VKM Report 2026: 07

Guidance on the scientific information required for risk assessment of genetically modified animals intended for field trials in Norway

Aimed at applicants seeking approval under the Norwegian Gene Technology Act

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Norwegian Scientific Committee for Food and Environment (VKM)

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Scientific Opinion of the Norwegian Scientific Committee for Food and Environment

26.08.2026

ISBN: 978-82-8259-465-3

ISSN: 2535-4019

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Cover photo: MostPhotos.com

Suggested citation: VKM, Johanna Eva Bodin, Paul Ragnar Berg, Knut Tomas Dalen, Nur Duale, Erik Georg Granquist, Anne Marthe Ganes Jevnaker, Tor Atle Mo, Kristian Prydz, Eli K. Rueness, Monica Sanden, Sjur Sandgrind, Guro Sandvik, Line Elisabeth Sundt-Hansen, Ville Erling Sipinen; Truls Nesbakken, Kaare Magne Nielsen, Espen Rimstad, Eva B. Thorstad, Amin Sayyari (2026). Guidance on the scientific information required for risk assessment of genetically modified animals intended for field trials in Norway. Scientific Opinion of the Norwegian Scientific Committee for Food and Environment. VKM Report 2026: 07, ISBN: 978-82-8259-465-3, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed two separate project groups to draft the opinion. The project group on molecular characterisation consisted of five VKM members, one external expert and one from the VKM staff. The project group on environmental risk assessment consisted of five VKM members, two external experts and one from the VKM staff. An interdisciplinary VKM approval group appointed specifically for the assignment, assessed, and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2023b). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the interdisciplinary VKM approval group, appointed specifically for the assignment.

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Expertise of VKM experts

Individuals working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The provisions on impartiality in the Norwegian Public Administration Act apply to all work carried out by VKM.

Acknowledgement

VKM is grateful to hearing expert Kjetil Hindar for sharing his views and experiences with the project group regarding environmental risk assessment of genetically modified Atlantic salmon. VKM would like to thank prof. Dag Inge Våge of The Norwegian University of Life Sciences (NMBU) for valuable input on the draft guidance document. VKM emphasizes that the referee is not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment (VKM, 2024), VKM received comments from the external referee before the final evaluation, and approval by the interdisciplinary approval group before the opinion was finalized for publication.

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Summary

The Norwegian Environment Agency has tasked The Norwegian Scientific Committee for Food and Environment (VKM) with developing generic guidance documents for environmental risk assessment of genetically modified organisms (GMOs) to be used in field trials. GMOs used in limited field trials may have effect on the environment, and human and animal health. All experiments involving the use of GMOs in field trails for research require approval under the Norwegian Gene Technology Act. The Norwegian Environment Agency is the decision-making authority for deliberate release of GM plants in field trials.

VKM performs health and environmental risk assessments (ERAs) of GMOs for the Norwegian Environment Agency. This guidance document is intended as a support for applicants seeking approval for field trials under the Gene Technology Act and identifies the scientific documentation and data necessary to facilitate an ERA of a genetically modified animal (GM animal) conducted by VKM.

The risk assessments conducted by VKM generally follow the step-by-step approach outlined in EU Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, starting with hazard identification, hazard characterization, exposure characterization and risk characterization. The Gene Technology Act and the Regulations on impact assessment under the Gene Technology Act implements Directive 2001/18/EC into Norwegian legislation. Appendix 2 of the Regulation contain the principles for environmental risk assessment, which corresponds to the principles of Annex II in the Directive. The European Food Safety Authority (EFSA) has developed further guidance on the risk assessment of genetically modified organisms based on Annex II.

ERAs of GM animals involve the collection, assessment and, where appropriate, generation of information on a GM animal to determine its potential impacts on the environment and on human and animal health, compared with non-GM animals or appropriate comparators.

VKM performs risk assessments that include the following six steps: hazard identification, hazard characterisation, exposure characterisation, risk characterisation, risk reducing measures, and overall risk evaluation of the use of the GM animal in a field trial. Applicants should identify the scientific documentation and data necessary for VKM to perform an ERA based on these steps. Applicants should also consider general risk reducing measures in relation to the identified risks, taking into consideration the type of GM animal, the intended management regime (confinement), the scale of the field trial, the characteristics of the genetic modification, the characteristics of the identified hazard(s), and the potential consequences for the environment and for human and animal health.

This document provides guidance for the assessment of the potential environmental effects of GM animals, based on data from the required molecular characterisation, consideration of the potential impacts of the modified characteristics of the GM animal, and any risk mitigating measures implemented in the event of escape of GM animals into the environment during field trials. The guidance is intended as a dynamic document to be amended in accordance with future scientific and regulatory developments.

Sammendrag

Vitenskapskomiteen for mat og miljø (VKM) har, på oppdrag fra Miljødirektoratet, utviklet generisk veiledningsmateriell for helse- og miljørisikovurdering av genmodifiserte organismer (GMO) til utsetting feltforsøk eller andre typer forsøksutsetting etter genteknologiloven. GMO som brukes i begrensede feltforsøk kan ha effekt på miljøet, og på menneskers og dyrs helse. Alle forsøk som innebærer bruk av GMO i feltforsøk til forskning, krever godkjenning fra Miljødirektoratet.

VKM utfører helse- og miljømessige risikovurderinger av GMO for Miljødirektoratet. Dette veiledningsdokumentet er ment som støtte for søkere som søker om godkjenning av GMO til feltforsøk under den norske genteknologiloven. Veiledningsdokumentet definerer den vitenskapelige dokumentasjonen og de dataene som er nødvendige for at VKM skal kunne utarbeide en miljørisikovurdering av genmodifiserte dyr (GM dyr).

VKMs risikovurderinger av GMO følger generelt den sak til sak tilnærmingen som er skissert i EUs Direktiv 2001/18/EF (utsettingsdirektivet), som regulerer bevisst utsetting og omsetning av GMO i miljøet. VKMs risikovurderinger identifiserer og karakteriserer fare, karakteriserer eksponering og karakteriserer risiko. Genteknologiloven og forskrift om konsekvensutredning etter genteknologiloven implementerer Direktiv 2001/18/EF i norsk lovgivning. Vedlegg 2 til forskriften inneholder prinsippene for miljørisikovurdering, som samsvarer med prinsippene i Annex II i direktivet. Den europeiske myndighet for næringsmiddeltrygghet (EFSA) har utviklet ytterligere veiledning om risikovurdering av genmodifiserte organismer basert på Annex II.

Miljørisikovurdering av GM dyr innebærer innsamling, vurdering og, der det er hensiktsmessig, generering av data om et GM dyr for å fastslå dyrets mulige effekt på miljøet, og på menneskers og dyrs helse, sammenlignet med ikke-GM-dyr.

VKM utfører risikovurderinger av GM dyr til bruk i feltforsøk etter følgende seks trinn: fareidentifikasjon, farekarakterisering, eksponeringskarakterisering, risikokarakterisering, risikoreduserende tiltak og samlet risikovurdering. Søkere bør identifisere den vitenskapelige dokumentasjonen og dataene som er nødvendige for at VKM skal kunne utføre en miljørisikovurdering basert på disse trinnene. Søkere bør også vurdere generelle risikoreduserende tiltak i forhold til identifisert risiko, med hensyn til typen GM dyr, det tiltenkte hold og avgrensning av GM dyret, omfanget av feltforsøket, genetisk endring og nye egenskaper hos GM dyret, karakteriseringen av de identifiserte farene, og de mulige konsekvensene for miljøet, og for menneske- og dyrehelse.

Dette veiledningsdokumentet skal bidra til at søkere skal kunne levere nødvendig vitenskapelig informasjon for en vurdering knyttet til GM dyrs mulige effekter på miljø, basert på data fra molekylære karakterisering, mulige konsekvenser av nye egenskaper, og eventuelle risikoreduserende tiltak som bør iverksettes for å unngå rømning til miljøet under feltforsøk. Veilederen skal være et dynamisk dokument som kan oppdateres i tråd med framtidig vitenska pelig og regulatorisk utvikling.

Background and Terms of Reference (ToR)

The Norwegian Environment Agency has requested VKM to develop generic guidance documents for health and environmental risk assessment of GMOs used in field trials or other types of experimental release under the Gene Technology Act. The background to the assignment is that the Norwegian Environment Agency has recently received two applications for experimental release of GMOs. The guidance documents should apply to different types of organisms, including plants and animals developed with genome editing techniques. The guidance documents should be based on the current regulations, the Gene Technology Act, and relevant guidance material available in the EU. Further, the guidance should be based on VKM's knowledge and experience with risk assessment of applications for field trials under the Gene Technology Act.

Introduction

The Norwegian Environment Agency has tasked VKM with developing generic guidance documents for environmental risk assessment of genetically modified organisms (GMOs) to be used in field trials. All experiments involving the use of GMOs in field trails for research require approval under the Norwegian Gene Technology Act. The Norwegian Environment Agency is the decision-making authority for deliberate release of GM animals in field trials.

A GMO field trial is a controlled, small-scale, experimental release of GMOs into the environment to evaluate their performance, safety, and stability under real conditions before potential commercialization. A field trial may range from high confinement using closed units to semi-confinement using fenced terrestrial enclosures or open net pens in the sea or a lake with water flow through.

GMOs used in limited field trials may have effect on the environment, and human and animal health. This guidance document is intended as a support for applicants in identifying the scientific documentation and data necessary to facilitate the risk assessment conducted by VKM.

This document provides guidance for the assessment of potential effects of genetically modified (GM) animals on the environment, based on data from the required molecular characterisation, considerations of potential impact of the modified characteristics of the GM animal and any risk mitigating measures taking in case of escape of the GM animals into the environment during field trials.

The use of GM animals in research is strictly regulated under the Animal Welfare Act. All animal research in Norway requires approval from the Norwegian Food Safety Authority, and applications must be submitted electronically through The Application and Oversight System (FOTS). GMs animal health and welfare aspect are not covered in this document, as the Norwegian Food Safety Authority handles these aspects through the FOTS system.

The risk assessments that VKM performs follow at the general level the step-by-step approach as outlined in EU Directive 2001/18/EC starting with hazard identification, hazard characterization, exposure characterization and risk characterization. The EU Directive 2001/18/EC on deliberate release into the environment of genetically modified organisms is implemented in the EEA Agreement (European Economic Area Agreement) and transposed into the Norwegian Gene Technology Act and underlying regulations. Regulations relating to impact assessment pursuant to the Gene Technology Act describes the requirements for field trial applications of GM plants.

Appendix 1 of the Regulation relating to impact assessments pursuant to the Gene Technology Act provides the information that the applicant is asked to submit in the application, together with an environmental risk assessment. Appendix 2 of the Regulation relating to impact assessments pursuant to the Gene Technology Act provides the principles and methodology to be included in the environmental risk assessment.

The EFSA guidance on risk assessment of GMOs is based on the requirements and step-by-step approach (step 1-6 described below) for risk assessment outlined in EU Directive 2001/18/EC. Directive 2001/18/EC is implemented in the European Economic Area (EEA) agreement and transposed into the Norwegian Gene Technology Act and underlying regulations. Regulations relating to impact assessment pursuant to the Gene Technology Act describes the requirements for field trial applications of GM plants.

There are two main EFSA guidance documents available for health- and environmental risk assessment of GM animals:

1. Guidance for risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects (EFSA 2012)

2. Guidance on the environmental risk assessment of genetically modified animals (EFSA 2013)

The applicability of the comprehensive EFSA guidance to the environmental risk assessment (ERA) of field trial of limited extent will be case by case based and this guidance focus on the parts of the EFSA guidance relevant for field trials.

This guidance document specifically focuses on field trials with farmed GM fish and farmed GM mammals in Norway. However, the document may also serve as a guideline for other types of GM fish and GM mammals.

To facilitate an efficient and conclusive risk assessment, it is important that the submitted documentation is sufficient. The extent of documentation needed to conclude a risk assessment will vary from case to case depending on e.g. the GM animal in question and the specific characteristics of the field trial(s).

The guidance is divided into four main chapters: 1) Molecular characterisation, 2) Cross-cutting considerations, 3) Specific areas of risk to be addressed in the ERA and 4) Risk reducing measures. Chapter 1 – 3 is subdivided to highlight important topics for applicants to consider when gathering pre-field trial data, as well as identifying and evaluating potential environmental concerns that should be included in the compiled application documentation. The guidance also states the importance of detailed descriptions of risk reducing measures when and where such are indicated to mitigate identified risks.

In line with current regulations, VKM performs risk assessments that include the following six steps:

1. Hazard identification, i.e. identification of characteristics of the GM animal and potential adverse effects that may be caused by the GM animal in a field trial.

2. Hazard characterisation, i.e. evaluation of the potential consequences of each adverse effect, should they occur.

3. Exposure characterisation i.e. evaluation of the likelihood of the occurrence of each identified potential adverse effect.

4. Risk characterisation, i.e. estimation of the risk posed by each identified characteristic of the GM animal depending on the likelihood and consequence of adverse effects.

5. Risk reducing measures to mitigate identified risks to the environment, such as hybridisation with wild species or spread beyond the trial site.

6. Overall risk evaluation of the use of the GM animal in a field trial.

Applicants should identify the scientific documentation and data necessary for VKM to perform an ERA based on these steps.

Differences caused by intended or unintended effects are identified in a comparative risk assessment based on the use of comparator(s) (see section 2.4).

Intended effects are effects that are designed to occur from the introduction of the genetic modification in question, and which fulfil the original objective(s) of the genetic modification. Alterations in the phenotype may be identified through a comparative analysis of, for example, growth, development, performance, reproduction, disease resistance and behaviour using appropriately selected comparator(s). The genetic difference to a comparator is usually characterized in laboratory settings prior to a field trial and the information from such experiments is essential for addressing the questions in this VKM guidance. Data from other field trials that could be relevant for environmental risk assessment of the actual GM animal can be used as supportive information.

Unintended effects are biologically relevant differences between the GM animal and the comparator(s) that go beyond the primary intended effect(s) of the genetic modification. Unintended effect(s) could potentially be linked to genetic rearrangements or metabolic alterations and may be predicted through the comparison of the biological and compositional characteristics of the GM animal with its comparator(s) reared and tested, where possible, under the same environmental conditions.

In Norway, farmed fish for human consumption are mostly domesticated native fish species with some exceptions (e.g. rainbow trout), while farmed mammals are mostly without wild relatives with some exceptions (e.g. pig, deer). Consequently, escaped GM fish and some mammals may interbreed with wild relatives. Depending on the genetic modification, GM animals (fish and mammals) may have adverse effects on wild relatives, other animals or plants if they escape. Transmission of infective organisms directly or indirectly to wild relatives or other animals may be one adverse effect and can occur even without escape.

The guidance describes the following specific areas of risk to be considered for potential effects on the environment in case of escape from field trials:

- Persistence and invasiveness of the GM animal and progeny
- Changes in fitness of the GM animal
- Horizontal and vertical gene transfer to microorganisms (HGT) or wild type conspecifics and related species (VGT)
- Interactions of the GM animal with target organisms (TO)
- Interactions of the GM animal with non-target organisms (NTO)
- Impacts of the GM animal on biogeochemical processes
- Environmental impacts of the techniques used to manage the GM animal
- Impacts of the GM animal on animal health and welfare
- Impacts of the GM animal on human health

The guidance considers effects of GM fish and mammals on human health through routes of exposure other than ingestion or intake; these include ocular and nasal exposure as well as dermal contact and inhalation relevant when handling the animals (occupational exposure).

The guidance covers the following intended management regimes (confinement) for farmed GM fish and mammals to be used in field trials; (1) confined and (2) semi-confined (see section 2.1).

Important note to applicants:

- In this document the term 'GM-animal' refers to genetically modified mammals and fish, unless specified otherwise.

- Use of the term 'should' in this document may have different interpretations; it can be understood as a suggestion or recommendation, not as a formal requirement. The type/extent of data needed will be case dependent to enable risk characterisation and conclude the risk assessment.

- The extent of data needed to resolve the areas of risk and conclude a risk assessment will depend on the properties of the GM animal and its environmental context and hence will vary from case to case.

- References to publications describing similar or parallel experiments (including with the same animal) may be used as supporting documentation but cannot be used as a substitute for the required scientific data described in this guidance. The extent of data needed to resolve the areas of risk and conclude a risk assessment will depend on the properties of the tested animal and its environmental context and hence will vary from case to case.

- All required scientific data necessary for the risk assessment should be presented with sufficient details in the submitted application. Provided experimental data must relate to the specific modified animal intended for use in the field trial(s).

- Unlike more traditional techniques used to develop GMOs, New Genomic Techniques (NGTs) e.g. CRISPR, enable developers to introduce a wide variety of targeted genetic modifications, ranging from subtle mutations (genome editing) to long genetic inserts such as new genes. In June 2026, the EU Commission adopted new regulations for genetically modified plants produced using NGTs. The regulations distinguish between two categories of plants developed by NGTs, NGT-1 and NGT-2. Plants categorised as NGT-1 are considered equivalent to conventional varieties (traits including herbicide tolerance and the production of known insecticidal substances not included), while plants categorised as NGT-2 remain subject to existing EU GMO-legislation. The regulation is under assessment by Norwegian authorities for possible incorporation into the EEA Agreement, and as such, this type of distinction of GM plants is not yet implemented in Norwegian regulations.

- The VKM guidance is intended as a dynamic document to be amended in accordance with future scientific and regulatory developments and requirements.

- A separate document elaborates further on the information required for molecular characterization and ERA of GM animals, using different techniques. A hypothetical application for the use of a GM pig in a field trial is presented to inform the applicant about the amount of information required for an ERA associated with potential risks. Information regarding an assessment performed by VKM (VKM 2023a) on genetically modified sterile VIRGIN® Atlantic salmon for use in research trials are also included. Note, that the application and assessment of the salmon was completed prior to the finalisation of this VKM guidance, the structure of the assessment therefore differs somewhat from that of the guidance.

1 Molecular characterisation

The molecular characterization covers a description of the animal used including characteristics, possibilities to survive and reproduce in the environment outside the field trial (in case of escape) and presence of relatives/conspecifics in the environment (part 1.1). The applicant should describe the characteristics that are intended introduced in the GM animal (1.2), the origin/source of all nucleic acids introduced (1.3), the techniques used (1.4), the type(s) of genetic modification(s) performed (i.e short or longer deletion/insertion and/or transgenic insertion (1.5)), the final characteristics of the GM animal to be used in the field trial (i.e are any antibiotic resistance genes present and has the GM animal to be used in the field trial the expected genotype (1.6)), and if relevant, information on the expression level of the transgenic insert or synthesis of new metabolites.

1.1 Information relating to the GM-animal

Information regarding intended use of the animal should be described. Innate properties of the animal should also be provided (independent of modification), such as species and subspecies and known properties of importance, e.g., whether it is already present in Norwegian fauna or agriculture, can reproduce under natural conditions in Norway, hybridise with wild relatives, or has known or suspected invasive properties.

For this information, a descriptive text in an application with referenced literature is sufficient.

1.2 Description of the intended trait(s) and characteristics to be introduced or modified

The molecular characterisation should include a general description of the introduced trait(s) and its mode of action. Resulting changes to the phenotype of the animal should also be described.

1.3 Information on nature and source of nucleic acid sequences and/or genes intended for insertion, use of vectors and genome editing

The application should include a description of all nucleic acid sequences intended for insertion and their origin and/or a description of genomic regions to be mutated or deleted. The documentation should encompass complete nucleic acid sequence(s) of the intended inserts or modifications, including information on any deliberate alteration(s) to the corresponding sequence(s) in the donor organism(s) or other templates.

The application should include physical maps of all applied plasmids/vectors indicating their size and components. A description of all nucleic acid sequence elements of the vectors should be included, not only the nucleic acid sequences intended for insertion, but also genetic markers and vector backbone sequences.

Likewise, information should be provided on the nature and source of the enzymes used for genome editing and how these were synthesized/produced. It must be clarified whether any of the reagents used were pre-assembled prior to being introduced to the cells/embryo to be

manipulated) or were synthesized by the cells/embryos by transfecting nucleic acid-based reagents into cells/embryos. If any enzymes are synthesized after transfection, the complete nucleic acid sequence(s) and type(s) of DNA or RNA sequences introduced to the cells/embryos should be provided.

Intended and actual modification(s) may differ. The information required to describe the outcome of the planned genetic manipulates is further described in section 1.6.

1.4 Techniques used for the genetic modification

The application should include scientific documentation that describes the method(s) used for the genetic modification. All involved techniques used for untargeted approaches or targeted modification approaches (such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein (Cas), Transcription Activator-Like Effector Nucleases (TALENs), zinc finger nucleases (ZFN), microinjection or other), reagents, nucleic acid sequences, guide-RNA sequences etc., should be described in detail by the applicant. This also includes techniques used for transient application with DNA-reagents that may unintentionally be integrated into the host genome.

Actual experimental protocols used to generate the GM animal should be described.

1.5 Type(s) of intended genetic modification(s)

Types of intended genetic changes can be insertions, substitutions, deletions and various recombination's. EFSA has defined GMOs developed by genome editing techniques (e.g. CRISPR/Cas9 and TALEN) into three different categories based on alternative usage of site-directed endonucleases (SDN, restriction enzymes): SDN1, SDN2, and SDN3 categories (EFSA 2012b). A description of these categories is included in this document. The SDN1-3 categories of genetic modifications spans from generating knockouts, substitutions/mutations, short insertions/deletions (indels), to insertion of cis- or transgenes (see Table 1, Box 1 and Figure 1).

Independent of the SDN category, an application must include a detailed description of all intended genetic modifications. All available experimental data, nucleic acid sequence information, and type(s) of modification(s) must be related to the specific animal(s) intended for use in field experiments.

1.6 Information on the sequences actually inserted/deleted or altered

The intended modification(s) (described in Section 1.5) and the actual modification(s) may differ. Information of all obtained genetic modification(s) should be presented as detailed as possible.

For transgenes, relevant information includes a description of the number of integration sites in the animal genome, and the size, copy number, and nucleic acid sequence of the insert for each integration site. This includes unintentional insertions of genetic markers and vector backbone sequences. For non-transgenes, such information includes a description of nucleic acid sequence alteration(s) and their genomic location(s).

Unintended insertion of nucleic acid sequence(s), deletions or alterations/rearrangements, and their position in the genome may occur. It will be useful if such alterations are described as detailed as possible around the edited site(s). Information on whether insertions have unintentionally interrupted the regulation and/or expression of existing gene(s) in the animal will also be of importance.

To be able to distinguish GM animals from wild individuals (e.g. in a scenario with escape), a reliable method(s) for detection of the obtained genome modification(s) in the GM animal should optimally be described.

1.7 Information on the expression of insert(s)

If the introduced modification(s) results in expression of new gene product(s) in the animal, information should be available as to whether the inserted or modified sequence(s) result in intended changes at the RNA, protein, and/or metabolite levels. Information on the levels of newly expressed proteins and/or other products and metabolites should be available. When relevant, the modified sequence(s) should be compared to sequences of known toxins, anti-nutrients, and allergens and documented with searches in up-to-date and relevant databases. This is most relevant for transgenesis including some SDN3 modifications and/or other modifications leading to new open reading frames (ORFs).

If relevant, a brief description of expected animal welfare issues caused by the genetic modification in the GM animal can be described, as this may affect fitness of the GM animal compared to wild counterparts and thus be relevant for the ERA.

Table 1. Terms and definitions

Cisgenesis	Cisgenesis is the genome modification of a recipient organism with a gene from a crossable sexually compatible organism (same species or closely related species). This gene includes its introns and is flanked by its native promoter and terminator in the normal sense orientation (EFSA, 2012b).
Comparator	The nearest conventional non-modified relative
ERA	Environmental risk assessment
Field trial	A controlled, small-scale, outdoor experiment testing genetically modified organisms in a natural environment.
Gene modification	The process of inserting novel DNA/genes from the same or another species or deleting genes. Common to all is the use of recombinant DNA technology. For detailed definitions and exceptions, we refer to the Norwegian Gene Technology Act: LOV-1993-04-02-38
Genome editing	The process of editing DNA with biotechnological techniques such as CRISPR, ZNF, and TALEN to target genetic changes to a specific location in a genome. Most often with the aim to change single nucleotides or produce short insertions/deletions (indels).
GM animal	Genetically modified animal
GMO event	A specific, single instance of modifying an organism's genome
GMO stack	A combination of two or more distinct genetic modifications—either through additional editing or breeding—into a single organism
Horizontal gene transfer (HGT)	Any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism.
Intragenesis	Intragenesis is a genome modification of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of the same or a sexually compatible species as the recipient. These may be arranged in a sense or antisense orientation compared to their orientation in the donor organism.
Keystone species	An organism that has a disproportionately large, essential effect on its natural environment relative to its low abundance or biomass.
KO	Knockout
New Genomic Techniques (NGTs)	NGTs refer to a collection of modern biotechnological methods that enable targeted modifications of an organism's genetic material. NGTs include techniques that have emerged or been developed since 2001, and they differ from older genetic modification techniques by offering greater precision, efficiency, and control, often without inserting foreign DNA. Examples of NGTs: • Site-directed nucleases (SDNs) like CRISPR-Cas, TALENs, ZFNs • Base editing and prime editing • RNA-dependent DNA methylation • Epigenome editing See Box 1. for details.
Non-target organism (NTO)	A on-target organism is any plant, animal, or insect that a GMO is not meant to affect but could be impacted by its presence or use.
Receiving environment(s)	The range of environments into which the GM animal(s) and their effluents (e.g. faeces, urine, gametes or zygotes) will be released or may escape to, or be distributed to through active or passive spread, and into which genetic modification may spread.

Site-directed nucleases	Group of enzymes that are capable of targeted cleavage of a double-stranded DNA molecule/genome, based on recognition of a defined nucleotide sequence. The main site-directed nucleases (SDN) are ZFNs, TALENs, and Cas of the CRISPR system. These are usually engineered versions of the endogenous enzymes found in bacteria. The outcome of their use has been categorised in 3 groups by EFSA (EFSA, 2012c): SDN1, SDN2, and SDN3.
SDN1	Category of genome-edited organisms where the edited genome contains a single or a few base-pair changes after random repair of targeted double-strand breaks in the genome.
SDN2	Category of genome-edited organisms where the edited genome contains single or a few defined base-pair changes after template-based repair of targeted double-strand breaks in the genome.
SDN3	Category of genome-edited organisms where the edited genome contains longer DNA fragments inserted after template-based homologous repair of targeted double-strand breaks in the genome. This type of edit may resemble classic transgene-based modification, but will typically avoid issues with random DNA insertions, vector sequences, and unintended foreign DNA.
Target organism (TO)	A target organism is the specific pest, disease-causing agent (pathogen), or unwanted organism that a GMO is designed to control, kill, or inhibit
Transgenesis	Transgenesis is a genetic modification introducing an exogenous or modified gene (transgene) of one species into a recipient organism of another species.
Vertical gene transfer (VGT)	Any process in which genetic material/genetic modifications passed to offspring

Box 1. New Genomic Techniques (NGTs) - main categories

- NGTs using site-directed nucleases (SDNs) that create a double-strand break in DNA. These include applications of site-directed nuclease-mediated genome editing (SDNs: endonucleases, zinc finger nucleases, TALEN and CRISPR-Cas), site-specific recombinase-mediated engineering, site-specific DNA transposition.
- NGTs achieving genome editing without breaking the DNA double helix or generating only a single-strand DNA break. These include applications of base editing and prime editing.
- NGTs inducing epigenomic changes or changes affecting DNA transcription into RNA. These NGTs include applications of site-specific modulation of epigenetic state (DNA methylation modifiers, histone modifiers), site-specific activators and repressors (CRISPRa and CRISPRi).
- NGTs acting specifically on RNA. These NGTs include RNA base editing, oligonucleotide-meditated RNA interference, CRISPR-Cas-mediated PAM-independent RNA interference, RNA splice isoform manipulation. Adopted from Broothaerts et al. (2021).

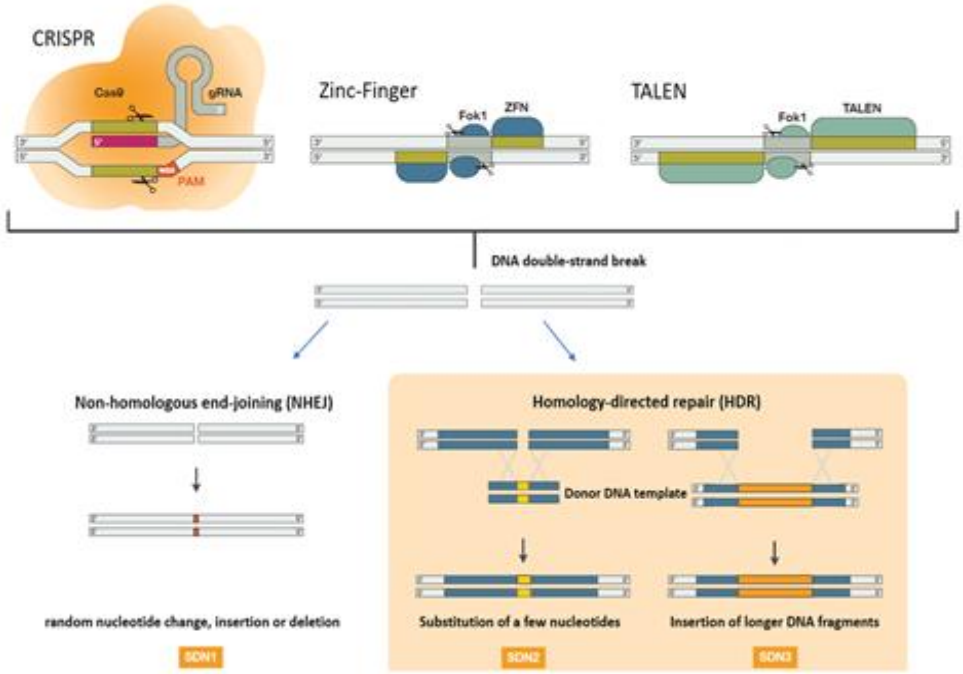

Figure 1. The outcome of genome editing with engineered site-directed nucleases (CRISPR, ZFNs and TALENs). The starting point for each genome editing is that site-directed nucleases (SDN) - "molecular scissors" - cut DNA at specific sites directed by their DNA-binding moiety, introducing a double-strand break (DSB) which triggers cellular DNA repair mechanisms. If no template (donor DNA) is added, the induced break is repaired using NHEJ (non-homologous end joining) pathway leading to gene disruption (SDN1). If a homologous repair template containing one or several single nucleotide variants is added, the break is repaired using HDR (homology directed repair) pathway resulting in gene correction (SDN2). If the added template contains longer DNA insertions flanked by sequences homologous to the target DNA site, the construct is inserted by either HDR or NHEJ (SDN3) (VKM, 2021).

2 Cross-cutting considerations

Cross-cutting considerations are fundamental considerations that permeate the individual parts of an environmental risk assessment (ERA) and constitute key information that risk assessors need to perform a sound risk assessment. An ERA is carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM animal concerned, the confinement level and the potential receiving environments. **The following sections elaborate on these areas of concern and describe key documentation that should be submitted by an applicant.**

2.1 Confinement (management regime)

The level of confinement used in field trials must be determined case by case. Information regarding confinement should be described by the applicant according to the following section. The applicants should discuss the choice and scale of experimental environment and of confinement measures.

The level of confinement should reflect

- the knowledge about the GM animal compared to its nearest conventional non-modified relative (comparator)
- the introduced trait(s) and its mode of action
- the mobility of the GM animal within the experimental environment
- the likelihood of escape of the GM animal, the potential number of escapees and the feasibility of recapture
- the tentative geographical distance of spread of an escaped GM animal
- the ability of the escaped the GM animal to become feral i.e. survive on its own
- the ability of the escaped the GM animal to crossbreed with wild relatives of the same, or in some cases, closely related species that could lead to introgression of the modification into a wild population
- the potential negative effects of the GM animal on the ecosystem

It should be noted that intrinsic properties, such as growth, survivability, spread and reproduction, of a GM animal are very important for the ERA. There are several factors to be considered, e.g. if the animal has conspecifics already present in the environment where it will be introduced (crossbreeding potential) or has high or low climate adaptability. The ERA includes concerns of the introduced traits(s) may influence survivability, fitness, fecundity and potential spread of the GM animal, with implications for the ecosystem and biodiversity.

The applicant should identify management regime measures (level of confinement) for the GM animal and should demonstrate that they are appropriate to reduce exposure and risk. The proposed measures should work efficiently and reliably under relevant rearing conditions and

in relevant receiving environments. The applicant should describe the risk-reducing measures in terms of reducing exposure and/or hazard and quantify such reductions (when possible).

Fish

1. Confinement refers to the use of closed units indoor or outdoor. There are two levels of confinement depending on the location of the field trial units and the handling/treatment/disinfection of outlet water from these units.

    A. High confinement is the use of closed units in land-based facilities, and the outlet water from the units is handled/treated/disinfected. GM fish kept under high confinement cannot escape during the trial or transport, and excreta, mucus, fish cells and infective organisms are not released to the environment.

    B. Medium confinement is the use of closed units on land or in the sea or a lake, but the outlet water from the units is not handled/treated/disinfected. GM fish kept under medium confinement cannot escape during the trial, but may escape to the environment during transport, and excreta, mucus, fish cells including gametes or zygotes and infective organisms may be released to the environment.

2. Semi-confinement refers to the use of open net pens in the sea or a lake with water flow through. Under semi-confined conditions GM fish may escape and excrement, cells including gametes or zygotes, and infectious organisms from the GM fish are released into the environment. Under semi-confined conditions, especially in open net pens, infective organisms from wild fish may be transmitted to the GM fish and opposite. For example, GM fish that have been modified to improve the resistance to an infectious disease, may still release such infective organisms that affect non-GM fish.

Mammals

1. Confinement refers to the use of indoor management systems designed to prevent any escape of GM mammals during field trials or transport. Although air exchange - and thus the movement of insects, microbes, and other biological material, cannot be entirely prevented, confinement facilities are constructed to exclude rodents, birds, and other animals. All waste streams, including carcasses, manure, and wastewater, should be collected in sealed systems and treated appropriately before disposal to minimize the risk of gene transfer or environmental release of viable microorganisms. Confinement further requires stringent security measures and continuous surveillance to eliminate any possibility of animal escape.

2. Semi-confinement resembles a farm-like environment in which GM mammals may have access to fenced terrestrial enclosures. Under semi-confined conditions, GM mammals may escape. Although environmental exposure to excreta is reduced, infectious agents originating from GM mammals may still reach surrounding ecosystems, and wildlife-derived pathogens may enter the semi-confined area. Semi-confinement does not prevent the movement of aerosols, pathogens, or other

biological or mechanical vectors. Genes or pathogens might therefore spread to the receiving environment (see section 2.2). Manure, wastewater, carcasses, and other waste are treated appropriately before disposal.

2.2 Receiving environments

Information regarding the receiving environment should be described by the applicant according to the following sections.

2.2.1 Definition of receiving environments

Receiving environments are defined as the range of environments into which the GM animal(s) and their effluents (e.g. faeces, urine, gametes or zygotes) will be released or may escape to, or be distributed to through active or passive spread, and into which genetic modification may spread.

There is a broad range of environments in terms of fauna and flora, climatic conditions, and habitat composition. The receiving environments for GM animals in field trials will vary in spatial scale from a very limited number of animals in enclosed areas (high or medium confinement) to potentially large regions due to escape. The extent of management will also vary from those that are subject to some level of management (semi-confined), to those that are enclosed (e.g. confined aquaculture facilities).

2.2.2 Identification and characterization of the receiving environments

The potential receiving environments for each GM animal should be described at three levels:

a) **The GM animal itself:** populations of the animal species, ecological requirement of the animal species, wild conspecifics (and other species with which it can hybridize) and genetic modification(s) (GM trait(s)). The applicant should consider the influence of the GM trait in determining the range of environments the GM animal may inhabit. Traits that confer tolerance to, for example, heat, cold, dehydration, salinity or disease may allow the GM animal to establish in environments not occupied by its conventional counterpart (described in 2.3). The characteristics of the GM animals will determine their behaviour and interactions with other biotic and abiotic factors in the receiving environments.

   The accessible ecosystem(s): e.g. marine (coastal and oceanic), brackish water, fresh water, cultivated agricultural habitats, natural and semi-natural habitats, rural and urban areas. Factors to be considered: physico-climatic conditions, altitude, depth, native and introduced fauna and flora. An accessible ecosystem includes all the living organisms and the abiotic conditions.

b) **Management systems:** i.e. production units, including rearing, breeding, animal welfare, transport and disposal of waste and carcasses and handling of escape. This will also include pathogen management, nature conservation activities, with e.g. chemicals that may be released in confined environments. When considering receiving environments of a GM animal, applicants should also consider (1) the use and/or

spread of waste products (i.e. effluents) of the GM animal and (2) the pests and pathogens associated with the GM animal.

2.2.3 Selection of relevant sites in receiving environments

The applicant should consider the diversity and complexity of the potential receiving environments, for each issue of concern. The full geographic range of the GM animal and the receiving environments in which these hazards could occur, including hazards caused by escape, should be evaluated.

2.3 Experimental environment

Information regarding the experimental environment should be described by the applicant according to the following sections.

The environmental concerns pertain to the complexity of the GM animal assessed and to the complexity of its interactions with components of the environment. Information regarding the experimental environment should be described by the applicant according to the following section.

Animals display complex behaviours, and this should be reflected in the applicant's choice of an appropriate experimental environment. The choice of the spatial scale of the experimental units, and the level of confinement required to avoid escape of the GM animals (fish /mammals) should be addressed by the applicant. For fish and mammals, the experimental environment may range over a continuum from a small-scale in vivo study within a confined management, up to a level that simulates an ecosystem.

In choosing a suitable level of confinement, the mobility of the GM animal within the experimental environment, the likelihood of escape, the geographic distance the GM animal may spread if escaping, the feasibility of recapture, the ability of the GM animal to become feral and to crossbreed with wild populations, and the consequences of crossbreeding should be considered. The potential for irreversible effects and the potential for negative impacts on wild populations, such as competition for resources or maladaptation and reduced fitness of wild types, should be considered.

Potential crossbreeding between escaped GM animals and wild -or domesticated animals must be considered specifically, and risk mitigating strategies to avoid gene transfer (by horizontal or vertical modalities) should be particularly emphasized.

2.4 Choice of comparator

A key scientific concept in risk assessment of GM animals is the comparative approach, where the GM animal in question should be compared to its nearest conventional non-modified relative. The non-modified organism from which the GM animal is derived is often termed the "conventional counterpart". The conventional counterpart is defined by EFSA as a non-GM animal, of the same species, with a genetic background that is as close as possible to that of the GM animal. Hence, when feasible and appropriate, similarities and differences in the interactions between the GM animal and the receiving environment (due to genetic changes

or modified management) should be evaluated in relation to a conventional counterpart. The application should include a description of the comparator(s), and justification for the choice of comparator(s).

Differences in management of the GM animals compared with the non-GM animals should also be discussed in the application. Here “management” covers all aspects of the rearing, breeding, transport and disposal of waste and carcasses.

Additional data from animals exhibiting traits analogous to those of the GM animal can support ecological risk assessment. The purpose is to generate or contextualize data on trait–environment interactions and potential implications for the ecosystem.

Note that according to the EFSA guidance (EFSA 2013), in cases where escape to the natural environment is possible, more comparators may be included (e.g. wild type of the same species and or related species).

2.5 Experimental design

The applicant should describe the design of the field trial, i.e. the planned experiments. All information that could be relevant should be described as accurately as possible to ensure reproducibility and reliability.

2.5.1 Principles of experimental design and statistical analysis

General statistical principles encompass relevant hypotheses, experimental designs and methodologies to generate high quality data. The statistical expectations as outlined in EFSA (2013) mainly relate to the quality of data presented at later stages of an ERA, i.e., in applications of large-scale production of GMOs to be used as food or feed. For the early phase field trials of a GM animals, the statistical principles would mainly concern the applicant’s need to ensure that informative data can be collected after repeated field trials.

A case-by-case approach depending on the characteristics of the GM animal justifies the design decisions, including environmental conditions and husbandry practices. Consideration should also be given to phenotypic plasticity, data reliability and uncertainty. Environmental influences during both rearing and experimentation must be considered, as they shape trait sensitivity to environmental variation and determine how traits may manifest under natural conditions.

The applicant should explicitly justify the selection of abiotic conditions (e.g., confinement measures, water flow, temperature, light), biotic interactions (competition, reproduction, predation), and life-history variables (life-stage, maturity, reproductive state).

For rapidly growing GM animals that reach maturity earlier than comparators, matching individuals by size or developmental stage rather than chronological age may be necessary.

2.6 Long-term effects

Information regarding potential long-term effects due to escape of the GM animal from the field trial to the surrounding environment should be described by the applicant according to the following sections to identify the magnitude of potential adverse effects. Long-term effects of GMOs are defined as adverse effects that occur over an extended period. The applicant should evaluate the possibility of escape and persistence and/or invasiveness in the receiving environment outside the field trial and potential adverse effects associated with escape.

The applicant should evaluate potential adverse effects of the GM animal and its offspring (including their waste products) on human and animal health, and on the environment. The applicant should consider the entire life cycle of the GM animal and the receiving environments of the animal's different life stages to determine possible adverse effects over time.

Knowledge of the baseline conditions in the receiving environments (and temporal changes in these conditions), prior to and following GM animal presence will help in the evaluation of potential long-term effects.

The potential long-term effects of the GM animal compared with its conventional counterpart and other appropriate comparators should be assessed.

A delay may be expected between escape of a GM animal to the environment and the observed effects. The length of the delay will differ among species depending on species attributes such as generation time, reproductive capacity and mobility. In addition, the number of GM individuals that escape, and the frequency of escape should be assessed.

For instance, effects caused by interbreeding between escaped GM animals and wild type conspecifics (or related species if interbreeding is possible) can be observed only after long time periods, depending on the reproduction rate.

2.6.1 Categories of long-term effects

Long-term effects are divided into two categories:

Category I: Direct exposure to a GM animal, or management practice, may result in a delayed response by target organisms (or their offspring). An example of such a long-term effect is the development of resistance against a genetic modification in the pest-target organism.

Category II: There might also be indirect long-term effects on the receiving environment that are unanticipated due to spatial or temporal complexity. Examples include interactions of escaped GM animals with other species, including pathogens, and conspecifics.

The probability and magnitude of long-term effects on the environment may differ among confined and semi-confined settings. Depending on the GM animal, applicants should estimate possible long-term effects of both category I and category II on a case-by-case basis.

Long-term effects of the GM animal should not be considered in isolation but compared with the long-term effects of its conventional counterpart or another appropriate comparator, if

present in the receiving environments. If no appropriate comparator is present, long-term effects should be compared between the presence and absence of the GM animal.

2.7 Uncertainties

The identification of hazard, the likelihood and the consequences are all terms characterised by, described with, and measured with various types and degrees of uncertainty. Although it may be impossible to identify all the uncertainties, each scientific output should describe the types of uncertainty encountered and considered, and relative importance should be indicated.

This should include:

- assumptions and extrapolations
- any conflicting scientific literature and viewpoints
- specified uncertainties

Uncertainty identification should include:

1. Linguistic uncertainty caused by different understanding of language used to describe environments, events and processes, or vague expressions (e.g. moderate, unlikely, rare)
2. Variability caused by fluctuations or differences in a quantity or process, occurring over time (e.g. seasonal changes in prey species)
3. Incertitude caused by limitations of scientific knowledge and systematic bias, censoring, measurement error, missing data, lack of suitable comparators, and other causes of incomplete understanding of mechanisms

Suggestion for identification and treatment of uncertainties:

- Clearly define predictive terms related to the description of risk (e.g. high, medium or low likelihood and consequence)
- Identify critical uncertainty and propose treatment
- Ensure appropriate endpoint selection to minimise complexity in data collection
- Avoid predictive bias caused by limited sample size
- Maintain transparency in the identification and treatment of sources of uncertainty

2.8 Aspects of animal health and welfare

Aspects of GM animal health and welfare will be covered in a separate application to the Norwegian Food Safety Authority, through The Application and Oversight System (FOTS). However, evaluating the GM animals' interaction with their receiving environment is essential, and possible alterations due to the GM trait should be assessed in health- and environmental risk assessments under the Gene Technology Act.

Disease resistance is a major target in GM animals. These traits can influence health and welfare of other animals, population dynamics and environmental interactions. Applicants should evaluate whether the health and welfare of the GM animal present a new hazard for wild animals' health and welfare compared with appropriate comparator. For example, if the genetic modification causes a GM animal to be more susceptible to disease, and there is a risk that the GM animal may spread disease to a greater extent than non-GM animals.

The applicant should describe infective organisms and susceptible animals in the receiving environment, identify direct and indirect interactions, discuss immediate and delayed ecological effects, and assess risks associated with carcass disposal and if the GM trait can alter potential interactions. GM animals may pose environmental risks if infected. Disease resistant animals may act as asymptomatic carriers, and resistance traits may drive pathogen evolution. Extended lifespan may increase risks for chronic diseases with long incubation periods, and behavioural changes may alter transmission dynamics.

Animals coexist with diverse microorganisms and parasites that vary in host range and virulence. Infection outcomes depend on behavioural, physiological, and developmental factors. Resistance or tolerance may impose selection pressures on pathogens, and management driven microbiome shifts may facilitate pathogenicity. Applicants should discuss possibilities for tolerance development, when relevant.

Other traits—such as those affecting growth, productivity, or reproduction—may modify susceptibility to infection. Alterations in digestion, metabolism, or gut microbiota may influence immunity, pathogen shedding and dissemination to the environment.

3 Specific areas of risk to be addressed in the ERA

The following chapter addresses specific areas of risk and constitutes key information that risk assessors need to perform a sound risk assessment. The chapter elaborate on these areas of risk and generally describe the documentation that should be included in an application. An ERA is carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM animal concerned, the confinement level and the potential receiving environments.

The specific areas of risk addressed in ERAs of GM animals intended for field trials are:

- Persistence and invasiveness
- Vertical gene transfer (VGT)
- Horizontal gene transfer (HGT)
- Impacts of the GM animal on biotic components and processes
- Interactions of the GM animal with target organisms (TO)
- Interactions of the GM animal with non-target organisms (NTO)
- Interactions of the GM animal with the abiotic environment
- Pathogens, infections and diseases
- Environmental impacts of the specific techniques used for the management of the GM animal
- Impacts of the GM animal on non-GM animal health and welfare
- Effects on human health

The chapter is divided into specific areas of risk for the ERA of GM fish and specific areas of risk for the ERA of GM mammals.

3.1 Specific areas of risk for the ERA of GM fish

3.1.1 Persistence and invasiveness of GM fish, including vertical gene transfer (VGT)

Step 1: Problem formulation including identification of hazard and exposure pathways

This section focuses on the genetic and population effects of escaped GM fish and possible recipients of the genetic modification. Applicants should address the consequences of escape, establishment, gene transfer and changes in the fitness of the GM fish (see step 2) and any recipient of genetic modification. The modification might result in changes in persistence, competitiveness and invasiveness of the GM fish line itself, of hybrids between GM and wild individuals due to escape, that might lead to environmental harm (Figure 2). The possible introgression of the genetic modification from the GM fish into wild species raises the need to assess how introgression affects conservation of genetic diversity in any affected wild population, including changes to allelic frequencies, population genetic structure and variation. It is important to assess effects of introgression on survival and reproductive capability and hence on local adaptation of the wild population.

One potential fitness consequence of a self-reproducing GM fish population or of a GM fish hybridising with wild conspecifics is enhanced fitness of the reproducing GM fish population. Other consequences could be that introgressed wild conspecifics and closely related species may create feral GM populations, or that hybrid or backcrossed populations, or decreased fitness of hybrid or backcrossed descendants, may cause decline or local extinction of wild fish populations.

Initially, basic information is required that enables characterising of the GM fish line and identifying possible hazards associated with escape. The information provided should be used to establish whether:

a) at least one GM fish or gametes or fertilized eggs from GM fish can escape from confined or semi-confined facilities and therefore have the potential to contribute to feral populations.

b) the GM fish is capable of reproduction.

c) the GM fish can hybridise with wild types of the same species that may occur in the receiving environments and produce viable and fertile first-generation hybrid offspring, or the GM fish can hybridise with other species of fish and produce fertile interspecific hybrids.

d) sufficient fertile GM fish can establish as new or enlarged feral populations in receiving environments.

e) at least one GM fish, or first-generation hybrid offspring or later-generation backcrossed descendants, may enter and survive in new receiving environments not occupied by wild types.

If introgression can occur, the applicant should assess the extent to which loss of genetic diversity is likely to pose a hazard and should characterise the risk of this hazard in practice and assess the magnitude of the environmental harm potentially caused by a decrease in genetic diversity.

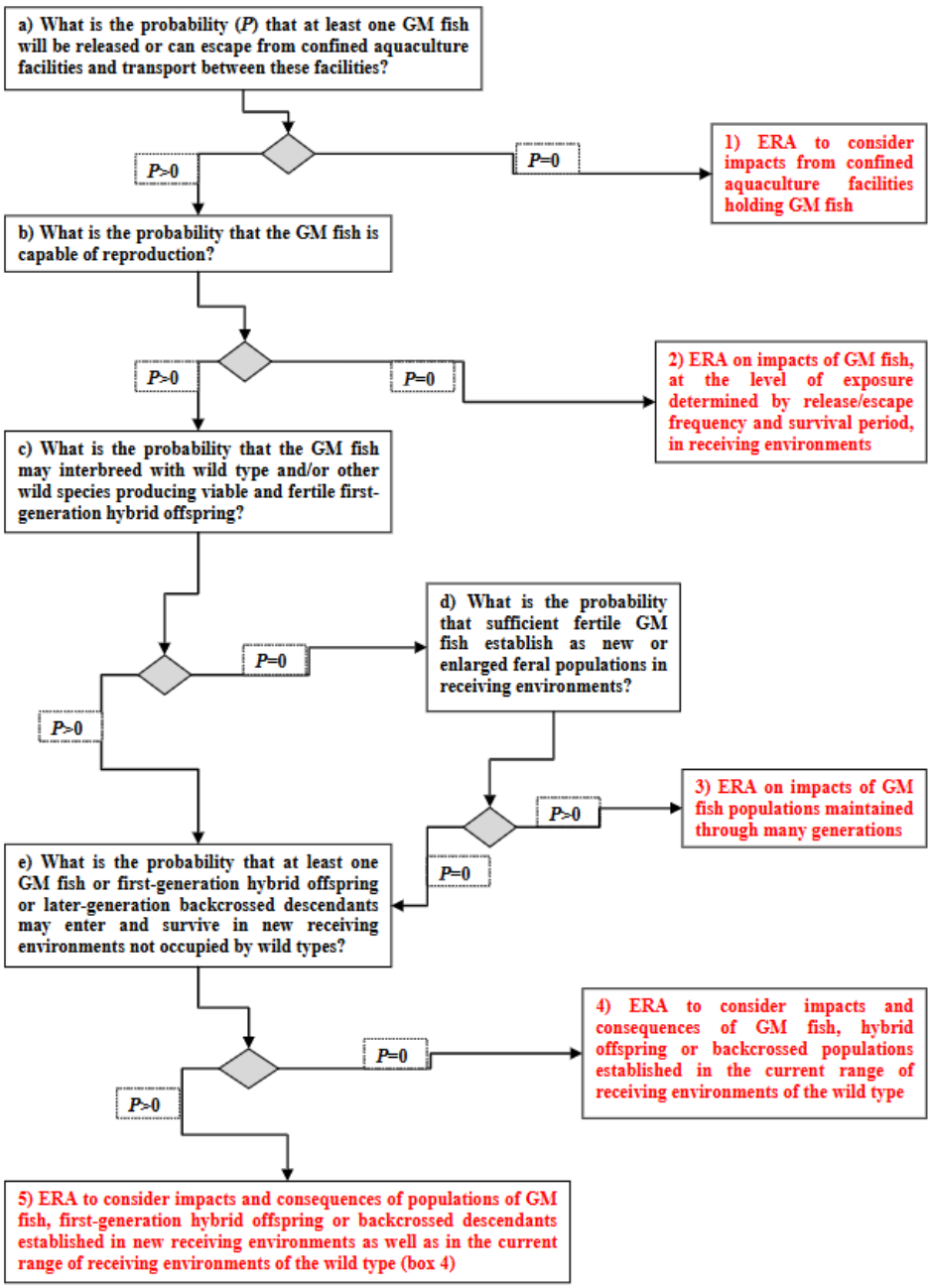

Figure 2 Example of a staged approach to problem formulation for the identification of hazards associated with the dispersal of GM fish and gene introgression and environmental exposure. These characteristics provide information on the extent of the ERA required for GM fish in each receiving environment. Figure excerpted from the Guidance on the environmental risk assessment of genetically modified animals, that also cover hazards associated with release and transportation between confined facilities (EFSA 2013).

Step 2: Hazard characterisation

If a hazard which might lead to adverse effects because of altered survival and reproductive success in GM fish and any offspring from outcrosses has been identified in the previous step, this needs to be described and characterized as detailed as possible. If the GM fish has more than one genetic modification giving rise to several new characteristics (e.g. stacked GM fish events), the applicant should consider whether the combination of them may lead to altered survival and reproductive success that is more than the simple product of the single GM trait.

It is advisable to pursue a stage-wise approach to assess measurable endpoints in a chain of events that must occur, to end up with incorporation of genetic modification from GM fish into a population of conspecifics in a receiving environment. The assessment should address two major endpoints: entry of sexually mature, fertile GM individuals into a receiving environment; and introgression of genetically modified genotypes into the gene pool of wild conspecifics and closely related species.

Step 3: Exposure characterisation

The environmental exposure should be related to the potential of the GM fish to escape into other environments, i.e. identify how likely it would be that GM fish escape and how often this could occur (e. g. when handling the fish), and if possible, how many fish might escape. Environmental exposure should be related to the life cycle of the GM fish and potential recipients of the genetic modification, considering the habitats of different stages and migration routes and interactions between the GM fish and wild conspecifics in these different environments. The exposure characterisation should also consider the size of any populations which might be at risk and the degree to which the environment locally may be marginal, fragmented and/or unfavourable. In addition, any mitigation measures to reduce gene flow (e.g. reduced fertility) and environmental exposure (e.g. confinement strategies) should be considered.

Step 4: Risk characterisation

In this step, applicants should quantitatively or semi-quantitatively estimate the probability of occurrence and magnitude of harmful effect(s) based on problem formulation, hazard and exposure characterisation. Applicants should characterise the risk by combining the magnitude of the consequences of each hazard and the likelihood of the consequences related to these hazards occurring in the receiving environments. Furthermore, applicants should assess the overall uncertainty for each identified risk.

Step 5: Risk reducing measures

If risks are identified related to reproduction or behaviour (e.g. survival and invasion), strategies to manage these risks may be required and should be suggested by applicants. These strategies should focus on improved physical, geographical or biological confinement if such risks are identified. For example, biological confinement could involve lowering mating frequency and/or sexual fertility or be directed at controlling the progeny of GM fish resulting from gene flow. Applicants should evaluate the efficacy and reliability of any risk mitigation measures and conclude on the final level of risk resulting from their application.

Step 6: Uncertainties

Uncertainties associated with persistence and invasiveness should be discussed, particularly with reference to the difficulties of conducting and interpreting experiments designed to demonstrate how changes in fish biology are likely to result in population effects in a range of environmental situations. The applicant should discuss if applying appropriate risk reducing measures would reduce risks due to uncertainties.

3.1.2 Impacts of GM fish on biotic components and processes including non-target organisms (NTOs)

This section seeks to determine if the GM fish has a different biotic interaction compared to its comparator and identify possible NTOs.

Step 1: Problem formulation including identification of hazard and exposure pathways

Direct effects are those effects that the fish itself generates, through means such as predation, competition, habitat alteration, inter- and intraspecific hybridisation and introduction of new parasites and diseases that influence behaviour and/or survival of the wild biota. Depending on the characteristics of the GM fish, there may be changes in the secretion of substances, actively or passively, or release of substances upon death of the GM fish or as metabolites, should the GM fish be consumed by a predator.

Indirect effects are those effects occurring when the GM fish is not in contact with the individual being affected. It is particularly important to examine whether this can give rise to trophic cascades whereby an initially small direct effect, caused by the GM fish, can lead to larger effects on the ecosystem by shifting the balance in the system. These effects typically occur through a limited number of species, so-called keystone species, and it is therefore especially important to identify such species (population sizes and population status) in the receiving environments and to assess to what extent escaped GM fish affect such species.

The potential ecological effects of the GM fish upon escape should be assessed from the perspective of the environment in which they may escape to. For this assessment, at least one of four appropriate populations in prioritized order should be considered:

1) Wild population of the same species as used in the field trial. For example, if the experimental fish is farmed Atlantic salmon and wild Atlantic salmon is found in the receiving environment, the ecological effect of escaped GM fish on the wild Atlantic salmon population must be considered.

2) Wild population of species closely related to the GM fish, and which occurs in possible accessible ecosystems.

   For example, if the GM fish is a rainbow trout and the accessible ecosystem contains wild brown trout and/or Atlantic salmon, the ecological effects of the escaped GM fish on these species should be assessed.

3) Wild populations of other fish species in the accessible ecosystems exploiting a similar ecological niche.

For example, if the genetic modification could have an effect on the ability for an escaped GM fish to compete with other fish species in the receiving ecosystems. The choice of the wild fish populations should be justified, and if information is missing about the ecology of the species, this should be assessed under uncertainties.

4) Potential non-target organisms (NTOs) of any other species that may be affected by the escape of the GM fish to the receiving environment(s) should also be assessed. These may include any macro- or microorganisms.

Step 2: Hazard characterisation

If a hazard has been identified during the problem formulation process which might lead to adverse effects, the magnitude and consequences of these hazards should be discussed.

To obtain a preliminary indication of whether competitive interactions might occur, applicants should assess similarity in resource use between potentially invasive GM fish and wild species, in order to determine the degree to which the GM fish and wild species utilise the same range of resources. Competition experiments in the laboratory under semi-natural conditions should be considered.

Applicants should determine whether the abundance of native species is likely to decrease after introduction of GM fish, through direct competition for resources, predation or indirect effects. These should include potential physical competition for some habitat requirements (e.g. shelter, refuge, breeding sites) and territorial behaviour, with the same or other species, whose change may lead to increased stress to potentially affected species and ultimately their decline. Applicants should also consider the effects of the additional number of individuals added to an environment, as well as novel GM trait(s).

Applicants should examine whether any change in behaviour, competition, dominance, feeding behaviour and predation of the GM fish relative to wild fish lead to food chain effects that in turn have ecological consequences, for example by depleting certain resources, thus depriving other biota of these resources (e.g. food, shelter) and hence driving down their populations. Conversely, changes in resource use could increase the supply of a resource, allowing certain biota to flourish.

An assessment is also required of whether the GM fish and its effluents present a new hazard for the health of other animals (section 3.1.4) and this should also be taken into consideration while assessing consequences of biotic interactions.

Step 3: Exposure characterisation

It is important to not assume that a physiological capacity to migrate will necessarily lead to a behavioural decision to actually migrate, so the link between physiology and behaviour requires examination. Dispersal can also be through involuntary transport by birds or animals capture, fishing or other human activities that facilitate dispersal. The management, transport and handling of the GM fish need to be considered fully.

Applicants should also consider the nature of the different receiving environments and determine whether these environments will sustain and support the GM fish. For example, GM fish may be able to survive for only limited time but may have a negative impact during this time and/or after dying. Also, GM fish may be able to survive for longer periods but may not be able to reproduce; thus, the frequency of invasions and the numbers of fish invading should be taken into consideration. Further, applicants should consider the possibility that the GM fish may change the ecology of the receiving environments and thereby expose the GM fish to novel biotic conditions. These can include products of the metabolism (e.g. carbon dioxide, ammonia), effluents (e.g. faeces) and products from decaying plants and animals. This includes microorganisms (primarily algae) that can influence fish survival by secreting bioactive toxins into surrounding water or by causing physical irritation to gill membranes. Some GM fish will have been developed to better endure certain biotic/abiotic factors, but it also becomes important to assess if this comes at a cost of enduring other factors in receiving environments.

Applicants should consider methods for assessing dispersal behaviour under confined conditions and consider testing different dispersal and migration scenarios in order to assess the full geographic range of the GM fish and hybridising species, and the ecological niches they are likely to influence.

Step 4: Risk characterisation

Applicants should consider the biota present in the receiving environments of the GM fish and determine the likely direct interactions that will occur in terms of food, prey, predation, competition, displacement, disease, local population change, etc. The indirect effects from these direct effects should then be considered in terms of food chain effects and the possible consequences for different biota in these ecosystems in the medium term and hence the long-term prospects for these environments.

Step 5: Risk reducing measures

Applicants should propose methods to reverse or reduce adverse impacts on receiving environments if such risks are identified.

Step 6: Uncertainties

Uncertainties associated with the efficiency or implementation of mitigation measures should be discussed. Uncertainties due to gaps in information, the limited scope of experimental studies and the need to extrapolate results to long-term exposure of the range of receiving environments should be discussed (see section 2.5 and 2.6). The applicant should also discuss if applying appropriate risk reducing measures could mitigate the risks due to uncertainties.

3.1.3 Horizontal gene transfer

Step 1: Problem formulation including identification of hazard and exposure pathways

Horizontal gene transfer (HGT) from fish is rare, and it has been shown that HGT from fish to fish has occurred a limited number of times during the evolutionary history of fishes (Policarpo

et al. 2025). Heritable HGT between multicellular eukaryotes is limited by the need for transformation of germline cells. However, genetic material from the fish might be taken up by microorganisms, and HGT events can increase in frequency if the genome modification includes addition of a mobile genetic element such as a transposon. Therefore, the initial problem identification should clarify if the genetic change leads to more mobile genes. If this is the case, the problem formulation should further consider 1) the presence of a defined mechanism that could facilitate horizontal transfer, uptake, and integration of the modified element in a new host, at biologically relevant levels; and 2) the potential of occurrence of HGT, depending on presence of homologous gene sequences and potential recipients.

Step 2: Hazard characterisation

If a hazard has been identified in step 1, the magnitude of adverse effects should be further characterized. This characterization should establish the nature and range of potential (short- and long-term) consequences. Information on the prevalence and distribution of genes similar to those introduced in GM fish should be considered.

Step 3: Exposure characterisation

If a hazard has been identified, the exposure characterization should consider the characteristics of the inserted genes, and the levels and routes of exposure to other organisms, including the likelihood for this to occur. For instance, cells with a mobile genetic element inserted will be released from shed epithelial cells in the gut and skin and could be present in faeces.

Step 4: Risk characterisation

Applicants should focus the risk characterisation on the identified hazards and its impact that may potentially occur in the various receiving environments. Any identified risk should be characterized by estimating the probability of occurrence, any positive selection conferred by the horizontally transferred trait and the magnitude of the consequences of the adverse effect(s).

Step 5: Risk reducing measures

Based on the outcome of the risk characterisation, applicants should suggest appropriate risk management strategies. Potential strategies may be related to the avoidance of conditions allowing exposure or positive selection.

Step 6: Uncertainties

Identified knowledge gaps and uncertainties should be briefly summarised and a clear statement on the absence/presence of selective conditions should be provided. The applicant should also discuss if applying appropriate risk management strategies would reduce risk due to uncertainties.

3.1.4 Pathogens, infections and diseases

Fish live in an environment together with numerous infectious organisms that may attach to the outside of the fish or live inside the fish. Most of these infectious organisms cause small negative effects to the fish but some can cause significant damages or disease. Here the term "pathogen" refers to infectious organisms that can cause disease.

Fish can be genetically modified with the primary goal of making them disease resistant or tolerant (direct effects), either to a specific disease or to many diseases (group 1). Fish may also be genetically modified to express other traits which may change their susceptibility to infectious diseases more indirectly (group 2). GM fish in group 1 are created with the intention of increasing resistance to pathogenic organisms while GM fish in group 2 are not created with the primary intention of increasing resistance to pathogens, but a consequence of the genetic modification is an effect on the susceptibility of the GM fish to infection. The existence of GM fish with altered susceptibility to pathogens could have consequences for the GM fish itself, for the fish population of which the GM fish is a part, for other organisms in the environment and in some cases for human health.

Step 1: Problem formulation including identification of hazard and exposure pathways

Applicants should consider whether the genetic modification could alter interactions between the GM fish and pathogens. Applicants should compare a GM fish with its appropriate comparator(s) under the range of receiving environments. Applicants need to justify the environmental conditions used in their studies to capture a range of receiving environments into which the GM fish and their effluents may be released both intentionally and unintentionally. The key question is: might the GM fish differently influence pathogens, in comparison with its comparator, in its confined environments and all other potential receiving environments?

Step 2: Hazard characterisation

If a hazard has been identified during the problem formulation process which might lead to adverse effects, the magnitude of such adverse effects should be discussed.

Factors influencing disease resistance and immune response of fish include genetics (e.g. species or strains), physiological state of a fish (e.g. age, size, sexual maturity), environment (e.g. temperature, season, photoperiod), stress (e.g. water quality, pollution, density, handling and transport, breeding cycles), nutrition (feed quality and quantity, nutrient availability, use of immune-stimulants, anti-nutritional factors in feed), pathogen (e.g. exposure level, types of pathogen and virulence) and disease management (e.g. use of antibiotics). All these interacting factors should be considered when characterising disease resistance and immune response of GM fish and the ability of the GM fish to transmit pathogens to NTOs.

Step 3: Exposure characterisation

This step is to evaluate the likelihood and/or frequency of occurrence for each identified hazard, and it is important that applicants consider the specific trait of the GM fish itself (e.g. group 1 or group 2), the receiving environments of the GM fish and the presence of non-GM fish in the receiving environments. For confined GM fish, factors affecting the introduction and

exposure to pathogens within aquaculture units should be considered, such as e.g. stocking density. In addition, the likelihood and frequency of escape need to be estimated. For semi-confined GM fish, the time fraction and developmental stage for confinement and non-confinement periods should be estimated. Applicants should describe in detail the different steps of handling fish in different stages of life and during transport. Other pathogen dispersal routes, such as urine, faeces, farm runoff and disposal of fish carcasses, shall also be considered.

Step 4: Risk characterisation

The risk characterisation should focus on the characterised hazards that may potentially occur in the various receiving environments. Risks should be characterised by discussing their probability of occurrence, any potential alteration in pathogen transmission due to the GM trait and the magnitude of identified consequences of the adverse effect(s), considering the characteristics of the recipient species, their life cycles and interactions with different receiving environments and other stressors. Estimates of impacts on recipient fish populations should be made in terms of their reproduction and growth and final population size. The broader environmental consequences of changes in fish populations should be assessed.

Step 5: Risk reducing measures

Applicants should propose methods to reverse or reduce identified risks, by removing hazards or reducing exposure. For example, to remove the hazard of pathogen transmission from GM to non-GM fish within a farm, an obvious risk management strategy is to cultivate only GM fish. Moreover, to reduce the frequency of transmission of pathogens from a farm housing GM fish to other farms and wild populations, stringent bio-security measures can be implemented on the farm to prevent release of pathogens. These can include sufficient levels of confinement to prevent animal escape, adequate waste treatment to prevent release of GM materials through farm runoff, adequate disposal of carcasses from diseased fish, etc. For disease-resistant or -tolerant GM fish, applicants should consider that dead fish may be carriers of pathogens with the ability to infect the GM fish and therefore implement strategies of handling carcasses to prevent the further spread of pathogen and disease (e.g. incineration).

Applicants should also describe if practices have to be adopted for GM fish rearing that are additional to the normal range of general good hygiene, and husbandry practices that should be implemented in confined aquaculture facilities to minimise disease and stress levels. These could include specific requirements for isolation, treatment, stocking density, nutrition, etc.

Step 6: Uncertainties

Any uncertainties encountered regarding the effect of the modification on pathogens and infectious diseases should be discussed. The applicant should also discuss if applying appropriate risk management strategies would reduce risk due to uncertainties.

3.1.5 Interactions of GM fish with the abiotic environment

Step 1: Problem formulation including identification of hazard and exposure pathways

Two aspects of abiotic interactions are relevant for the ERA of GM fish, and must be considered by the applicants:

If the GM fish have an altered tolerance to abiotic factors (intended or unintended), and if the GM fish can affect the abiotic environment in a different way from the non-GM fish. The second aspect can be divided into direct effects of the GM fish itself, and indirect effects cascading from the direct effects, which may act either on abiotic factors or on biotic components. These aspects should be considered both when the fish are enclosed in the experimental environment, and in the case of an escape.

The genetic modification can alter the sensitivity and behavioural response of GM fish to the abiotic environment, such as water depth, water flow, temperature, oxygen level, light or salinity. This could affect the ability of the GM fish to disperse or sustain in specific environments or behave in a different way. For example, a genetic modification of cod could alter the response to light, affecting the maturation process, and a possible risk could be that the GM cod could spawn at a different time than the conventional counterpart. Direct abiotic effects are those that the modified fish itself generates, for example by different digging behaviour when building nests or consuming more oxygen or releasing substances that could influence the abiotic environment. Indirect abiotic effects can arise from the direct effects and can act on the biotic or abiotic components of the environment. For example, increased digging behaviour of escaped GM salmon could increase the release of silt from the riverbed, which can accumulate further downstream and change the abiotic conditions for the biota downstream.

Step 2: Hazard characterisation

If a hazard has been identified, the applicants should discuss if this can lead to a changed abiotic environment in the accessible ecosystems. Applicants should discuss whether the GM fish may have changed behaviour or physiology that can affect its tolerance and response to abiotic factors, or if the GM fish can affect the abiotic environment differently than the comparator(s).

Step 3: Exposure characterisation

This step should consider the duration and the magnitude of interactions between the GM fish and the abiotic environment. For example, the escape of GM fish needs to be considered, and the ability of the GM fish to endure and exploit different abiotic conditions than the comparator(s).

Step 4: Risk characterisation

Applicants should consider whether key components of the environment are affected, the reversibility of these effects, and the level of harm associated with them. Because of the complex nature of the ecological interactions, applicants should clearly identify assumptions made and the level of uncertainty associated with the conclusions of risks. In addition, applicants should explain why identified impacts on the abiotic environment are considered acceptable and do not present risks.

Step 5: Risk reducing measures

Applicants should propose methods to reduce or reverse adverse impacts on the abiotic factors and key ecological functions identified in the risk characterisation.

Step 6: Uncertainties

Any uncertainties encountered regarding altered interaction between the GM fish and the abiotic environment compared to the comparator should be described. The applicant should also discuss if applying appropriate risk management strategies would reduce risk due to uncertainties.

3.1.6 Impacts of GM fish on human health

This guidance document considers primarily effects of GM fish on human health through routes of (occupational) exposure, mainly dermal, nasal and ocular contact, but also unintended consumption.

Applicants should evaluate whether the GM fish presents a new hazard for human health compared with appropriate comparators. Applicants should consider both immediate and delayed effects on human health resulting from potential direct and indirect interactions with GM fish. Fish are capable of carrying pathogens and parasites that can infect humans, and these may be present in the water as well as in the fish. Furthermore, some fish can produce proteins and other compounds that can cause irritations or allergenic responses to exposed humans working with fish.

Step 1: Problem formulation including identification of hazard and exposure pathways

Some pathogens from wild and/or cultured aquatic species are reported to cause illness or disease in humans. Applicants should assess whether GM fish have an increased capacity to cause human disease, e.g. because they are a more efficient reservoir of a specific pathogen and consequently a more efficient disease vector for human disease. Applicants should also assess whether the GM fish may become a carrier of different pathogens that can cause human disease. Furthermore, applicant should assess potential toxicity and allergenicity for humans resulting from exposure to the GM fish.

Step 2: Hazard characterisation

If a hazard has been identified which might lead to adverse effects, the applicant should discuss the magnitude of these adverse effects.

Applicants should discuss whether the load of a specific pathogenic agent may reach levels that can cause human diseases. If the GM fish express a new protein or there is altered composition or expression of components known to be associated with allergenicity or toxicity, applicants should provide an up-to-date search for homology of the amino acid sequence of the proteins and altered constituents to known allergenic or toxic substances.

Step 3: Exposure characterisation

The possible impacts of GM fish on human health may occur at different stages in the development and processing of the GM fish. Applicants should discuss if the conditions of breeding, rearing, transport, storage and processing of the GM fish could affect the levels of

occupational exposure (dermal, nasal, oral, ocular and inhalation exposure) in relation to the characterised hazards associated with the GM fish.

Step 4: Risk characterisation

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects on human health should be discussed for each characterised hazard based on levels of human exposure through all exposure routes, and particularly at critical points identified in the exposure analysis. The magnitude of the consequences of the hazard and the likelihood of its occurrence should be discussed.

Step 5: Risk reducing measures

Where risks have been identified in step 4, applicants shall describe measures intended to minimise risks to humans handling the GM fish. These could include measures to reduce the hazard (e.g. by better disease management) or to reduce exposure (e.g. with protective clothing or work processes).

Step 6: Uncertainties

Any uncertainties encountered regarding impact on human health due to exposure to the GM fish compared to the comparator should be described. The applicant should also discuss if applying appropriate risk reducing measures could reduce the risks due to uncertainties.

3.2 Specific areas of risk for the ERA of GM mammals

3.2.1 Persistence and invasiveness of GM mammals, including vertical gene transfer (VGT) to farmed or wild conspecifics

Step 1: Problem formulation including identification of hazard and exposure pathways

This section addresses potential environmental effects resulting from altered biological properties of GM mammals, e.g. being more persistent or invasive than the comparator. Including when sexual reproduction with related species or breeds makes vertical gene transfer possible, as could be the case for e.g. pig or farmed deer. Thus, the applicant must address the potential for GM mammals used in the field trials to escape, spread, persist, and become invasive. The propagule pressure, including the potential number of escapees should be considered. If hybridisation with wild conspecifics may occur, the potential loss of genetic diversity and its consequences in the wild population should be assessed.

Applicants should utilise existing data to clarify how interactions between the GM animal and its environment, including other organisms, may affect invasiveness of the GM animal. Where existing data are insufficient, targeted experimental studies to supply estimates of particularly important parameters (e.g. estimates of changes in growth, survival, fecundity, development, behaviour) may be required before field trials are authorised. However, survival and fitness of the GM animal in natural environment may be part of the analyses in the field trial to obtain reliable data prior to large scale production. If the modification is expected to affect health or welfare impairment in other animals, confined studies should be considered.

The intended confinement regime and the presence of sexually compatible wild conspecifics must be considered when assessing risks of persistence, establishment, and invasiveness. For traits intended to induce sterility, applicants should demonstrate both effectiveness and durability. Disease resistant phenotypes should also be evaluated for their potential to impose selection pressures on target or non-target pathogens, potentially altering their infectivity or pathogenicity, taking the scale and duration of the field trial into account. Confinement and mitigation strategies must prevent the emergence or spread of such pathogens.

Step 2: Hazard characterisation

GM mammals for which the modified trait already is present in farmed or wild populations, or are inherently safe and require no altered management, generally require no further assessment beyond hazard identification.

Potential ecological implications of behavioural changes in GM animals should be discussed, including potential disruptions to food webs, competitive interactions, and disease transmission. Since GM mammals may escape, the receiving environment must be clearly defined according to species and farming system. For example, GM cattle may be housed indoors or managed on pasture. For GM pigs, the domestic pig serves as the appropriate comparator in farm-based (intended) environments, whereas wild boar is the most relevant comparator for wild (unintended) environments.

GM mammals may have invasive potential, depending on their phenotype, reproductive capacity, and ecological interactions. The applicant should describe if the genetic modification affects any of these characteristics. Uncertainty and data gaps should be described.

The applicant should consider mechanisms of action, direct and indirect effects, spatial and temporal scales, and the magnitude of potential impacts. Direct immediate effects may include competition, predation, or herbivory, and disease transmission. Indirect immediate effects may involve HGT including via faecal microorganisms. Long term direct effects may include hybridisation with conspecifics, while long term indirect effects may involve alterations of soil structure, nutrient cycles, or disease dynamics, such as changes in the spread of vector-borne pathogens.

Potential adverse environmental effects should be assessed both in the intended production systems and in the wild.

Step 3: Exposure characterisation

Applicants should characterise the environmental exposure by describing the conditions under which the GM mammals are kept and the potential for movement or escape into other environments, and the magnitude of adverse effects if escape occurs. The level of confinement strongly influences the likelihood of escape and must be clearly defined.

The assessment must also consider whether the GM trait affects accessibility to the environment.

For mammals, propagule pressure is the strongest predictor of establishment success in a novel environment. Although escapes from confined systems are expected to involve few individuals, species kept in large groups—such as livestock—pose a higher risk of generating transient or persistent populations. Escaped GM mammals may also experience high propagule pressure; therefore, the number of individuals that escape, and the potential frequency of escape events must be theoretically assessed to ensure that propagule pressure remains below levels associated with persistence or invasiveness.

Step 4: Risk characterisation

A risk characterisation includes a discussion on the magnitude of identified risk, which could be escape events, and the likelihood of adverse effects to occur if the GM animal escape. This should be discussed for each potential adverse effect. Because multiple adverse effects may occur, both the magnitude and likelihood of each should be assessed. Uncertainty associated with each identified risk must also be described.

The assessment should identify the weakest step in the sequence of events leading to persistence (escape, survival, reproduction, gene spread) and invasion (dispersal, population increase, ferality). Identifying this weakest link helps determine the point at which the overall risk is greatest.

Step 5: Risk reducing measures

GM mammal species differ in how readily they can be controlled or managed, and this variability directly influences their risk of persistence and invasion. These risks can be

minimised through careful genetic design, appropriate expression of the modification, and robust planning and regulation of confinement. Reducing propagule pressure at all stages is essential, as is implementing measures that limit vertical gene transfer (VGT) and environmental exposure.

Escape risks can be mitigated through secure physical confinement and fully licensed, monitored transport. Persistence can be further reduced by enhancing the ability to detect and recover escaped individuals and by limiting their reproductive potential in the wild—for example, through tagging, diagnostic marking, or maintaining single-sex groups. Dispersal risks may be lowered by improving detectability (e.g., phenotypic markers). Avoiding interactions between GM and non-GM mammals will reduce the risk of hybridisation and VGT.

Step 6: Uncertainties

Uncertainties and knowledge gaps regarding GM invasiveness and gene transfer and its likely consequences should be described and the applicant should discuss if applying appropriate risk management strategies would minimize risks due to uncertainties.

3.2.2 Vertical and horizontal gene transfer

3.2.2.1 VGT to animals in production systems

The most common form of VGT is sexual reproduction. VGT of a genetic modification from a GM mammal into wild species is not considered an environmental risk in itself; however, there is a potential risk associated with any phenotypic and biotic effects of VGT.

Step 1: Problem formulation including identification of hazard and exposure pathways

The applicant should consider effects not only on the dynamics of populations in the receiving environments but also on their genetic diversity. The maintenance of genetic diversity is increasingly seen as a vital component of environmental policy.

Applicants should assess the extent to which loss of genetic diversity is likely to represent a hazard and should characterise the risk of this hazard in practice and assess the magnitude of the environmental harm potentially caused by a decrease in genetic diversity.

Step 2: Hazard characterisation

Applicants should clearly describe the breeding strategy for the development of the GM animal. An assessment should be made if the vertical transfer of the genetic modification would create a hazard.

Any differences in the generation time and/or reproductive period between the GM animal and the parental species from which it was derived should be accounted for. The focus should be on differences in breeding strategies and/or in their likely outcomes between the GM animal and its non-GM comparator. For the latter, the strategies should represent current conventional practice. If there are no such differences, then there is no need for the applicant to proceed to the next step.

Applicants should evaluate the magnitude of the potential harm caused by a loss in genetic diversity.

Step 3: Exposure characterisation

To determine the likelihood of the potential loss of genetic diversity, the exposure assessment needs to consider a potentially large uptake of the GM animal and the number of genetic backgrounds into which the trait is likely to be bred.

The exposure characterisation should also consider if there are any populations which might be at risk in the receiving environment and the degree to which the environment locally may be marginal, fragmented and/or unfavourable.

Applicants should consider the potential effects of environmental stress, including disease, on selection and genetic diversity.

Step 4: Risk characterisation

An estimate of the risks of the potential loss of genetic diversity should be made. Following this, the risks of any adverse effects from any of this loss should be stated. Since there may be more than one potential adverse effect, the magnitude and likelihood of each individual adverse effect should be assessed.

Step 5: Risk reducing strategies

Applicants may need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions that foster the loss of genetic diversity.

Step 6: Uncertainties

Uncertainties and knowledge gaps regarding vertical gene transfer should be described and the applicant should discuss if applying appropriate risk management strategies would minimize risks due to uncertainties.

3.2.2.2 Horizontal gene transfer

The evaluation of the impact of horizontal gene transfer (HGT) from GM mammals includes analysis of the potential for the transfer of the genetic modification and further dissemination to other organisms. Furthermore, if HGT can occur, the consequences of such transfer events for human and animal health and the environment should be evaluated.

Step 1: Problem formulation including identification of hazard and exposure pathways

HGT from GM mammals to other mammals and to other multicellular eukaryotes are rare, and the initial problem formulation should focus on characteristics of genetic modifications that can lead to changed mobility. If changes in the potential for mobility of genetic material have been identified, a further detailed ERA is necessary, including magnitude and likelihood of adverse effects due to HGT.

The probability and frequency of HGT from mammals (including the genetic modification) to exposed microorganisms is determined by the following factors: (1) the amount and quality of DNA accessible to microorganisms in relevant environments; (2) the presence of microorganisms with a capacity to develop genetic competence, i.e. to take up extracellular DNA; and (3) the existence of genetic recombination processes by which the mammal DNA can be incorporated and thus stabilised in the microbial genome (including chromosomes or plasmids). In addition, positive selection of rare transfer events is required for long-term impact on the population.

The applicant should consider alternative sources of exposure, such as if the introduced genes in the GM animal is already present in the environment, e.g. from wild relatives. If the introduced genetic modification does not lead to changes in the horizontal mobility of the DNA into microbial populations beyond any other chromosomal mammals DNA (non-mobile), applicants should provide a short statement that substantiates the absence of a HGT potential beyond other non-mobile mammals' genes.

Step 2: Hazard characterisation

The probability of occurrence of a HGT of the altered genetic material in the GM animal and its consequences should be described.

Step 3: Exposure characterisation

If a hazard has been identified, the probability of HGT to occur should be discussed. The exposure characterization should take into account the limited scale and duration of the field trials.

Step 4: Risk characterisation

The risk characterization for focuses on the likelihood and consequences of an identified hazard from HGT events, taking into account current knowledge of the trait in question and to what extent the genetic modification has altered its potential for horizontal mobility.

Step 5: Risk reducing strategies

A case-by-case approach should be applied if a risk is identified. Appropriate risk management strategies should be considered.

Step 6: Uncertainties

Uncertainties and data gaps regarding horizontal gene transfer should be described and the applicant should discuss if applying appropriate risk management strategies would minimize risks due to uncertainties.

3.2.3 Pathogens, infections and diseases

Any changes in susceptibility or interactions of GM mammals with pathogens, infections and disease compared with their non-GM comparator(s) should be considered. Infected GM

animals may pose risks to other animals, humans and the environment potentially giving rise to secondary effects, including indirect welfare impacts.

Step 1: Problem formulation (including identification of hazard and exposure pathways)

The first step is to determine the likelihood of introduced changes in susceptibility of the GM animal to infections and diseases or any other alteration in pathogen interaction compared with the non-GM comparator. Potential consequences of altered pathogen interactions may occur as acute effects or as delayed or cumulative effects over time. Both immediate and long-term consequences should therefore be considered in the identification of hazards and exposure pathways.

Step 2: Hazard characterisation

If a hazard has been identified regarding GM animal and pathogen interaction; its nature and potential magnitude of harm especially for animals in the receiving environment should be discussed. This includes potential adverse effects on:

- non-GM animal welfare, such as pain, suffering, distress, or long-term impairment
- non-GM animal health, including severity, duration, and reversibility of disease
- human health and the environment, where relevant

Direct adverse effects on the GM animals and indirect or secondary effects, including those arising from pathogen management or transmission dynamics that may influence welfare outcomes should be discussed.

Step 3: Exposure characterisation

Exposure characterisation should evaluate the likelihood of occurrence for each identified hazard and the extent of potential exposure. Factors to be considered include:

- characteristics of the GM trait,
- the receiving environment (confined or semi-confined),
- and the presence or absence of non-GM animals or other susceptible species.

Where GM animals are kept under confined or semi-confined conditions, the likelihood of escape should be assessed. The proportion of time spent in confinement should be estimated, as this may influence both exposure pathways and indirect welfare outcomes.

Potential routes of infection transmission (e.g. aerosols, urine, faeces) should be discussed. Acute and chronic exposure scenarios should be considered separately, and exposure via farm waste products should also be discussed. In addition to epidemiological considerations, the

implications for number of animals affected, duration of exposure, and potential welfare impacts should be considered in the extent of the field trial.

Step 4: Risk characterisation

Risk characterisation should discuss the probability and magnitude of adverse effects. This includes consideration of both primary welfare effects in GM mammals and possible secondary effects in other animals due to transmission or disease control measures, when relevant.

Step 5: Risk reducing measures

Where risks are identified appropriate risk-reducing measures should be described. Measures may include containment and biosecurity practices, health and welfare monitoring, management interventions, and predefined criteria for intervention or removal of affected animals. Such measures should aim to reduce risks to animal welfare, animal and human health, and the environment.

Step 6: Uncertainties

Uncertainties and data gaps regarding altered pathogen interaction due to the modified trait should be described and the applicant should discuss if application of appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.4 Impacts of GM mammals on target organisms (TOs)

A target organism (TO) is an organism on which specifically designed characteristics of a GM mammal are intended to act. For mammals TOs will typically be pathogens or pests. TOs should be defined by the applicants. All other organisms (except the GM mammal itself) should be considered as NTOs (see below).

The intended use of the GM mammal and the level of confinement (as defined in section 2.1) should be considered when identifying interactions with TOs.

Steps 1 and 2: Problem formulation, hazard identification and characterisation

The focus of the problem formulation is to determine potential adverse environmental effects of the interactions between the GM mammal and TOs. An important first step is to identify any TOs of the GM mammal in the receiving environments. The likelihood of the TOs to evolve mechanisms reducing the efficacy of the genetic modification, and potential consequences for management of the GM mammal, should be discussed. Hazard characterisation should include consideration of the a) likelihood that the TOs could switch to different hosts and b) the likelihood of secondary pests or pathogens infecting the GM mammal. On a case-by-case basis, an assessment of c) environmental effects of the TOs induced by the GM mammal might also

be required. This is necessary if the GM mammal causes suppression or elimination of the TO population(s).

Step 3: Exposure characterisation

The likelihood of negative effects on the environment via the GM animal's effects on the TOs should be assessed.

Step 4: Risk characterisation

The risk should be characterised for TOs for a) the evolution of mechanisms to reduce efficacy, b) the potential for development of undesirable changes caused by indirect effects in the interaction between the TOs and the GM mammal in the receiving environments, and/or c) the overall effects of the GM mammal in suppression or elimination of TO population(s).

Step 5: Risk reducing measures

Where risks are identified appropriate risk reducing measures should be described.

The applicant should also describe monitoring measures that are required to assess the actual efficacy of the GM animal in managing the TO population(s).

Step 6: Uncertainties

Uncertainties and data gaps regarding effects on the TOs should be described and the applicant should discuss if application of appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.5 Impacts of GM mammals on non-target organisms (NTOs)

NTOs are defined as all species that are directly and/or indirectly exposed to the GM mammal except TOs (see section 3.2.4). The applicant should consider potential environmental impacts, both immediate and/or delayed, and all direct and indirect interactions between the GM mammal and NTOs (including competitors, prey, hosts, symbionts, predators, parasites and pathogens) where the genetic modification might play a role.

The intended use of the GM mammal and the level of confinement (as defined in section 2.1) should be considered when identifying interactions with NTOs.

Applicants should first consider the GM mammal itself, e.g. the genetic modification, the donor and recipient organism, the new/modified traits, and the phenotypic and reproductive characteristics of the GM mammal and whether there are TOs (see section 3.2.4).

Second, applicants should define the accessible ecosystems and potential effects on NTOs if GM mammals should escape.

Step 1: Problem formulation including identification of hazard and exposure pathways

NTOs present in ecosystems both inside and outside of a GM mammal's body are of relevance. For example, if the manure of a GM mammal contains a different composition of microorganisms (NTOs) than the manure of the comparator.

A crucial step in problem formulation is the identification of aspects of the receiving environments (e.g. valued entity, ecosystem services/functions) that need to be protected from harm, and to translate those into concrete measurable phenomena (i.e. assessment endpoints).

Typical assessment endpoints can be the distribution, abundance, and species richness of certain groups of organisms at a relevant life stage within a landscape or region over a specific time period.

Problem formulation should be supported by all relevant available data and information sources (from literature, as well as from previous laboratory and field experiments). Applicants should justify why the data used are relevant to the receiving environments where the GM mammal could have an impact (indirectly or if escaped). The sources of data should be properly justified and described.

There are two different scenarios for potential hazards:

Scenario 1)

For receiving environments where the comparator is present.

Adverse effects on NTOs can be caused by morphological, behavioural, biochemical, physiological, developmental and reproductive changes in the GM animal. They may include modified responses to husbandry and dietary regimes, in bioactivity (endocrine, pharmacological, or immunological activity) and toxicity of newly expressed substances. A special case of scenario 1 could be if a GM mammal with the purpose of reducing the abundance of specific target organism (e.g. a pest) should escape into the wild. The intended reduction of the target organism could cause unintended effects on NTOs for instance if the target organism is an important food source for an endangered species.

Scenario 2)

For receiving environments where neither the comparator is not present.

NTOs may belong to different trophic levels (e.g. primary producers, herbivores, predators, decomposers, parasites/pathogens or mutualists/symbionts) and will as such have different direct and indirect interactions with the GM mammal.

The modified trait may increase the invasive potential of the GM mammal. In a three-level food chain, the GM mammal indirectly affects a competing NTO via a resource species they are sharing (competition). Multitrophic effects are here defined as more complex indirect effects via two or more species (see further below in this section).

Impacts on NTOs can be caused by changes to the GM mammal, to its management as well as the effects of the introduced traits. As GM mammals and their biology differ, depending on the modifications and intended use, a case-by-case-approach should be followed.

As all interactions between the GM mammal and other species of different trophic levels in the receiving environment cannot be assessed (even for semi-confined conditions), applicants need to select a representative subset of NTO species for consideration. These are called focal NTOs.

Identification of NTOs

Then a four-step process needs to be followed for the identification of NTOs either for scenario 1, scenario 2, both scenarios, or not at all.

For scenario 1, where a comparator species is present in the receiving environments, applicants should discuss how the genetic modification may lead to different (direct or indirect) species interactions of the GM mammal as compared with the comparator species (comparative assessment). If applicants clearly show that the GM mammal has no different species interactions from the comparator species further steps to identify focal NTOs will not be required. If differences in species interaction cannot be excluded, or if there are one or more TOs, it should be assessed if the GM animal might escape into accessible ecosystems where the comparator species is not present (scenario 2 applies) and the four-step selection process for scenario 2 should be followed.

- Step A: Identification of functional groups (trophic levels) of NTOs that are directly exposed to or indirectly interacting with the GM mammal.
- Step B: Identification of NTO species from each functional group in the receiving environments. It should also be considered that different life stages of a given species may have different ecological roles (e.g. different feeding habits).
- Step C: Ranking species based on ecological criteria.

  Applicants shall prioritise NTO species from each relevant functional group. Keystone species (see definition in the Glossary) and particularly vulnerable species (i.e. are certain populations already threatened and thus more vulnerable to additional pressures?) should be prioritised. A food web with the GM mammal and all prioritised species should be made to obtain an overview of all direct interactions. For scenario 1, an additional food web with the comparator species instead of the GM mammal should be constructed (all NTO species remaining the same). Effects on any relevant NTOs not included in the food web (e.g. endosymbionts) should also be identified. Unknown interactions should be indicated, e.g. by question marks. Details and further information are provided in step 2, hazard characterisation, below.
- Step D: Selection of focal species for in-depth investigation.

  A restricted number of focal species identified in step C needs to be selected for in-depth investigations. Prioritised species from step C and the number of direct and indirect food-web interactions with the GM mammal are typical selection criteria. It is expected that applicants select at least one focal species from each relevant functional group for an in-depth investigation. Applicants should explain and justify their selection. The details of the in-depth investigation are outlined in hazard characterisation below.

Additional considerations:

The presence of GM mammals may cause a selective pressure on NTOs and thus affect their evolution. NTOs that have changed evolutionarily might if transported to other regions and, because of their novelty, change species interactions there as well.

If cross-breeding between escaped GM mammals and wild relatives, or within the offspring of the GM mammal, is likely to occur, then exposure of NTOs to these GM offspring over life cycles should be assessed as well as environmental consequences of such exposure.

Knowledge gaps and scientific uncertainties are especially relevant for this section, as it will be hard to identify all possible direct and indirect interactions of GM mammals with NTOs. The uncertainty resulting from excluding non-focal NTOs and their direct and indirect interactions with GM mammals should be assessed by applicants.

Step 2: Hazard characterisation

Step C: Construct food webs for relevant NTO species (identified in Step A and B described above).

For scenario 1, an additional food web with all relevant NTO species and their interactions with the comparator should be drawn. The relevant NTO species are expected to be the same in the food web with the GM mammal and the food web with the comparator species, but the species interactions might be different. To construct such food webs, a thorough understanding of the biology and ecology of the species will be required. Information about the species can, for example, be acquired from literature sources or from experts. A table should identify if the species is directly or indirectly affected by the GM mammal. Effects on any relevant NTOs not included in the food web (e.g. endosymbionts) should also be identified.

The effects of the GM mammal on relevant NTOs should be compared with effects of the comparator (scenario 1 above). For scenario 2 (where no comparator species is present), the comparison is between a food web without the GM mammal (the current situation) and a food web with the GM mammal (the possible future situation). Hence, the effects of the GM mammal on relevant NTOs are indicating possible future changes, caused by the GM mammal, in the populations of relevant NTOs.

Step D: Focal NTO species investigation

Measurement endpoints for hazard characterisation should be those that quantify (1) direct effects on focal NTOs; (2) one-way indirect effects on focal NTOs (via one other species), and (3) multitrophic effects (e.g. change in biodiversity, ecosystem functions and services). Due to increasing complexity, it may not be practical to quantify effects for (2) or (3), and qualitative effects should then be identified.

As outlined in step C above, the effects should be compared with those of the comparator or wild conspecifics, if present in the receiving environment (scenario 1). If conspecifics are not present in the receiving environment (scenario 2), then the comparison should be between absence and presence of the GM mammal.

The aim is to ensure that adequate data exist to assess the effects of interactions between the GM mammal and each focal NTO.

1. Direct effects on NTOs

If not known from step C, direct interactions of the GM mammal on focal NTOs need to be identified. For scenario 1, information on the comparator species' direct interactions can be used together with information on how the genetic modification may affect these interactions (if the latter is known). For scenario 2, the first step for identifying potential direct interactions with NTOs should be to identify species that are directly interacting with the comparator in all the ecosystems within its geographic range. The second step should be to compare these species with NTOs in the receiving environment.

2. One-way indirect effects on NTOs

If one-way indirect interactions between the GM mammal and focal NTOs are identified in step C, their qualitative effects should be indicated and quantified.

For scenario 1 one-way indirect interactions between the GM mammal and focal NTOs should be compared with those of the comparator species, and it should be indicated if they are expected to be quantitatively similar or different. For scenario 2, applicants should indicate qualitative effects of indirect interactions, and quantifying the effects should be done depending on the focal GM mammal.

3. Multitrophic effects on NTOs

In a food web it is usually impossible to predict the combined effects of all multitrophic interactions by theoretical means. The change in biodiversity, ecosystem functions and services due to release of the GM mammal can then be compared with changes due to the comparator (scenario 1) or to the existing situation if the comparator is not present (scenario 2).

Step 3: Exposure characterisation

To evaluate the likelihood that identified hazards will pose a risk on NTOs, it is important that the applicant consider the scope and scale of the field trial, including the confinement level and potential for escape.

The likelihood of escape needs to be estimated, based on the characteristics of the level of confinement and the facilities where the GM mammals will be held and on the characteristics of the GM mammals themselves, e.g. their mobility. The life expectancy of GM mammals is also important here.

For semi-confined GM mammals, the likelihood of their escape and their invasive potential require estimation. For receiving environments, the likelihood that the genetic modification affects the internal ecosystem of GM mammals (e.g. gut microbiota) in addition to external accessible ecosystems, needs to be evaluated.

For GM mammals that can reproduce with wild conspecifics it will be required to determine whether interbreeding with any NTOs can be expected.

Estimates of maximum numbers of GM mammals that can escape, should be provided by the applicants. The exposure assessment should account for a worst- case scenario in space and time.

Step 4: Risk characterisation

Applicants should estimate risks posed by the GM mammal for NTOs for each hazard identified, quantitatively wherever possible. It should be discriminated between the risk for NTOs in the confined / semi-confined environment and the risk posed by escaped GM mammals for NTOs in the wild.

Step 5: Risk reducing measures

For risks caused by the GM mammals on NTOs, identified and characterised, applicants should propose appropriate risk management strategies for each risk. Possible risk management strategies include measures to reduce the probability that confined, and semi-confined, GM mammals escape and interact with NTOs.

Step 6: Uncertainties

Uncertainties regarding interactions with NTOs should be described and the applicant should discuss if applying appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.6 Interactions of GM mammals with the abiotic environment

Step 1: Problem formulation including identification of hazard and exposure pathways

The GM mammal may exhibit altered tolerance to abiotic factors, whether intended or unintended, and may interact with abiotic processes in ways that differ from those of the non-GM comparator. Interactions with the abiotic environment include processes mediated by GM mammals that influence the movement, transformation, and storage of energy, water, carbon, nitrogen, and other elements within ecosystems.

Examples of such interactions include intake and release of carbon dioxide, alterations of plant or aquatic material, modification of soil organic matter, and changes in nitrogen transformation pathways. These processes may affect greenhouse gas fluxes (e.g. CO_2, CH_4, N_2O) and thereby influence climate-relevant processes. Effects of GM mammals on soil biota may be of relevance, as soil organisms play a key role in soil structure, biogeochemical cycling, immobilisation and mobilisation of nutrients, degradation of organic matter, and emissions of greenhouse gases.

Problem formulation should address both immediate receiving environments and the wider receiving environment, including relevant environmental compartments (soil, water, air), considering the scale, duration, and scope of the field trial. Indirect effects associated with

altered management or husbandry practices applied to the GM mammal should also be considered where relevant.

Step 2: Hazard characterisation

If a potential hazard to abiotic processes has been identified, its nature and potential magnitude should be further characterised, including relevant exposure pathways.

With respect to the receiving environment, the evaluation should consider potential impacts of GM mammals through factors such as:

- GM-specific metabolites, or other compounds into environmental compartments that may influence soil organic matter
- effluents (e.g. faeces or urine) that decompose differently from those of non-GM mammals due to the presence of specific compounds or altered concentrations of substances resistant to decomposition
- abiotic habitat modification (e.g. disturbance of physical structures resulting from burrowing, grazing, trampling, or other mechanical effects)
- alterations to biogeochemical processes, including nutrient cycling and nitrogen transformation

Applicants should assess whether the GM mammal, and its associated management practices, may have adverse effects on abiotic processes in comparison with the non-GM comparator. Where changes in abiotic processes may influence biological components of the receiving environment, such interactions should be evaluated in relation to the characteristics of the introduced trait.

The level of details required in the assessment should be proportionate to the characteristics of the GM trait, the GM mammal, and the scope of the application, including the size and duration of the field trial.

Step 3: Exposure characterisation

Exposure characterisation should assess the likelihood that abiotic processes may be adversely affected because of the GM mammal or its associated management practices, including potential exposure following escape.

The assessment should cover relevant spatial and temporal scales and account for variability among possible receiving environments. Factors such as containment conditions, duration of the field trial, and management intensity should be considered.

Step 4: Risk characterisation

Risk characterisation should compare conditions under current conventional management systems with those anticipated for the GM mammal, to identify any adverse effects on abiotic processes. The assessment should consider the time- and scale limited nature of field trials, as well as the reversibility of potential effects.

Where risks are identified, the likelihood and magnitude of effects, including their spatial and temporal extent, should be described.

Step 5: Risk reducing measures

Where risks to abiotic processes have been identified and characterised, applicants should consider targeted risk management measures aimed at minimising unintended environmental effects. Such measures may include adaptations of management practices, containment measures, or monitoring of relevant abiotic parameters.

Step 6: Uncertainties

Uncertainties and data gaps encountered should be described, including uncertainties related to indirect, cumulative, or long-term effects on abiotic processes and environmental compartments. The applicant should also discuss if application of appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.7 Environmental impacts of the specific techniques used for the management of GM mammals

GM mammals may require specific management techniques during field trials that differ from those applied to non-GM comparators. Applicants should assess the potential environmental impacts associated with such management techniques, including housing, handling, biosecurity, feeding, treatment, disposal of animals, and management of waste products. Any changes in husbandry or management practices required due to the introduced trait, and their potential environmental consequences, should be identified and evaluated.

Step 1: Problem formulation including identification of hazard and exposure pathways

Problem formulation should consider the receiving environment(s) in which the GM mammal is managed during the field trial, including the range of husbandry and management practices applied and if these need to be altered due to the GM mammal. Applicants should identify and describe difference in management techniques that may have impact on environmental compartments (soil, water, air) and that may give rise to environmental exposure pathways.

Examples of management-related factors that may require consideration include practices associated with altered susceptibility to pathogens, enhanced biosecurity measures, vaccination programmes, containment systems, treatments, or practices that may contribute to the establishment of new reservoirs or vectors. The assessment should follow a case-by-case approach, recognising that management-related environmental effects depend on the characteristics of the GM mammal, the introduced trait, the field trial design, and the specific techniques applied.

Step 2: Hazard characterisation

Where changes in management techniques have been identified, the nature and potential magnitude of environmental effects associated with such changes should be discussed. Applicants should describe the specific management practices applied during the field trial and discuss whether these practices may result in greater or different adverse environmental effects compared with current conventional practices for the non-GM comparator.

Management techniques that may be relevant for hazard characterisation include, but are not limited to, vaccination and treatment protocols, biosecurity measures, housing systems, containment practices, and disposal procedures. The assessment should focus on how these practices may affect abiotic or biotic components of the receiving environment.

Step 3: Exposure characterisation

Where changes in management practices are expected, applicants should discuss the scale, frequency, and duration of such changes and the resulting potential exposure of the receiving environment in the limit of the field trial. Both short-term and longer-term exposure scenarios should be considered, considering the duration of the field trial and the intensity of management interventions.

Exposure characterisation should address relevant spatial and temporal scales and consider whether management-related exposures are confined to the immediate receiving environment or may extend beyond it.

Step 4: Risk characterisation

Risk characterisation should assess the likelihood and magnitude of adverse environmental effects resulting from altered management practices due to the field trial with the GM mammal. This assessment should integrate the findings from hazard and exposure characterisation and consider the time- and scale-limited nature of field trials.

Any identified risks associated with management techniques should be described.

Step 5: Risk reducing measures

If risks related to management practices have been identified and characterised, applicants should describe risk management measures aimed at preventing or minimising unintended environmental effects. Such measures may include adaptations of management techniques, containment approaches, waste handling procedures, or monitoring of environmental parameters.

Step 6: Uncertainties

Uncertainties and data gaps associated with the environmental impacts of specific management techniques used for the GM mammal should be described. The applicant should also discuss if applying appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.8 Impacts of GM mammals on non-GM animal health and welfare

This section addresses the assessment of whether the GM mammal present a new hazard for the health and welfare of other animals. Applicants should consider the present section in conjunction with sections – 3.2.3. Pathogens, infections and diseases, 3.2.5 Interactions of GM mammals with NTOs and 3.2.7 Environmental impacts of the specific techniques used for the management of GM mammals.

A generalised risk assessment for the impact of GM animals on the health and welfare of non-GM animals is not feasible. Each new genotype or phenotype requires a case-specific, stepwise assessment to identify and characterise hazards to conspecific and heterospecific non-GM animals.

Step 1 and 2: Problem formulation including identification and characterisation of hazard and exposure pathways

The assessment should begin with problem formulation defining plausible pathways through which the GM animal or its products could adversely affect other animals (e.g., infection, competition, toxicity, behavioural change).

Hazard identification and characterisation should determine whether the GM animal introduces new hazards or alters the probability, magnitude, or distribution of existing ones.

Step 3: Exposure characterisation

Exposure assessment must specify which target and non-target animals may be exposed to the GM animal and its products, the exposure routes, and the intensity and duration of exposure. These components must be integrated into a risk characterization and uncertainty analysis.

The evaluation should encompass the GM animal, its products, and the management practices required for its husbandry, as well as their ecological and epidemiological implications.

A critical element is determining whether the GM animal or its husbandry alters susceptibility or resistance to pathogens. Such changes may shift infection reservoirs or transmission

dynamics, affecting animals in shared environments. The assessment must consider potential modifications in pathogen load, shedding patterns, and transmission routes (fecal, airborne, vector-borne), as well as impacts on pathogen evolution through altered host–pathogen interactions or selective pressures. Comparative pathogen profiling against appropriate non-GM comparators is essential.

The assessment must evaluate whether the GM animal inadvertently alters host range, becomes a competent host for new pathogens, or modifies interactions with vectors through changes in physiology or behaviour. Pathway analyses should examine whether such changes could establish new transmission cycles affecting domestic or wild mammals.

Potential ecological and behavioural interactions with non-GM animals must be assessed, including:

- Competition or displacement due to altered fitness.
- Predator–prey dynamics, where changes in size, behaviour, or distribution influence predation patterns and predator health.
- Reproductive interactions, including introgression of GM traits into non-GM populations, with implications for population health, disease susceptibility, reproductive success, local adaption and genetic diversity. These considerations are particularly relevant for species with escape potential (e.g., pigs, farmed deer).

Non-GM animals may be exposed to excreta, secretions, carcasses, or contaminated feed and bedding containing GM-encoded proteins, metabolites, or altered microbiota. Such exposures may induce toxicological, immunological, or physiological effects, increase susceptibility to infection, or facilitate indirect disease transmission.

Where GM mammals are kept under confinement or may escape, the assessment must evaluate biosecurity effectiveness during the complete field study and life stages and the consequences of escape. Potential establishment of feral GM populations must be examined for impacts on ecosystems, disease reservoirs, and wildlife health and welfare. Management systems (e.g., high-density indoor housing, outdoor systems, and research facilities) may create novel interfaces between GM animals, domestic animals, and wildlife, altering disease and ecological risks.

Step 4: Risk characterisation

Risk characterisation includes an assession on the likelihood and degree to cause environmental harm for the risks identified of the GM mammals on non-GM animal health and welfare. The uncertainty for each risk should also be described.

Step 5: Risk reducing measures

Risk reducing measures include description of strategies to mitigate the adverse effects to the environment from escaped GM animals, if any risks are identified.

Step 6: Uncertainties

The applicant should describe any uncertainties encountered related to health effects of non-GM animal due to interaction with or exposure to the GM mammal and discuss if applying appropriate risk reducing measures would reduce risks due to uncertainties.

3.2.9 Impacts of GM mammals on human health

An assessment of potential impacts on human health related to the GM mammal should be conducted, with particular attention to occupational exposure, handling, management practices, and unintended exposure, including escape or accidental consumption. Applicants should consider both immediate and delayed effects on human health resulting from direct or indirect interactions with the GM mammal.

Potential changes in the production of biologically active compounds, toxic or allergenic substances, as well as alterations in disease transmission dynamics, including zoonotic pathways, arising from the genetic modification should be identified and assessed.

Step 1 Problem formulation including identification of hazard and exposure pathways

Problem formulation should identify potential human health hazards associated with the GM mammal in relation to the receiving environment(s) and the conditions under which the field trial is conducted. This includes potential hazards arising from physiological, behavioural, or metabolic changes in the GM mammal due to the introduced trait.

Consideration should be given to potential pathogenic or toxicological impacts on human health resulting from escape of the GM mammals, including situations involving direct handling or indirect environmental exposure. The problem formulation should follow a case-by-case approach, considering the characteristics of the GM mammal, the introduced trait, and the design and duration of the field trial.

The following aspects should be considered where relevant:

- altered capacity for disease transmission to humans due to physiological or behavioural changes
- potential to contribute to the emergence or selection of new pathogens or pathogen strains, including those with altered host ranges that may include humans
- introduction or altered levels of toxic, allergenic, or biologically active compounds produced by the GM mammal or its metabolic processes
- phenotypic changes observed during development that may increase human exposure potential
- consequences of changes in management or husbandry practices required for the GM mammal that may affect human exposure pathways

Step 2: Hazard characterisation

Identified hazards to human health should be characterised in terms of their nature and potential severity. This includes assessing whether the GM mammal exhibits characteristics that may increase the likelihood of adverse effects on human health compared with the non-GM comparator under conventional practice.

Special attention should be given to risks relevant to workers involved in the handling, care, or management of the GM mammal, particularly were changes in behaviour, physiology, or containment conditions may alter the frequency or intensity of contact.

Hazards identified for the field trial should be evaluated in comparison with exposures associated with non-GM animals under conventional management, to support a comparative risk assessment.

Step 3: Exposure characterisation

Exposure characterisation should assess plausible human exposure scenarios associated with GM mammals. These may vary depending on:

- different stages of development, management, or processing of the GM mammal
- intended and foreseeable uses of the GM mammals during the field trial
- characteristics of the receiving environments
- contact with live or dead animals, including prior to and following slaughter or disposal

Applicants should assess management practices applied to the GM mammal to estimate occupational exposure levels, including frequency and duration of contact. Exposure routes such as dermal, inhalation, nasal, ocular, or oral exposure should be considered where relevant, and exposure rates should be estimated where feasible.

Approaches for the disposal of dead animals should also be assessed as part of exposure characterisation.

Step 4: Risk characterisation

Risk characterisation should integrate hazard and exposure information to estimate the likelihood and magnitude of adverse effects on human health for each identified hazard–exposure pathway. This assessment should distinguish between occupational exposure and potential non-occupational or accidental exposure scenarios.

Step 5: Risk reducing measures

If risks to human health have been identified, applicants should propose risk management and mitigation measures aimed at minimising adverse effects. Such measures may include

adaptations to management practices, handling procedures, containment systems, personal protective measures, or waste and carcass disposal protocols.

Step 6: Uncertainties

Uncertainties and data gaps related to human health impacts should be described and the applicant should discuss if applying appropriate risk reducing measures would reduce risks related to uncertainties.

4 Risk reducing measures

Risk reducing measures may be needed, depending on the risk identified, the type of GM animal, the confinement level and scale of the field trial, the genetic modification characteristics, and the characteristics of the identified hazard, and any consequences to the environment and human health.

The applicant should consider general risk reducing measures such as:

- Trained personnel and adequate resources
- Extent of confinement (is there need for changes in confinement level during the trial?)
- Adequate disposal of waste and carcasses
- Adequate size and duration of the field trial
- Description of safeguards in place during and post field experiment
- Recapture strategies
- Prevention of spread of infections and disease occurrence
- Prevention of zoonosis occurrence or allergic development in humans
- Follow up, e.g., additional monitoring period

5 Concluding remarks

The overall risk evaluation is assessed by VKM, based on the data provided by the applicant.

The risk assessment is based on the adversity of the risks identified for each area of concern and the sum of overall adverse effects on the environment or human and animal health considering risk reducing measures. Transparency regarding encountered uncertainties that may affect the risk conclusion is important. The applicant may address any encountered uncertainties with data from published studies with the same modified animal or other animals having the same characteristics or modification.

Data collected from field trials often forms the basis for further and larger scale experiments and applications for commercial production. In this context, it is important that the quality of the experimental design and collected data from the field trial is at the level ensuring proper statistical power for identifying relevant differences to comparator and revealing potential adverse effects.

Where adverse effects on the environment or human and animal health have been identified, risk reducing measures should be included in the field trial design to minimize the risks associated with the GM animal.

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