonucleoprotein (RNP)-mediated CRISPR genome editing). Therefore, these further clarifications are needed:

- if a DNA vector is not used, the information requested in this section is considered not applicable and insufficient and needs to be further complemented by a request for information on the methods and components for NGT strategies not involving DNA, including information on the NGT tool used, its target(s) and predicted off-target(s).
- if a vector is used, the information requested in this section is considered applicable but insufficient, as information on the NGT tool used, its target(s) and predicted off-target(s) will be needed.

BOX 4 Text from EFSA GMO and AHAW Panel (2012) – Section 2.1.2.1.1 – Description of the methods and vectors used for the genetic modification

The applicant should provide information on the following:

a. The method and the steps of genetic alteration, including relevant bibliographic references, the production method of the vector/fragment used for transformation, and the methods and criteria used for selection. When relevant this will include a description of the technologies used to remove part of the insert, to limit the chance of mobilisation of the insert or to drive the trait through the population;
b. The cellular or tissue material to be transformed;
c. Nature and source of vector(s) used for transformation, including:
    - a table identifying each component of the plasmid/vector, including the region intended for insertion, its size, its origin and its intended function;
    - a physical map of the functional elements and other plasmid/vector components together with the relevant information needed for the interpretation of the molecular analyses (e.g. restriction sites, the position of primers used in PCR, location of probes used in Southern analysis). The region intended for insertion should be clearly indicated.
d. The helper plasmids, if used during the genetic transformation process, including a detailed description of the cis/trans acting system;
e. The purity of the preparation containing the construct prior to introduction into recipient animals or cells.

a.2 Source and characterisation of nucleic acid intended to be inserted.

The information relating to the source and characterisation of nucleic acid intended to be inserted, described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products are partially

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applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed.

This section is originally intended to recommend information for the risk assessment of GM animals, based on the source and characterisation of nucleic acid intended to be inserted. In the case of NGT animals, the insertion of new genomic material does not always occur.

Therefore, the information is fully applicable and sufficient in cases where an exogenous sequence is inserted. It is not applicable when no exogenous sequences are inserted.

TABLE 5 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'Information relating to the genetic modification' for NGT animals, based on the selected case studies.

5a. Description of the methods and vectors used for the genetic modification.			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
3, 4, 5, 7	Strategy of editing does not involve vectors.	Fully applicable, insufficient	Application of NGTs does not always utilise vectors; as such application of certain aspects of the current guidance should be adapted on a case-by-case basis. The guidance should request a description of the NGT tools used and their method of delivery. The guidance should reconsider the information on the 'purity of the preparation' as this may not be known but should instead request information on the methodology used for purification of reagents used in the NGT process.
1, 2, 6	Strategy involves plasmid vectors	Fully applicable, sufficient	None
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None
5b. Source and characterisation of the nucleic acid intended to be inserted			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1, 2, 3, 4, 5, 7	Absence of exogenous foreign DNA in the edited animals.	Partially applicable, insufficient.	As nucleic acid is not always inserted using NGTs, the guidance should distinguish between recommendations for SDN-1, SDN-2 and SDN-3 approaches.
6	DNA template inserted at target site	Fully applicable, sufficient.	None
1–7	Targeted loci show different types of modifications.	Fully applicable, insufficient.	The guidance should request information on the specific DNA changes intended at the target site. The guidance should clarify the number of nucleotides that may be inserted or changed using an NGT approach, to accurately define the boundary between SDN-2 and SDN-3.
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None

b. Information relating to the GM animal.

b.1 General description of the traits and characteristics introduced or modified.

The information relating to the general description of the traits and characteristics introduced or modified, described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products includes a description of the generation of the GM animal to be marketed from the initial GM animals, including the breeding strategy, information on whether the initial GM animal was mosaic, whether the GM animals were to be marketed, are hemizygous or homozygous with regard to the sequence(s) actually inserted, and the ploidy of the GM animals to be marketed.

The GMO panel considers that this information is applicable and insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses. Some specific requirements referring to sequences '*actually inserted*' would not be relevant or may need to be adapted for gene edited sequences. In order to cover all technologies used, the term '*modification*' rather than '*insertion*' is more suited in the assessment of MC requirements.

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b.2 Information on the sequences actually inserted/deleted or altered.

The information (list a–g, Box 5) relating to the information on the sequences actually inserted/deleted or altered described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is partially applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed.

All points (a–g) are applicable and sufficient where NGTs have been used to insert an exogenous DNA sequence (SDN-3) and this concerns exogenous DNA sequence intended to be inserted or not intended to be inserted (from the vector(s) sequence(s)).

Point (a) is also applicable and sufficient where applications of NGTs have been used to modify an endogenous sequence (SDN-1 and SDN-2) and require use of exogenous DNA sequence (vector sequence and/or template DNA) and this concerns sequences not intended to be inserted (from the vector(s) sequence(s) or template DNA).

Point (a) is not applicable where applications of NGTs have been used to modify an endogenous sequence (SDN-1 and SDN-2) and that no exogenous DNA sequence (no vector sequence and no template DNA) has been used in the NGT process (e.g. base editing or prime editing through RNP-mediated CRISPR genome editing).

Points (b), (d), (e) and (f) are not applicable when no exogenous DNA sequence (no vector sequence and no template DNA) has been used as part of the NGT process.

When the intended genetic modification concerns modification/deletion of one or more endogenous sequence(s), points c–g, adapted to the characterisation of the modified sequence instead of the inserted sequence, would apply.

Points (c) and (g) are applicable and sufficient.

BOX 5 Text from EFSA GMO and AHAW Panel (2012) – Section 2.1.2.2.2. Information on the sequences actually inserted/deleted or altered

The applicant should provide the following information:

a. The size and copy number of the inserts, both complete and partial. The analysis should cover sequences that could be inserted into the host animal, such as any parts of the plasmid/vector. The analysis should span the entire insert locus/loci as well as flanking sequences;
b. The organisation and sequence of the inserted genetic material at each insertion site;
c. Size and function of the deleted/modified region(s), in the case of intended deletion/modification(s);
d. Sub-cellular location(s) of insert(s) (integrated in the nuclear or mitochondrial genome, or maintained in a non-integrated form) and methods for ascertaining those sub-cellular location(s) of the insert(s);
e. Sequence information for both 5′ and 3′ flanking regions at each insertion site, with the aim of identifying interruptions of known genes and functional elements, presence of genes in the vicinity of the insert and possible deletions in the recipient DNA. Bioinformatics analysis should be conducted using up-to-date databases with the aim of performing both intraspecies and interspecies homology searches. The characteristics and versions of the databases must be provided;
f. Open reading frames (ORFs) present within the insert and spanning the junctions. The ORFs should be analysed between stop codons, not limiting their lengths. Bioinformatics analyses should be conducted to investigate possible similarities with known toxins or allergens using up-to-date databases. The characteristics and versions of the databases should be provided;
g. Depending on the information gathered, further analyses may be needed to complete the risk assessment.

b.3 Information on the expression of the inserted/modified sequence.

The information (list a–d, Box 6) relating to the information on the expression of the inserted/modified sequence described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed.

- Point a is applicable and sufficient.
- Point b is applicable and sufficient for SDN-3. For cisgenesis strategies where the transferred genetic material replaces an endogenous DNA sequence of the host, in the same genomic context, it is applicable on a case-by-case basis. Concerning SDN-1 and SDN-2 point b would only be applicable and sufficient when a change in expression of the modified gene is expected. It would be applicable to the SDN-1 and SDN-2 strategies on a case-by-case basis. In cases where NGT application results in loss of expression of a protein, this point does not apply.
- Point c is applicable and sufficient for SDN-1, SDN-2 and SDN-3.

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BOX 6 Text from EFSA GMO and AHAW Panel (2012) – 2.1.2.2.3. Information on the expression of the inserted/modified sequence

The applicant should provide the following information:
a. Description of the method(s), including specificity and sensitivity, used for expression analyses;
b. The mean and range of concentrations of newly produced proteins or expression levels of endogenous animal proteins, deliberately modified in the GM food(s) and feed(s) to be placed on the market, together with the raw datasets;
c. When justified by the nature of the insert (e.g. gene silencing through RNA interference), information on the expression of targeted gene(s) and on possible effects to related endogenous genes (to be selected by in silico analysis) should be provided;
d. On a case-by-case basis, expression of genes situated near the inserted/modified sequence.

b.4 Inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the GM animal.

The information relating to the inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the GM animal described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed. However, the GMO Panel is of the opinion this information would only be needed on a case-by case basis (e.g. where there is a hypothesis suggesting genetic instability at the target locus).

- The information requested in this section is considered applicable and sufficient for the risk assessment of SDN-3 applications, but less information could be requested, on a case-by-case basis, for SDN-3 targeted into a previously assessed and considered safe harbour.
- Concerning SDN-1, SDN-2 animals and animals obtained through SDN-3-mediated cisgenesis strategies where the transferred genetic material replaces an endogenous DNA sequence of the host, in the same genomic context, this section is considered applicable and sufficient on a case-by-case basis only, when the intended modification could have consequences on the stability of the edited locus/loci (e.g. large inversion or deletion).
- It should be noted that the generation interval and/or reproductive capacity may be limited for certain animal species and that it may therefore be necessary to adapt the criteria for demonstrating genetic stability on a case-by-case basis.[16]

TABLE 6 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'Information relating to the GM animal' for NGT animals, based on the selected case studies.

6a. General description of the traits and characteristics introduced or modified			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–7	Targeted gene modification led to a given trait	Fully applicable, insufficient	The guidance should be expanded to explicitly include description of any sequence which has been altered by the NGT rather than only sequence which has been inserted.
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None
6b. Information on the sequences actually inserted/deleted or altered			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
2, 3, 4, 7	Targeted genes have been knockout and a new ORF created.	Partially applicable, insufficient	The guidance should request further information on the target site(s), including whether the target is part of a multi-gene family, and a computational prediction of any new peptide sequences expressed.
1, 2, 3, 4, 5, 7	Absence of exogenous foreign DNA	Partially applicable, insufficient	The guidance should request information on the inserted genetic element on a case-by-case basis as this information is only relevant to a subset of NGT applications.
6	Cisgene inserted at target site	Fully applicable and sufficient	None
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None

[16]A pathway to harm is a causal link of events that needs to be realised so that the genetic modification leads to a harm to a specific protection goal. Testable risk hypotheses can be formulated for each of the steps in the pathway, and they will allow the risk to be characterised (Devos et al., 2019).

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TABLE 6 (Continued)

6c. Information on the expression of the inserted/modified sequence			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1, 2, 3, 4, 5, 7	No evidence of production of a NEP that would present a plausible pathway to harm.	Partially applicable	Many endogenous genes have varied expression within and between breeds of a species, influenced by age, sex, husbandry and other environmental conditions, and the base genetics of the individual. The guidance should address the challenges of assessing changes to gene expression associated with NGTs as opposed to natural variation and should be on a case-by-case basis where there is an associated plausible pathway to harm. The guidance should indicate that the recommendations in this section should be applied on a case-by-case basis as NGT can introduce genetic variation associated with a phenotype without the underlying changes in protein expression being known. When the genetic variant is already present in the species and has a history of safe use then expression analysis may not be required.
6	The inserted cisgene is non-coding, and while the functional relationship between the genotype and phenotype is established, the causal relationship remains unproven	Not applicable	Where a cisgene has been inserted in the same genetic context as can be observed in other members of the same species, and the resulting phenotype has a history of safe use, the guidance should not require analysis of altered gene expression.
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None
6d. Inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the GM animal			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–7	No reason to anticipate a bias in the inheritance of the edited gene.	Partially applicable	The guidance should recommend that this be performed on a case-by-case basis where there is a hypothesis-driven reason to believe that the genetic modifications may be unstable.
1, 4, 5, 6	Difficulty to generate large progenies.	Partially applicable	The guidance should consider alternative methods for testing stability for species with long generation time. The current knowledge has expanded since the original guidance was produced such that in most cases the request for genetic/phenotypic stability is not meaningful.
8	Insect modified for sterility using two transgenic strains	Case not appropriate for the purpose of this section.	None

3.2.2.2.1.3 | *EFSA GMO and AHAW Panel (2012) – Section 2.1.3 – Comparative analysis*

The general considerations relating to the comparative analysis described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products are partially applicable and insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, with some clarifications needed, as follows:

- Guidance on the selection of appropriate comparators, on the parameters and components to be captured and on the design of animal trials is insufficient. For cases where no appropriate comparator can be identified, the guidance foresees a comprehensive safety and nutritional assessment for which it lacks sufficient recommendations.
- The selection of appropriate comparators is considered crucial for the comparative assessment of NGT animals. Considering the complexity of traits which can be obtained by NGTs, the open-ended nature of the current guidance on how to identify comparators is considered insufficient.
- The existing guidance presents the comparative analysis of the composition and of phenotypic characteristics based on animal trials as an essential component and as a starting point to structure the risk assessment for NGT animals. This is considered only partially applicable (see above Section 3.2.1).

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- The essential starting point to structure and conduct the risk assessment of NGTs applied to animals is the molecular characterisation. The need for additional steps to identify hazards should only be considered once the molecular characterisation is completed. In cases where no new genetic material has been introduced and the newly introduced trait already exists in conventionally-bred animals with a history of safe use, no further comparative analysis may be required to conclude on the safety. This may avoid the need to request additional experimental data.
- The application of NGTs to animals might produce a result that is indistinguishable from the comparator(s) (i.e. obtained by conventionally-bred animals).
- Where no appropriate comparator(s) can be identified, the current guidance recommends that a comprehensive safety and nutritional assessment of the products derived from the GM animal should be carried out. This is insufficiently clear and only partially applicable, and requires further clarification of the term 'comprehensive safety and nutritional assessment of the GM animal-derived food/feed' to be carried out where no comparator(s) could be identified.

a. Criteria for the selection of the comparator(s)

The information relating to the criteria for the selection of the comparator(s) described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is partially applicable but insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, lacking clarity in view of the broad spectrum of traits which can be obtained by means of NGTs. It is worth noting that while complementary guidelines on criteria for selecting comparators in the risk assessment of GM plants are available in several sources (i.e. EFSA GMO Panel, 2011a, 2011b; Regulation (EU) No 503/2013[17]), the only source of guideline specifically for a comparator selection in GM animals is EFSA GMO and AHAW Panel (2012).

The choice of the comparator(s) is a keystone in the risk assessment for hazard identification, and should be based on sound scientific principles and justified, in particular, with respect to the history of consumption as food and feed and the evidence that a conventional animal can be taken as a reference for history of breeding, rearing and animal health and welfare. The application of NGTs may result in the creation of animals exhibiting simple traits or more complex traits resulting in profound phenotypic and/or compositional changes. Depending, for example, on the complexity of the NGT trait, it may become increasingly challenging to identify an appropriate comparator for the food and feed, animal health and welfare and environmental risk assessments. A comparator of the NGT animal might be the conventionally-bred animal from which the NGT animal is derived, representing the closest comparable genetic background, or a non-NGT surrogate animals with similar characteristics or traits to those of the NGT animal being considered. The lack of an appropriate comparator may arise from the specifics of the receiving environment or management conditions (e.g. no suitable comparator can be reasonably raised in that environment or under those conditions), or because the new trait is sufficiently novel that no appropriate comparator exists within the species. Another challenge may be the difficulty of conducting experiments that are sufficiently realistic while using confinement measures. For such cases, the EFSA GMO and AHAW Panel (2012) suggests considering whether the use of additional comparators could help contextualise any effects of the NGT modification. This would allow for the assessment of whether husbandry practices, management conditions and/or receiving environments could influence the characterised endpoints. This approach may be particularly relevant for certain NGT modifications, where husbandry conditions, management practices or conditions of use are suitable for NGT animals but may be suboptimal or non-permissive for the conventional animals from which the NGT animals are derived, and vice versa (e.g. fish modified by NGT to be more cold-tolerant may be better assessed in the cold environment for which they are adapted rather than the environment in which their parental line is typically reared). In such cases, the extra comparators should be selected based on each identified pathway to harm, the hypothesis being tested and the impacts being assessed. Although principles are given in EFSA GMO and AHAW Panel (2012), the approaches to be followed and the data requirements to be fulfilled are insufficiently detailed, making this requirement partially applicable but insufficient.

When no appropriate comparator(s) can be identified for particular conditions (e.g. NGT modification, husbandry conditions, management conditions or receiving environment), following the recommendations of EFSA GMO and AHAW Panel (2012) for GM animals, a comprehensive safety and environmental risk assessment of the NGT animal and derived food and feed should be carried out. However, the approaches to be followed and the data requirements to be fulfilled are insufficiently detailed, making this requirement partially applicable but insufficient.

b. Animal trials for comparative analysis – experimental design and statistical analysis

The information relating to the experimental design and statistical analysis of animal trials for comparative analysis described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is partially applicable but insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, whenever animal trials are scientifically required, lacking clarity in view of the design and methodology of these studies.

[17]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

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b.1 Principles of experimental design and statistical analysis of data.

This section guides the applicant when performing experimental comparative trials with GM animals. Overall, it is partially applicable but insufficient for NGT animals due to following challenges:

- There is a lack of sufficiently detailed scientific guidance on animal trials with regard to the parameters to be investigated, sampling plans and the appropriate statistical design under practical conditions.
- The current recommendation to perform difference and equivalence testing faces potential practical challenges when considering different types of NGT animals and their generation times, as well as the number of experimental animals that may be required for statistical power. Animal trials with extra comparators under representative management and husbandry conditions may become unrealistically large, which adds to practical and animal welfare challenges. Depending on the trait and the animal species, equivalence testing following recommended procedures may not be feasible and sufficient statistical power may not be achievable.

If animal trials are scientifically necessary to complete the risk assessment, the experimental design must account for a statistical design that addresses the practical challenges and limitations outlined above. Indeed, reliable conclusions cannot be drawn from animal trials when statistically significant results cannot be obtained from well-designed experiments with an appropriately sized cohort. This highlights the importance of re-evaluating the scientific criteria that mandate animal trials, and, consequently, restructuring the risk assessment framework for NGT animals.

The EFSA GMO and AHAW Panel (2012) also suggested considering the use of prior records on conventionally-bred animals which may be retrieved from previous experiments, datasets from appropriate databases, or data from the scientific literature and research reports. Updated guidance is required to clarify how best to use prior records to complement or even eliminate the need for new experiments.

b.2 Information required

This section sets out comprehensive information requirements for experimental animal trials. It is fully applicable despite the comments above concerning the need for those trials and regarding the principles of experimental design and statistical analysis of the data. It is considered insufficient for those cases where prior records on conventionally-bred animals are integrated in the comparative assessment. Such data may be retrieved from previous experiments, datasets from appropriate databases, or data from scientific literature and research reports, but the validity of such data should then be fully justified.

The use of prior records as a component of the comparative safety assessment of NGT animals needs to be supported by availability of:

- Data from literature and/or previous control experiments which are reliable and representative of the NGT animal type.
- Accessible composition databases for food of animal origin
- Accessible animal health and welfare database
- Data collections on phenotypic traits under appropriate defined management and husbandry conditions.

c. Comparative analysis of phenotypic characteristics, including health, physiological and welfare parameters.

The information relating to the comparative analysis of phenotypic characteristics, including health, physiological and welfare parameters described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is fully applicable, but insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, as described below.

- A more complete definition of the parameters to be selected is needed, including e.g. ABMs described and validated since 2012 as welfare indicators of many of the 33 welfare consequences and for example indicators of cognitive capacity or affective states.
- More detailed guidance is needed on the selection of phenotypic characteristics to include as well as clarification of the terms 'health', 'physiological' and 'welfare' as there seems to be a significant overlap.
- Further clarifications are needed on how to retrieve reliable data for conventional populations, e.g. from genetic selection schemes in conventional breeding.
- In addition, the conditions under which the comparative analysis should take place, especially as part of the last phases of the assessment, needs further description and should be updated to the agricultural industry, including the global trade of live animals, where animals may be born/hatched in one country/region and live their life in another, characterised by quite large differences in the presence of novel stimuli and stressors related to for example climatic conditions, housing, husbandry, nutrition and other management conditions as well as infectious agents.

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- The choice of an appropriate comparator in terms of animal welfare is not straightforward as conventional breeding and traditional husbandry of farmed animals, whether for food or non-food production, are themselves associated with significant welfare consequences (e.g. broilers; EFSA AHAW Panel, 2023b).
- For animals other than mammals and birds, the above constitutes a significant challenge, as currently, no EFSA guidance exists for these and the level of scientific knowledge as well as EFSA animal welfare guidance about the welfare of non-mammalian/non-avian species is far less developed than for farmed mammals and birds.
- Due to the high individual variability in most ABMs that are relevant for animal welfare (e.g. level of expression of a certain behaviour, individual variability of physiological response), dilemmas exist regarding the ambition to reduce the number of animals used for scientific purposes versus the precautionary principle calling for high certainty in conclusions from studies comparing GM animals and the chosen comparator(s).

d. Comparative analysis of compositional characteristics

The information relating to the comparative analysis of compositional characteristics described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is fully applicable, but insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, as described below.

d.1 Selection of material for compositional analysis

This section is applicable for cases which require a comparative analysis, but insufficient. Further definitions are required as to appropriate material for compositional analysis under circumstances where the altered trait does not directly impact edible tissues (e.g. absence of horns).

d.2 Selection of compounds for compositional analysis

This section is applicable under the assumption that a comparative assessment is needed, but insufficient in regard to the selection of components. There is currently no consensus document of the Organisation for Economic Co-operation and Development (OECD) on key components of food of animal origin. Databases on food composition for foods of animal origin still need to be identified which may serve as sources for historical control data and/or to establish equivalence limits for the comparative compositional analysis. It appears straightforward to identify relevant macro- and micronutrients.

However, the request to include bioactive compounds is unclear, lacking a definition of what this encompasses and thereby being open to different interpretations. Depending on the species and the function of the trait, the selection of compounds for compositional analysis may be extended, on a case-by-case basis, to those regulating physiological processes in animals and humans. Pertinent examples are hormones, growth factors, biogenic amines and eicosanoids.

Whether no exogenous DNA is introduced and only endogenous genes are modified, it might be more appropriate to explore a more targeted analysis, focused on components that could be directly affected by the genetic modification, instead of a broad compositional analysis targeting a wide range of animal-endpoints.

d.3 Effects of processing

The information required to assess whether or not the processing and/or preserving technologies applied to NGT animal-derived food and feed products are likely to modify its characteristics, compared with their respective products from the non-NGT counterpart, is fully applicable. A case-by-case approach based on a plausible hypothesis of harm is considered applicable also for food and feed products derived from NGT animals.

TABLE 7 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'comparative assessment' for NGT animals, based on the selected case studies.

7a. Criteria for the selection of the comparator(s).

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8	To characterise the NGT animals, a comparison was made with conventionally-bred animals ('wild types' – WT)	Partially applicable, insufficient	The guidance should propose criteria for the selection of the comparator(s) considering the most updated knowledge on traits, related pathways to harm and NGT animal species at the pre-market stage. This would allow practical recommendations. The guidance should support the definition of scenarios where a comprehensive safety and nutritional assessment would need to be conducted in cases of the absence of an appropriate comparator.

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TABLE 7 (Continued)

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
7b. Animal trials for comparative analysis – experimental design and statistical analysis			
1–8	To characterise the NGT animals, comparative experimental trials were performed.	Fully applicable, insufficient	The guidance should highlight that experiments should be fit for purpose and may not be needed when no pathway to harm is identified, when data already exists or when the trait is already present in the gene pool or species and has a history of safe use. The guidance should include more practical and detailed guidance on conducting animal trials with adequate sample size in relation to the statistical power of the analysis, adequate methodology and improved modelling approaches, even when number of animals is limited due to conditions related to animal biology (e.g. generation time). Different information might be requested based on the trait. Depending on the scenario, the requirements may differ
7c. Comparative analysis of phenotypic characteristics, including health, physiological and welfare parameters.			
1, 2, 4, 5, 6	To characterise the NGT animals, phenotypic characteristics were compared to those of conventionally-bred animals ('wild types' – WT)	Fully applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, –endpoints) to allow compositional and phenotypic analyses. Update and refinement of the vocabulary used is needed to avoid overlapping and align with current knowledge on animal health and welfare. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals.
3, 7	To characterise the NGT animals, phenotypic characteristics were compared to those of conventionally-bred animals ('wild types' – WT)	Fully applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, –endpoints) to allow compositional and phenotypic analyses. Update and refinement of the vocabulary used is needed to avoid overlapping and align with current knowledge on animal health and welfare. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals. Risk assessment in animal health and welfare for fish need further developments to integrate previous existing guidance documents with current scientific knowledge.
8	To characterise the NGT animals, phenotypic characteristics were compared to those of conventionally-bred animals ('wild types' – WT)	Fully applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, –endpoints) to allow compositional and phenotypic analyses. Update and refinement of the vocabulary used is needed to avoid overlapping and align with current knowledge on animal health and welfare. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals. Risk assessment in animal health and welfare for insects need further developments in the absence of previous guidance, considering significant knowledge gap in this field.
7d. Comparative analysis of compositional characteristics			
1, 2, 3, 4, 5, 7	To characterise the NGT animals, key endpoints in various tissues were compared to those of conventionally-bred animals ('wild types' – WT)	Fully applicable, insufficient	The guidance should include a comprehensive and specific list of tissues and related compositional key endpoints based on the potential use of those tissues for food and feed purposes. Compositional key endpoints could include, as appropriate, macronutrients, micronutrients, anti-nutrients, known or potential allergens, toxins and other constituents of toxicological or nutritional relevance.
6, 8	Not edible tissues	Case not appropriate for the purpose of this section.	None

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3.2.2.2.1.4 | *EFSA GMO and AHAW Panel (2012) – Section 2.1.4 - Toxicological assessment*

The general principles of the toxicological assessment described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses.

The aim of the toxicological assessment is to address the potential toxicity of any novel protein(s) or other new constituent(s) due to the genetic modification, such as any possible changes in the levels of natural constituents beyond normal variation, and/or the impact of other changes in composition.

The requirement for toxicological testing on a case-by-case basis is applicable to NGT animals, driven by the outcome of molecular characterisation and comparative analysis; however, the information available in EFSA GMO and AHAW Panel (2012) to support the waiver or supplementation of toxicological studies is generally limited in detail.

a. Standardised guidelines for toxicity tests.

The standardised guidelines for toxicity tests described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses. However, based on recent experience on the risk assessment of GMOs, an important recommendation is to incorporate additional endpoints into experimental studies, where relevant, to better capture the anticipated effects of specific changes introduced by genetic alterations (both EGTs and NGTs). Further discussion on the criteria required for the selection of key endpoints is needed in the future.

b. Phenotypic comparison.

The information relating to the phenotypic comparison described in EFSA GMO and AHAW Panel (2012) is partially applicable but insufficient for the risk assessment of NGT animals for food, feed and other agricultural uses. Furthermore, this section would be more pertinent in the frame of the section on comparative analysis.

The text is applicable with regard to comparative aspects of animal phenotypes as indicators for: (i) potential adverse health and welfare effects of the newly expressed proteins, other new constituents and/or changed levels of natural constituents (intended changes), including effects due to specific biological activities of the respective constituents (e.g. endocrine, pharmacological or immunological activity); (ii) occurrence of unintended effects of the genetic modification.

Regarding the comparative aspects of animal health and welfare status (phenotype), it is not applicable and insufficient. Further discussion of the potential link between animal health and welfare and potential negative health effects on humans and animals consuming derived food or feed products could be relevant. As indicated in EFSA AHAW Panel (2012a, 2012b), the choice of indicators should be appropriate for the species and category of animals, and a wide range of animal-based-measures (ABMs) may be necessary. It is good practice in AHAW risk assessment to use several indicators and a multi-disciplinary approach. Since the publishing of the EFSA AHAW Guidance in 2012, numerous scientific studies have developed and validated new potential ABMs for 1-more of the 33 welfare consequences listed in the most recent EFSA guidance (EFSA AHAW Panel, 2022a). The indicators can be behavioural but can also be, for example, clinical or physiological. In addition, regarding behavioural recordings, in the EFSA Guidance (EFSA AHAW Panel, 2012a), the focus is on observations of non-evoked or undisturbed behaviour. The use of such indicators is highly relevant, but since the publishing of the guidelines, the knowledge about behavioural tools that can be used to infer animal emotional states (e.g. Weary et al., 2017) or cognitive capacity (e.g. Nawroth & Rorvang, 2022) has increased markedly and should be taken into account in the future guidance. In addition, the indicators chosen should reflect a potential susceptibility to certain diseases. In the case a certain welfare consequence is increased in gene edited animals, the applicant should provide measures to correct or mitigate it (if any) and the degree to which it could happen.

c. Assessment of newly expressed proteins.

The principles for the protein safety assessment outlined in EFSA GMO and AHAW Panel (2012) are generally applicable and sufficient to characterise the toxicological profile of newly expressed proteins in edible tissues. This applies when NGTs introduce or alter protein-coding sequences, leading to the expression of a protein not typically found in the species or modifying the amino acid sequence of an existing protein to confer a specific function (e.g. removal of pathogen binding sites).

Notably, as science evolves, the risk assessment requirements should be adapted to incorporate new developments. At the time of writing this report, the EFSA GMO Panel is exploring new approaches for the protein safety risk assessment in GMOs, ensuring that proteins present due to genetic alterations are assessed in an appropriate, consistent and proportionate manner. These aspects will need to be integrated in future guidance for the protein safety assessment of food and feed products derived from biotechnology (e.g. NGTs).

d. Assessment of new constituents other than proteins.

The principles for the assessment of new constituents other than proteins described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient to characterise their toxicological profile. When NGTs results in the expression of such constituents not typically found in the species under assessment, such as bioactive compounds with endocrine, physiological

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or immunological activity, pharmacological activity, or RNAi and similar molecules expected to exert a specific function, these should be evaluated on a case-by-case basis.

e. Assessment of altered levels of food and feed constituents.

The principles for the assessment of altered levels of food and feed constituents described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient to characterise their toxicological profile. When NGTs result in altered levels of food and feed constituents beyond their natural variation, these should be evaluated on a case-by-case basis.

f. Assessment of the whole food/feed derived from GM animals.

The principles for the assessment of the whole food/feed described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses. Animal feeding trials with rodents or other target animal species should be performed on a case-by-case basis, driven by the evidence derived from molecular characterisation, compositional analysis and toxicological evaluation of the identified intended and/or unintended effects related to NGTs.

TABLE 8 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'toxicological assessment' for NGT animals, based on the selected case studies.

8a. Phenotypic comparison			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–5	NGT targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Partially applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, endpoints) to allow phenotypic analyses (see Table 7) and on linking animal health and welfare status with potential negative health effects on humans and animals upon consumption of NGT animal-derived products
6	NGT not targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Case not fitting for the purpose of this section.	None
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8b. Assessment of newly expressed proteins			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–5	NGT targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Fully applicable, sufficient	Although the basis for the assessment is applicable and sufficient (e.g. HoSU, molecular characterisation, bioinformatics, in vitro and in vivo studies), new approaches are becoming available for use to streamline and strengthen the protein safety assessment. This might be further expanded.
6	NGT not targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Case not fitting for the purpose of this section.	None
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None

(Continues)

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TABLE 8 (Continued)

8c. Assessment of new constituents other than proteins			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–6	NGT targeted not to alter expression of new constituents other than proteins in edible tissues and/or derived products.	Fully applicable, sufficient	Although applicable and sufficient, a solid rationale/evidence that new constituents are not intended to be present in edible tissues, also in case of traits intended to express in non-edible tissues (e.g. case 6), might be further expanded.
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8d. Assessment of altered levels of food and feed constituents			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–6	NGT targeted not to alter expression of levels of food and feed constituents in edible tissues and/or derived products	Fully applicable, sufficient	Although applicable and sufficient, a solid rationale/evidence that altered levels of food and feed constituents are not present in edible tissues, also in case of traits intended to express in non-edible tissues (e.g. case 6), might be further expanded.
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8e. Assessment of the whole GM food/feed derived from GM animals			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–5	NGT targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Fully applicable, sufficient	None
6	NGT not targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Case not fitting for the purpose of this section	None
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None

3.2.2.2.1.5 | *EFSA GMO and AHAW Panel (2012) – Section 2.1.5–- Allergenicity assessment*

The general principles of the allergenicity assessment described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses.

The aim of the allergenicity assessment is to address the potential allergenicity of any novel protein(s), as well as of the whole food derived from the NGT animal. Thus, the specific allergy risk of NGT animals is associated with:

(i) presence of newly/differently expressed protein(s) in edible parts of the animals
(ii) alterations to the allergenicity of the whole animal and derived products (e.g. due to altered expression of natural endogenous allergens as an unintended effect of the genetic modification)

a. Assessment of allergenicity of the newly expressed protein.

The general principles of the allergenicity assessment described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses.

Notably, as science evolves, the risk assessment requirements should be adapted to incorporate new developments. In this context, the GMO Panel has previously published a scientific opinion on further developmental needs for the allergenicity and protein safety assessment in products derived from biotechnology (EFSA GMO Panel, 2022d). Furthermore, as

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explained above, at the time of writing this report, the EFSA GMO Panel is exploring new approaches for the protein safety risk assessment in GMOs.

The allergenicity assessment of newly expressed proteins does not apply when no new proteins are intended to be expressed (e.g. applications with no new amino acid combination in the final product). However, this section is applicable and sufficient when modifications involve introduction or alteration of protein-coding sequences into the animal genome.

b. Assessment of allergenicity of the tissues, organs and products from the GM animal.

The principles of the allergenicity assessment of the tissues, organs and products from animals described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses.

Thus, it is recommended that when the recipient of the genetic editing is an animal whose products are known to be common food allergens (e.g. milk, eggs, fish, etc.) and when there are no indications of possible interaction(s) between the metabolic pathway(s) involved in the expression of the trait protein encoded by the NGTs and those involved in the biosynthesis of endogenous allergenic proteins naturally present in the conventional animal tissues, the same management measures as for the non-NGT animal products should be applied. Likewise, if there are indications based on, for example, the compositional analysis that the allergenicity of the whole NGT animal-derived food might be substantially, qualitatively or quantitatively changed because of interactions between the metabolic pathways of synthesis of the newly expressed protein(s) and of endogenous or new allergens, the allergenic potential of NGT food and feed should be further investigated.

c. Adjuvanticity.

The principles of the assessment of adjuvanticity described in EFSA GMO and AHAW Panel (2012) are partially applicable and insufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, as described below.

The EFSA GMO and AHAW Panel (2012) does not cover in sufficient detail how to conduct the assessment of adjuvanticity. Therefore, the guidance is overall insufficient for NGT animals.

If there are no indications of relevant changes in the context of potential adjuvanticity from compositional analysis and/or toxicological/allergenicity evaluation of the identified intended and/or unintended effects, there is no need to address potential adjuvanticity. Moreover, adjuvanticity is currently assessed based on evidence provided by the scientific literature, and further research and validation of experimental methodologies are needed to better address adjuvanticity in a regulatory context (EFSA GMO Panel, 2024).

TABLE 9 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'allergenicity assessment' for NGT animals, based on the selected case studies.

9a. Assessment of allergenicity of the newly expressed protein			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–5	NGT targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Fully applicable, sufficient	Although the basis for the assessment is applicable and sufficient (e.g. HoSU, molecular characterisation, bioinformatics, in vitro and in vivo studies), new approaches are becoming available for use to streamline and strengthen the protein safety assessment. This might be further expanded.
6	NGT not targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Case not fitting for the purpose of this section.	None
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
9b. Assessment of allergenicity of the tissues, organs and products from the GM animal			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
1–5	NGT targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Fully applicable, sufficient	None

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TABLE 9 (Continued)

9b. Assessment of allergenicity of the tissues, organs and products from the GM animal			
Case study	**Relevant aspects of the case**	**Applicability and sufficiency of the guidance**	**Recommendation for future guidance updates**
6	NGT not targeted to alter expression or structure of proteins present in edible tissues and/or derived products.	Case not fitting for the purpose of this section.	None
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None

3.2.2.2.1.6 | *EFSA GMO and AHAW Panel (2012) – Section 2.1.6 – Nutritional assessment*

The general principles of nutritional assessment described in EFSA GMO and AHAW Panel (2012) are applicable and sufficient for the risk assessment of NGTs animals for food, feed and other agricultural uses.

The intended modification in NGT animals may alter the overall nutrient profile of the derived animal products, potentially affecting the nutritional status of specific consumer groups consuming the food or the feed. Unexpected changes in nutrient composition could also have similar impacts.

Animal feeding trials with rodents or other target animal species should be conducted on a hypothesis-driven case-by-case basis, whether justified based on a thorough characterisation of the inserted genetic modification identifying potential intended and unintended effects in edible tissues, a thorough compositional analysis, and/or toxicological identification of intended and/or unintended effects. If a hazard is identified, resulting in a plausible pathway to harm, endpoint measurements will vary depending on the target species, but could include specific measures of adverse effects in addition to more classical measures such as feed intake, body weight, animal performance and nutrient bioavailability.

a. Specific considerations for the nutritional assessment of GM animal-derived food.

The information covering specific considerations for the nutritional assessment of GM animal-derived food described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived food and feed products is applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, as described below.

In principle, on the one hand, if the genetic alteration does not impact the edible parts of animals, it might not be necessary to perform a nutritional assessment. On the other hand, it should be demonstrated that genetic modifications have no impact on edible tissues and its composition. This should be based on a thorough characterisation of the inserted genetic alteration to characterise both intended and unintended effects on edible tissues.

If it becomes necessary to conduct a compositional comparison, an appropriate list of compounds should be made available, similar to the risk assessment of GM plants with reference to OECD documents on compositional considerations for individual crops (e.g. OECD consensus document, 2001, 2002, 2009, 2012). This list should consider the knowledge available on species and traits.

b. Specific considerations for the nutritional assessment of GM animal-derived feed.

The information covering specific considerations for the nutritional assessment of GM animal-derived feed described in EFSA GMO and AHAW Panel (2012) for the risk assessment of GM animals and their derived feed products is applicable and sufficient for the risk assessment of NGTs applied to animals for food, feed and other agricultural uses, as described below.

At the time of writing of this report, there is a shift towards a circular economy, where products, materials and resources are kept in use for as long as possible and waste generation is minimised (COM/2020/98 Final).[18]This is central to the EU's goal of creating a sustainable, low-carbon, resource-efficient and competitive economy. In this perspective, a better use of resources from Animal Byproducts (ABP), including NGT animals, is relevant. The EU restrictions on use of most Processed Animal Protein (PAP) in farmed animal feed, introduced in 2001, are now in the process of being changed. Several restrictions have been lifted as of 2024, e.g. the use of insect meal in feed for aquaculture, poultry and pigs, as well as PAP of poultry origin in feed to pigs and vice versa (Regulation (EU) 2021/1372).[19] The use of ABPs for animal feed is relevant for NGT animals, since ABPs for feed are often produced from the residual carcass of the animal.

Animal feeding trials with target animal species should be considered on a hypothesis-driven case-by-case basis to assess the impact of the NGT animal-derived food/feed, based on a thorough characterisation of the inserted genetic

[18]https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1583933814386&uri=COM:2020:98:FIN.

[19]https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021R1372.

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modification identifying potential intended and unintended effects in edible tissues, a thorough compositional analysis, and/or toxicological identification of intended and/or unintended effects. If it becomes necessary to conduct a compositional comparison, an appropriate list of compounds should be made available, similar to the risk assessment of plants where reference to OECD documents on compositional considerations for individual crops are available (e.g. OECD consensus document, 2001, 2002, 2009, 2012). This list should consider the knowledge available on species, traits and specific processed products from NGT animals. If a hazard is identified, resulting in a plausible pathway to harm, endpoint measurements will vary depending on the target species but could include specific measures of adverse effects in addition to more classical measures such as feed intake, body weight, animal performance and nutrient bioavailability.

TABLE 10 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'nutritional assessment' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–6	The genetic modification could lead to an altered content of natural constituents in the NGT animal-derived food and feed beyond their natural variation.	Fully applicable, sufficient	Although applicable and sufficient, a species and tissue specific list of compounds should be recommended along with reference values for equivalence limits, might be further expanded.
7	Fish modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None
8	Insect modified for sterility, not intended for consumption.	Case not fitting for the purpose of this section.	None

3.2.2.2.2 | *EFSA GMO and AHAW Panel (2012), Section 2.2 – Exposure assessment – anticipated intake/extent of use*

The general principles outlined for exposure assessment are applicable for the risk assessment of NGTs animals, although mainly focused on animals intended for food and feed uses, as they were developed based on exposure assessments involving estimated dietary intake of GM food and feed and consumption data. This section is, therefore, insufficient to cover recommendations for NGT animals intended for other agricultural uses where the final product is not food or feed, in particular regarding exposure assessment through routes other than the dietary intake (e.g. respiratory and contact route of exposure). In such cases, exposure should be evaluated on a case-by-case basis. Furthermore, in the absence of any identified hazards, the requirement for an estimate of expected intake becomes questionable. However, when a hazard is identified, further clarifications should be integrated detailing the methodologies for data collection and quantification of the anticipated intake/extent of use and consequent exposure assessment.

3.2.2.2.3 | *EFSA GMO and AHAW Panel (2012), Section 2.3 – Risk characterisation*

The general principles outlined for risk characterisation are applicable and sufficient for NGTs animals, although mainly focused on those intended for food and feed uses, as they were developed based on exposure assessments involving estimated dietary intake of GM food and feed and consumption data. This section is, therefore, insufficient to cover recommendations for NGT animals intended for other agricultural uses where the final product is not food or feed, in particular regarding exposure assessment through routes other than the dietary intake (e.g. respiratory and contact route of exposure). In such cases, exposure assessment should be evaluated on a case-by-case basis.

3.2.2.2.4 | *EFSA GMO and AHAW Panel (2012), Section 2.4 – Other considerations*

(i) Risk assessment of animals with introduced non-heritable traits.

The EFSA GMO and AHAW Panel (2012) guidance does not apply for the safety assessment of food and feed products derived from GM animals with introduced non-heritable traits. The principles behind this are currently also applicable to NGTs applied to animals intended for agricultural, food and feed production, although this aspect may change in the future.

(ii) Assessment of the potential risk associated with horizontal gene transfer.

The applicant was required to assess any potential risk associated with horizontal gene transfer from the GM animal and its products to humans, animals and microorganisms. This is only applicable to NGTs applied to animals intended for food, feed and other agricultural uses in those cases in which genes/sequences of concern[20] have been introduced. Information

[20]Gene of concern: genes known to contribute to the production of toxins, toxic metabolites, antimicrobials of medical and veterinary importance, acquired genes conferring resistance to antimicrobials of medical and veterinary importance.

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on how to conduct the assessment of the potential risk associated with horizontal gene transfer have been addressed in the EFSA GMO Panel (2013) guidance, and its evaluation is described below (Section 3.3).

3.2.3 | EFSA GMO and AHAW panel (2012), Part D – Assessment of animal health and welfare

The EFSA GMO and AHAW Panel (2012) outlined the general principles for the assessment of animal health and welfare of GM animals, focusing on the strategy to evaluate potential effects of genetic modification on animal health and welfare. It includes principles of the comparative approach for the comparison of compositional and phenotypic characteristics, previously described and assessed in Sections 3.2.1 and 3.2.2, as well as the methodology to assess health and welfare across the three stages: Stage A – monitoring at the laboratory level; Stage B - experimental farm assessment; Stage C –: field trial assessment.

3.2.3.1 | *EFSA GMO and AHAW Panel (2012), Section 1 – General principles*

3.2.3.1.1 | *EFSA GMO and AHAW Panel (2012), Section 1.1 – Potential effects of genetic modification on animal health and welfare*

The principles guiding the evaluation of the potential effects of genetic modification on animal health and welfare are also applicable to NGT animals; however, the strategy and the methodology proposed to assess health and welfare across the three stages – A (monitoring at the laboratory level), B (experimental farm assessment) and C (field trial assessment) – is only partially applicable and insufficient, and not entirely adequate in the case of NGT animals.

Significant advances, for example in the understanding of the emotional and cognitive complexity of relevant animal species and in animal welfare assessment in general, that are important for risk assessment of animal health and welfare, have been made since the EFSA GMO and AHAW Panel guidance was published in 2012. Thus, even assuming that a comparative analysis is required, the methodology proposed in that document must be revised and significantly expanded, to incorporate recent knowledge on animal health and welfare, welfare consequences and the use of ABMs, as outlined in recent EFSA opinions (e.g. EFSA AHAW Panel, 2022b on the welfare of pigs and EFSA AHAW Panel, 2023a on the welfare of calves) and in scientific studies.

For example, the 2012 guidance document recommends using the so-called Welfare Quality® indicators, which are suited for assessing animal welfare at farm level, particularly for classifying farms for commercial purposes. However, given advancements in animal welfare science, these indicators may not provide a sufficiently targeted assessment. Additionally, EFSA's current methodology, developed when mandates of mammals and birds arrived, most probably does not adequately cover non-mammalian and non-avian species, such as fish, insects and invertebrates, and requires further development. Furthermore, the EFSA GMO and AHAW Panel guidance (2012) emphasises animal function, but overlooks affective states – how animals perceive their environment – which are now central to EFSA's definitions of animal welfare consequences (EFSA AHAW Panel, 2022a). To identify endpoints and adequate measures for animal health and welfare assessment in the case of NGT/EGT animals could pose challenges that would need to be further evaluated in all species, and uncertainty would need to be considered, especially in fish and insects.

Moreover, as discussed in Section 1.3.4, while risk assessments for animal health and welfare should encompass all in vivo phases, including animals at pre-commercial stages of the breeding process, EFSA's risk assessment in the context of this opinion focuses on NGT animals produced at the end of the breeding process – those that have achieved the desired traits and are ready for market entry, in accordance with the terms of reference of the mandate.

It is worth noting that pre-commercial development of EGT and NGT animals may involve procedural steps that are not commonly used in current conventional breeding processes. The earliest procedural steps may result in unintended phenotypes (e.g. still birth, deformity) that are sometimes associated with advanced breeding technologies (e.g. cloning, embryo transfer) resulting in hazards impacting animal health and welfare. These hazards are case-specific. Therefore, there is scope for future work identifying and characterising welfare consequences caused by the use of some advanced breeding technologies, including conventional breeding, EGT and NGT, in all types of animals at the pre- and post-commercial phases, to suggest possible preventive and mitigating measures to limit these welfare consequence and to suggest corrective measures to hazards along the in vivo phases.

EFSA animal health and welfare assessment in non-EGT/NGT animals is currently carried out considering separate phases of the production cycle. Assessing animal health and welfare hazards throughout the whole production cycle is not clearly detailed the present guidance (EFSA GMO and AHAW Panel, 2012), and the relevant phases of the risk assessment should be clarified in alignment with EU legal framework.

In this SO, the assessment focused on aspects related to risk for animal health and welfare that would be specific in relation to NGT animals. Aspects related to reduction of risk, or positive effect of EGTs and NGTs on animal health and welfare are not requested by the mandate.

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TABLE 11 Applicability and sufficiency of EFSA GMO and AHAW Panel (2012) section(s) on 'Potential effects of genetic modification on animal health and welfare', for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8	All case studies involve procedures to animals that can be relevant for animal health and welfare	Fully applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, endpoints) to allow analyses of relevant welfare consequences. Update and refinement of the vocabulary used is needed to avoid overlapping and to align with current knowledge on animal health and welfare. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals. The guidance should clarify which production stages are relevant for the animal health and welfare risk assessment in EFSA. Risk assessment in animal health and welfare for other vertebrates than the typical farmed mammalian and avian species, such as fishes, need further developments to integrate previous existing guidance documents with current scientific knowledge. Risk assessment in animal health and welfare for insects and other invertebrates need further developments in the absence of previous guidance, considering significant knowledge gaps in this field.

3.2.4 | EFSA GMO and AHAW Panel (2012), part E – post-market monitoring (PMM)

3.2.4.1 | *EFSA GMO and AHAW Panel (2012), Section 1 – Post-market monitoring and surveillance of health and welfare of GM animals*

The principles and objectives of post-market monitoring and surveillance of health and welfare of GM animals are applicable to NGT animals, although the EFSA GMO and AHAW Panel (2012) does not provide many details on when and how to conduct this activity, and therefore can be optimised in future revisions of the guidance.

3.2.4.2 | *EFSA GMO and AHAW Panel (2012), Section 2 – Post-market monitoring of the GM animals and their derived food and feed*

The principles and objectives of post-market monitoring of the GM animals and their derived food and feed are applicable to NGT animals, although the EFSA GMO and AHAW Panel (2012) does not provide many details on when and how to conduct this activity, and therefore can be optimised in future revisions of the guidance.

3.3 | ToR 3 – Evaluation of the applicability and sufficiency of the guidance on the environmental risk assessment of genetically modified animals (EFSA GMO Panel, 2013)

EFSA GMO Panel (2013) provides guidance for the environmental risk assessment of genetically modified (GM) animals within the framework of Regulation (EC) No 1829/2003. The evaluation of the applicability and sufficiency focused on the five parts of this guidance consisting of:

(i) Scope of the Guidance Document (see Section 3.3.1)
(ii) Strategies for the ERA of GM animals (see Section 3.3.2)
(iii) Cross-cutting considerations (see Section 3.3.3)
(iv) Specific areas of risk to be addressed in the ERA (see Section 3.3.4)
(v) Post-market Environmental Monitoring (see Section 3.3.5)

3.3.1 | EFSA GMO Panel (2013) – 1. Scope of the Guidance document

The Guidance covers animals developed for food/feed uses and for non-food/feed uses, except those for the production of pharmaceuticals. Animals for contained[21] and experimental uses are excluded, as are animals modified to contain a gene drive.

[21]'Contained uses' refers to any activity in which organisms are genetically modified or in which such GMOs are stored, transported, destroyed, disposed of or used in any other way and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment.

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3.3.2 | EFSA GMO Panel (2013) – 2. Strategies for the ERA of GM animals

In this section the basic principles of ERA are described. The ERA is case-by case, should be performed in a scientifically sound and transparent manner, makes use of the problem formulation approach and keystone is the comparative safety assessment e.g. comparison of the environmental impact(s) of the GM animal to those from a non-GM animal. Both intended and unintended effects of the genetic modification are to be assessed. This approach is also applicable to NGT animals. However, this section needs to be updated to refer to Directive 2018/350,[22] amending Directive 2001/18/EC to explicitly include the problem formulation approach for all GMOs. It should also be indicated that non-GM/non-NGT comparator(s) are not always available, and so experience with additional comparators (including non-animal comparators such as pest control methods) may also be useful for the ERA. Therefore, this section is considered to be partly applicable and insufficient for NGT animals.

3.3.2.1 | *EFSA GMO Panel (2013) – 2.1. Different steps of the environmental risk assessment*

In this section the different steps of the ERA are described, based on a six-step problem formulation approach. This section is considered to be fully applicable to NGT animals, but insufficient for the following reasons.

It is important to highlight that the ERA should be case-specific and hypothesis-driven. The aim is not to reduce uncertainty to zero for all potential areas of concern, but to test the most plausible and robust pathways to harm. It should be stressed that the first step in the ERA (problem formulation including hazard identification) is needed for all NGT animals, but that the next steps in the ERA are only needed if a hazard is identified. To come to a robust ERA and to establish the amount of data required, risk managers should set clear, specific protection goals and limits of concern. Acknowledging that not all risks and hazards can be foreseen in advance, post-market environmental monitoring is implemented in order to detect any adverse effects unanticipated in the ERA.

It is further recommended to highlight the value of statistics and scenario modelling for risk characterisation. Additionally, the efficacy of the new trait of the NGT animal should only be proven in those cases for which the new trait could be linked to safety concerns. For instance, in animals modified for sterility, low efficacy of the modification would imply release of some fertile animals, which could in turn lead to environmental risks. Another example is animals modified for disease resistance, which could lead to environmental harms if resistance is not complete. However, this potential harm would be connected to the trait and not to the specific means used to achieve it (i.e. it would be a concern also in case of resistance achieved by genomic selection).

3.3.2.2 | *EFSA GMO Panel (2013) – 2.2. Information to identify potential unintended effects*

This section describes that the ERA takes into account both intended and unintended effects of the genetic modification, which can be identified following a comparative approach (e.g. comparison to the non-GM/non-NGT animal), considering the information provided by the molecular characterisation (including off-target analysis), the compositional analysis, the phenotypic characteristics and the interactions of the GM animal with the receiving environments. Each identified (un) intended effect should be assessed for potential adverse effects on human health and the environment. This section is considered to be fully applicable and sufficient for NGT animals.

3.3.3 | EFSA GMO Panel (2013) – 3. Cross-cutting considerations

3.3.3.1 | *EFSA GMO Panel (2013) – 3.1 Receiving environments*

This section is fully applicable as it gives the scientific background to the complexities of considering receiving environments. However, it is insufficient as it is not clear on how applicants should deal with this complexity in a proportionate and targeted way. The data requirements are open ended and are not driven by the need to test a plausible pathway to an identified harm that is different to appropriate comparators, in nature or degree. The importance of focusing data requirements using a comparative, case-by-case approach is not made clear. For example, according to the Guidance, data are required on receiving environments and management systems, yet such data is not considered to be needed unless these are expected to change because of the genetic modification or in the case no interaction with receiving environments is expected. Also, the data requirements for ecosystems accessible to escaped animals are open ended, and are not limited by consideration of plausible pathways to harm arising from the animal-trait combination in relation to the appropriate comparator.

[22]Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms.

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TABLE 12 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Receiving environments', for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1, 2, 3, 6	(Semi-) confinement of the animals No sexually-compatible wild relatives in the EU	Fully applicable, insufficient	Characterisation of receiving environment(s) should focus on those receiving environments where plausible pathways to harm have been identified. The level of characterisation is dependent on the introduced trait, animal species, hybridisation potential and intended management system (confinement, semi-confinement, open release).
4, 5	Potential for hybrisation with wild relatives	Fully applicable, insufficient	See above
7	(Semi-) confinement of the animals Potential for hybrisation with wild relatives in case sterility is incomplete	Fully applicable, insufficient	See above
8	Open release Potential for hybrisation with wild relatives	Fully applicable, insufficient	See above

3.3.3.2 | *EFSA GMO Panel (2013) – 3.2 Experimental environment*

This section is partly applicable and insufficient as it covers the scale and experimental conditions of any experiment without first discussing its purpose and need. Indeed, the first sentence stresses the need to cope with complexity rather than to use experiments to test specific hypotheses in order to assess specific risks of harm; the appropriate experimental environment should be derived from the experimental hypothesis in a case-by-case manner. Furthermore, the text needs to reflect the importance of the principles of reduction, refinement and replacement (3R's principles) in animal experiments, in line with EU actions for the protection of animals used for scientific purposes[23] (Hubrecht & Carter, 2019). In particular, experiments are not always needed even when a potential harm has been identified, as appropriate data might already exist, as long as the robustness of the ERA is maintained.

TABLE 13 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Experimental environments' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–6	Hypoallergenic trait (milk, eggs) (cases 1,2), increased muscle mass trait (case 3, 5), disease resistance trait (case 4, 5) and hornless trait (case 6) Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species The hornless trait has a history of safe use in cattle	Partly applicable, insufficient	The guidance should highlight that experiments should be fit for purpose and may not be needed when no pathway to harm is identified, when data already exists or when the trait is already present in the gene pool or species and has a history of safe use, and no unintended effects are identified by means of the comparative assessment.
7, 8	Sterility trait (case 7, 8) Sterility trait is present in the gene pool of fish	Partly applicable, insufficient	In addition to the above, the guidance should highlight that in case of an introduced sterility trait, the efficacy of the trait should be demonstrated. Experiments and/or modelling may be needed to test potential pathways to harm linked to the introduced trait (sterility) in insects, linked to relevant unintended effects or in case the trait is not 100% effective.

3.3.3.3 | *EFSA GMO Panel (2013) – 3.3 Choice of comparators*

Aspects pertinent to the choice of comparators in the environmental risk assessment are discussed in Section 3.2.2.2.1.3 of this document.

[23] https://environment.ec.europa.eu/topics/chemicals/animals-science_en#:~:text=EU%20legislation%20on%20animals%20in,the%20use%20of%20live%20animals. Accessed: 26 October 2024.

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3.3.3.4 | *EFSA GMO Panel (2013) – 3.4 The use of non-GM surrogates*

This section covers the potential use of non-GM surrogates to provide data to inform the ERA; it is fully applicable and sufficient, as it notes that such work would be case-specific and provides options for applicants, rather than stating/implying/indicating it would be required in every case.

3.3.3.5 | *EFSA GMO Panel (2013) – 3.5 Experimental design and statistics*

The guidance provided for the experimental design and statistics of experiments involving GM animals is considered partly applicable but insufficient, as no clear and proper instructions are provided on when such experiments are needed, how they should be carried out and how to do the statistical analyses. It is recommended that updated guidance clearly establishes that due to welfare considerations, including the 3R's principle, animal trials should only be conducted when it is necessary to assess a particular risk hypothesis. Additionally, the guidance provided is difficult to apply in species with long generation times, and the GMO Panel recommends it is revised to better address the challenges posed by these species.

TABLE 14 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Experimental design and statistics' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8		Partly applicable, insufficient	The guidance should be further developed on experimental design, including statistics and modelling.

3.3.3.6 | *EFSA GMO Panel (2013) – 3.6 Long-term effects*

This section is applicable, in that it covers the issues to be considered, but insufficient as it does not give guidance as to how the issues should be addressed in a proportionate way. In particular, it does not show how effects should be considered in a case-specific, hypothesis-led manner, and suggests direct studies aimed at predicting any type of long-term effect should be performed, rather than focusing on testing hypotheses related to the risk assessment of specific harms that could occur over long timescales. Long-term effects that are not anticipated in the ERA would be identified by means of post-market environmental monitoring (PMEM).

TABLE 15 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Long-term effects' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1, 2, 3, 6	No sexually-compatible wild relatives in the EU	Fully applicable, insufficient	The guidance should reflect that assessment of long-term effects should only focus on potential harms. In cases where there is a need to assess these long-term effects, the magnitude of the potential harm should be considered.
4, 5, 7, 8	The species could hybridise with wild relatives in the EU	Fully applicable, insufficient	In addition to the considerations above, for any NGT animal species also other parameters might need to be considered such as the size of the wild population to be affected and its genetic diversity.

3.3.3.7 | *EFSA GMO Panel (2013) – 3.7 Further guidance on modelling*

This section is considered fully applicable, and it should be nested within section 3.5 of the EFSA guidance on experimental design and statistics (EFSA GMO Panel, 2013).

3.3.3.8 | *EFSA GMO Panel (2013) – 3.8 Uncertainty analysis*

This section describes the sources and types of uncertainty in ERA and how to analyse and assess those uncertainties. This section is fully applicable for NGT animals, but insufficient since reference should be made to EFSA's Guidance on uncertainty published in 2018 (EFSA Scientific Committee, 2018) that provides more information on how to properly assess uncertainty. This 2018 Guidance is applicable to the ERA of NGT animals as well as of GM animals.

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TABLE 16 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Uncertainty analysis' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8		Fully applicable, insufficient	The guidance should refer to EFSA Guidance on uncertainty (2018)

3.3.3.8.1 | EFSA GMO *Panel (2013) – 3.8.3 Interplay between ERA conclusions and PMEM*

The concept of PMEM is described as an approach to deal with uncertainties that are inherent to all risk assessments. Specific risks and critical uncertainties identified in the conclusions of the ERA form the basis of case-specific monitoring in PMEM plans, whereas General Surveillance aims to cover the remaining residual uncertainty. This is also applicable to NGT animals, therefore this section is considered fully applicable and sufficient. An important point covered by the Guidance which should be highlighted refers to the importance of establishing a feedback loop between PMEM and ERA e.g. how results of case-specific monitoring can be used to inform ERA in order to reduce earlier identified specific risks or critical uncertainties. Currently there is little integration between pre-market environmental risk assessment, risk management measures resulting from them and post-market monitoring. It is recommended that updated guidance provides instructions how to strengthen the interconnection between these elements.

TABLE 17 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Interplay between ERA and PMEM' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8		Fully applicable, sufficient	None

3.3.3.9 | *EFSA GMO Panel (2013) – 3.9 Aspects of GM animal health and welfare*

This section describes the need to ensure the health and welfare of GM animals, and how to document this based on the EFSA AHAW guidance (EFSA AHAW Panel, 2012a). Generally, the principles are applicable but insufficient, given the progresses in the field of animal welfare since 2013 in both science, regulation and EFSA guidance. Reasons for this conclusion are presented in Section 1.3.3 above in the assessment of the EFSA GMO and AHAW Panel guidance document published in 2012 (EFSA GMO and AHAW Panel, 2012).

3.3.4 | Specific areas of risk to be addressed in the ERA

EFSA GMO Panel (2013) 4.1. Specific areas of risk for fish; 4.2 Specific areas of risk for insects; 4.3 Specific areas of risk for birds and mammals.

The sections on 'areas of risk' of fish, insects, mammals and birds lack focus on case-by-case assessments that identify the most plausible pathways to harm, based on the biology of the animal species (for example, whether it can hybridise with feral species or establish in the wild) and the novel trait in question. The GMO Panel recommends that updated guidance should reflect that modifications resulting in phenotypes already present in natural or farmed populations (which have a history of safe use and do not require a change in management) do not have to be addressed further in the ERA. In such a case, unless an unintended effect resulting from the genetic modification is identified, risk assessment should stop at the level of hazard identification.

The term 'recombinant DNA' should be replaced throughout the document by 'genetic change' or 'genetic alteration' to cover the use of NGTs such as gene editing. Moreover, several essential tasks, such as how to estimate risks quantitatively or how to minimise certain risks, lack clear instructions in the current text. Some sections might only be relevant in specific cases, so the updated Guidance should clarify where its content applies, such as risk management strategies only being relevant when a risk has been identified. Finally, the use of pre-existing knowledge should be encouraged, particularly to avoid redundant studies such as disease trials, in line with the 3R's principles, when sufficient data already exists.

Unless indicated otherwise, most sections in the Guidance on areas of risk are considered to be partly applicable or fully applicable, since all areas of risk need to be addressed, but the Guidance also addresses animals that act as vectors of human disease, which are outside the scope of the current mandate. In most cases the guidance provided in these sections is considered insufficient for the reasons mentioned above.

For the specific areas of risk mentioned below, the focus of the ERA is on the intended (novel) trait. In case unintended effects are identified that could lead to a hazard, these will also be part of the risk assessment.

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3.3.4.1 | *Persistence and invasiveness, including vertical gene transfer*

EFSA GMO Panel (2013) Section 4.1.1 (fish), Section 4.2.1 (insects) and Section 4.3.1 (mammals and birds).

In addition to the general issues mentioned earlier, it is recommended that more emphasis is given to the impact that the intended confinement regime of the modified animal (confined, semi-confined, open release) and the presence of sexually-compatible wild relatives in the receiving environment will have on the risk of persistence and invasiveness. Additionally, in cases where the intended novel trait leads to sterility (i.e. cases 7 and 8), the effectiveness of the trait needs to be confirmed. If this is the case, sterility will most probably result in reduced fitness corresponding to a reduced likelihood of persistence and invasiveness. In general, farming/breeding is known to influence animal behaviour and reduce the fitness of both the animal and their offspring in the wider environment, regardless of whether they are GM, NGT or conventionally-bred.

TABLE 18 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Persistence and invasiveness' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1, 2, 3, 6	Hypoallergenic trait (milk, eggs) (cases 1,2), increased muscle mass (case 3) and hornless trait (case 6) No sexually-compatible wild relatives in the EU Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species The hornless trait has a history of safe use in cattle	Fully applicable, insufficient	The guidance should specify that this area of risk does not have to be addressed further in the ERA* if the introduced trait: – cannot result in phenotypes that enhance reproductive success and/or survival, which would result in increased persistence and invasiveness, or – the trait is present in the gene pool of the species or has a history of safe use in the species.
7	Sterility trait The species could not hybridise with wild relatives in the EU if effective sterility is demonstrated	Fully applicable, insufficient	See above
4, 5	Disease resistance trait (case 4,5), increased muscle mass trait (case 5) The species could hybridise with wild relatives in the EU Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species	Fully applicable, insufficient	The guidance should specify that this area of risk only needs to be addressed when the introduced trait: – increases the likelihood that the animals persist, hybridise with wild relatives and produce fertile offspring with increased fitness, or – is not present in the gene pool of the species or does not have a history of safe use
8	Sterility trait in insects The species could hybridise with wild relatives in the EU Intended for open release	Fully applicable, insufficient	See above

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm, unless an unintended effect is identified that could lead to a hazard.

3.3.4.2 | *Horizontal gene transfer*

EFSA GMO Panel (2013) Section 4.1.2 (fish), Section 4.2.2 (insects) and Section 4.3.2 (mammals and birds).

In addition to the general issues mentioned earlier, the Guidance should reflect that horizontal gene transfer should only focus on genes/sequences of concern[24] (e.g. acquired antimicrobial resistance genes, AMR) that result in traits for which selection pressure may exist in the environment. If no such genes/sequences are introduced, risk assessment should stop at the level of hazard identification for this area of risk.

Furthermore, in the case of GM mammals and birds, this section of the Guidance also addressed vertical gene transfer, focusing on potential loss of genetic diversity within a species as a result of the genetic modification. This section is considered not applicable as potential loss of biodiversity is considered a result of animal breeding and not an environmental risk as such.

[24]Gene of concern: Gene known to contribute to the production of toxins, toxic metabolites, antimicrobials of medical and veterinary importance, acquired genes conferring resistance to antimicrobials of medical and veterinary importance.

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TABLE 19 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: (Vertical and) horizontal gene transfer' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1 2, 4, 5, 6	Introduced traits in mammals and birds are obtained by gene editing Myostatin (*mnstn*) knockout is present in the gene pool of multiple species The hornless trait (knock-in of Celtic *POLLED* allele) has a history of safe use in cattle	Partly applicable, insufficient Vertical gene transfer is not applicable	The guidance should specify that this area of risk does not have to be addressed further in the ERA* in case the genetic alteration does not introduce a gene/sequence of concern (e.g. AMR gene), for example when the genetic alteration: – only consists of edited and/or deleted genes, or – is present in the gene pool of the species or has a history of safe use in the species.
3, 7, 8	Introduced traits in fish and insects are obtained by gene editing Sterility trait by knockout of dead end (*dnd*) gene is present in the gene pool of fish	Partly applicable, insufficient	See above

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm, unless an unintended effect is identified that could lead to a hazard.

3.3.4.3 | *Pathogens, infections and diseases*

EFSA GMO Panel (2013) Section 4.1.4 (fish), Section 4.2.3 (insects) and Section 4.3.3 birds and mammals).

This section covers the potential effects of the genetic modification on the susceptibility of the modified animals to pathogens, infections and disease. Apart from the considerations above, it is important to note that the guidance on GM insects referring to vectors of human diseases is not applicable to the current mandate.

TABLE 20 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Pathogens, infections and diseases' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8	Hypoallergenic trait (milk, eggs) (cases 1, 2), increased muscle mass trait 3, 5), hornless trait (case 6) and sterility trait (case 7, 8)	Partly applicable, insufficient	The guidance should specify that this pathway to harm should only be addressed further in ERA* in cases where the introduced trait may be expected to result in a modified susceptibility to disease in the animals
4, 5	Disease resistance trait	Partly applicable, insufficient	The guidance would benefit from allowing for the use of pre-existing knowledge instead of conducting new disease trials, in line with the 3R's principles.

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm, unless an unintended effect is identified that could lead to a hazard.

3.3.4.4 | *Interaction with target organisms*

EFSA GMO Panel (2013) Section 4.2.4 (insects), Section 4.3.4 (birds and mammals).

In addition to the general issues mentioned earlier, the guidance considers a major hazard to be the failure to achieve the intended effects on the target population, for example the failure of population suppression. Resistance evolution can be considered both an environmental harm, since trait failure could lead to the application of more environmentally harmful interventions and an economic one. Animals that are resistant to a pathogen, potentially leading to the evolution of more virulent pathogens or the animal becoming a reservoir for the pathogen, resulting in a hazard to non-modified animals, are covered in the Section 3.3.4.3 on 'pathogens, infections and diseases'. Furthermore, the guidance on GM insects referring to vectors of human diseases is not applicable to the current mandate.

TABLE 21 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Interaction with target organisms' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1, 2, 6	The animals are not targeting any organism	Applicable, sufficient	None

(Continues)

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TABLE 21 (Continued)

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
4, 5	The animals are virus-resistant	Not applicable	The guidance should specify that hazards resulting from potential evolution of the virus in virus-resistant animals should be addressed in the section 'pathogens, infections and diseases'
8	The insect is targeting wild type *D. suzukii* populations (sterility trait)	Not applicable, insufficient	The guidance should specify that resistance development in target populations results in loss of efficacy, which could lead to trait failure and the need to deploy more environmentally harmful strategies. The guidance could indicate that applicants should make reference in their dossier to good management practices to ensure the trait maintains its efficiency and potential environmental benefits of the NGT animal.

3.3.4.5 | *Interactions with non-target organisms (NTOs)*

EFSA GMO Panel (2013) Section 4.2.5 (insects) and Section 4.3.5 (birds and mammals).

In addition to the aspects described earlier, updated guidance should consider that effects on NTOs can be transient (i.e. revert to levels of no biological significance after a limited duration) and very difficult to detect in the context of the highly dynamic nature of agroecosystems, which are typically poorly characterised. Furthermore, the guidance should emphasise that data should be preferentially from European countries, as the text now only refers to data coming from non-European countries and laboratory experiments.

TABLE 22 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Interactions with non-target organisms (NTOs)' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8	Hypoallergenic trait (cases 1, 2), increased muscle mass (cases 3, 5), disease resistance trait (cases 4, 5), hornless trait (case 6) and sterility trait (case 7, 8) Semi-containment of animals (case 1–7), open release of insects (case 8) Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Sterility trait is present in the gene pool of fish The hornless trait has a history of safe use in cattle	Partly applicable, insufficient	The guidance should explain that this area of risk only needs to be addressed further in the ERA* in case: – plausible pathways to harm can be identified resulting from the introduced trait, – there is relevant exposure to NTOs or – the trait is not present in the gene pool of the species, or it does not have a history of safe use in the species. The guidance should explain how to focus data collection on studies that are needed to evaluate an identified harm, rather than to look for any environmental change which does not affect protection goals

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm, unless an unintended effect is identified that could lead to a hazard.

3.3.4.6 | *Environmental impacts of the specific techniques used for the management of the animals*

EFSA GMO Panel (2013) Section 4.1.6 (fish), Section 4.2.6 (insects) and Section 4.3.7 (birds and mammals).

Apart from the considerations listed above, the assessment should mainly focus on situations in which the specific management techniques applied to the NGT animals differ from those used for conventionally-bred animals, although potential effects associated to the use of current management techniques on NGT animals should also be considered.

TABLE 23 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Environmental impacts of the specific techniques used for the management of the animals' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–7	Different management techniques may/may not be needed to prevent or mitigate specific welfare consequences in NGT animals (fish, birds, mammals) as compared to conventional bred animals	Applicable, sufficient	Although applicable and sufficient, the guidance should indicate that applicants make reference in their dossier to good management practices that reduce the likelihood of environmental risks.

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TABLE 23 (Continued)

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
8	Sterility trait in *D. suzukii* to suppress wild type populations Open release	Applicable, sufficient	Although applicable and sufficient, the guidance should indicate that applicants make reference in their dossier to good management practices that reduce the likelihood of environmental risks.

3.3.4.7 | *Impact on human health*

EFSA GMO Panel (2013) Section 4.1.7 (fish), Section 4.2.7 (insects) and Section 4.3.9 (birds and mammals).

Apart from the considerations listed above, the guidance on GM insects regarding the modification of insects for the control of vectors of human disease is not applicable to the current mandate.

TABLE 24 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Impact on human health' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–3, 6–8	Hypoallergenic trait (milk, eggs) (cases 1, 2), increased muscle mass trait (case 3), hornless trait (case 6) and sterility trait (case 7, 8) Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Sterility trait is present in the gene pool of fish The hornless trait has a history of safe use in cattle	(Partly) applicable, sufficient	The guidance should explain that this area of risk does not need to be further addressed in the ERA* in cases where the introduced trait is already present in the gene pool of the species, or has a history of safe use in the species.
4,5	Disease resistance trait (case 4, 5)	Fully applicable, sufficient	The guidance should explain that this area of risk does not need to be further addressed in the ERA* in cases where the introduced trait is already present in the gene pool of the species, or has a history of safe use in the species.

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm unless an unintended effect is identified that could lead to a hazard.

3.3.4.8 | *Interactions with the abiotic environment*

EFSA GMO Panel (2013) Section 4.1 (fish) and Section 4.3.9 (birds and mammals).

TABLE 25 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Interactions with the abiotic environment' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–7	Hypoallergenic trait (milk, eggs) (cases 1, 2), increased muscle mass trait (case 3, 5), hornless trait (case 6), disease resistance trait (case 4, 5) and sterility trait (case 7) Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Sterility trait is present in the gene pool of fish The hornless trait has a history of safe use in cattle	Partly applicable, insufficient	The guidance should clearly explain, based on concrete examples, what adverse effects on the abiotic environment can be expected resulting from NGT animals The guidance should explain that this area of risk does not need to be further addressed in the ERA* in cases where the introduced trait is already present in the gene pool of the species, or has a history of safe use in the species.

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm unless an unintended effect is identified that could lead to a hazard.

3.3.4.9 | *Impacts of GM fish on biotic components and processes*

EFSA GMO Panel (2013) Section 4.1.3 (fish)

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TABLE 26 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Impact on biotic components and processes' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
3,7	Increased muscle mass and the sterility trait in fish Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Sterility trait is present in the gene pool of fish	Partly applicable, insufficient	The guidance should explain how to focus data collection on studies that are needed to evaluate an identified harm, rather than environmental change which does not affect protection goals. The guidance should explain that this area of risk does not need to be further addressed in the ERA* in cases where the introduced trait is already present in the gene pool of the species, or has a history of safe use in the species.

*Risk assessment stops at the level of hazard identification, since no hazard is identified for this pathway to harm unless an unintended effect is identified that could lead to a hazard.

3.3.4.10 | *Impacts of GM mammals and birds on non-GM animal health and welfare*

EFSA GMO Panel (2013) Section 4.3.8 (GM Mammals and birds).

The section is considered partially applicable but insufficient for NGT mammals and birds. The primary reasons for the lack of sufficiency are: (1) that the text refers to EFSA Scientific Opinions (e.g. EFSA AHAW Panel, 2007, 2010, 2012c, 2012d) that were based on knowledge on animal welfare, which has since evolved significantly (see Section 1.3.3); and (2) that the text refers to the EFSA AHAW Panel (2012a) guidance, which is still in place but has been complemented with a more recent guidance in 2022 (EFSA AHAW Panel, 2022a). It is important to mention that EFSA guidance documents for risk assessment in animal welfare have not been applied yet to fish or insects. Especially regarding insects, the limited scientific knowledge on this topic might constitute a limitation in the welfare assessment.

To identify endpoints and adequate measures for animal health and welfare assessment in the case of NGT- and EGT animals could pose challenges that would need to be further evaluated in all species, and uncertainty would need to be considered, especially in fish and insects.

Moreover, as discussed in Section 1.3.4, while risk assessments for animal health and welfare should encompass all in vivo phases, including animals at intermediate stages of the breeding process, EFSA's risk assessment in the context of this opinion, focuses on NGT animals produced at the end of the breeding process – those that have achieved the desired traits and are ready for market entry, in accordance with the terms of reference of the mandate.

It is worth noting that pre-commercial development of EGT and NGT animals may involve procedural steps that are not commonly used in current conventional breeding processes. The earliest procedural steps may result in unintended phenotypes (e.g. still birth, deformity) that are sometimes associated with advanced breeding technologies (e.g. cloning, embryo transfer) resulting in hazards impacting animal health and welfare. These hazards are case-specific. Therefore, there is scope for future work identifying and characterising welfare consequences caused by the use of some advanced breeding technologies, including conventional breeding, EGT and NGT, in all types of animals at the pre- and post-commercial phases, to suggest possible preventive and mitigating measures to limit these welfare consequences and to suggest corrective measures to hazards along the in vivo phases.

EFSA animal health and welfare assessment in non- EGT/NGT animals is currently carried out considering separate phases of the production cycle. Assessing animal health and welfare hazards throughout the whole phases of the production cycle is not clearly detailed the present guidance (EFSA GMO and AHAW Panel, 2012), and the relevant phases of the risk assessment should be clarified in alignment with EU legal framework.

In this SO, the assessment focused on aspects related to risk for animal health and welfare that would be specific for in relation to NGT animals. Aspects related to reduction of risk, or positive effect of EGTs and NGTs on animal health and welfare are not requested by the mandate.

TABLE 27 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) for the 'Area of risk: Impacts of GM mammals and birds on non-GM animal health and welfare' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8	All case studies involve procedures to animals that can be relevant for animal health and welfare	Applicable, insufficient	The guidance should include more specifications on selecting measures (especially ABMs, endpoints) to allow analyses of relevant welfare consequences. Update and refinement of the vocabulary used is needed to avoid overlap and to align with current knowledge on animal health and welfare. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals.

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TABLE 27 (Continued)

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
			The guidance should clarify which production stages are relevant for the animal health and welfare risk assessment in EFSA. Risk assessment in animal health and welfare for vertebrates other than the typical farmed mammalian and avian species, such as fishes, need further developments to integrate previous existing guidance documents with current scientific knowledge. Risk assessment in animal health and welfare for insects and other invertebrates needs further development in the absence of previous guidance, considering significant knowledge gaps in this field.

3.3.5 | EFSA GMO Panel (2013) – 5 Post-market environmental monitoring

This section describes the two elements of PMEM, case-specific monitoring and general surveillance. This section is considered fully applicable but insufficient for NGT animals (as well as for GM animals). It should be highlighted that case-specific monitoring should be proportionate and consider the specific characteristics of the event (animal-trait-management system combination). Moreover, the guidance should emphasise the importance of the feedback loop from monitoring data obtained from PMEM to inform the ERA.

3.3.5.1 | *EFSA GMO Panel (2013) – 5.1 Case-specific monitoring*

This section describes that where the ERA identifies specific risks or critical uncertainties, case-specific monitoring should be carried out after placing the NGT animal on the market to inform the ERA and ensure that the risk management measures are working effectively. This section is considered fully applicable but insufficient for NGT animals, as it should clearly indicate that CSM should be hypothesis-driven, and should be proportionate to the specific risk or critical uncertainty identified in each case. It is recommended that updated guidance states case-specific monitoring should always include monitoring the efficacy of the risk management measures implemented to reduce exposure.

TABLE 28 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'Case-specific monitoring' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1–8		Fully applicable, insufficient	The guidance should explain that CSM is only required in certain cases, should be hypothesis-driven, and should be proportionate to the specific risk or critical uncertainty identified.

3.3.5.2 | *EFSA GMO Panel (2013) – 5.2 General Surveillance*

This section describes the objectives of general surveillance (GS), which are to detect unanticipated adverse effects, to determine harm to protection goals and to determine the causality between the detected unanticipated adverse effects and the placing on the market of the GM animal. This section is considered fully applicable but insufficient for NGT animals. It is considered that GS is very difficult to implement because GS is hypothesis-free and entails several methodological challenges that should be addressed.

While the principles of GS are fully applicable, the practical implementation of GS activities could be adjusted considering the particular characteristics of the NGT animal-trait-use combination, including the following cases:

- In cases where NGT animals that are (1) contained or semi-contained, (2) do not express traits that could pose adverse environmental effects and (3) these animals, after potential escape, cannot persist in the environment or cannot hybridise with wild relatives (e.g. animals genetically modified for sterility).
- In cases where the trait already exists in the natural or the breeders' gene pool and has a history of safe use.
- In specific cases for certain NGT animals. This could apply to NGT animals that only contain deletions, insertion of endogenous genes (cisgenesis) or edits (SDN-1/SDN-2), depending on the outcome of the ERA.

Clear guidance on fit for purpose approaches for GS should be provided.

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TABLE 29 Applicability and sufficiency of EFSA GMO Panel (2013) section(s) on 'General surveillance' for NGT animals, based on the selected case studies.

Case study	Relevant aspects of the case	Applicability and sufficiency of the guidance	Recommendation for future guidance updates
1, 2, 3, 6	(Semi-) confinement of the animals Hypoallergenicity trait (case 1, 2), increased muscle mass (case 3), hornless trait (case 6) No sexually-compatible wild relatives in the EU Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Hornless trait has a history of safe use in cattle Traits are obtained by gene editing	Fully applicable, insufficient	The guidance should explain that the practical implementation of GS activities could be adjusted considering the particular characteristics of the NGT animal-trait-use combination, including the following cases: – NGT animals that are (1) contained or semi-contained, (2) do not express traits that could pose adverse environmental effects and (3) these animals, after potential escape, cannot persist in the environment or cannot hybridise with wild relatives (e.g. animals genetically modified for sterility). – In case the trait already exists in the natural or the breeders' gene pool and has a history of safe use – NGT animals that only contain deletions, insertion of endogenous genes (cisgenesis) or edits, depending on ERA Clear guidance on fit for purpose approaches for GS should be provided
4, 5	(Semi-)containment of pigs Increased muscle mass trait (case 5), disease resistance trait (case 4, 5) Potential for hybrisation with wild relatives Myostatin knockout (increased muscle mass trait) is present in the gene pool of multiple species Traits are obtained by gene editing	Fully applicable, insufficient	See above
7	(Semi-) confinement of the fish Sterility trait The species could not hybridise with wild relatives in the EU if sterility is demonstrated Sterility trait is present in the gene pool of fish Trait is obtained by gene editing	Fully applicable, insufficient	See above
8	Open release of insects Sterility trait Potential for hybridisation with wild relatives Trait is obtained by gene editing	Fully applicable, insufficient	See above

4 | SPECIFIC AREAS AND ASPECTS OF THE EFSA GUIDANCE DOCUMENTS (EFSA GMO and AHAW PANEL, 2012; EFSA GMO PANEL, 2013) WHICH SHOULD BE UPDATED, ADAPTED OR COMPLEMENTED

Following a thorough evaluation of EFSA GMO and AHAW Panel (2012) and EFSA GMO Panel (2013) the EFSA GMO Panel concluded that their principles and recommendations provide, partially, basis for assessing the risks of NGT animals for food, feed and other agricultural uses. Furthermore, further updates or modifications to the existing text are required on an ad hoc basis in specific areas, and details are given in the Sections 4.1 and 4.2.

4.1 | ToR 4 – Specific areas and aspects of the EFSA guidance (EFSA GMO and AHAW Panel, 2012) which should be updated, adapted or complemented

4.1.1 | Comparative approach

The principle of the comparative approach as introduced in the existing guidelines is considered fully applicable for the safety assessment of NGT animals covering all aspects related to molecular characterisation, food and feed safety, animal health and welfare and environmental risk assessment. It is however recommended that its implementation is more clearly and cohesively described for greater clarity, given the current guidelines does not sufficiently describe how to conduct the comparative approach for NGT animals.

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This may be achieved by defining different scenarios, e.g. by means of a decision tree, on how the comparative assessment should be conducted. Such scenarios could depend on existing knowledge on the function of the trait, the nature of the genetic modification and the outcomes of a comprehensive molecular characterisation of the NGT animal.

The development consensus documents on the biology of food producing animal species and on compositional considerations for new breeds of relevant livestock, fish, birds and invertebrates should be considered, in line with OECD documents for plants.

In line with this recommendation, the consolidation of databases on key phenotypic and compositional parameters as well as animal health and welfare indicators should be supported in view of their use in regulatory risk assessment.

The selection of appropriate comparators is an essential step in risk assessment, and key for the implementation of the comparative approach, and in some cases more than one comparison may be needed across the assessment as a whole, for example one for the impacts on human nutrition and another for potential environmental impacts of commercial production. The guidance should propose more clearly the criteria for the selection of the comparator(s) considering the most updated knowledge on traits, related pathways to harm and NGT animal species at the pre-market stage. This would allow practical recommendations.

Should a comparative analysis of the characteristics of the NGT animal be needed, a future guidance for experimental design, modelling and the use of pre-existing data needs to be updated to address the requirements both for statistically robust assessments and for the minimisation of the use of animals in experiments. The guidance should set-out the criteria for the selection of key parameters, recommendations for the statistical design and data sources on the characteristics of conventionally-bred animals to structure the hazard identification in the areas of food/feed, animal health and welfare and environment.

For such scenarios where animal field studies would be required for comparative analysis, a future guidance should provide practical recommendations on how they should be conducted by addressing experimental design, selection of tissues and key endpoints as well as indicators of animal health and welfare (ABMs) and relevant welfare consequences.

4.1.2 | Molecular characterisation

Part of the EFSA GMO and AHAW Panel (2012) dedicated to the information relating to the genetic modification should be adapted to be suitable to the SDN-1, SDN-2 and SDN-3 applications.

The main difference between the SDN-3 technique and EGTs is that the insertion of the cisgene, intragene or transgene is targeted to a predefined region of the genome. Therefore, SDN-3 reduces the risks associated with potential unintended modifications at the insertion site of the host genome when compared to EGTs. Consequently, aspects of the EFSA GMO and AHAW Panel (2012) addressing risks associated with potential unintended modifications are not fully applicable to the SDN-3 and should be adapted.

Concerning the sequences inserted, in the case of cisgenesis, the potential hazards are of the same nature as conventional breeding, and are reduced compared to intragenesis or transgenesis. In addition, hazards are reduced for intragenesis compared to transgenesis. Consequently, some aspects of the EFSA GMO and AHAW Panel (2012) addressing risks associated with the inserted sequence are not applicable to cisgenic and not fully applicable to intragenic animals produced via EGTs or SDN-3 and should be adapted.

Additional function(s) (e.g enzymatic activity, signalling, gene regulation) introduced via cisgenesis are of the same nature and present the same hazards as those introduced via conventional breeding. In addition, additional function(s) introduced via intragenesis present reduced hazards compared to transgenesis. Consequently, aspects of the molecular characterisation of EFSA GMO and AHAW Panel (2012) addressing risks associated with newly expressed proteins (NEPs), are not fully applicable to cisgenic or to intragenic animals produced via EGTs or SDN-3 and should be adapted.

SDN-1 and SDN-2 techniques aim to modify an endogenous DNA sequence in a targeted manner, meaning that these NGT animals (genome-edited animals) are less likely to present hazards associated with unintended modifications of the host genome than animals obtained by EGTs. Consequently, aspects of the EFSA GMO and AHAW Panel (2012) addressing risks associated with potential unintended modifications are not fully applicable to the SDN-1, SDN-2 techniques.

Genome-edited animals produced via SDN-1 or SDN-2 techniques do not contain any transgenes, intragenes or cisgenes, and genetic changes introduced by these techniques are of the same nature as those introduced through conventional breeding. For these reasons, animals produced via SDN-1 or SDN-2 techniques will not present the hazards associated with NEPs produced by transgenes, intragenes or cisgenes in animals obtained using EGTs or the SDN-3 approach. In consequence, aspects of the EFSA GMO and AHAW Panel (2012) addressing risks associated to NEPs are not valid for the SDN-1 and SDN-2 techniques.

As NGTs provide a spectrum of outcomes (single base alteration through to targeted insertion of large DNA elements) additional refinement is required to define a threshold between SDN-1 and SDN-2 modification of endogenous sequences and SDN-3 approaches.

SDN-1, SDN-2 and SDN-3 techniques can induce off-target changes in the genome of the recipient animal, which are similar in nature to the genetic changes that occur naturally through conventional breeding techniques. Data resources pertaining to the genomes of most farmed animal species have increased since the previous guidance, allowing off-target sites to be computationally predicted and off-target events can be detected during breed development and if necessary screened out before regulatory approval is sought.

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Further, less information may be required on a case-by-case basis with respect to the stability of either inserted foreign DNA or altered endogenous DNA sequences. More information may be provided by the applicant on the relative position and function of known functional elements (e.g. genes and associated regulatory sequences) at the NGT target site.

Where gene editing by SDN-1 of SDN-2 is used to alter an endogenous gene, quantitation of expression of the altered gene (either intentionally or not) should only be required on a case-by-case basis when there is an associated plausible pathway to harm.

Some specific requirements referring to exogenous sequences inserted in the GM animals would not be relevant or may need to be adapted for gene edited sequences in order to cover the use of NGTs in applications; the term 'modification' is more suited in the assessment of MC requirements.

It is recommended that future updated guidance documents include the request of information on the specific DNA changes intended at the target site.

4.1.3 | Food and feed risk assessment

4.1.3.1 | *Toxicology and Allergenicity assessment*

Whereas the principles of the classical approach for the toxicology and allergenicity assessment of new proteins described in the 2012 guidance for GM animals are still considered applicable and sufficient, the GMO Panel foresees the potential need to review future guidelines as new approaches are becoming available for use in risk assessment to streamline and strengthen the protein safety assessment, such as in silico prediction tools and in vitro testing for protein toxicity and allergenicity (EFSA GMO Panel, 2022a, EFSA GMO Panel, 2022b) and a targeted use of in vivo studies according the principles of the 3R's.

While reaffirming that the principles described by the EFSA GMO and AHAW Panels guidance (2012) remain applicable and sufficient for characterising the toxicological profiles of new constituents other than proteins, as well as altered levels of endogenous food and feed constituents, it is important to emphasise that these assessments should be conducted on a case-by-case basis. Additionally, future updated guidelines could benefit from including more specific recommendations, informed by currently available examples.

It is recommended that future updated guidance documents include more detailed methodological approaches and/or indications, applicable beyond the assessment of NGT animals, to evaluate (i) newly expressed proteins, (ii) altered levels of food and feed constituents, (iii) the whole food/feed derived from NGT animals, (iv) adjuvanticity, (v) nutritional aspects and (vi) Post-Market Monitoring.

Moreover, adjuvanticity is currently assessed based on evidence provided by the scientific literature, and further research and validation of experimental methodologies are needed to better address adjuvanticity in a regulatory context (EFSA GMO Panel, 2024).

4.1.3.2 | *Nutritional assessment*

While the general principles are applicable and sufficient for the assessment of NGT animals, the EFSA GMO and AHAW Panel (2012) could provide more details on how to conduct this evaluation. Consequently, future updated guidelines should offer greater precision in detailing methodological aspects. Animal feeding trials involving target species should be considered on a case-by-case basis to evaluate the impact of NGT animal-derived food or feed. This assessment should be informed by a comprehensive characterisation of the genetic modification, including the identification of potential intended and unintended effects in edible tissues, a detailed compositional analysis and toxicological evaluation of any identified effects. If a hazard is identified with a plausible pathway to harm, endpoint measurements should be tailored to the target species and include specific indicators of adverse effects in addition to conventional metrics such as feed intake, body weight, growth performance and nutrient bioavailability. For compositional comparisons, a minimum set of species and tissue specific reference list of compounds should be established. This could include, as appropriate, macronutrients, micronutrients, anti-nutrients, known or potential allergens, toxins and other constituents of toxicological or nutritional relevance. This approach aligns with existing frameworks for the risk assessment of genetically modified plants, as outlined in OECD consensus documents on compositional considerations for individual crops (e.g. OECD, 2001, 2002, 2009, 2012).

4.1.4 | Animal health and welfare

The general principles of the EFSA GMO and AHAW Panel (2012) are partially applicable but insufficient for the assessment of conventional and GM animals, including EGT and NGT animals, and an update in line with the approach described in AHAW 2022 guidance is recommended. The methodology to assess health and welfare must be revised and significantly expanded to incorporate recent knowledge on animal welfare, welfare consequences and the use of animal-based measures (ABMs), as outlined in recent EFSA AHAW opinions (e.g. EFSA AHAW Panel, 2022b in pigs and EFSA AHAW Panel, 2023a in calves) and scientific studies.

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Current EFSA methodology on health and welfare was developed considering mandates received on mammals and bird species, therefore methodology on fish and invertebrates needs to be further addressed.

The guidance focuses mainly on animal function and does not mention for example affective states (the valence of how animals perceive the world), which are now the core of the definition of the EFSA animal welfare consequences (EFSA AHAW Panel, 2022a), and need to be considered for GM animals including EGTs and NGT animals.

The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals.

Risk assessments for animal health and welfare should encompass all in vivo phases under the remit of EFSA and need to focus on guidance to applicants and assessors which is case-specific, hypothesis driven and proportionate.

Guidance should be given in terms of statistical analyses, modelling and experimental design and be reviewed in light of 3R's principles and potential applications of artificial intelligence etc. EFSA methodology on health and welfare of fish and invertebrates should be further developed to be fit for purpose. This should include, for all animal species, that the focus of the guidance should be moved from 'animal function' to affective states (the valence of how animals perceive the world). Part of this work involves the incorporation of recent knowledge on animal welfare, welfare consequences and the use of animal-based measures (ABMs). In addition, the assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals. Identification of measures and endpoints to evaluate animal health and welfare is deemed needed, as well harmonisation in the EU assessment.

4.1.5 | Post-market monitoring

The principles and objectives of post-market monitoring (PMM), as outlined in the EFSA GMO and AHAW Panel (2012) guidance, are applicable to NGT animals. However, the guidance lacks detailed instructions on when and how to conduct PMM, leaving room for optimisation in future revisions. Furthermore, there is a need to strengthen the interconnection between pre-market risk assessment and PMM. Monitoring data is valuable to complement the pre-market risk assessments by tracing real-world impacts on human and animal safety and on animal welfare, and it can help to detect risks or long-term effects that may not have been anticipated during controlled trials, which could lead to changes in the risk assessment conclusions.

4.2 | ToR 4 – Specific areas and aspects of the EFSA guidance (EFSA GMO Panel, 2013) which should be updated, adapted or complemented

4.2.1 | Environmental risk assessment

The GMO Panel recommends that updated guidance highlights that the ERA should be case-specific and hypothesis-driven, with the aim to test the most plausible and robust pathways to harm. It should stress that only the first step in the ERA (problem formulation including hazard identification) is needed for all NGT animals, whereas the next steps are only needed if a plausible hazard is identified. In particular, ERA can stop at hazard identification if the trait is already present in the gene pool of the species, has a history of safe use in the species and/or no unintended effects are identified that could lead to a hazard. Any hazard should be specified in relation to specific protection goals and limits of concern clearly defined by risk managers. The guidance should be updated to indicate that the comparative approach cannot always rely on non-GM/non-NGT comparator(s) and clearly indicate that experience with additional comparators, including non-animal comparators, may also be useful for the ERA as part of a weight-of-evidence approach.

Updated guidance should highlight that receiving environment(s) only have to be characterised in cases where phenotypic differences are found that could lead to plausible pathways to harm. The level of characterisation required is based on specific factors such as level of intended confinement or potential for hybridisation of the modified animal with wild relatives, and it should be clearly defined.

The updated guidance should clearly specify that data should be collected or generated only to test hypotheses to harms identified in the problem formulation, and not to measure environmental change that does not impact defined protection goals.

Should experimental data be required to complete the risk assessment, updated guidance should clearly state that experimental designs should be powerful enough to provide the required data whilst considering the importance of the 3R's principle in animal experiments (Hubrecht & Carter, 2019). Clear guidance should be provided on how to perform and report the statistical analyses. Additionally, the value of modelling for risk characterisation should be highlighted.

Updated guidance should reflect that assessment of long-term effects should only focus on potential harms, which may involve quantitative forecasts.

4.2.2 | Aspects of GM animal health and welfare

The methodology to assess health and welfare should be revised and significantly expanded to incorporate recent knowledge on animal welfare, welfare consequences and the use of animal-based measures (ABMs). For all animal species the

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focus of the guidance should be moved from 'animal function' to affective states (the valence of how animals perceive the world), which are the core of the definition of the EFSA animal welfare consequence. The assessment should encompass the agricultural practices in which the animals are kept, including the global trade of live animals. Risk assessments for animal health and welfare should encompass all in vivo phases under the remit of EFSA. EFSA methodology on health and welfare of fish, and insects and other invertebrates should be further developed to be fit for purpose.

Different sections of the guidance regarding the specific areas of risk should be updated:

- The guidance should clearly state that in cases where the introduced trait leads to sterility, the efficiency of the trait should be confirmed, as this will be highly relevant for the assessment of the risk of increased persistence and invasiveness.
- The GMO Panel/WG further recommends updating the guidance to clearly state that only in those cases in which the introduced trait leads to modified susceptibility to pathogens should this area of concern be assessed in the pre-market risk assessment.
- Updated guidance should clarify that the assessment of potential HGT is only relevant in cases in which the genetic modification includes the introduction of a gene/sequence of concern (e.g. AMR gene) which could be selectively retained and lead to a harm.
- Updated guidance regarding potential interactions with target organisms should specify that the evolution of resistance in target pests is regarded both as an environmental harm and an economic one. Additionally, hazards to non-GM animals as a result of the evolution of resistance of the target pathogen in a disease-resistant NGT animals should be addressed under the areas of concern related to pathogens, infections and diseases.
- Updated guidance on potential interactions with NTOs should focus on data collection to test risk hypotheses connected to protection goals, and emphasise that in cases where field data is deemed necessary for the risk assessment, it should preferentially come from studies performed in EU environments.
- Updated guidance should specify that an assessment of the potential environmental impacts associated with the management of NGT animals should only be carried out in those cases in which an altered regimen which could be associated with the potential for greater environmental harms is applied to the NGT animals as compared to current practices in conventionally-bred animals.
- Updated guidance should provide concrete examples to illustrate the potential harms to the abiotic environment that could result from NGT animals as compared to conventionally-bred animals.

4.2.3 | Post-market environmental monitoring

Updated guidance in this area should clearly indicate that case-specific monitoring is only required in cases in which either a risk or significant levels of critical uncertainty have been identified during the ERA. When needed, CSM should be proportionate and hypothesis-driven.

The feedback loop that integrates monitoring data and environmental risk assessment should be improved. Updated guidance should acknowledge that general surveillance is related to the evolution of agroecosystems and consequently ideally it should cover the environmental impacts of the range of stressors and systems interacting therein, and not be limited to a specific one (i.e. GMOs). However, the GMO Panel acknowledges that the current legislative framework requires applicants to monitor potential adverse effects related only to the regulated product they intend to place on the market, and it recommends risk managers to discuss the feasibility of an approach that considers the range of environmental stressors affecting agroecosystems. Guidance should be applicable and sufficient to allow applicants to implement general surveillance activities.

Finally, updated guidance should refer to the most recent regulatory framework and applicable EFSA guidances, which includes citing Directive 2018/350, EFSA guidance on uncertainty analysis (EFSA Scientific Committee, 2018) and the most recent EFSA guidance on Animal Health and Welfare (e.g. EFSA AHAW Panel, 2022a).

5 | OVERALL CONCLUSIONS

When the existing EFSA guidance documents (EFSA GMO and AHAW Panel, 2012; EFSA GMO Panel, 2013) for risk assessment of GM animals were first developed, genetic modification primarily involved the random insertion of exogenous gene cassettes into the genomes of recipient animals. At that time, limited knowledge of whole genome sequences and gene functions in animals used for food, feed and other agricultural uses introduced considerable uncertainty about the potential effects of these modifications. These uncertainties formed the basis for the development of the existing guidelines.

Since then, the field has progressed considerably. NGTs, such as CRISPR/Cas and other precision-editing tools, have enabled targeted genetic alterations, often without requiring the insertion of exogenous gene cassettes. These technologies, which do not themselves pose additional risks, allow for more predictable outcomes, minimising hazards associated with earlier methods.

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As the potential risks associated with the genetic modification process are increasingly well understood, future guidelines could place greater emphasis on evaluating the introduced trait(s) or product as well as their interaction with receiving environment, rather than focusing on the method used to create the genetic modification.

A horizon-scanning exercise identified a variety of animals obtained with NGTs (NGT animals), with the potential to reach the EU market in the short, medium and long term, based on the current stage of market development (commercial, pre-commercial, research and development).

No novel hazards have been identified that are linked to either the modification process or the newly introduced trait, when SDN-1, SDN-2 and comparable techniques (e.g. base editing or prime editing) were compared to established genomic techniques (EGTs) or conventional breeding. Hazards posed by SDN-3 are of the same nature as those posed by EGTs and the targeted insertion may reduce the potential hazards associated with the disruption of endogenous genes and/or regulatory elements in the recipient genome. Hazards posed by the new trait resulting from the introduced transgenic or intragenic DNA sequence are of the same nature as those posed by EGTs. Hazards posed by the new trait resulting from the introduced cisgenic DNA sequence are of the same nature as those posed by conventional breeding. Off-target mutations from genome editing are similar in nature to those from conventional breeding and do not pose novel hazards. Consequently, based on the currently available data, no new potential hazards, and thus, no new risks to humans, animals or the environment have been identified.

Animal health and welfare hazards in EGT and NGT animals related to pre-commercial phases should be addressed through case-by-case evaluations when informative to the risk assessment, as earliest procedural steps may result in unintended phenotypes that are sometimes associated with advanced breeding technologies.

A thorough evaluation of existing EFSA guidance documents for the risk assessment of GM animals revealed that their principles and recommendations provide the basis for assessing the risks of NGT animals for food, feed and other agricultural uses; however, the current text covers only partially the topics in several areas (e.g. animal health and welfare), and might require further updates, adaptations or enhancements on an ad hoc basis, to address the risk assessment of NGT animals, as outlined in this opinion.

ABBREVIATIONS

ABMs	animal-based measures
ABP	animal byproducts
DSB	double-strand break
EGTs	established genomic techniques
ESCs	embryonic stem cells
IGF2	insulin-like growth factor-2
iPSC	induced pluripotent stem cells
MSCs	mesenchymal stromal cells
MSTN	myostatin
NGTs	new genomic techniques
OECD	Organisation for Economic Co-operation and Development
PAP	processed animal protein
PEDV	porcine epidemic diarrhoea virus
PGCLCs	primordial germ cell-like cells
pgSIT	precision guided sterile insect technique
PMEM	post-market environmental monitoring
RNPs	ribonucleoproteins
SCENIHR	European Commission's Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SDNs	site-directed nucleases
SSCs	spermatagonial stem cells
TAL	transcription activator-like (TAL) effector nuclease
TALEN TAL	transcription activator-like (TAL) effector nuclease
TGEV	transmissible gastroenteritis virus
ToRs	Terms of References

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REFERENCES

Atkins, A., Chung, C. H., Allen, A. G., Dampier, W., Gurrola, T. E., Sariyer, I. K., Nonnemacher, M. R., & Wigdahl, B. (2021). Off-target analysis in gene editing and applications for clinical translation of CRISPR/Cas9 in HIV-1 therapy. *Frontiers in Genome Editing*, *3*, 673022. https://doi.org/10.3389/fgeed.2021.673022

Bishop, T. F., & Van Eenennaam, A. L. (2020). Genome editing approaches to augment livestock breeding programs. *The Journal of Experimental Biology*, *223*(Pt Suppl 1). https://doi.org/10.1242/jeb.207159

Broothaerts, W., Jacchia, S., Angers, A., Petrillo, M., Querci, M., Savini, C., Van den Eede, G., & Emons, H. (2021). New Genomic Techniques: State-of-the-Art Review, EUR 30430 EN, Publications Office of the European Union, Luxembourg, ISBN 978–92–76-24696-1, JRC121847.

Burger, B. T., Beaton, B. P., Campbell, M. A., Brett, B. T., Rohrer, M. S., Plummer, S., Barnes, D., Jiang, K., Naswa, S., Lange, J., Ott, A., Alger, E., Rincon, G., Rounsley, S., Betthauser, J., Mtango, N. R., Benne, J. A., Hammerand, J., Durfee, C. J., … Cigan, A. M. (2024). Generation of a commercial-scale founder population of porcine reproductive and respiratory syndrome virus resistant pigs using CRISPR-Cas. *CRISPR Journal*, *7*(1), 12–28. https://doi.org/10.1089/crispr.2023.0061

Burkard, C., Lillico, S. G., Reid, E., Jackson, B., Mileham, A. J., Ait-Ali, T., Whitelaw, C. B., & Archibald, A. L. (2017). Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLoS Pathogens*, *13*(2), e1006206. https://doi.org/10.1371/journal.ppat.1006206

Carlson, D. F., Lancto, C. A., Zang, B., Kim, E. S., Walton, M., Oldeschulte, D., Seabury, C., Sonstegard, T. S., & Fahrenkrug, S. C. (2016). Production of hornless dairy cattle from genome-edited cell lines. *Nature Biotechnology*, *34*(5), 479–481. https://doi.org/10.1038/nbt.3560

Devos, Y., Craig, W., Devlin, R. H., Ippolito, A., Leggatt, R. A., Romeis, J., Shaw, R., Svendsen, C., & Topping, C. J. (2019). Using problem formulation for fit-for-purpose pre-market environmental risk assessments of regulated stressors. *EFSA Journal*, *17*(Suppl 1), e170708. https://doi.org/10.2903/j.efsa.2019.e170708

Dong, Y., Li, H., Zhao, L., Koopman, P., Zhang, F., & Huang, J. X. (2019). Genome-wide off-target analysis in CRISPR-Cas9 modified mice and their offspring. *G3 (Bethesda)*, *9*(11), 3645–3651. https://doi.org/10.1534/g3.119.400503

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2007). Scientific Opinion of the panel on animal health and welfare on a request from the commission on animal health and welfare aspects of different housing and husbandry systems for adult breeding boars, pregnant, farrowing sows and unweaned piglets. *EFSA Journal*, *5*(10), 572. https://doi.org/10.2903/j.efsa.2007.572

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2010). Scientific Opinion on welfare aspects of the management and housing of the grand-parent and parent stocks raised and kept for breeding purposes. *EFSA Journal*, *8*(7), 1667. https://doi.org/10.2903/j.efsa.2010.1667

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2012a). Guidance on risk assessment for animal welfare. *EFSA Journal*, *10*(1), 2513. https://doi.org/10.2903/j.efsa.2012.2513

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2012b). Statement on the use of animal-based measures to assess the welfare of animals. *EFSA Journal*, *10*(6), 2767. https://doi.org/10.2903/j.efsa.2012.2767

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2012c). Scientific Opinion on the use of animal-based measures to assess welfare of dairy cows. *EFSA Journal*, *10*(1), 2554. https://doi.org/10.2903/j.efsa.2012.2554

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2012d). Scientific Opinion on the use of animal-based measures to assess welfare in pigs. *EFSA Journal*, *10*(1), 2512. https://doi.org/10.2903/j.efsa.2012.2512

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022a). Scientific Opinion on the methodological guidance for the development of animal welfare mandates in the context of the farm to fork strategy. *EFSA Journal*, *20*(7), 7403. https://doi.org/10.2903/j.efsa.2022.7403

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022b). Scientific Opinion on the welfare of pigs on farm. *EFSA Journal*, *20*(8), 7421. https://doi.org/10.2903/j.efsa.2022.7421

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022c). Scientific Opinion on the welfare of small ruminants during transport. *EFSA Journal*, *20*(9), 7404. https://doi.org/10.2903/j.efsa.2022.7404

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022d). Welfare of cattle during transport. *EFSA Journal*, *20*(9), 7442. https://doi.org/10.2903/j.efsa.2022.7442

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022e). Scientific Opinion on the welfare of equidae during transport. *EFSA Journal*, *20*(9), 7444. https://doi.org/10.2903/j.efsa.2022.7444

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2022f). Scientific Opinion on the welfare of pigs during transport. *EFSA Journal*, *20*(9), 7445. https://doi.org/10.2903/j.efsa.2022.7445

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2023a). Scientific Opinion on the welfare of calves. *EFSA Journal*, *21*(3), 7896. https://doi.org/10.2903/j.efsa.2023.7896

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2023b). Scientific Opinion on the welfare of broilers on farm. *EFSA Journal*, *21*(2), 7788. https://doi.org/10.2903/j.efsa.2023.7788

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2023c). Scientific Opinion on the welfare of laying hens on farm. *EFSA Journal*, *21*(2), 7789. https://doi.org/10.2903/j.efsa.2023.7789

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare). (2023d). Scientific Opinion on the welfare of ducks, geese and quail on farm. *EFSA Journal*, *21*(5), 7992. https://doi.org/10.2903/j.efsa.2023.7992

EFSA GMO and AHAW Panel (EFSA Panel on Genetically Modified Organisms and EFSA Panel on Animal Health and Welfare). (2012). Scientific Opinion on the guidance on the risk assessment of food and feed from genetically modified animals and animal health and welfare aspects. *EFSA Journal*, *10*(1), 2501. https://doi.org/10.2903/j.efsa.2012.2501

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011a). Scientific Opinion on guidance for risk assessment of food and feed from genetically modified plants. *EFSA Journal*, *9*(5), 2150. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011b). Scientific Opinion on guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. *EFSA Journal*, *9*(5), 2149. https://doi.org/10.2903/j.efsa.2011.2149

18314732, 2025, 8, Downloaded from https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2025.9566 by Universita'Degli Studi Di Mila, Wiley Online Library on [04/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2012). Scientific Opinion addressing the safety assessment of plants developed using zinc finger nuclease 3 and other site-directed nucleases with similar function. *EFSA Journal*, *10*(10), 2943. https://doi.org/10.2903/j.efsa.2012.2943
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2013). Guidance on the environmental risk assessment of genetically modified animals. *EFSA Journal*, *11*(5), 3200. https://doi.org/10.2903/j.efsa.2013.3200
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2020a). Applicability of the EFSA opinion on site-directed nucleases type 3 for the safety assessment of plants developed using site-directed nucleases type 1 and 2 and oligonucleotide-directed mutagenesis. *EFSA Journal*, *18*(11), 6299. https://doi.org/10.2903/j.efsa.2020.6299
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2020b). Scientific Opinion on the adequacy and sufficiency evaluation of existing EFSA guidelines for the molecular characterisation, environmental risk assessment and post-market environmental monitoring of genetically modified insects containing engineered gene drives. *EFSA Journal*, *18*(11), 6297. https://doi.org/10.2903/j.efsa.2020.6297
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2021). Scientific Opinion on the evaluation of existing guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified plants obtained through synthetic biology. *EFSA Journal*, *19*(2), 6301. https://doi.org/10.2903/j.efsa.2021.6301
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2022a). Scientific Opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology. *EFSA Journal*, *20*(7), 7410. https://doi.org/10.2903/j.efsa.2022.7410
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2022b). Updated scientific opinion on plants developed through cisgenesis and intragenesis. *EFSA Journal*, *20*(10), 7621. https://doi.org/10.2903/j.efsa.2022.7621
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2022c). Statement on criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis. *EFSA Journal*, *20*(10), 7618. https://doi.org/10.2903/j.efsa.2022.7618
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2022d). Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. *EFSA Journal*, *20*(1), 7044. https://doi.org/10.2903/j.efsa.2022.7044
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2024). New developments in biotechnology applied to microorganisms. *EFSA Journal*, *22*(7), 8895. https://doi.org/10.2903/j.efsa.2024.8895
EFSA Scientific Committee. (2018). Guidance on uncertainty analysis in scientific assessments. *EFSA Journal*, *16*(1), 5123. https://doi.org/10.2903/j.efsa.2018.5123
EFSA Scientific Committee. (2020). Scientific Opinion on the evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology. *EFSA Journal*, *18*(10), 6263. https://doi.org/10.2903/j.efsa.2020.6263
EFSA Scientific Committee. (2022). Scientific Opinion on the evaluation of existing guidelines for their adequacy for the food and feed risk assessment of microorganisms obtained through synthetic biology. *EFSA Journal*, *20*(8), 7479. https://doi.org/10.2903/j.efsa.2022.7479
Ezaki, R., Sakuma, T., Kodama, D., Sasahara, R., Shiraogawa, T., Ichikawa, K., Matsuzaki, M., Handa, A., Yamamoto, T., & Horiuchi, H. (2023). Transcription activator-like effector nuclease-mediated deletion safely eliminates the major egg allergen ovomucoid in chickens. *Food and Chemical Toxicology*, *175*, 113703. https://doi.org/10.1016/j.fct.2023.113703
Fraser, D. (2009). Assessing animal welfare: Different philosophies, different scientific approaches. *Zoo Biology*, *28*(6), 507–518. https://doi.org/10.1002/zoo.20253
Fujihara, R., Katayama, N., Sadaie, S., Miwa, M., Sanchez Matias, G. A., Ichida, K., Fujii, W., Naito, K., Hayashi, M., & Yoshizaki, G. (2022). Production of germ cell-less rainbow trout by dead end gene knockout and their use as recipients for germ cell transplantation. *Marine Biotechnology (New York, N.Y.)*, *24*(2), 417–429. https://doi.org/10.1007/s10126-022-10128-w
Gui, T., Zhang, J., Song, F., Sun, Y., Xie, S., Yu, K., & Xiang, J. (2016). CRISPR/Cas9-mediated genome editing and mutagenesis of EcChi4 in Exopalaemon carinicauda. *G3 (Bethesda)*, *6*(11), 3757–3764. https://doi.org/10.1534/g3.116.034082
Hennig, S. L., Owen, J. R., Lin, J. C., Young, A. E., Ross, P. J., Van Eenennaam, A. L., & Murray, J. D. (2020). Evaluation of mutation rates, mosaicism and off target mutations when injecting Cas9 mRNA or protein for genome editing of bovine embryos. *Scientific Reports*, *10*(1), 22309. https://doi.org/10.1038/s41598-020-78264-8
Hruscha, A., Krawitz, P., Rechenberg, A., Heinrich, V., Hecht, J., Haass, C., & Schmid, B. (2013). Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. *Development*, *140*(24), 4982–4987. https://doi.org/10.1242/dev.099085
Hubrecht, R. C., & Carter, E. (2019). The 3Rs and humane experimental technique: Implementing change. *Animals (Basel)*, *9*(10), 754. https://doi.org/10.3390/ani9100754
Idoko-Akoh, A., Goldhill, D. H., Sheppard, C. M., Bialy, D., Quantrill, J. L., Sukhova, K., Brown, J. C., Richardson, S., Campbell, C., Taylor, L., Sherman, A., Nazki, S., Long, J. S., Skinner, M. A., Shelton, H., Sang, H. M., Barclay, W. S., & McGrew, M. J. (2023). Creating resistance to avian influenza infection through genome editing of the ANP32 gene family. *Nature Communications*, *14*(1), 6136. https://doi.org/10.1038/s41467-023-41476-3
Kandul, N. P., Liu, J. R., Buchman, A., Shriner, I. C., Corder, R. M., Warsinger-Pepe, N., Yang, T., Yadav, A. K., Scott, M. J., Marshall, J. M., & Akbari, O. S. (2022). Precision guided sterile males suppress populations of an invasive crop Pest. *Genetic Biotechnology*, *1*(4), 372–385. https://doi.org/10.1089/genbio.2022.0019
Kim, J. W., Kim, J., Cho, J. Y., Shin, Y., Son, H., Sathiyamoorthy, S., Kim, B. S., Kim, Y. O., Kang, B. C., & Kong, H. J. (2024). Association between muscle growth and transcription of a mutant MSTN gene in olive flounder (*Paralichthys olivaceus*). *Marine Biotechnology*, *26*, 599–608. https://doi.org/10.1007/s10126-024-10322-y
Kishimoto, K., Washio, Y., Murakami, Y., Katayama, T., Kuroyanagi, M., Kato, K., Yoshiura, Y., & Kinoshita, M. (2019). An effective microinjection method for genome editing of marine aquaculture fish: Tiger pufferfish *Takifugu rubripes* and red sea bream *Pagrus major*. *Fisheries Science*, *85*(1), 217–226. https://doi.org/10.1007/s12562-018-1277-3
Kishimoto, K., Washio, Y., Yoshiura, Y., Toyoda, A., Ueno, T., Fukuyama, H., Kato, K., & Kinoshita, M. (2018). Production of a breed of red sea bream *Pagrus major* with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. *Aquaculture*, *495*, 415–427. https://doi.org/10.1016/j.aquaculture.2018.05.055
Koslova, A., Trefil, P., Mucksova, J., Reinisova, M., Plachy, J., Kalina, J., Kucerova, D., Geryk, J., Krchlikova, V., Lejckova, B., & Hejnar, J. (2020). Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(4), 2108–2112. https://doi.org/10.1073/pnas.1913827117
Kou, Z., Wang, S., Luo, X., Xu, J., Tomberlin, J. K., & Huang, Y. (2024). Wingless strain created using binary transgenic CRISPR/Cas9 alleviates concerns about mass rearing of Hermetia illucens. *Communications Biology*, *7*(1), 1652. https://doi.org/10.1038/s42003-024-07254-7
Lee, J., Kim, D. H., & Lee, K. (2020). Muscle hyperplasia in Japanese quail by single amino acid deletion in MSTN Propeptide. *International Journal of Molecular Sciences*, *21*(4), 1504. https://doi.org/10.3390/ijms21041504
Mellor, D. J. (2017). Operational details of the five domains model and its key applications to the assessment and Management of Animal Welfare. *Animals*, *7*(8), 60. https://doi.org/10.3390/ani7080060
Mellor, D. J., & Beausoleil, N. J. (2015). Extending the 'five domains' model for animal welfare assessment to incorporate positive welfare states. *Animal Welfare*, *24*(3), 241–253. https://doi.org/10.7120/09627286.24.3.241

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Nawroth, C., & Rorvang, M. V. (2022). Opportunities (and challenges) in dairy cattle cognition research: A key area needed to design future high welfare housing systems. *Applied Animal Behaviour Science*, *255*, 105727. https://doi.org/10.1016/j.applanim.2022.105727

Nie, H. Y., Liang, L. Q., Li, Q. F., Li, Z. H. Q., Zhu, Y. N., Guo, Y. K., Zheng, Q. L., Lin, Y., Yang, D. L., Li, Z. G., & Su, S. K. (2021). CRISPR/Cas9 mediated knockout of gene results in melanization defect of the cuticle in adult *Apis mellifera*. *Journal of Insect Physiology*, *132*. https://doi.org/10.1016/j.jinsphys.2021.104264

Norris, A. L., Lee, S. L. S., Greenlees, K. J., Tadesse, D. A., Miller, M. F., & Lombardi, H. A. (2020). Template plasmid integration in germline genome-edited cattle. *Nature Biotechnology*, *38*(2), 163–164. https://doi.org/10.1038/s41587-019-0394-6

OECD (Organisation for Economic Co-operation and Development). (2001). Consensus document on key nutrients and key toxicants in low erucic acid rapeseed (canola). Series on the Safety of Novel Foods and Feeds, No. 1-ENV/JM/MONO(2001)13.

OECD (Organisation for Economic Co-operation and Development). (2002). Consensus document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti- nutrients and secondary plant metabolites. Series on the safety of novel foods and feeds, No. 6-ENV/JM/MONO(2002)25.

OECD (Organisation for Economic Co-operation and Development). (2009). Consensus document on compositional considerations for new varieties of cotton (Gossypium hirsutum and Gossypium barbadense): key food and feed nutrients and anti-nutrients. Series on the Safety of Novel Foods and Feeds, No 11, ENV/JM/MONO(2004)16.

OECD (Organisation for Economic Co-operation and Development). (2012). Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [Glycine max (L.) Merr.]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens. No. 25-ENV/JM/MONO(2012)24.

Parisi, C., & Rodríguez-Cerezo, E. (2021). Current and future market applications of new genomic techniques. EUR 30589 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978–92–76-30206-3. https://doi.org/10.2760/02472

Peterson, K. A., Khalouei, S., Hanafi, N., Wood, J. A., Lanza, D. G., Lintott, L. G., Willis, B. J., Seavitt, J. R., Braun, R. E., Dickinson, M. E., White, J. K., Lloyd, K. C. K., Heaney, J. D., Murray, S. A., Ramani, A., & Nutter, L. M. J. (2023). Whole genome analysis for 163 gRNAs in Cas9-edited mice reveals minimal off-target activity. *Communications Biology*, *6*(1), 626. https://doi.org/10.1038/s42003-023-04974-0

Proudfoot, C., Carlson, D. F., Huddart, R., Long, C. R., Pryor, J. H., King, T. J., Lillico, S. G., Mileham, A. J., McLaren, D. G., Whitelaw, C. B. A., & Fahrenkrug, S. C. (2015). Genome edited sheep and cattle. *Transgenic Research*, *24*(1), 147–153. https://doi.org/10.1007/s11248-014-9832-x

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J. S., Ringkamp, M., Sluka, K. A., Song, X. J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain*, *161*, 1976–1982. https://doi.org/10.1097/j.pain.0000000000001939

Rodriguez-Villamil, P., Ongaratto, F., Bostrom, J., Larson, S., & Sonstegard, T. (2021). 13 generation of SLICK beef cattle by embryo microinjection: A case report. *Reproduction, Fertility and Development*, *33*, 114.

Seruggia, D., Fernández, A., Cantero, M., Pelczar, P., & Montoliu, L. (2015). Functional validation of mouse *tyrosinase* non-coding regulatory DNA elements by CRISPR-Cas9-mediated mutagenesis. *Nucleic Acids Research*, *43*(10), 4855–4867. https://doi.org/10.1093/nar/gkv375

Shirai, Y., Piulachs, M. D., Belles, X., & Daimon, T. (2022). DIPA-CRISPR is a simple and accessible method for insect gene editing. *Cell Reports Methods*, *2*(5), 100215. https://doi.org/10.1016/j.crmeth.2022.100215

Song, L., Xu, Y. F., Zhao, Y. Q., Wang, J. W., & Guo, H. R. (2024). Identification of active shrimp U6 promoters for efficient CRISPR/Cas9 gene editing in penaeid shrimps based on a VP28-pseudotyped baculovirus expression system. *Aquaculture*, *591*, 741128. https://doi.org/10.1016/j.aquaculture.2024.741128

Song, R. G., Wang, Y., Zheng, Q. T., Yao, J., Cao, C. W., Wang, Y. F., & Zhao, J. G. (2022). One-step base editing in multiple genes by direct embryo injection for pig trait improvement. *Science China Life Sciences*, *65*(4), 739–752. https://doi.org/10.1007/s11427-021-2013-8

Sui, Z., Wu, Q., Geng, J., Xiao, J., & Huang, D. (2024). CRISPR/Cas9-mediated efficient white genome editing in the black soldier fly Hermetia illucens. *Molecular Genetics and Genomics*, *299*(1), 5. https://doi.org/10.1007/s00438-023-02088-0

Sun, Y., Zhang, J., & Xiang, J. (2017). A CRISPR/Cas9-mediated mutation in chitinase changes immune response to bacteria in Exopalaemon carinicauda. *Fish & Shellfish Immunology*, *71*, 43–49. https://doi.org/10.1016/j.fsi.2017.09.065

Tan, W. F., Carlson, D. F., Lancto, C. A., Garbe, J. R., Webster, D. A., Hackett, P. B., & Fahrenkrug, S. C. (2013). Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(41), 16526–16531. https://doi.org/10.1073/pnas.1310478110

Van Eenennaam, A. L. (2023). New genomic techniques (NGTs) animals and their Agri/food/feed products. *EFSA Supporting Publications*, *20*(9), EN-8311. https://doi.org/10.2903/sp.efsa.2023.EN-8311

Wang, X., Liu, J., Niu, Y., Li, Y., Zhou, S., Li, C., Ma, B., Kou, Q., Petersen, B., Sonstegard, T., Huang, X., Jiang, Y., & Chen, Y. (2018). Low incidence of SNVs and indels in trio genomes of Cas9-mediated multiplex edited sheep. *BMC Genomics*, *19*(1), 397. https://doi.org/10.1186/s12864-018-4712-z

Weary, D. M., Droege, P., & Braithwaite, V. A. (2017). Behavioral evidence of felt emotions: Approaches, inferences, and refinements. *Advances in the Study of Behavior*, *49*(49), 27–48. https://doi.org/10.1016/bs.asb.2017.02.002

Wei, J. W., Wagner, S., Maclean, P., Brophy, B., Cole, S., Smolenski, G., Carlson, D. F., Fahrenkrug, S. C., Wells, D. N., & Laible, G. (2018). Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin. *Scientific Reports*, *8*, 7661. https://doi.org/10.1038/s41598-018-25654-8

Whitelaw, B. (2019). We have entered the era of genome-edited farmed animals. *Emerging Topics in Life Sciences*, *3*(5), 645–649. https://doi.org/10.1042/ETLS20190057

Whitworth, K. M., Rowland, R. R. R., Petrovan, V., Sheahan, M., Cino-Ozuna, A. G., Fang, Y., Hesse, R., Mileham, A., Samuel, M. S., Wells, K. D., & Prather, R. S. (2019). Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Research*, *28*(1), 21–32. https://doi.org/10.1007/s11248-018-0100-3

Wu, Y., Wu, T. F., Yang, L. Y., Su, Y., Zhao, C. H., Li, L., Cai, J., Dai, X. Y., Wang, D. S., & Zhou, L. Y. (2023). Generation of fast growth Nile tilapia (*Oreochromis niloticus*) by myostatin gene mutation. *Aquaculture*, *562*, 738762. https://doi.org/10.1016/j.aquaculture.2022.738762

Young, A. E., Mansour, T. A., McNabb, B. R., Owen, J. R., Trott, J. F., Brown, C. T., & Van Eenennaam, A. L. (2020). Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. *Nature Biotechnology*, *38*(2), 225–232. https://doi.org/10.1038/s41587-019-0266-0

Yu, H., Li, H. J., Li, Q., Xu, R., Yue, C. Y., & Du, S. J. (2019). Targeted gene disruption in Pacific oyster based on CRISPR/Cas9 ribonucleoprotein complexes. *Marine Biotechnology*, *21*(3), 301–309. https://doi.org/10.1007/s10126-019-09885-y

Zhang, J. Q., Song, F. G., Sun, Y. Y., Yu, K. J., & Xiang, J. H. (2018). CRISPR/Cas9-mediated deletion of *EcMIH* shortens metamorphosis time from mysis larva to postlarva of *Exopalaemon carinicauda*. *Fish & Shellfish Immunology*, *77*, 244–251. https://doi.org/10.1016/j.fsi.2018.04.002

Zhang, X. H., Tee, L. Y., Wang, X. G., Huang, Q. S., & Yang, S. H. (2015). Off-target effects in CRISPR/Cas9-mediated genome engineering. *Molecular Therapy--Nucleic Acids*, *4*, e264. https://doi.org/10.1038/mtna.2015.37

SUPPORTING INFORMATION

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APPENDIX A

List of welfare consequence and negative affective states as reported in EFSA AHAW panel guidance (2022a)

TABLE A.1 List and description of 33 welfare consequences used for all animal species as produced via expert discussion (EFSA AHAW Panel, 2022a).

	Welfare consequence	Description
1	Bone lesions (incl. fractures and dislocations)	The animal experiences negative affective states such as pain, discomfort and/or distress due to fractures or dislocations of the bones (excluding those fractures leading to locomotor disorders).
2	Cold stress	The animal experiences stress and/or negative affective states such as discomfort and/or distress when exposed to low effective temperature.
3	Eye disorders	The animal experiences negative affective states such as discomfort, pain and/or distress due irritation or lesion or lack of function of at least one eye.
4	Group stress	The animal experiences stress and/or negative affective states such as pain, fear and/or frustration resulting from a high incidence of aggressive and other types of negative social interactions, often due to hierarchy formation and competition for resources or mates.
5	Gastro-enteric disorders	The animal experiences negative affective states such as discomfort, pain and/or distress due to impaired function or lesion of the gastrointestinal tract resulting from for example nutritional deficiency, infectious, parasitic or toxigenic agents.
6	Handling stress	The animal experiences stress and/or negative affective states such as pain and/or fear resulting from human or mechanical handling (e.g. sorting and vaccination of newly hatched chicks, loading/unloading, catching and crating of animals to be transported, inversion).
7	Heat stress	The animal experiences stress and/or negative affective states such as discomfort and/or distress when exposed to high effective temperature.
8	Inability to avoid unwanted sexual behaviour	The animal experiences stress and/or negative affective states such as pain and/or fear resulting from inability to avoid forced mating.
9	Inability to perform exploratory or foraging behaviour	The animal experiences stress and/or negative affective states such as frustration and/or boredom resulting from the thwarting of the motivation to investigate the environment or to seek for food (i.e. extrinsically and intrinsically motivated exploration).
10	Inability to express maternal behaviour	The animal experiences stress and/or negative affective states such as frustration resulting from the thwarting of the motivation to care for offspring, including during the pre-partum/pre-laying phase.
11	Inability to perform sucking behaviour	The animal experiences stress and/or negative affective states such as frustration resulting from the thwarting of the motivation to suck from an udder.
12	Inability to chew and/or ruminate	The animal experiences stress and/or negative affective states such as frustration resulting from the thwarting of the motivation to ingest sufficient amounts of fibrous feed or the inhibition of rumination.
13	Inability to perform play behaviour	The animal experiences stress and/or negative affective states such as frustration resulting from the thwarting of the motivation to engage in social/locomotor or object play.
14	Inability to perform sexual behaviour	The animal experiences stress and/or negative affective states such as frustration resulting from the thwarting of the motivation to engage in sexual activities.
15	Inability to perform comfort behaviour	The animal experiences stress and/or negative affective states such as discomfort and/or frustration resulting from the thwarting of the motivation to maintain the function and integrity of the integument (e.g. cannot keep clean, scratch, dust bathe).
16	Isolation stress	The animal experiences stress and/or negative affective states such as frustration and/or fear resulting from the absence of or from limited social contact with conspecifics.
17	Locomotory disorders (including lameness)	The animal experiences negative affective states such as pain, discomfort and/or due to impaired locomotion induced by e.g. bone, joint, skin or muscle damage.
18	Mastitis	The animal experiences negative affective states such as pain and/or discomfort due to the inflammation of at least one of the mammary glands.
19	Metabolic disorders	The animal experiences negative affective states such as inappetence, weakness, fatigue, discomfort, pain and/or distress due to disturbed metabolism (e.g. acidosis and ketosis), deficiencies in several nutrients (e.g. anaemia) or induced by ectoparasites affecting metabolism (anaemia due to red mites) or poisoning
20	Motion stress	The animal(s) experience motion sickness, stress and/or fatigue due to the forces exerted as a result of acceleration, braking, stopping, cornering, gear changing, vibrations and uneven road surfaces during transport.
21	Muscle disorders	The animal experiences negative affective states such as discomfort and/or pain due to a disorder or lack of function of the muscles (e.g. myopathy in broilers).

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TABLE A.1 (Continued)

	Welfare consequence	Description
22	Predation stress	The animal experiences stress and/or negative affective states such as fear and/or pain resulting from being attacked or perceiving a high predation risk.
23	Prolonged hunger	The animal experiences craving or urgent need for food or a specific nutrient, accompanied by a negative affective state and eventually leading to a weakened condition, as metabolic requirements are not met.
24	Prolonged thirst	The animal experiences craving or urgent need for water, accompanied by an uneasy sensation (a negative affective state) and eventually leading to dehydration as metabolic requirements are not met.
25	Restriction of movement	The animal experiences stress and/or negative affective states such as pain, fear, discomfort and/or frustration due to the fact that it is unable to move freely, or is unable to walk comfortably (e.g. due to overcrowding, unsuitable floors, gates, barriers).
26	Respiratory disorders	The animal experiences negative affective states such as discomfort, pain, air hunger and/or distress due to impaired function or lesion of the lungs or airways.
27	Resting problems	The animal experiences stress and/or negative affective states such as discomfort, and/or frustration due to the inability to lie, rest comfortably or sleep (e.g. due to hard flooring, inability to perch or vibration during transport). This may eventually lead to fatigue.
28	Reproductive disorders	The animal experiences negative affective states such as pain and/or discomfort due to a disorder of the reproductive system resulting from physical injury or infection (including dystocia and metritis).
29	Sensory under- and/or overstimulation	The animal experiences stress and/or negative affective states such as fear, discomfort due to visual, auditory or olfactory under/overstimulation by the physical environment.
30	Separation stress	The animal experiences stress and/or negative affective states such as fear and/or frustration resulting from separation from conspecifics.
31	Skin disorders (other than soft tissue lesions and integument damage)	The animal experiences negative affective states such as pain, discomfort and/or distress due to e.g. infections (e.g. dermatophytosis/ringworm, pseudomonosis, staphylococcosis, viral diseases), ectoparasites (e.g. mange or red mites), inflammation of the skin or sunburn.
32	Soft tissue lesions and integument damage	The animal experiences negative affective states such as pain, discomfort and/or distress due to physical damage to the integument or underlying tissues, e.g. multiple scratches, open or scabbed wounds, bruises, ulcers, abscesses and feather or hair loss. This welfare consequence may result from negative social interactions such as aggression, tail-biting or feather pecking, from handling or from damaging environmental features or from mutilation practices (e.g. beak trimming, de-toeing, dehorning, tail docking).
33	Umbilical disorders and hernias	The animal experiences negative affective states such as discomfort and/or pain due to inflammation of the navel or any type of hernias.

TABLE A.2 List and description of negative affective states (EFSA AHAW Panel, 2022a).

Negative affective state	Description
Boredom	Boredom is an unpleasant emotion including suboptimal arousal levels and a thwarted motivation to experience almost anything different or more arousing than the behaviours and sensations currently possible.
Discomfort	Discomfort can be physical or psychological and is characterised by an unpleasant feeling resulting in a natural response of avoidance or reduction of the source of the discomfort. Pain is one of the causes for discomfort, but not every discomfort can be attributed to pain. Discomfort in non-communicative patients is assessed and measured via behavioural expression, also used to describe pain and agitation, leading to discomfort being interpreted as pain in some conditions.
Stress[a] and distress	STRESS[a]: Stressors are events, internal or external to the body involving real or potential threats to the maintenance of homeostasis. When stressors are present, the body will show stress responses (biological defence to re-establish homeostasis – for example behavioural, physiological, immunological, cognitive and emotional). Stress is a state of the body when stress responses are present. DISTRESS: Distress is a conscious, negatively valenced, intensified affective motivational state that occurs in response to a perception that current coping mechanisms (involving physiological stress responses) are at risk of failing to alleviate the aversiveness of the current situation in a sufficient and timely manner.
Fatigue	Physiological state representing extreme tiredness and exhaustion of an animal.
Fear	The animal experiences an unpleasant emotional affective state induced by the perception ofa danger or a potential danger that threaten the integrity of the animal.
Frustration	Negatively valenced emotional state consecutive to the impossibility to obtain what is expected or needed. Frustration is very often triggered by restriction of natural behaviours thus resulting in thwarted motivation to perform these behaviours.
Pain	An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja et al. 2020).

[a]The term stress does not describe a negative affective state in itself, but it is mentioned and defined in the table as it is a prerequisite of distress.

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