SCIENTIFIC OPINION

Guidance on the environmental risk assessment of genetically modified animals[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

This document provides guidance for the environmental risk assessment (ERA) of genetically modified (GM) animals to be released into the environment and placed on the EU market according to Regulation (EC) No 1829/2003 or Directive 2001/18/EC. It provides guidance for assessing potential effects of GM animals on animal and human health and the environment and the rationales for data requirements for a comprehensive ERA. The ERA should be carried out on a case-by-case basis, following a step-by-step assessment approach. This document describes the six steps for the ERA of GM animals, as indicated in Directive 2001/18/EC, starting with (1) problem formulation including hazard and exposure identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) an overall risk evaluation. The Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority follows Annex II of Directive 2001/18/EC, considering specific areas of risk to be addressed by applicants and risk assessors during the ERA of GM fish, GM insects and GM mammals and birds. Each specific area of risk is considered in a structured and systematic way following the aforementioned six steps. In addition, this Guidance Document describes several generic cross-cutting considerations (e.g. choice of comparators, use of non-GM surrogates, experimental design and statistics, long-term effects, uncertainty analysis) that need to be accounted for throughout the whole ERA.

KEY WORDS

Birds, Directive 2001/18/EC, Environmental Risk Assessment (ERA), fish, genetically modified, insects, mammals, release.

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SUMMARY

This Guidance Document (GD) provides guidance for the environmental risk assessment (ERA) of GM animals to be released into the environment and placed on the EU market according to Regulation (EC) No 1829/2003 or Directive 2001/18/EC. It provides guidance to risk assessors for assessing potential adverse effects of GM animals on the environment and the rationales for data requirements for a comprehensive ERA. It also provides general guidance for drawing conclusions on the post-market environmental monitoring (PMEM).

The ERA of GM animals involves collecting, assessing and, where appropriate, generating information on a GM animal in order to determine its impact on the environment, human and animal health compared with non-GM animals or appropriate comparators.

The ERA should follow a step-by-step assessment approach. In accordance with Directive 2001/18/EC, the EFSA Panel on Genetically Modified Organisms (GMO Panel) describes the six steps for the ERA of GM animals: (1) problem formulation including hazard and exposure identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) overall risk evaluation. As a general principle, the use of a step-by-step approach beginning with problem formulation is required whereby scientifically reliable evidence, based on qualitative and, whenever possible, quantitative analyses, is combined with an explicit uncertainty analysis in order to support the final conclusions of the ERA.

In accordance with Annex II of Directive 2001/18/EC, the EFSA GMO Panel considers specific areas of risk that applicants should address following systematically the six steps of the ERA. This GD addresses for GM fish, GM insects and GM mammals and birds the following areas of risk: (1) persistence and invasiveness of the GM animal, including vertical gene transfer; (2) horizontal gene transfer; (3) interactions of the GM animal with target organisms; (4) interactions of the GM animal with non-target organisms; (5) environmental impacts of the specific techniques used for the management of the GM animal; (6) impacts of the GM animal on biogeochemical processes; and (7) impacts of the GM animal on human and animal health.

In addition, this GD describes several generic cross-cutting considerations (e.g. choice of comparators, use of non-GM surrogates, experimental design and statistics, long-term effects, uncertainty analysis) that need to be accounted for throughout the whole ERA. The EFSA GMO Panel provides guidance to applicants on the selection of relevant receiving environments in which the GM animal is likely to be released, the choice of adequate comparators and, where appropriate, the use of non-GM surrogates with similar characteristics that can inform the ERA of the GM animal. Applicants should follow the experimental design, the general statistical principles outlined in this document such as specification of effect size, limits of concern, power analysis and the reporting recommendations. If experimental studies are being used they should allow testing for difference and equivalence. Moreover, applicants should communicate results and conclusions from the uncertainty analyses, as well as communicate how each type of uncertainty was treated, eliminated at a specific step, or further assessed and carried throughout the ERA. This GD also addresses the assessment of long-term effects requiring specific information sources and techniques, including experimental or theoretical methodologies, as well as aspects of the health and welfare of GM animals to be released into the environment.

The ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the type of GM animals and trait(s), the potential receiving environments and their intended use(s). The ERA may benefit from the comparative safety assessment of food and feed derived from GM animals, including data on the molecular characterisation, on the compositional analysis and on the phenotypic characterisation of the GM animal. The outcome of this comparative assessment will inform the initial steps of the ERA of GM animals and, in particular, the identification of possible unintended effects due to the transformation process and/or the trait. For the sake of a comprehensive ERA, information related to interactions between the GM animal and its receiving

environments should be collected (e.g. desk and literature studies), assessed and, where appropriate, generated (e.g. studies, modelling).

Therefore, the ERA should be carried out in a scientifically sound manner based on available scientific and technical data and on common methodology for the identification, gathering and interpretation of the relevant data. Tests, measurements, and data generated should be clearly described as well as the assumptions made during the ERA. In addition, the use of scientifically sound modelling approaches could provide further useful information for the ERA. Thus, sufficient scientific data enabling qualitative/quantitative risk estimates, must be available in order to draw a conclusion on the possible environmental risk posed by a given GM animal.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

Following a request of the European Commission (DG Environment and DG SANCO, ENV.B3 D(2007) 2004, DG SANCO, SANCOJK/dj D5 D(2010) 450066), EFSA initiated the development of a Guidance Document for the safety assessment of GM animals that would address both food and feed and environmental safety as well as animal health and welfare issues.

To address the request of the European Commission with respect to environmental safety issues, EFSA embarked on various initiatives. By the end of 2008, an external open call for tender on GM fish was launched, which was followed in the beginning of 2009 by open calls on GM insects, GM mammals and birds. Early 2011, external contractors submitted their reports (see Umweltbundesamt, 2010; FERA, 2010 and Hull, 2010) which provided criteria for the Environmental Risk Assessment (ERA) of GM fish, GM insects, and GM mammals and birds. The reports by external contractors served as basis for the identification of scientists with relevant expertise and the development of this Guidance Document. Between mid-2010 and mid-2011, three Working Groups were established to provide applicants with guidance on the ERA of GM fish, GM insects and GM mammals and birds, respectively. To prepare a de novo Guidance Document for applicants, the aforementioned Working Groups of the EFSA GMO Panel considered various sources of information, including the reports by external contractors, relevant comments from stakeholders on previous EFSA Guidance Documents, scientific literature, conference reports, and expert consultation. Seminars and workshops were also organised to support the development of this Guidance Document.

A draft Guidance Document is now submitted for public comments during an appropriate period of time (mid-June 2012 – end August 2012). The EFSA GMO Panel will consider all scientifically relevant comments from the public when finalising the present document. The EFSA GMO Panel will not consider issues related to risk management (e.g. traceability, labelling, coexistence). Ethical and socio-economic issues are also outside the remit of the EFSA GMO Panel.

EFSA will regularly review this Guidance Document in the light of experience gained, technological progress and scientific developments. By establishing a harmonised framework for the ERA of GM animals, this document provides useful guidance both for applicants and risk assessors.

Guidance on the risk assessment of food and feed from GM animals and on animal health and welfare aspects was developed by the EFSA GMO Panel, in close collaboration with the EFSA Panel on Animal Health and Welfare (AHAW Panel), and is provided in a separate document (EFSA, 2012a).

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

On 13 February 2007, the EFSA GMO Panel received a mandate from the European Commission (ENV.B3 D(2007) 2004) with the request to 'develop, building on the work done in the context of the Codex Alimentarius, a guideline on the safety evaluation of GM animals that would address both, food/feed safety and environmental safety of this technology. It is envisaged that this guidance will be used as input to discussions with the competent authorities dealing with Directive 2001/18/EC towards the adoption of the annexes to technical progress'. EFSA responded to this mandate with its letters [Ref. CGL/SR/DC-SM/cz(2007) 2269682; Ref. CGL/PB/SM-YD/md(2008) 3187837; Ref. CGL/PB/SM-YD/md (2008) 3187581; Ref.RM/PB/EW/shv/lg(2009)3701902]. In these letters, EFSA presented its work plan informing the European Commission that the ERA and the safety assessment of food and feed products derived from GM animals would have been addressed in parallel.

On 25th March, 2010, the European Commission (DG SANCO, SANCO JK/dj D5 D(2010) 450066) requested a revision of the mandate on the guidance on the human health and environmental risk assessment of GM animals, to include animal health and welfare aspects, in order to produce a final comprehensive package of information on the use of GM animals.

On 14th March 2011, the European commission (DG SANCO, SANCO/E1/GL/lh Ares (2011) 247006) requested EFSA to issue one single GD on the ERA of GM animals rather than three separate documents on GM fish, GM insect and GM mammals and birds.

ASSESSMENT

This document provides guidance to applicants on how to conduct the ERA of GM animals to be released into the environment and placed on the EU market according to Regulation (EC) No 1829/2003 (EC, 2003) or Directive 2001/18/EC (EC, 2001). It provides detailed guidance to assist applicants in the preparation and presentation of the ERA part of their applications. This GD also includes environmentally-related animal health and welfare aspects of GM animals.

Guidance on the risk assessment of food and feed derived from genetically modified animals and on animal health and welfare aspects, within the framework of Regulation (EC) No 1829/2003, is provided in a separate document (EFSA, 2012a). That GD addresses the molecular characterisation, which provides information on the structure and expression of the insert(s) and on the stability of the intended trait(s); the toxicological and allergenicity assessment of the novel protein(s) as well as of the whole food derived from the GM animal; and the nutritional assessment to evaluate whether food and feed derived from a GM animal are as nutritious to humans and/or animals as food and feed derived from traditionally bred animals. That GD also addresses the scientific requirements for the assessment of health and welfare of GM animals bred for food and feed uses, but it does not cover the ERA of GM animals for food and feed uses which is addressed in this document. Genetically modified animals can be placed on the EU market for food/feed uses (e.g. GM cattle) or non-food/feed uses (like GM 'ornamental' fish or GM insects). Both types of uses are covered in this GD, except the use of GM animals for the production of pharmaceuticals.

Applicants should also comply with all legal requirements applicable to animals, being GM and non-GM (e.g. health & welfare standards, quarantine standards, requirements for animal testing). In this respect, the European Union legislation requires that animals are not caused avoidable pain and distress and obliges the owner/keeper of animals to respect minimum welfare requirements (EC, 2010). This Guidance Document also addresses aspects of the health and welfare of GM animals to be released into the environment.

1. Scope of this Guidance Document

This document provides guidance to applicants on how to conduct the ERA of GM animals[4] to be released into the environment and placed on the EU market according to Regulation (EC) No 1829/2003 (EC, 2003) or Directive 2001/18/EC (EC, 2001).

The scope of this Guidance Document includes GM animals whose genetic material has been altered in a heritable way through the techniques of genetic modification (see Annex IA, part 1, Article 2(2) of Directive 2001/18/EC) allowing for the combination and/or introduction of genetic material into host animal genomes in a way that does not occur naturally by mating and/or natural recombination. It does not preclude the possibility that this Guidance Document can also be applied to animals produced by other (existing or novel) techniques that require a safety assessment.

This Guidance Document covers the commercial and any associated unintended or accidental release of GM animals into the environment but excludes their release for experimental purposes under Part B of Directive 2001/18/EC (EC, 2001). Ethics, socio-economic aspects as well as issues linked to traceability, labelling, or co-existence of production and supply systems are not addressed in this Guidance Document.

Developments and scientific activities in the area of GM animals indicate that future applications may include traits related to disease resistance, growth enhancement, sterility, population suppression, cold tolerance, dietary performance, including increased food conversion efficiency, ornamental uses and production of industrial goods. Taking such information into consideration when developing this Guidance Document, the EFSA GMO Panel decided to address the following animals likely to be transformed:

- fish[5] as poikilothermic animal with vertebrae;
- insects (e.g. mosquitoes, agricultural pests, bees);
- mammals, whatever their degree of domestication and breeding (e.g. cattle, pigs, goats, rabbits, companion animals);
- birds, whatever their degree of domestication and breeding (e.g. hens, ducks).

Genetically modified animals can be placed on the EU market for (1) food/feed uses (e.g. GM cattle) or (2) non-food/feed uses (like GM 'ornamental' fish or GM insects). Both types of uses are covered in this Guidance Document, except for the use of GM animals in the production of pharmaceuticals. Furthermore, although this Guidance Document does not give detailed guidance on the risk assessment of the accidental intake of GM animals not intended for food and feed uses by humans (e.g. GM insects), applicants should assess this likelihood and assess any risk by implementing principles in the Guidance Document on the risk assessment of food and feed from GM animals and on animal health and welfare aspects (EFSA, 2012a). Potential impacts of such GM animals on human health, through other routes of exposure (than ingestion), are addressed in sections 4.1.7, 4.2.6 and 4.3.9.

Furthermore, this Guidance Document covers the following groups of GM animals: (1) captive, (2) semi-captive and (3) non-captive:

(1) GM animals that are intended to be kept in captivity include, for example, domesticated species and companion animals held indoors or in a fenced area or animals held in zoological gardens. It is expected that most captive GM animals will be intended for use in farming and production systems;

[4] In this Guidance document the term 'GM animal' refers to the specific GM animal carrying single or stacked event(s) for which approval is requested.

[5] Excluding tetrapods (amphibians, reptiles), shellfish (Mollusca [including Cephalopoda], Crustacea, Echinodermata). To find out if a specific species is considered a fish, consult www.fishbase.org

(2) Semi-captive GM animals are those that are under human control, yet are not always in captivity but can freely browse at times (e.g. cattle browsing on an unfenced pasture or cats exploring their owners' neighbourhood); most of them are expected to be mammals and birds;

(3) Non-captive GM animals are intended to be directly released into specific environments (e.g. managed releases of sterile insects or rabbits that are intended to control wild insect or rabbit populations, respectively).

2. Strategies for the ERA of GM animals

As described in Directive 2001/18/EC (EC, 2001), the ERA should be carried out in a scientifically sound and transparent manner based on available scientific and technical data and on common methodology for the identification, gathering and interpretation of the relevant data. The ERA should include any relevant data (e.g. unpublished research data, scientific publications, scientific and expert opinions) obtained prior to and/or during the ERA process. The relevance of all studies and reports in reaching final conclusions on risks should be described and areas of uncertainty identified. The ERA should be carried out on a case-by-case basis, meaning that the required information will vary depending on the type of GM animal concerned, its GM trait(s), the intended use, the potential receiving environments (see section 3.1) taking into account, specific husbandry and management requirements, biotic and abiotic interactions, including other GM organisms already in the environment.

As a general principle, the use of a step-by-step approach (see section 2.1) beginning with problem formulation is required whereby scientifically reliable evidence, based on qualitative and, whenever possible, quantitative analyses, is combined with an explicit uncertainty analysis in order to support the final conclusions of the ERA (see section 3.7).

In developing this Guidance Document, the EFSA GMO Panel benefited from the structured problem formulation approach developed for the risk assessment of GM plants (EFSA, 2010a). A key element in the risk assessment of GM animals is the comparative approach (see section 2.2). Associated with this is the identification of differences between the GM animal and its appropriately selected comparator(s), caused by both intended and unintended effects.

Intended effects are effects that are designed to occur from the introduction of the genetic modification(s) in question and which fulfil the original objectives of the genetic modification. Alterations in the phenotype may be identified through a comparative analysis of growth, development, performance, reproduction and disease resistance, etc. with the appropriately selected comparator(s).

Unintended effects are considered to be consistent differences between the GM animal and the appropriately selected comparator(s), which go beyond the primary intended effect(s) of the genetic modification(s).

The overall ERA strategy for GM animals seeks to deploy appropriate methods and approaches to compare the GM animal and by-products (e.g. manure) with their non-GM comparators and with other farmed and wild types with some history of familiarity in order to determine environmental effects. Thus appropriately selected non-GM animals can serve as comparators for assessing the environmental impacts of each GM animal in its different receiving environments (see section 3.3).

In an ERA, it could be useful to draw on previous knowledge of and experience with the non-GM animal and to assess differences associated with the GM animal in the different receiving environments. Familiarity might also derive from the knowledge and experience available from conducting risk assessments on releases or introductions of non-GM animals (e.g. irradiated sterile mosquitoes) and from previous applications for similar GM and non-GM traits and GM constructs in similar or different animals.

2.1. Different steps of the Environmental Risk Assessment

The objective of the ERA is to identify and evaluate on a case-by-case basis potential adverse effects of the GM animal, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environments. The ERA should be carried out according to the six following steps described in Annex II of Directive 2001/18/EC (EC, 2001):

1. Identification of characteristics which may cause adverse effects
2. Evaluation of the potential consequences of each adverse effect, if it occurs
3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect
4. Estimation of the risk posed by each identified characteristic of the GMO(s)
5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)
6. Determination of the overall risk of the GMO(s)

In this Guidance Document, and according to the aforementioned six steps, we advise that the ERA should be performed following these six steps also outlined in Figure 1:

1. Problem formulation including identification of hazard and exposure pathways
2. Hazard characterisation
3. Exposure characterisation
4. Risk characterisation
5. Risk management strategies
6. Overall risk evaluation and conclusions

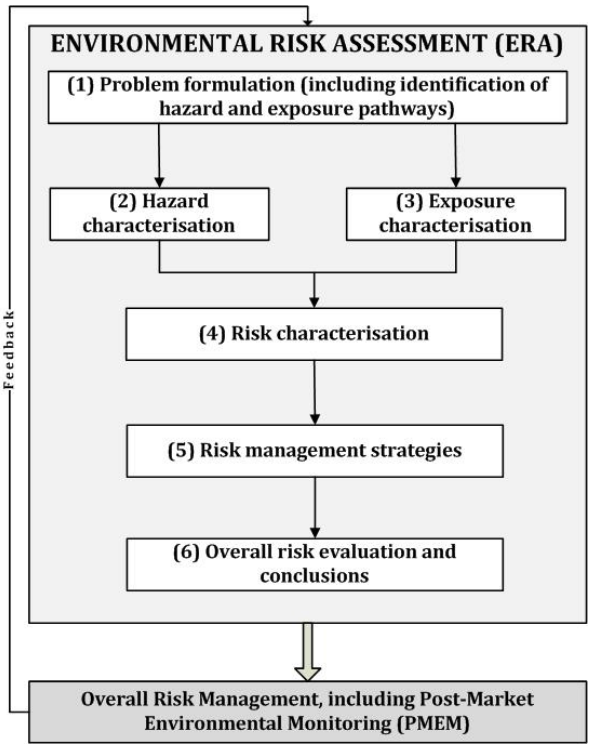


Figure 1: Six steps within the Environmental Risk Assessment (ERA) and relationship to risk management including post-market environmental monitoring (Chapter 5) according to Directive 2001/18/EC and Regulation (EC) No 1829/2003. The comparative safety assessment embraces this step-by-step approach to ERA.

Applicants should consider uncertainties relevant to each of the six steps of the ERA and these should be qualitatively and, when possible, quantitatively assessed by applicants in order to support final conclusions of the ERA (EFSA, 2009a). Section 3.7 refers to appropriate methodology to identify, describe and subsequently address the different types of uncertainties throughout the ERA.

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

Each risk assessment begins with a problem formulation in which the most important questions that merit detailed risk characterisation are identified. Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment.

Problem formulation starts with the identification of the aspects of the environment that need to be protected from harm according to environmental protection goals set out by Directive 2001/18/EC and other environmentally-related legislation (see Table 1).

Problem formulation includes the identification of the characteristics of the GM animal that can cause harm to or adverse effects on human health and/or the environment (hazards), of the nature of these effects, and of the exposure pathways through which the GM animal may adversely affect the environment (identification of hazard and exposure pathways). It also includes defining assessment endpoints and setting of specific hypotheses to guide the generation and evaluation of data in the next risk assessment steps (i.e. hazard and exposure characterisation). In this process, both existing scientific knowledge and knowledge gaps (see section 3.7 on uncertainties) should be considered.

Table 1: Examples of environmental protection goals in EU. Directive 2001/18/EC specifically applies to GM animals. Other regulatory documents, as listed below, should be considered by applicants, even though GM animals may not be specifically mentioned.

Examples of protection goals			
Areas of protection		**Background**	**Scope**
Biodiversity conservation	Species of conservation or cultural value	Directive 2004/35/EC	Environmental liability
		Directive 92/43/EEC	Conservation of natural habitats and of wild fauna and flora
		Directive 2009/147/EC	Conservation of wild birds
		Regulation (EC) 338/1997	Protection of endangered wild fauna and flora
		Action plan for biodiversity	Conservation of biodiversity
	Protected habitats	Biodiversity strategy	Conservation of biodiversity
		Biodiversity action plan for the conservation of natural resources	Conservation of natural resources
		Biodiversity action plan for agriculture	Conservation of biodiversity
		Bern convention	Conservation of European wildlife and natural habitats
		Convention on biological diversity	Conservation of biological diversity
Ecological functions	Land	Directive 2004/35/EC	Environmental liability
		Thematic strategy for soil protection	Preservation of soil functions
	Water	Directive 2000/60/EC	Water protection
		Directive 2008/56/EC	Strategy for the marine environment
	Production systems	Regulation (EC) 708/2007	Use of alien and locally absent species in aquaculture
		Biodiversity strategy	Sustainable use of biodiversity
		Thematic strategy on the sustainable use of natural resources	Sustainable use of natural resources

A crucial step in problem formulation is the identification of hazards through a comparative safety assessment (hazard identification). A comparison of the characteristics of the GM animal with those of the appropriately selected comparator(s) enables the identification of differences in the GM animal that may lead to harm. These differences are theoretically assessed in the problem formulation process in order to identify the potential environmental consequences of these differences. Those differences which have the potential to cause harm will need to be assessed while other differences which have no environmental consequences may be deemed irrelevant and require no further assessment. More detailed guidance for applicants on how to apply problem formulation on specific areas of risk to be addressed in the ERA is provided in Chapter 4 of this document.

Problem formulation considers also available information on qualitative exposure through which the GM animal may adversely affect the environment (identification of exposure pathways). Depending upon the intended uses of a GM animal, the pathways and levels of exposure of the GM animal to the environment will vary.

In the case where the GM animal use includes the release of the live animal into the environment (in any stage of the life cycle) and/or its breeding and rearing in the EU, the problem formulation will consider exposure pathways, including exposure resulting from the expected management of the GM animal and its possible escape into other receiving environments. Concerning the latter, possible pests and pathogens associated with the (GM) animal should also be considered as the accidental escape of the animal would also result in the release of any infectious or non-native agents into the wild.

In the case where the GM animal use does not include the release of the live animal into the EU environment nor its breeding and rearing in the EU, the problem formulation will consider the following possible routes of exposure: 1) via the accidental release into the environment of viable eggs and animals during transportation and processing; 2) unintended exposure, for example, through consumption of raw GM animals and raw or processed GM animal products or 3) indirect exposure via the use of GM animal waste (e.g. manure, animal carcasses) or by-products used for non-food/feed purposes (e.g. as fertiliser).

Subsequently, within the problem formulation, the identified potential adverse effects need to be linked to assessment endpoints in order to derive testable hypotheses that allow quantitative evaluation of the harm posed to those assessment endpoints. The hypotheses are of importance as they will further guide the setting up of a methodological approach[6] on how to evaluate the magnitude of harm. Measurable assessment endpoints can be derived from the protection goals provided in the EU legislation (see Table 1). Defining assessment endpoints is necessary to focus the risk assessment on assessable/measurable aspects of the environment: 'a natural resource (e.g. food species) or natural resource service (e.g. population control functions of predator populations) that could adversely be affected by the GM animal and that require protection from harm'. Through hypotheses, assessment endpoints are translated into quantitatively measurable endpoints, termed measurement endpoints (such as measurements of mortality, reproduction, abundance). A measurement endpoint can be regarded as an indicator of change in the assessment endpoint, and constitute measures of hazard and exposure.

Finally, the environmental quality to be preserved is defined by setting limits of concern, which enable the definition and identification of the minimum level of difference between the GM animal and its conventional counterpart that may lead to harm. Baselines of the receiving environments, should, as far as possible and based on available data, be established before any (harmful) characteristics of the GM animal can be identified. The baselines serve as points of reference against which future changes can be compared (see section 3.3).

[6] Problem formulation is generally performed on the basis of a conceptual model and an analysis plan (EPA, 1998; Hill and Sendashonga, 2003; Nelson and Banker, 2007; Raybould and Cooper, 2005; Raybould, 2006, 2007; Romeis et al., 2008; Storkey et al., 2008; Raybould, 2009; Raybould et al., 2009; Wolt, 2009; Wolt et al., 2010; Kapuscinski et al., 2007a, 2007b, 2007c).

Therefore, in the problem formulation process, applicants should on a case-by-case basis:

1. Identify relevant aspects of human and animal health and the environment that need to be protected from harm according to protection goals (Table 1) set by risk managers according to EU legislation (including suitable protection units e.g. individuals, populations, communities, guilds as well as the spatial and temporal scale of protection);
2. Identify characteristics of the GM animal and, where appropriate, the associated production and management systems that can cause harm to the identified protection goals;
3. Assess the intended uses of the GM animal and the level of physical and biological confinement (see Chapter 1) that will be applied to the GM animal in order to identify environmental exposure pathways for the GM animal;
4. Identify the potential adverse effects linked to those harmful characteristics;
5. Define assessment endpoints being representative of the previously identified protection goals;
6. Define measurement endpoints as measurement units for both hazard and exposure;
7. Describe interrelationships between assessment and measurement endpoints and relate these to protection goals;
8. Define relevant baselines used as points of reference to determine the minimum relevant ecological effect that is deemed of sufficient magnitude to cause harm;
9. Set the limits of concern for each assessment endpoint in order to define the minimum relevant ecological effect that is deemed *biologically relevant* (see EFSA, 2011c), and is deemed of sufficient magnitude to cause harm;
10. Formulate specific testable hypotheses that are clearly phrased and easily transferable to data to be generated or evaluated;
11. Consider possible uncertainties (e.g. knowledge gaps).

The information considered in problem formulation can take many forms, including published scientific literature, scientific and expert opinions, unpublished research data, obtained prior to and/or during the ERA process. It should also include available data from analyses performed to characterise the GM animal including molecular, compositional and phenotypic analysis (for further details, see section 2.2). Data on interactions with biotic and abiotic factors generated outside Europe with the GM animal itself, or closely related species, might be used by applicants, if its relevance for the European environments has been justified. Data from risk assessments on releases or introductions of non-GM animals with similar phenotypes (e.g. irradiated sterile mosquitoes) and from previous applications for similar GM and non-GM traits and GM constructs in similar or different animals species might also inform the ERA.

In the case where no hazard is identified at the end of the problem formulation (step 1) in relation to any of the areas of risk described in Chapter 4, applicants are not requested to further address the remaining five steps described below. Applicants should then discuss and explicitly justify the rationales behind their decision.

2.1.2. Step 2: Hazard characterisation

Hazard characterisation in this Guidance Document is defined as the qualitative and/or quantitative evaluation of environmental harm associated with the hazard as set out in one or more hypothesis(e)s derived from the problem formulation (step 1).

The magnitude of each potential adverse environmental effect should be evaluated in relation to defined comparative baselines and assessment endpoints (see section 2.2 and also section 2.2.2 in EFSA, 2010a). The magnitude should be expressed, if possible, in quantitative rather than qualitative terms. Ordered categorical descriptions such as *'high'*, *'moderate'*, *'low'* or *'negligible'*, where the

ordering is from *'high'* at one end to *'negligible'* at the other, may be used to place potential adverse effects on a scale of magnitude.[7] These terms should themselves be defined in quantitative terms as precisely as possible. In case it is not possible to identify an adverse effect in a particular environment, the associated potential consequences (harm) could be assessed as *'negligible'* or *'insignificant'* (EC, 2002).

2.1.3. Step 3: Exposure characterisation

In the problem formulation (step 1), the possible routes by which exposure may occur are evaluated. The next step is the estimation of the likelihood of occurrence of adverse effects (EC, 2002). The aim of the exposure characterisation is the quantitative estimation of the likely exposure of other biota and the environment to the GM animal. Therefore applicants should perform an exposure characterisation which includes the nature, magnitude, frequency and duration of the exposure to the GM animal. The environmental exposure assessment should be related to the intended use of the GM animal and its level of release. Propagule pressure as the combined effect of the number of individuals released into the environment and the number of release events over a specified period of time can be a useful element to assess exposure. Applicants should also estimate escape frequencies, if applicable. Applicants should provide estimates of effluents generated by the GM animals in the specified management and production systems.

For each hazard identified and characterised, it may not be possible to estimate precisely the likelihood of exposure. Likelihood of exposure can be expressed either qualitatively using an ordered categorical description (such as *'high'*, *'moderate'*, *'low'* or *'negligible'*) or quantitatively as a relative measure of probability (from zero to one, where zero represents impossibility and one certainty). If qualitative terms are used, the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of zero to one, to which the term refers.

2.1.4. Step 4: Risk characterisation

In this Guidance Document, risk characterisation is described as the quantitative or semi-quantitative estimate of the probability of occurrence and magnitude of harmful effect(s) based on problem formulation, hazard and exposure characterisation.

The applicant should characterise the risk by combining:

- The magnitude of the consequences of each hazard ("*high*", "*moderate*", "*low*" or "*negligible*");
- The likelihood of the consequences related to hazard occurring ("*high*", "*moderate*", "*low*" or "*negligible*") in the receiving environments.

[7] The following classifications are extracted from the Commission Decision 2002/623/EC {EC, 2002 #144} and are suggested as illustrative and qualitative examples in a very broad sense. They are not intended to be definitive or exclusive, but to give an indication of the considerations that might be taken into account when weighing up the consequences:

"*high level consequences*" might be significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species in the short or long-term. Such changes might include a reduction in or complete eradication of a species leading to a negative effect on the functioning of the ecosystem and/or other connected ecosystems. Such changes would probably not be readily reversible and any recovery of the ecosystem that did take place would probably be slow;

"*moderate consequences*" might be significant changes in population densities of other organisms, but not a change which could result in the total eradication of a species or any significant effect on endangered or beneficial species. Transient and substantial changes in populations might be included if likely to be reversible. There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem;

"*low level consequences*" might be non-significant changes in population densities of other organisms, which do not result in the total eradication of any population or species of other organisms and have no negative effects on functioning of the ecosystem. The only organisms that might be affected would be non-endangered, non-beneficial species in the short or long-term;

"*negligible consequences*" would mean that no significant changes had been caused in any of the populations in the environment or in any ecosystems.

The applicant should assess the overall uncertainty for each identified risk (see section 3.7), possibly including consideration of:

- Assumptions and extrapolations made at various levels in the ERA;
- Any conflicting scientific literature and viewpoints;
- Specified uncertainties.

It is also recommended to consider, where appropriate, representative exposure scenarios including a worst-case scenario for which applicants consider factors that can lead to high level of exposure such as high rates of uptake, high mobility and the potential for escape, survival and hybridisation with wild or feral relatives.

The risk characterisation should indicate whether the problem formulation (including hazard identification), hazard characterisation and exposure characterisation are complete or not. This would allow determining if the characterisation of the risk may be finalised, or if further data should be generated in order to complete the risk characterisation of the GM animal.

2.1.5. Step 5: Risk management strategies

The risk characterisation (step 4) may identify risks and uncertainties that require measures to manage them. Therefore, risk management should be described and/or proposed by applicants based on the conclusions of risk characterisation of the GM animal performed under steps one to four. These risk management strategies should aim to reduce the identified risks associated with the GM animal to a level falling within the limits of concern and should consider defined areas of uncertainty (see section 3.7).

Applicants should describe the risk management in terms of reducing exposure and/or hazard, and quantify such reduction (when possible). Where applicants have identified risk management measures (e.g. physical confinement, infertility) for the GM animal which can reduce these risks, then the reliability and efficacy of these measures should be justified.

Therefore if a risk is characterised at the end of step 4, risk mitigation measures, proportionate to the level of the identified risk, should be proposed to reduce it. If the characterised risk is not considered significant, risk mitigation measures might not be needed. In the latter case, applicants should then discuss and explicitly justify the rationales behind their decision.

Applicants should consider specific management strategies to ensure quality control of the GM animals produced, so that the animals conform to the description in the applications. For instance, appropriate management and control measures should be put in place prior to the releases into the environment of mass reared GM sterile mosquitoes in order to ensure the consistency and efficacy of the release systems and to achieve the intended outcome (e.g. suppression of the wild population when it is a pest or vector of human disease). Such measures would identify possible programme failures (e.g. untransformed mosquitoes in the reared GM population, occurrence of females).

Applicants should also state the post-commercialisation measures they will put in place in order to monitor and verify the efficacy of the risk management measures and to allow changes in risk management strategies in case circumstances change, or new data indicating the need for changes to the risk management become available (see section 5.1).

2.1.6. Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM animal should be made taking into account the results of steps one to four of the ERA and their levels of uncertainty, the weight of evidence and the risk management strategies proposed (step 5) in the different receiving environments.

The overall risk evaluation should result in informed qualitative, and if possible quantitative, guidance to risk managers. Applicants should explain clearly what assumptions have been made during the ERA and what is the nature and magnitude of uncertainties associated with the identified risk(s) (see section 3.7).

When risks or uncertainties are identified at step 4 of the ERA, applicants should propose and describe the risk management strategies (step 5) that will be associated with the production and release of the GM animal taking into account the range of scenarios (including worst-case scenarios) studied in the ERA. The risk management strategies proposed should be proportionate to the results of the different scenarios studied, to the specific protection goals in the receiving environments and to the levels of uncertainty and risk identified in the ERA. Applicants should assess to what extent the proposed risk management strategies will reduce risks. In addition, applicants should identify any uncertainty associated with the efficacy and implementation of risk management strategies and their potential implications.

When risks are identified, the scale and likelihood of harm associated with these risks needs to be described. Applicants should indicate why these levels of risk might be acceptable in assessing the net overall environmental impact of the GM animal.

Finally, an evaluation of the overall risk of the GM animal should be made, taking into account the results of the risk characterisation (step 4), the proposed risk management strategies (step 5) and the associated levels of uncertainty. The overall risk evaluation and conclusions determine the requirements for the Post-Market Environmental Monitoring (PMEM) of the GM animal. Monitoring results may confirm the assumptions of the ERA or may lead to its re-evaluation (see Chapter 5).

The ERA is an iterative process. If new information on the GM animal and its effects on human and animal health or the environment becomes available, the ERA may need to be re-addressed in order to (1) determine whether the risk characterisation has changed; and (2) determine whether it is necessary to amend the risk management.

2.2. Information to identify potential unintended effects

A key element in the risk assessment of GM animals is the comparative approach in accordance with Annex II of Directive 2001/18/EC (EC, 2001). Associated with this is the identification of intended and possible unintended differences between the GM animal and its appropriately selected comparator(s).

Unintended environmental effect(s) could potentially be linked to genetic rearrangements or metabolic perturbations and may be predicted through the comparison of the biological and compositional characteristics of the GM animal with its appropriately selected comparator(s) reared under the same environmental conditions. Each identified unintended effect should be then specifically assessed for possible environmental effects in Chapter 4.

Sources of data may include:

- Molecular characterisation: a starting point in the identification of potential unintended effects is analysis of the DNA construct and insertion site to establish whether the insertion is likely to have potential effects other than those of the intended modification (e.g. unintended effect(s) could be due to loss of function of an endogenous gene at the insertion site) (EFSA, 2012a).

- Targeted compositional analysis: unintended effects may be detected through a comparative targeted compositional analysis between the GM animal and its products with the appropriately selected comparators (e.g. unintended effect(s) could potentially be linked to metabolic perturbations) (EFSA, 2012a).

- Phenotypic and behavioural characteristics: unintended effects may also be detected through the comparison of the phenotypic (i.e. morphological and physiological) and behavioural characteristics of the GM animal with the appropriately selected comparator(s) (e.g. unintended effect(s) could be potentially linked to morphological alterations) (EFSA, 2012a). Phenotypic characteristics should be evaluated taking into account various environments.

- Interactions between the GM animal and its receiving environments: unintended effects may be detected through comparisons of biotic and abiotic interactions of the GM animal and the appropriately selected comparators with components of their receiving environments (for examples see Table 2 in section 3.1).

Genetically modified animals can be placed on the EU market for (1) food/feed uses (e.g. GM cattle, GM pigs) or (2) non-food/feed uses (like GM 'ornamental' fish, most GM insects and GM companion animals). Both types of uses are covered in this Guidance Document (except the use of GM animals for the production of pharmaceuticals), but the background information available for the comparative assessment varies between them:

1. In the safety evaluation of food and feed from GM animals (EFSA, 2012a), the comparative assessment includes a comprehensive molecular characterisation of the GM animal (e.g. expression, stability of the recombinant DNAs), a compositional analysis and a phenotypic (i.e. morphological and physiological) characterisation of the GM animal. The outcome of this comparative assessment will inform the initial steps of the ERA of those GM animals and, in particular, the identification of possible unintended effects due to the transformation process and/or the trait.

2. GM animals that are not intended for food and feed uses, but to be deliberately released into the EU environment, need to comply with principles laid down in Annex II and Annex IIIA of Directive 2001/18/EC. Therefore, each GM animal must be characterised and descriptive information need to be provided prior its deliberate release into the environment according to Annex IIIA of Directive 2001/18/EC. Applicants may find it helpful to consider the principles laid down in the EFSA GMO Panel Guidance Document on the risk assessment of food and feed from GM animals (EFSA, 2012a) concerning molecular characterisation of the GM animal. The EFSA GMO Panel reiterates that the risk assessment is done on a case-by-case basis and different amounts of data may be required in different cases. Applicants should thus provide a detailed rationale for any deviation from the full set of requirements on molecular characterisation of a GM animal.

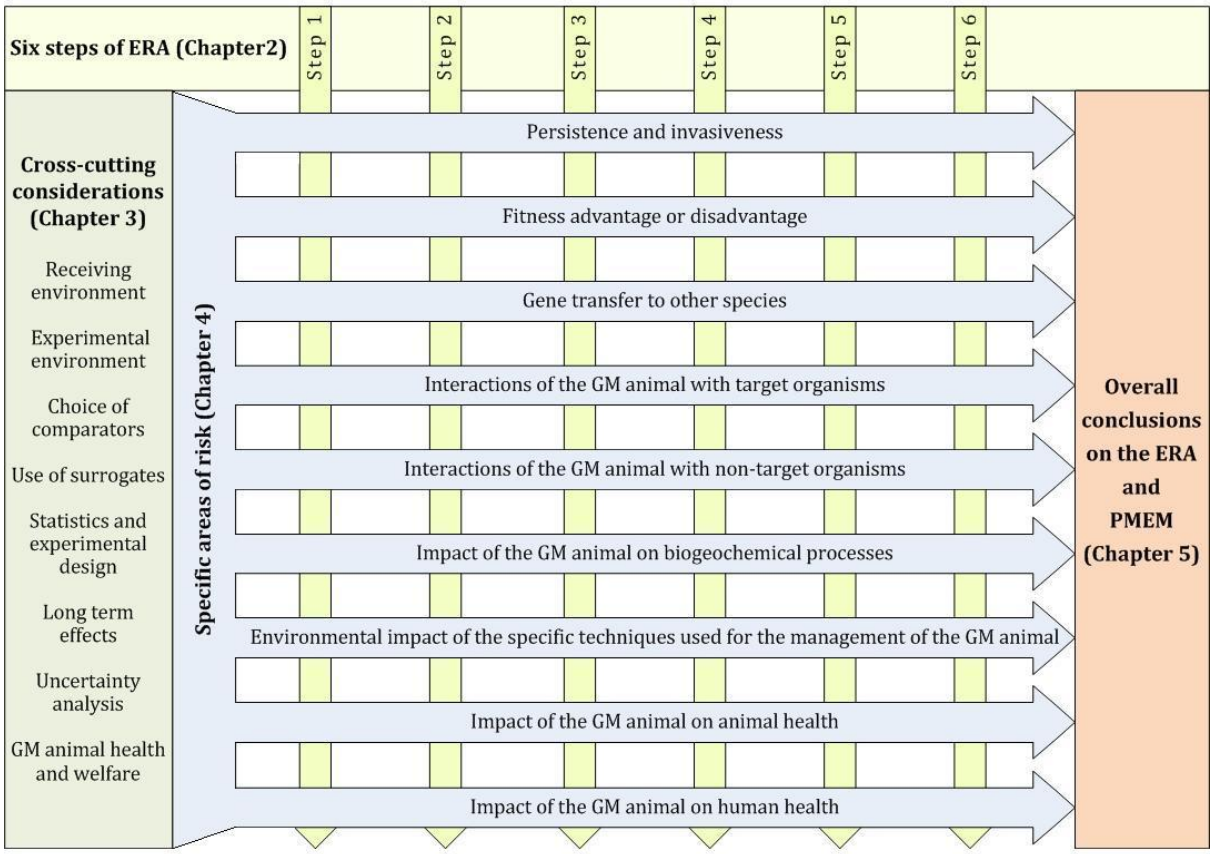
2.3. Structural overview of this Guidance Document

As explained in the previous sections, the ERA of GM animals should be carried out according to the six steps laid down in Annex II of Directive 2001/18/EC (EC, 2001).

Annex II of Directive 2001/18/EC also identifies nine specific areas of risk that should be addressed by applicants in the ERA of GM animals. For each specific area of risk (see Chapters 4), applicants are requested to provide information in a clear and concise way following systematically the six steps of the ERA. Detailed guidance for applicants on how to apply the six-step approach to the specific areas of risk is provided in sections 4.1 for GM fish, 4.2 for GM insects and 4.3 for GM mammals and birds.

In addition, Chapter 3 of this Guidance Document describes the generic cross-cutting considerations (e.g. choice of comparators, use of non-GM surrogates, experimental design and statistics, long-term effects, uncertainty analysis) that applicants should take into account throughout the entire ERA.

Figure 2 depicts the structural overview of this Guidance Document and the interplay between the different parts of it, mainly the six systematic steps of the ERA (see Chapter 2), the cross-cutting considerations (see Chapter 3), the specific areas of risk (see Chapter 4) and the Post-Market Environmental Monitoring (PMEM, see Chapter 5).



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632 **Figure 2:** Structural overview of this Guidance Document and the interplay between its different
633 parts.

634

3. Cross-cutting considerations

This chapter describes the generic considerations that applicants should take into account throughout the whole ERA process of GM animals. When deemed appropriate, further guidance for specific categories of GM animals is provided in dedicated sub-sections (see sections 3.3 and 3.8).

3.1. Receiving environments

According to Directive 2001/18/EC (EC, 2001), the ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM animal concerned, their intended use and the potential receiving environments, taking into account inter alia other GMOs already in the environment. Further, this Directive provides details on required information relating to the conditions of release, the receiving environments and the interactions between the GMOs and the environment. Commission Decision 2002/623/EC (EC, 2002) provides further details related to potential receiving environments.

3.1.1. Definition of receiving environments

The range of environments into which the GM animal(s) and their by-products will be released or may escape or be distributed to through active or passive spread and into which the recombinant DNA may spread are defined as receiving environments.

A broad range of environments in terms of fauna and flora, climatic conditions, habitat composition and ecosystem functions and human interventions occur in the EU. The receiving environments for GM animals will vary in spatial scale from a very limited number of captive locations to large regions within the EU. They will also vary in the extent of management, from those that are wild and natural, those that are subject to some level of management, to those that are completely synthetic, where the environment is designed for the production of the GM animal. Accordingly, GM animals will potentially interact with widely differing environments (see Figure 3).

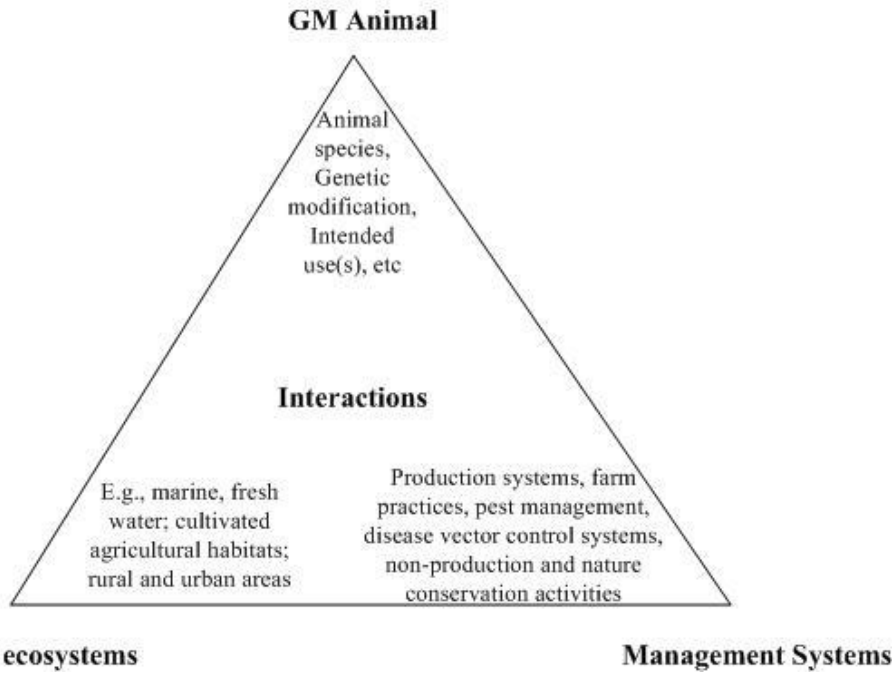
3.1.2. Identification and characterization of the receiving environments

The receiving environments of each GM animal will be characterised by three components (Figure 3):

- The GM animal - factors to be considered: e.g. wild populations of the animal species, ecological requirement of the animal species, wild relatives, genetic modification(s) and intended uses(s);

- Accessible ecosystem(s) (e.g. marine, fresh water, cultivated agricultural habitats, natural and semi-natural habitats, rural and urban areas) – factors to be considered: physic-climatic conditions, altitude, depth, native and introduced fauna and flora. An accessible ecosystem is here defined as a biological system within a receiving environment to which the GM animal, and its by-products have access and may interact with;

- Management systems (e.g. production systems, management practices, pest and disease management, non-production and nature conservation activities, release in enclosed environments i.e. captive and semi-captive conditions).



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672 **Figure 3:** The receiving environments of each GM animal will be characterised by: (A) the GM
673 animal including its intended uses, (B) the Accessible Ecosystem(s), and (C) the Management
674 Systems. Examples of attributes of (A), (B), and (C) that could interact are provided in the figure.

675 In some highly controlled management systems (e.g. intensive housed livestock units), the receiving
676 environments, the accessible ecosystem and the management system may be one and the same.

677 The first component is the GM animal itself (see figure 3). Both the animals and the GM trait(s)
678 determine where the GM animals will most likely be released. Some GM animals (e.g. GM olive fly)
679 can realistically be released in some geographical zones only, while others, like GM pigs and GM
680 salmon, may be released or become more widely established in the EU. GM traits such as biotic (e.g.
681 disease resistance) and abiotic (e.g. cold) stress tolerance will also determine which GM animals are
682 likely to survive and where they establish. Consideration should be given to the influence of the GM
683 trait in determining the range of environments which the GM animal may inhabit, as traits which
684 confer tolerance to e.g. heat, cold, dehydration, salinity, disease may allow the GM animal to be
685 produced or establish in environments not occupied by the conventional counterpart. Therefore, all
686 these elements should be taken into account when defining the receiving environments for the ERA of
687 each GM animal.

688 GM animals have widely different characteristics of reproduction, spread and invasiveness. Also, they
689 may be developed for different uses (e.g. food production in the case of mammals, birds and fish;
690 suppression or replacement of plant pest species or disease vector populations in the case of insects).
691 The intended use(s) and the characteristics of the GM animals will determine their behaviour and
692 interactions with other biotic and abiotic factors in the receiving environments (see Table 2).

693 Accessible ecosystem(s) (see Figure 3) may contain a wide range of varying habitats at various scales
694 (e.g. marine, fresh water, cultivated agricultural habitats, natural and semi-natural habitats, rural and
695 urban areas) and are characterized by specific conditions (e.g. physic-climatic conditions, altitude,
696 water quality) where native and other animal species may live and interact with the GM animals.
697 Certain animals migrate and some reproduce in different environments. Some may have different life
698 stages in different environments. Therefore the whole life cycle of the GM animal and the receiving
699 environments of these different stages require consideration. Interactions between GM animals and
700 non-GM animals such as herbivores, predators, parasitoids, decomposers, pollinators, pathogens,
701 conspecifics are influenced by biotic (e.g. food sources) and abiotic (e.g. climate, water quality)

factors in the receiving environments. Furthermore GM animals might change abiotic factors of the receiving environments e.g. due to organic waste products (see Table 2).

The management systems component (see Figure 3) should include consideration of land and water use, livestock husbandry or rearing facilities and their management, since these production/management/control systems can differ significantly between regions. For example, GM disease/pest/parasite resistance could allow GM animals to be kept at higher stocking densities but this may pose a risk to other animals in the production system as the GM animals could still be a source of infection. This is well known for farm livestock but can also be the case for farmed fish where the occurrence of infections can have severe consequences and may require significant use of antimicrobials (e.g. salmon farming). When considering receiving environments for the ERA of a GM animal, applicants should also consider: (1) the use of by-products of the GM animal (e.g. faeces, urine, waste materials, excreta, respiratory output) and (2) the pests and pathogens associated with the GM animal. Identifying the receiving environments of products of the GM animals confined to captive conditions may be a more important factor than the distribution of the living GM animal itself for the risk assessment. Therefore interactions of such by-products with the biotic and abiotic factors (see Table 2) in receiving environments should be considered. Furthermore, GM animals with enhanced resistance may acts as vectors, carriers or reservoirs of pests/pathogens or may change the nature of pests/pathogens (e.g. change their virulence or resistance). The receiving environments of these pest/pathogens may be additional to that of the GM animal and its by-products and interactions of these organisms with the biotic and abiotic factors in receiving environments should also be considered.

The three components listed above (see Figure 3) result in biotic and abiotic interactions that should be considered by applicants when identifying and characterising receiving environments for carrying out the ERA of GM animals (see Table 2).

Table 2: Examples of biotic and abiotic factors important in identifying and characterising receiving environments.

Ecosystem resources and functions	Biotic and abiotic ecosystem factors and attributes	Biotic and abiotic ecosystem sub-factors interacting with GM animal
Required from the ecosystem by the animal	Food sources	Prey, host, food materials
	Mates	Conspecifics (both sexes) and other species in case of hybridization
	Feeding, mating and breeding territory/sites	Space use and requirements for different life stages, migratory requirements
	Climate	E.g. temperature, wind, sunlight, precipitations
	Chemical and physical properties	E.g. O_2, salinity, turbidity, temperature, water flow
	Security	E.g. shade, shelter, *refugia*
Contributed to the ecosystem by the animal	Conspecifics	Population characteristics (Genetics, demographics, etc.)
	Predators, consumers	Species which may use the GM animal as a prey/food item
	Pests (e.g. pathogens, parasites) and diseases	Pathogen abundance and distribution
	Organic waste products	Faecal and respiratory outputs t (e.g. CH_4, NH_4, CO_2); post-mortem decomposition; toxic compounds
	Habitat restructuring	E.g. Stream bed structure, habitat alteration, nest building

3.1.3. Selection of the relevant receiving environments

The ERA should take account of the diversity and multivariate nature of the characteristics of the potential receiving environments of each GM animal for each issue of concern. However in practice it will not be feasible to study all the receiving environments of a GM animal so that in many cases applicants will have to select specific study sites. Applicants should consider selecting sites where the exposure and impacts are expected to be highest and where it is anticipated that if effects exist they will be detected.

In order to select appropriate receiving environments in which to study each issue of concern, applicants need to consider the full geographic range of the GM animal, the issues of concern and the receiving environments in which these issues occur. For example, if a NTO is potentially at risk from a GM animal, then studies should be conducted in environments where there are/will be high levels of exposure of the NTO to the GM animal and where there are measurable numbers of the NTO, in order to study population effects. Applicants should consider following the steps shown in Table 3 in order to select these relevant receiving environments.

Since not all receiving environments, where the GM animal and its by-products (e.g. manure, animal carcasses) will be intentionally or might be unintentionally released and spread, can be considered in detail, applicants should discuss and justify the applicability of studies outcomes obtained in some relevant receiving environments to all identified receiving environments as described in section 3.1.2. In order to do this it may be useful to classify regional data, reflecting aspects of the receiving environments relevant to the GM animals (e.g. data on the occurrence of sexually compatible relatives of GM animals in different habitats of the EU, or effects of production systems on the interactions between the GM animal and the environment). Some categorizations of regions or habitats into geographical, climatic or bio-geographical zones which could be used for this purpose exist. In addition, applicants might consider useful information on animal species and their distribution as well as online databases for specific taxa of *Kingdom Animalia*, such as the *Fauna Europaea* website (http://www.faunaeur.org/) and the *EUNIS* website (http://eunis.eea.europa.eu/).

Table 3: Selection process of relevant receiving environments for ERA

Step 1 Animal	Consider the present distribution range of the (non-GM) animal species
Step 2 Animal × trait	Revise present distribution areas and their management systems according to the nature of the GM trait (including by-products, pests and pathogens associated with the GM animal): - add potential future release or escape, and establishment/invasion in an area; - where relevant, consider changes in production/management systems, according to the nature of the trait, concentrate on those areas and management systems in which the GM animal is most likely to be present.
Step 3 Animal × trait × environmental	Select appropriate scenarios representative of interactions in receiving environments for each environmental issue of concern identified in the problem formulation, taking into consideration assessment endpoints.

Relevant baseline(s) of the receiving environments, including production systems, indigenous biota and their interactions, should be established to identify any potentially harmful characteristics of the GM animals (EC, 2002). Relevant baselines refer to current production and management systems for which published literature is available. These baseline(s) serve as a point of reference against which future changes can be compared (also, see section 3.3 on selection of comparators). The baseline(s) will depend to a considerable extent on the receiving environments, including biotic and abiotic factors (for example, natural preserved habitats, agricultural farmland or contaminated land).

Furthermore applicants should take into account the potential risk implications for the presence of any other GM animals and other introduced species that have been placed on the market and released in

the same receiving environments, including interactions between the specific production, management and control characteristics associated with the different GM animals. In addition, applicants should consider likely and/or predicted trends and changes to receiving environments, and how these might interact with the GM animals.

For the set of selected receiving environments identified in step 3 of Table 3, applicants should describe:

- The characteristics of these receiving environments where the GM animal is likely to occur (e.g. that might induce users to adopt it), also taking into consideration the receiving environments where GM animal by-products (e.g. manure, animal carcasses) are likely to be spread;
- The representative management systems (e.g. use of the animal, other (GM) animals, production and breeding systems, disease control practices);
- The range of relevant biotic and abiotic interactions likely to occur in the receiving environments taking into consideration the range of environmental conditions, protection goals (including those related to species differences across Europe) and production systems. Where appropriate, the presence of cross-compatible wild relatives, the ability of the GM animal to form feral populations and hence the potential impacts on the receiving environments should be considered.

Ecological niche modelling (Thackeray et al., 2010; Sutherst et al., 2011) may be an additional method for predicting the spread of a GM animal into natural habitats. For example, future receiving environments, corresponding with the ecological niche of the GM animal concerned, could be estimated together with the implications of the GM animal occurring within accessible ecosystem(s) in these receiving environments. Also trophic interactions between the GM animal and the biotic factors in such accessible ecosystems could be considered. For further specific details see sections 4.1.1, 4.2.1 and 4.3.1 on persistence and invasiveness.

These considerations of receiving environments should be accounted for each step of each specific area of risk (see Figure 2) for each GM animal. Therefore the overall ERA should conclude on risk(s) identified in each receiving environment at both national and regional scales.

3.2. Experimental environment

The complexity of the environmental concerns requiring study in any risk assessment is related to the complexity of the substance or organism assessed and to the complexity of its interactions with components of the environment. These complexities are generally more pronounced in animals, less so in plants and least in substances. For example, animals generally exhibit more complex behaviour (and maybe sociality) than plants; the mobility of an individual animal and its population will generally exceed that of a plant within a life-time, and whereas plants are usually at the bottom of the food chain an animal may be either a predator or a prey item, or may be both. Hence, it might be expected that, firstly, the ERA of a GM animal would be more varied and complex, and encompasses a wider range of issues than the ERA of a plant or a substance. Secondly, that the mobility of animals would focus the ERA more on questions related to invasiveness and persistence and thus draw on the considerable scientific literature concerning alien species.

Hence, it is reasonable for the ERA of a substance, such as a pesticide, to be restricted largely to studies of its eco-toxicological effects, using a simple tiered approach (e.g. EFSA, 2010b). For a GM plant, which is at the base of the food chain, toxicity remains important but the tiered approach is widened somewhat and there is a greater focus on indirect ecological effects, possibly at higher trophic levels (EFSA, 2010a). For the ERA of a GM animal, potential environmental impacts are more likely to be examined in the ecological interactions within the multi-trophic hierarchy in which the animal exists. Therefore the tiered eco-toxicological approach (e.g. Andow et al., 2006; Romeis et al., 2008) promulgated through standardized methodologies (developed by, for example, OECD, ISO, EPPO and

EFSA, 2010b), in which studies performed within laboratories may trigger further studies in wider environments, has less relevance for the ERA of GM animals.

For any identified risk, once the rationale and hypotheses of an experimental study on a GM animal prior to commercial release have been formulated clearly (see Section 2.1) one of the first decisions must be the choice of an appropriate experimental environment, to define the spatial scale of the experimental units, and of the containment measures to deploy to avoid unintentional release of the GM animal. For fish, insects and mammals and birds the experimental environment may range over a continuum from an in vitro study, through a small-scale in vivo study within a completely-contained laboratory, up to larger scales that may include, respectively: ocean mesocosms of many thousands of cubic metres (van der Meeren & Lønøy, 1998); screened enclosures of thousands of cubic metres (Miller et al., 2010; Richard et al., 2009); fenced fields of tens of hectares. In rare circumstances where the likelihood of escape is minimal and recapture relatively assured, studies might be possible on even larger-scale arenas such as: remote islands or lakes, where potential harm is not considered a problem.

In choosing suitable containment measures, applicants should consider the mobility of the GM animal within the experimental environment, the likelihood of escape, the feasibility of recapture, the ability of the GM animal to become feral and to cross-breed in the wild if it escapes (see the use of non-GM surrogates as discussed in section 3.4). Applicants should consider the use for which the GM animal is intended; relevant factors might include whether it is a domesticated species and/or companion animal (e.g. growth-enhanced fish or neon-mice); whether it usually remains under human control (e.g. avian influenza-resistant chicken); whether it is usually confined within some enclosure (e.g. farmed salmon); whether it is sometimes given liberty to roam and over what area (e.g. organically-reared, free-ranging enviropig), and whether it will be released directly into a non-captive environment (e.g. mosquito) (see also Chapter 1).

Applicants should discuss and justify explicitly the choice and scale of experimental environment and of containment measures. Applicants should consider the arguments for and against small- and large-scale experimentation (EFSA, 2010a). The control and manipulation of experimental conditions at the small-scale by isolating organisms and excluding extraneous factors can thereby limit complexity, lessen variability and facilitate the identification of causal relationships while potentially reducing its generality. However, there is a need to incorporate realistic evaluation of certain factors that can only be addressed at the large-scale, such as: animal mobility, multi-trophic interactions (including behavioural responses), indirect effects, chronic and/or sub-lethal effects, abiotic factors (such as ambient weather and light conditions), and variability in responses to different receiving environments, ecosystem functionality and population-level effects. Applicants deploying mathematical or other modelling techniques should seek to verify those models and justify explicitly their validation (see also section 3.7) and should consider to what extent this may be facilitated by limited experimentation within semi-natural environments. Applicants should consider the use of surveys to provide relevant data where there is no experimental imposition of treatments, especially within the intended receiving environments.

Experimental conditions also need to take into consideration variation over time such as seasonal or annual variation in conditions, taking into account winter and summer as well as rain season and dry season.

3.3. Choice of comparators

The ERA of a GM animal is based on the comparative approach (see Chapter 2, above) as prescribed by Directive 2001/18/EC on the deliberate release into the environment of GMOs. Regarding comparators, the section on general principles in Annex II of Directive 2001/18/EC specifies that "identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations". The non-modified organism from which the GM animal is derived is often termed the 'conventional counterpart'. Hence, where feasible and appropriate,

similarities and differences in the interactions between the GM animal and the environment due to the genetic modification and induced changes in management should be estimated in relation to a conventional counterpart. In general, the conventional counterpart is defined (as in EFSA, 2011a) as a non-GM animal with a genetic background that is as close as possible to that of the GM animal. In such cases, the comparator should be from the same species as the GM animal and the selection of appropriate comparator animals may be aided by considering genetic distance and/or pedigree.

The term 'GM animal' generally refers to the specific GM animal carrying single or stacked event(s) for which approval is requested. However, in practice, commercially available GM animals will often be produced as the offspring from crosses between a GM animal carrying the event with other individuals of the same species. Applicants should consider the genetic background of those individuals which might subsequently include the GM trait(s) and also how these should be studied in comparison with conventional types. On a case-by-case basis, depending on the nature of the event and according to the scope of the application, comparative data may be required on the environmental impacts of the event when present in different genetic backgrounds. In particular, applicants should consider and discuss breeding in which the recombinant DNA could be introduced or introgressed into genetic backgrounds of domesticated, captive-bred and wild individuals. This extends to consideration of maternal and paternal effects typical for specific females and males.

The ERA should cover the full range of GM animals that might arise from the event being assessed; these include, but are not necessarily restricted to: the transformed animal itself; the offspring of animals of the same species with which it can hybridise; the offspring of feral types with which it can hybridise; and the offspring of wild types (including other (sub-)species) with which it can hybridise. Each of these types may require a different comparator(s) to determine environmental effects. In cases where recombinant DNA(s) may be passed to feral or wild types of the same or different species it may be necessary to generate wild type populations possessing the recombinant DNA and to compare their phenotypic and environmental impacts with those of the wild type. Applicants should fully describe the wild types selected for these studies and indicate how they and the environments studied can be used to represent the impacts of transformed wild types in a range of receiving environments.

There is a potential problem for the comparative approach described in Directive 2001/18/EC (EC, 2001) if no individual of the species, for which the application is made, is present in the receiving environments being considered (and therefore no non-modified organism or conventional counterpart is available for comparison with the GM animal). Annex III of Directive 2001/18/EC (covering information required in the notification) acknowledges that: "future developments in genetic modification may necessitate adapting this Annex to technical progress or developing guidance notes on this Annex. Further differentiation of information requirements for different types of GMOs, for example […] fish or insects […] may be possible once sufficient experience with notifications for the release of particular GMOs has been gained in the Community." However, such adaptations would apply only to the provision of information and not to Annex II, which deals with the general principle of comparison. Commission Decision 2002/623/EC (EC, 2002), establishing guidance notes to Annex II, commented on the general principle of comparison with the non-modified organism. It concerns the need to establish baseline data in each receiving environment that may serve as a point of reference, against which, future changes may be compared; these data may be pre-existing or gathered explicitly. Nevertheless, the problem remains, because again, what is discussed is the provision of information on which the comparison may be based, and not the form of the comparison itself.

When no such conventional counterpart organism is available, there are two main components influencing the potential environmental impacts of the GM animal. The first is the introduction of the species itself into the receiving environments in which it currently does not exist. In this case it must be considered as an alien species with the potential to establish and possibly invade this and other similar environments (EC, 2007). The second is whether, over and above the introduction of new conventional (traditionally-bred or non-GM wild) animals of this species into receiving environments, there are additional effects attributable to the genetic modification of the animal, compared to traditionally-bred, conventional counterpart. A literal reading of Directive 2001/18/EC could contend

that the ERA should be restricted exclusively to consideration of the second component, but this might greatly underestimate the effect of releasing the GM animal into the environment. Therefore, this guidance recommends that the ERA considers the full package of potential effects, including both components. Ideally, an ERA would identify and quantify, separately, these two components. However, in cases where the GM animal will be introduced into environments not occupied by a conventional counterpart, no empirical environmental data can exist on the first component, and it is not feasible to gather environmental data by the introduction of the traditionally-bred, conventional animal. Therefore, the separation of the effects into the two components may not be possible and is not a mandatory requirement since it is the total environmental impact of the GM animal that requires assessment. The main function of the ERA in this case must be the identification, study and characterization of the aggregate of all adverse environmental effects as a consequence of the release of the GM animal into the receiving environments and the comparison must be with the state of the receiving environments prior to release.

In such cases (where the conventional counterpart is not present), a possible comparator might be a non-GM animal from the same species to that of the GM animal, that occurs already in the receiving environments (e.g. wild types of the GM animal). An alternative choice might be a non-GM animal from a different species, but one that occupies the same (failing that, a similar) ecological niche and that has similar ecological characteristics to the GM animal. It may well be necessary for different elements of the ERA to employ one or more different comparators in order to place environmental impacts into context. Again, the selection of appropriate comparators may be aided by considering genetic distance and/or pedigree.

Because it may not be feasible to conduct experiments that are sufficiently realistic using confinement measures (see section 3.2, above), it may be appropriate to study instead non-GM surrogate animals with similar characteristics or traits to those of the GM animal being considered, in natural uncontained environments, together with appropriate comparators for the non-GM surrogate. In such cases the study should consider using comparators that are as similar as possible to the conventional counterpart and/or wild type of the GM animal, to avoid the difficulty of inferences from a chain of indirect comparisons. This is explored in more detail in section 3.4, below.

Moreover, information should be provided on the breeding scheme and/or pedigree applied to the GM animal, and to all the comparators and non-GM surrogates used that are bred (not wild). In addition as much information as available should be supplied on the origins, history, evolution, phenotype and genetics of wild/feral comparators used in studies. Explicit justification for the choice of all the selected comparator(s) and surrogate(s) should be provided with a full discussion of the issues.

Finally, whatever information is generated, collected and assessed and whatever additional comparators and non-GM surrogates contribute to that information, applicants should draw final conclusions on potential adverse environmental impacts either in relation to the conventional counterpart, if it exists in the receiving environments, or to the overall environmental consequences of release, if it does not exist in the receiving environments.

Directive 2001/18/EC also requires that differences in the use or management of the GMO compared to similar non-genetically modified products should be highlighted (see sections on management 4.1.6, 4.2.5 and 4.3.7). Here, ‘use’ includes the functions of companion animals and ‘management’ includes all aspects of production systems such as aquaculture, rearing and husbandry for, respectively, fish, insects and mammals & birds. For certain assessment issues such as the effects of differences in use and management, the inclusion of additional comparator(s) may be particularly appropriate, because it is necessary to place any effects of the genetic modification into context by assessing whether use or management practices may influence the expression of the studied endpoints (EFSA, 2009a). However, if more than one management technique is employed, the principal comparisons for inferences regarding environmental harm should be those which represent typical commercial practices. Where practicable, management should follow standard practices and deviations should be

961 documented clearly; practices should conform to the latest EU regulations and guidance concerning
962 sustainability, e.g. aquaculture,8 husbandry,9 pesticides.10

963 **3.3.1. Choice of comparators for ERA of GM fish**

964 The ERA of GM fish should compare the GM fish to: (1) its non-GM source progenitor line; (2) one
965 or more populations of wild fish within the same species originating from the location or locations into
966 which it is proposed to release the GM fish; (3) one or more populations of wild fish species filling a
967 similar ecological niche as the GM fish in accessible ecosystems, as explained below; and (4)
968 aquaculture lines of the same species as the GM fish, whenever an aquaculture line is currently
969 produced in aquaculture in the accessible ecosystems.

970 In addition to the non-GM line, applicants should use at least one wild population as comparator
971 where the risk assessment has predicted that escape into environments occupied by wild types is a
972 possibility.

973 For each comparator used, the risk assessment should apply appropriate statistical methods to test for
974 differences between the GM fish line and the comparator line (EFSA, 2010c).

975 i. <u>For initial characterization of the GM fish line</u>

976 The comparator for characterization of the gene construct, gene expression, and whole-organism
977 phenotype of the GM line should be the non-GM line, that is, the line used to produce the GM fish
978 (EFSA, 2012). Applicants should use this comparator to characterise, in a statistically sound manner,
979 all the intended and unintended phenotypic changes in the GM fish line (see Gong et al., 2007;
980 Kapuscinski et al., 2007a and Devlin et al., 2007).

981 The non-GM line provides an initial but not a sufficient comparison for a reliable environmental risk
982 assessment. Applicants should compare the GM fish line to one or more additional fish populations, as
983 outlined below.

984 ii. <u>For assessing ecological effects, including genetic effects, of GM fish that might enter</u>
985 <u>receiving environments</u>

986 It is necessary to assess ecological differences and similarities between the GM fish line and wild fish
987 populations that fill a similar ecological niche in the accessible ecosystems (Devlin et al., 2007 and
988 references therein). Following this fundamental ecological principle, and depending on the wild
989 species composition in the accessible ecosystems (Moreau et al., 2010, 2011), appropriate comparator
990 specimens include one or more of the following types:

991 1. Wild population of the same species as GM fish, and that occur in possible escape zones and
992 accessible ecosystems.

993 2. Wild population of species closely related to the GM fish, and that occur in possible escape zones
994 and accessible ecosystems.

995 For example, if the GM line is a rainbow trout and accessible ecosystem has wild brown trout,
996 compare to wild brown trout population or populations from the accessible ecosystem.

997 3. Wild populations of other fish species in the accessible ecosystems filling a similar ecological niche
998 and, thus, with which the GM fish could compete.

999 The applicant should support the choice of the wild population they use with relevant information on
1000 differences in quantitative traits and local adaptation. In the case this information is missing,

[8] http://ec.europa.eu/research/fp6/p5/pdf/biosoc-library-brochreports2-food_ca.pdf
[9] See http://www.eu2011.hu/news/agricultural-ministers-discussed-sustainable-animal-husbandry-informal-meeting,
[10] See http://ec.europa.eu/environment/ppps/home.htm

applicants can either provide that information or consider this in the uncertainty analysis (see section 3.7).

The applicant should consider whether or not to use all the above three types of wild fish comparators unless the GM fish will be propagated or somehow used in aquaculture only near ecosystems that clearly lack a particular type. Applicants should provide ecological justifications for their choice of comparators (see Kapuscinski et al., 2007a, b; Devlin et al., 2007 for detailed guidance on selection of appropriate comparators).

iii. When accessible ecosystems also involve aquaculture of non-GM line of same species

It is additionally appropriate to compare the GM fish to a farmed line or a line of the same species, if such a line (or lines) is currently used in aquaculture operations from which fish could enter the accessible ecosystems. The objective of this comparison is to assess if the GM fish pose different ecological risks than those posed by the farmed, non-GM line, or lines. Risks that the GM fish pose to the aquaculture farms themselves should also be examined.

There are two reasons to make sure that the farmed line, does not replace wild population comparators, as recommended above. Firstly, in most aquaculture contexts, important gaps of knowledge exist regarding the ecological effects of non-GM farmed species and lines within species that are in current use (Kapuscinski et al., 2007b; Devlin et al., 2007; Svasand et al., 2007). Secondly, GM fish lines are unlikely to pose the exact same environmental risks as non-GM lines currently grown in commercial aquaculture, particularly in respect to (1) their impact over multiple generations following an incident of a single escape, and (2) their impact from recurrent escape incidences. In most cases, conventionally bred strains will express altered phenotypes due to changes in a range of genes with additive effects, whereas in GM strains a single transgene will be responsible for the phenotypic change from wild type. Therefore the genetic consequences of GM fish interbreeding with wild relatives are very different from those of non-GM, domesticated fish. In the first case any individual inheriting the transgene maintains its phenotypic expression across generations; whereas in the latter, the effect of integration of domesticated genotypes into wild populations is halved at each generation and therefore the related phenotype will eventually disappear from the wild population.

Applicants should provide information and justification for omitting one or more of the aforementioned comparators.

3.3.2. Choice of comparators for ERA of GM insects

For the initial characterisation of the GM insect, the appropriate comparator would be the non-GM organism.

For the environmental risk characterisation of GM insects, the most appropriate comparator will depend on the GM application and may consist of either the conventional counterpart (i.e. the non-GM insect with a genetic background as close as possible to the GM insect) or comparison of alternative management scenarios (e.g. insecticides). GM insects form part of a system of management, often aimed at population control, which includes rearing and release technologies and management processes that are integral to the overall quality and impact of the system.

Hence, depending on the type of GM application, the appropriate comparator consists of:

- for control systems based on GM sterility or inherited lethality: another alternative control scenario (e.g. insecticides) that suppresses the natural population with as much specificity as possible;
- for preventative releases of GM sterile or inherited lethality technology: the pre-release baseline scenario in which the target pest organism is not yet present, with any alternative prevention measures in place;

- for GM replacement strategies, which reduce the vector capability of a population without suppressing the population: a wild population in a disease-free location, with any appropriate management scenario (for nuisance impacts, for example);
- for GM pollinators: the pollination system based on unmodified insect, which may be of different species if that is the conventional management system.

Further details are provided in section 4.2.3: interactions of the GM insects with target organisms.

3.4. The use of non-GM surrogates

ERA of GM animals involves collecting, assessing and, where appropriate, generating information on a GM animal in order to determine its impact on the environment, human and animal health. Applicants might use alternative methods to collect relevant scientific and technical data informative for their ERA. One solution might be to gather data from experimental studies using GM animals performed in confined and controlled conditions (see section 3.2 above). However, for many animals such an approach is limited by how closely experiments are able to mimic natural conditions and hence encompass the complexity of factors interacting with the animal in its receiving environments.

Consequently, non-GM surrogate animals with similar characteristics or traits to those of the GM animal being considered, could be used to replace the GM animal so that experiments can be carried out in nature in order to determine environmental impacts (Kapuscinski et al., 2007a).

The selection of the non-GM surrogate animal will depend on the interaction(s) being assessed. For example, there may be several types of non-GM surrogate animals that can be used, depending on the traits expressed by the GM animal:

(1) GM animals carrying genes that induce sterility may be replaced with sterile animals genetically altered through other means than GM, and that are not prohibited from being released into the receiving environments (e.g. polyploid fish or radiation sterilized insects), in order to assess ecological interactions and genetic interactions not associated with introgression of the recombinant DNA;

(2) selectively bred and domesticated strains that express phenotypes similar to that of GM animals (e.g. fast-growing farmed salmon can replace GM salmon with similar phenotypic growth) in order to assess ecological interactions and genetic interactions not associated with introgression of the recombinant DNA;

(3) induced phenotype in the wild animal (e.g. by slow-release implants of the hormone or regulatory factor otherwise produced by the recombinant DNA) to assess ecological interactions associated with introgression of transgene into wild type(s) (Kapuscinski et al., 2007c and Hull, 2010).

Valuable data for the ERA may be obtained from the consideration of non-GM surrogates. The suitability of non-GM surrogates – and of derived data – needs to be considered on a case-by-case basis. For example, prediction of the likely handling procedures and environmental impact of GM sterile insects may usefully be informed by the current and historic use of radiation-sterilised, non-GM insects for similar purposes. These are likely to represent close surrogates, especially where the same or similar species is involved.

Non-GM surrogates are likely to be particularly useful as a source of historic or parallel data (e.g. literature) to inform risk assessment rather than as experimental models from which to derive new information that can be related to the specific to the GM animal under consideration. However, in the case where applicants decide to carry out specific experiments, they should consider the use of non-GM surrogates to obtain *de novo* data without using the GM animal. Applicants should describe and justify the selection criteria used for the surrogate.

Effects of non-GM surrogates should be compared with appropriate comparators in order to determine any differences in effects. The choice of comparators should follow the same approach as described in

section 3.3. Given the very large amount of data in the scientific literature on the effects of introduced animals into new environments, their use as surrogates for GM animals may be advantageous because their impacts are already well documented.

The applicant shall describe and justify:

- the objectives of each study and the hypotheses to be tested using non-GM surrogates and comparators;
- the selection criteria used for the non-GM surrogate and any comparators;
- the specific design of each study including the assessment and measurement end points, and statistical power;
- the adequacy and relevance of each study for extrapolations from surrogate to the GM animal being assessed;
- the number and range of environments studied;
- the interpretation and extrapolation of the surrogates to the GM animal, including the use of statistical models (see section 3.5 hereunder);
- the reliability and uncertainty associated with the data, assumptions made in the models and non-GM surrogates used and extrapolation to impact on receiving environments.

3.5. Experimental design and statistics

3.5.1. General Principles

This section applies to data collected from experiments in which specific hypotheses are tested to ascertain whether there are adverse environmental effects due to the GM animal when compared to its comparator(s) and to measure their magnitude. When such experiments are conducted outdoors, whether they be terrestrial in a field environment or aquatic in a marine environment, they are termed 'trials' throughout this chapter. Also, where the term GM animal is used in this section, the text applies equally to any surrogate for the GM animal (see section 3.4, above). This section does not apply to data obtained from surveys such as those conducted for post-market environmental monitoring (see Chapter 5), or to observational data. Neither does this chapter apply to a comparison of whole ecosystems, where there is, by definition, no possible replication.

Comparative analysis is performed in order to assess similarities and differences between the GM animal and its appropriate comparators. The comparative analysis referred to above shall involve two approaches: (1) a proof of difference, to verify whether the GM animal is different from its comparator(s) and might therefore be considered a potential risk depending on the type of the identified difference, extent and pattern of exposure; and (2) a proof of equivalence (EFSA, 2010c) to verify whether the GM animal is equivalent or not to its comparator (EFSA, 2010a) within certain bounds (see definition of so-called 'limits of concern' below). When a surrogate for a GM animal is used instead of the GM animal itself (see section 3.4, above) the analysis shall include, in addition, and if appropriate data is available a proof of equivalence between the non-GM surrogate and the GM animal.

The principles underlying these statistical tests are to provide information with quantified uncertainty that may be used by biologists in risk characterisation of those endpoints for which differences or lack of equivalence are found. Hazard characterization should be used to place identified differences into biological context. In this process, allowance must be made for the distinction between statistical and biological significance as discussed in EFSA (2011). The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant on safety grounds (see limits of concern, below). For risk

assessment it is not the function of statistical analysis to provide results that lead automatically to a particular decision; instead, the case-by-case approach shall remain paramount.

For each measurement endpoint, the level of environmental protection to be preserved shall be expressed, directly or indirectly, through the setting of thresholds termed 'limits of concern' in EFSA (2010). For small scale studies (e.g. in a laboratory or small netted enclosure) the limits of concern will be more likely to reflect environmental protection goals indirectly. These may, if exceeded, lead to further studies at larger scales, if appropriate. For larger scale trials, the limits of concern should reflect more directly the minimum ecological effects (in positive and negative directions) that are deemed biologically relevant. For such trials, at least one of the limits of concern shall represent the minimum effect that is considered by applicants potentially to lead to environmental harm. If this limit is exceeded then detailed quantitative modelling of exposure may be required to scale up adverse effects at the field level both temporally (to seasons, generations) and spatially (to production units, local environments, larger regions and ecosystems) (EFSA, 2008). Data from previous experiments, or data from the scientific literature and research reports, can be used to define the limits of concern. This must be done on a case-by-case basis. However, purely as a guide for trials, where the endpoint is species abundance, several ecological studies, both in the USA and in the EU (Heard et al., 2003), have adopted a multiplicative effect size of 50% as a limit of concern. Whilst this may be a reasonable level, care is required to define the population which is potentially affected. Unless there is explicit justification, limits of concern for small scale studies shall usually be less than those for larger scale studies. Again, as an indication for laboratory studies, a multiplicative effect size of 20% has sometimes been taken as a threshold, while 30% has been employed for semi-field experiments. For field studies, several studies, both in the USA and in the EU (Heard et al., 2003), have adopted 50% as a limit of concern, which is a reasonable level.

As a hypothetical example, consider a behavioural experiment in which the aggressiveness of a GM growth-enhanced cat (see introduction in chapter 4 of GM M&B) is compared to its conventional counterpart. Each might separately be confined with a trained human volunteer and the measurement endpoint might be, say, the number of observations of aggressive encounters per ten minute period. Here thresholds might be set at a multiplicative difference of ±15% for a small room or ±25% for a larger, outdoor enclosure. Whilst these thresholds are arbitrary, they were chosen on the basis of whether they reflect adequately a potential environmental impact; if exceeded they may lead to further, more realistic or detailed experimentation.

Whatever are the limits of concern adopted, applicants shall state their value and justify the choice explicitly, for each measurement endpoint. For trials, it will usually be the lower limit, which might correspond for example to a decrease in the abundance of a particular species in the presence of the GM animal relative to that for its comparator, that will be defined as the threshold effect deemed to be of just sufficient magnitude to cause environmental harm. Notwithstanding this general approach, it is acknowledged that the multiplicity and diversity of questions that might be posed in an ERA may demand alternative statistical approaches, on a case-by-case basis.

3.5.2. Principles of experimental design

For many GM animals, particularly for larger species, it is recognized that the available number of animals may be limited. In addition many are sexually reproducing species with variable numbers of offspring of varying genetic uniformity. In addition their phenotypes may be very plastic so that the source of the materials used for comparative purposes is important. However, experiments should be adequately replicated wherever possible. General recommendations for experimental design may be found in Cochran and Cox (1957), Quinn and Keough (2002) and Crawley (2005). For animal experiments, principles of experimental design have been set out for laboratory experiments in the ILAR Journal (2002), see especially the papers of Festing and Altman (2002) and of Johnson and Besselsen (2002), and for fish in several papers by Underwood (e.g. Underwood, 2000). These principles often apply equally well to trials. When many comparator individuals are represented in an

experiment, care should be exercised to ensure that between-animal variation is representative of the genetic variability present in typical populations of the comparator (and see Taylor, 1985).

In the statistical theory of the design of experiments, the causes that are thought to contribute towards the value of the variables measured by the experiment are often termed 'factors', especially when they are controllable in the experiment (fixed factors) and take a limited number (termed 'levels') of different values. 'Treatment factors' are those of primary interest and relate directly to the questions the experiment is designed to address. For example, experiments to inform risk assessment might have a treatment factor with two levels: a GM animal and a conventionally-reared comparator. In addition, most experiments would include additional factors, such as feed level, predation risk, light conditions, season, sex, temperature etc., the interaction of which with the treatment factor may be of interest. For example, temperature may have different effects on the GM animal and the non-GM animal (Lõhmus et al., 2010).

'Blocking' is the arranging of experimental units in groups (blocks) that are similar to one another. Typically, a 'blocking factor' is a source of variability that is not of primary interest to the experimenter and should be treated as random factor in the statistical analysis. An example of a blocking factor might be the husbandry/cultural conditions that the animals are kept under. Usually an experimental unit is represented by a single animal. However, these will often be kept within a group of animals (as for poultry and fish) and one of the blocking factors will be the housings for those groups (such as cages and pens). In such cases, the variation captured by the blocking factor may be of importance because it may reveal aspects of the experimental conditions that were not expected. Care also should be taken to ensure adequate separation between groups to avoid unwanted interaction between them (i.e. to ensure statistical independence unless this is part of the experimental design). The blocking factors in the design should be chosen to be appropriate for the experimental units and should help to maximise the statistical power of the experiment to detect treatment effects (Richardson et al., 2004). All treatments in the experiment (usually the relevant treatment factor will have two levels: the GM animal and its comparator, but may also include a third level if a surrogate is involved) shall be fully randomised to the experimental units to avoid systematic bias.

It may be important to keep animals that are being compared under the same management conditions. On a case-by-case basis, it should be considered whether to include different management practices or environmental conditions (for example, temperature) as factor(s) within the experimental design, to assess whether the effects of the genetic modification are influenced by such practices/environments. In this way the interaction between, for example, the main effect (GM *versus* comparator) and a factor of interest, such as temperature, may be estimated. Similarly, and on a case-by-case basis, it should be considered whether to include in the design other factors where appropriate, such as age, sex, feed levels, predation risk level, habitat complexity, parity, lactation, laying cycle, etc. (but see Mead, 1990). The chosen experimental design and management conditions should ensure that any confounding of the main effect of GM versus comparator with other factors is minimized. Applicants should explain the choice of conditions to rear and manage the animals, as well as other distinctive factors included, or excluded, in the experimental design. Applicants should discuss any possible effects of plasticity with regard to the experimental design, the reliability and uncertainty associated with the data, and any assumptions made in the models (see section 3.7 on uncertainty).

Since GM animals cannot be deliberately released into the environments, for which ERA is being conducted, applicants will either need to perform studies under confined conditions from which the animals cannot escape or use non-GM surrogates (see section 3.3 and also section 3.4) in order to inform the risk assessment. Ecologically relevant information about GM animals can be derived from: (1) experimental studies under confined conditions or (2) field data on non-GM surrogates which share characteristics with the GM animals (Devlin et al., 2006). In both cases, there is a need to extrapolate from the experimental results into effects of the GM animal on the environment under unconfined conditions. Experimental conditions should try to mimic as closely as possible the natural habitat (e.g. stream, lake, ocean, field, meadow, forest) which the GM animal is likely to experience. These conditions are critical for identifying phenotypic differences between the GM animal and its non-GM

comparator; they can also provide background information for designing more complex experiments (Devlin et al., 2006).

A range of responses of GM animals are likely to be environment-dependent. This presents the problem of extrapolating findings under a specific set of experimental conditions to those which would be experienced by the GM animal following deliberate release or escape into the receiving environments. Further, conditions in nature are inherently diverse and variable in time and space, presenting a major obstacle in providing reliable data for ERA.

It can therefore be important to consider that experiments conducted in the laboratory expose GM animals to different environmental conditions, both within and between generations. Hence, it is essential to record the variation in their phenotypic responses, i.e. to assess plasticity and identify gene-environment interactions. Applicants should consider the influence of the environment during rearing of experimental animals and the influence of environmental conditions during the experiment itself. These responses will be used to assess how sensitive a specific trait is to environmental influence (plasticity) to understand how it may or may not change once the animal is exposed to other natural conditions (Sundström et al., 2009). Because plasticity is an effect of the genotype of an animal interacting with the environment, it is important to assess how other genotypes are affected by the same environmental conditions. Hence, the addition of a genetic modification to an animal may dramatically alter its response to environmental conditions. This extends to maternal and paternal effects typical for specific females and males (Mousseau and Fox, 1998). Here it is also important to note that transformation of one trait can affect other traits (pleiotropy) so that studies should address not only the modified trait (e.g. growth rate), but also other potential effects (e.g. activity level, aggression, disease resistance, fertility, longevity etc). Trade-offs between the transformed state and other characteristics also need to be identified so that they can be examined (e.g. feeding-risk taking). Further, different factors in the environment may act as antagonists or in synergy in their effects on phenotype, so experiments need to take this into consideration. Hence, while confined laboratory experiments cannot completely mimic actual environmental conditions, they are necessary in order to identify those phenotypic differences that are likely to occur between the GM and its comparator and which will form the basis of the risk-assessment.

Studies conducted in confined space can provide a useful understanding of the phenotypic effects of a recombinant DNA molecule under more complex environmental conditions. However, such studies should mimic as far as is practical, natural conditions that the GM animal is likely to experience. These conditions could include, for example, habitat structure, other ecosystems species, live natural prey items, natural predators and opportunities for pathogen effects. Such conditions allow multiple factors to operate simultaneously in a spatial context more representative of nature, minimizing phenotypic effects resulting from artificial conditions in cultures, small arenas and the laboratory (Sundström et al., 2004, 2005, 2007; Devlin et al., 2006, 2007). Confined studies can provide useful data necessary for an environmental risk assessment in relatively small and simple environments. For species with greater ranges over their full life cycle, applicants should attempt to conduct confined studies for critical life stages, including early life, reproductive adult, and transitional life stages in between these such as when a migratory species moves over considerable distances (e.g. Devlin et al., 2006, 2007). Applicants should record and discuss changes in phenotypic and ecological traits (e.g. changes in quantity of faeces excreted when modifying a food conversion or feed assimilation trait). For confined studies, applicants shall justify explicitly the choice and use of the following: abiotic factors such as the containment structure, wind or water movement (lotic, lentic), temperature, salinity in aquatic environments, light; biotic interactions within- and between-species including competition, reproduction, predation; life-history factors including age, maturity, development, migration, reproductive state.

Certain genetic modifications may result in management or production conditions that are appropriate for the GM animals, being suboptimal or non-permissive for the comparator, and vice-versa. An example of this might be cold-tolerant GM fish that express antifreeze proteins; these can be farmed at locations where the comparator cannot be reared. Suppose the husbandry conditions can be

summarized by some critical variable T, where the ranges of non-permissiveness, sub-optimality and optimality are defined by the values *l*, *r*, *s* and *u*, as displayed in Table 4.

Table 4: Definition of ranges of husbandry conditions

Values of critical variable, T, for husbandry condition					
GM animal	non-permissive: T < l(GM)	suboptimal: l(GM) < T < r(GM)	optimal: r(GM) < T < s(GM)	suboptimal: s(GM) < T < u(GM)	non-permissive: u(GM) < T
Comparator (C)	non-permissive: T < l(C)	suboptimal: l(C) < T < r(C)	optimal: r(C) < T < s(C)	suboptimal: s(C) < T < u(C)	non-permissive: u(C) < T

These values differ for the GM animal and for its comparator; a graphical example is given in Figure 4.

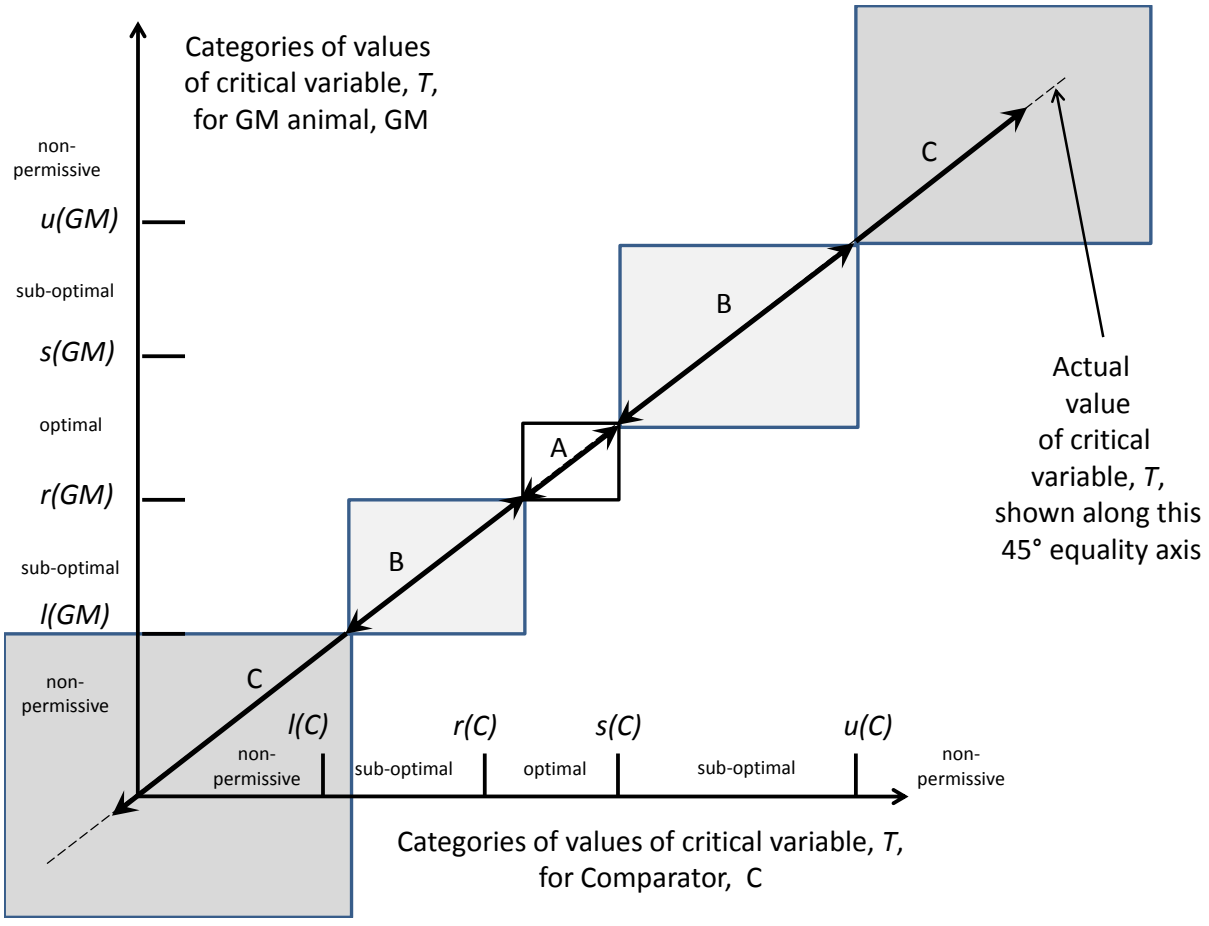


Figure 4: Comparative approach allowing for management conditions that may be optimal, sub-optimal or non-permissive. Values of the critical variable, T, for the management condition considered are those assumed in Table 4, above. Dashed line is the equality line at 45° to each axis, representing the actual value of the critical variable, T. When the ranges {r(GM), s(GM)} and {r(C), s(C)} overlap, there is a region labelled 'A' (unshaded) in which the management condition is optimal for both C and GM. Experiments done within this region lead to valid comparisons between the two treatments. Values of T within the regions indicated by 'B' (light shading) are sub-optimal for either or both of C and GM and comparisons between the two treatments require care (see text). Values of T within the

regions indicated by 'C' (dark shading) are non-permissive for either or both of C and GM; experimentation should not be done and no valid comparisons between the two treatments may be made.

The ideal situation is where the optimal conditions reflected by typical commercial practice for the GM animal and its comparator overlap (region 'A' in Figure 4). Applicants should seek to perform experiments within this range whenever possible because the comparative approach is appropriate. The two regions 'B' in Figure 4 represent conditions that are sub-optimal for at least one of the treatments, GM and/or its comparator. The interpretation of data from experiments within this region requires care and applicants should justify explicitly why conclusions drawn concerning comparisons from such experiments can be made validly. The two regions labelled 'C' in Figure 4 represent management conditions that are non-permissive on health or welfare grounds for one or other of the treatments GM and/or its comparator. No comparative experimentation within this region should be performed as the only valid conclusion between the two treatments is survival and/or non-survival. In some rare cases the two regions 'C' may overlap such that it is impossible to identify a value of variable *T* that is not non-permissive for one or other of the treatments. For example, if *T* were temperature and the upper value for the GM, *u*(GM) was 5.0 and the lower value for the comparator, *l*(C), was 8.0. In that case, an additional comparator(s) may be required, such as an animal of another species (e.g. non-GM surrogate: see section 3.4) with similar characteristics to those of the GM under consideration. Of course, it should be realised that optimality conditions may be one-side rather than two-sided as in the example portrayed here. Hence, for example, toxic substances will not usually be optimal at larger values than sub-optimal; resources will not be optimal at smaller values than the sub-optimal, etc.

A similar problem may occur with rapidly-growing GM animals that reach maturity or marketable sizes earlier than their comparators. In that case, a comparator with the same size or weight rather than the same age may have to be chosen in order to represent an appropriate comparator, especially for the developmental stage at which they are marketed as ready for consumption. It is recommended that the experimental design represents a range of management conditions, including feeding regimes suitable for the GM and its comparator. However, it is vital that both the GM and comparator can be reared without unacceptable risk of mortality or adverse welfare issues. Care should be taken to choose an experimental design that does not suffer unduly from loss of animals during the experiment. Both GM and comparator should be reared prior to experimentation under conditions that allow the experimenter to assess how rearing conditions may affect the development of the GM animal and its comparator. For example, management conditions may be very different from conditions in the receiving environments (e.g. in term of food availability and predation risk), and the phenotype of the GM animal after rearing in both environments needs to be considered in the comparative study (Sundström et al., 2007). These conditions may influence the results of the experiment and this should be considered.

Applicants shall state explicitly the size of the effect that it is desired to detect in the study by the difference test, for each measured endpoint. Usually, this size will relate directly to the limits of concern (see above). The effect size may be asymmetric, and in particular may be set as zero in one direction to yield a non-inferiority form of the equivalence test (Laster and Johnson, 2003). The magnitude of the effect size that the study is designed to detect will generally be greater for trials designed to provide confirmatory data for the assessment of unintended effects than for specific hypotheses (see Chapter 2). The effect size will often be placed on the multiplicative scale; however, the natural scale or some other scales are admissible alternatives, on a case-by-case basis. In principle, where more than one comparator is used, different effect sizes may be specified for the different comparators; however, this is unlikely to be necessary in practice. Applicants shall provide a full justification for all effect sizes chosen.

Based on such effect sizes, power analyses aid transparency and may engender public confidence that the risk to the consumer or the environment is well-defined and low (Marvier, 2002); these require specification of the magnitude of the effect size that the study is designed to detect. For each study,

applicants should ensure that the design is such that the main effect for the difference test (assuming there are no interactions between this and other factors) has sufficient statistical power to provide a reasonable level of credible evidence and should seek to attain as close to 80% power for a 5% size of test as is feasible. Applicants shall provide an analysis that estimates the statistical power for each difference test on each endpoint, based on the stated effect size and assuming a 5% type I error rate. The analysis should be done at the planning stage of the study. The power analysis should use only information verifiable as available prior to the study; under no circumstances should data from the study itself be used. It is recommended that applicants prepare an experimental design protocol for each study (see Appendix to Perry et al., 2009 for a suggested checklist).

It may be necessary to consider the use of some form additional control in order to demonstrate post-hoc that the study was capable of detecting the desired effects. For example, in a predator-prey experiment with insects, a knock-down insecticide might be used on a single plot to demonstrate that there was a sufficient population density of the prey species available in the experimental area to be sampled). If such a control is external to the experiment, for example on a single unrandomised plot, then data from the control should not enter the statistical analysis in any form.

3.5.3. Statistical analysis

Recommended procedures for statistical analysis involving difference and equivalence tests are discussed in EFSA (2010c) and EFSA (2010). If possible, applicants should follow the recommendation to calculate a confidence interval for each endpoint and to display all endpoints on the same graph(s). Care must be taken that the analysis is appropriate if the experimental unit is a group rather than an individual animal. In such cases, data must be presented from replicated groups to provide information on between-group variability.

Data transformation should be considered to ensure normality and to provide an appropriate scale on which statistical effects are additive; in particular, potential non-linear responses such as *probit*, or quadratic should be allowed for. As is routine in ecological applications, for many measurement endpoint response variables, a logarithmic transformation (or a generalized linear model with a logarithmic link function) may be appropriate. In such cases, any difference between two means on the logarithmic scale may be interpreted as a ratio on the natural scale. Consideration should be given to the possible need to analyse males and females separately, where appropriate, preferably by including sex as a factor in the analysis. Allowance should be made, usually through analyses involving statistical mixed models, for possible temporal autocorrelation when repeated measurements are taken from the same animals. Rejection of outliers should only be done for biological reasons, which should be stated explicitly. Statistical tests for outliers should never be applied for automatic outlier removal. Any discarded outliers should be identified, and analyses should be provided both with and without outliers. It is recommended that applicants prepare a statistical analysis protocol for each study (see Perry et al., 2009 for a suggested checklist).

Other recommended procedures for statistical analysis can be found in EFSA (2011a).

3.5.4. Information required

A full and explicit justification should be given for the choice of animals and other biota in the experiment, including rearing background and experimental conditions (e.g. temperature, light conditions, structural complexity, feed levels etc). Applicants should provide any data analysed and all programming code used for analyses and simulation, in an editable form, together with a full description of the statistical model used, listing any assumptions made, and the software used for the analysis. In addition, applicants should provide a table or graph categorised by the factors in the experimental design, giving, for each (possibly transformed) endpoint, the means and standard errors of means of the GM animal and its comparator(s), and any other test material, where applicable. The husbandry and cultural conditions selected should be comprehensively described and fully justified. The use of all veterinary drugs and other biocides should be described fully.

For a particular measurement endpoint, the mean difference(s) between the GM animal and its comparator(s) shall be reported, together with a 90% confidence interval constructed around it. This mean (or these means), these confidence limits and all equivalence limits shall be displayed on a graph(s) similar to Figure 1 of EFSA (2010c), but where values are plotted relative to a zero baseline. (Note that the line of zero difference on the logarithmic scale corresponds to a multiplicative factor of unity on the natural scale). The horizontal axis shall be labelled with values that specify the change on the natural scale. In the case of logarithmic transformation, changes of 2× and ½× will appear equally spaced on either side of the line of zero difference. Both the difference test and the equivalence test may be implemented using the well-known correspondence between hypothesis testing and the construction of confidence intervals. In the case of equivalence testing the approach used shall follow the two one-sided tests (TOST) methodology (e.g. Schuirmann, 1987) by rejecting the null hypothesis when the entire confidence interval falls between the equivalence limits. The choice of the 90% confidence interval corresponds to the customary 95% level for statistical testing of equivalence. Since the confidence interval graph is used also for the test of difference, each difference test will have a 90% confidence level. Although 1 in 10 of these tests is expected to yield a significant result by chance alone, applicants shall report and discuss all significant differences observed between the GM animal, its comparator(s) and, where applicable, any other test material, focussing on their biological relevance within the context of risk characterisation (see above). Regarding the simultaneous tests of difference and equivalence, each outcome from the graph shall be categorised and the respective appropriate conclusion shall be drawn, as described in EFSA (2010c).

The applicant should clearly describe in words all the questions that each experiment is designed to address. In addition, each of these questions should be re-stated in formal terms, in the form of the precise null hypothesis that was tested to answer the question. Applicants should give details of any alternative statistical models considered and specify why the model chosen for analysis was deemed most appropriate. Any departures from the experimental design and statistical analysis protocols referred to above should be specified.

3.6. Long-term effects

According to Directive 2001/18/EC, the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM animal on human and animal health and the environment. Effects can be direct and indirect, immediate and delayed, including cumulative long-term effects (EC, 2001).

Predicting and assessing (adverse) long-term effects requires information about the GM animal, its intended uses, and the receiving environments (see also section 3.1), both in terms of the baseline conditions in the receiving environments and temporal changes in these conditions independently of the GM animal and following GM animal introduction. The rate and degree to which the baseline is likely to change independently of the GM animal will vary among management systems. Long-term effects of the GM animal should not be considered in isolation but compared to the long-term effects of its conventional counterpart or another suitable comparator, if present in the receiving environments (see also section 3.3). If no suitable comparator is present in the receiving environments, long-term effects should be compared between the presence and absence of the GM animal (again, see section 3.3).

Long-term effects are poorly investigated for most existing animal species, including invasive species (Strayer et al., 2006). However, published figures of time delays between the introduction of invasive species to an exotic range, their establishment, and spread with associated harmful effects can be informative to get some first ideas about expected time delays for GM animals (Henry et al., 2010). Some data are currently available for animals and suggest delays of approximately ten years for insects, >10 years for fish, and > 60 years for mammals and birds (Jeschke and Strayer, 2005; Szalai et al., 2010). Although these are rough estimates, delays in this order of magnitude may also be expected for GM animals. Of course, they are expected to differ among species and their specific attributes, e.g. generation time. The number of GM individuals that escape or are released into the wild, and the

frequency of escape/release events – i.e. propagule pressure – will also importantly influence such delays (shorter delays are expected for high propagule pressure). The spread of GM animals will start where they escape or are released, and it very much depends on the GM animal's dispersal abilities how fast it will spread to other locations. Finally, effects caused by interbreeding between escaped or released GM animals and 'wild type' conspecifics (or related species where interbreeding can occur; Henry et al., 2010) can only be observed after long time periods, depending (among other factors) on generation time.

Therefore, applicants should consider the whole life cycle of the GM animal and the receiving environments of the different life stages to determine possible adverse effects over time. The analysis should be conducted on a case-by-case basis and applicants should provide information and justification for their approach.

3.6.1. Categories of long-term effects

Long-term effects might result from a diversity of primary causes and secondary interactions, which make it difficult to generalise on methods of investigation. Nevertheless, long-term effects may differ from a GM animal's effects before its placing on the market due to several reasons which may be classified into two categories (EFSA, 2010a):

> Category I: long-term or chronic exposure to a particular GM animal or practice may result in a delayed response by organisms or their progeny. An example for such a long-term effect is the development of a resistance in the pest-target organism of a genetic modification.
>
> Category II: long-term effects may also occur due to increases in spatial and temporal complexity. Before placing on the market, only certain spatial and temporal scales can be empirically tested, hence there might be long-term effects as a result of increased spatial or temporal complexity after placing on the market. Examples include interactions of GM animals with other species, as the complexity of species interactions increases with spatial complexity.

Over longer time periods, evolutionary, behavioural, and other changes of species will cause further changes of species interactions. Climate also differs across spatial and temporal scales: increasing spatial complexity increases the combinations of environmental variables that individuals of a GM animal are confronted with. Increasing temporal complexity further increases the range of environmental variables that GM animals are confronted with, e.g. due to climate change. Climate change also affects other GM and non-GM species, so species interactions are affected in this way, too. In fact, climate change is likely to change whole species communities and will lead to "no-analogue communities" and ecological surprises (Williams and Jackson, 2007).

Over time, new management or production systems may arise. Such changes and their potential effects on the GM animal must be addressed in the application as well, on a case-by-case basis (see sections 4.1.6, 4.2.5 and 4.3.7 on management systems).

3.6.2. Guidance to applicants

Long-term effects may differ among captive, semi-captive, and non-captive GM animals (see above Chapter 1). Depending on the GM animal, applicants should estimate possible long-term effects of category I and II on a case-by-case basis.

Some long-term effects of Category I might already have been investigated within confined experimental systems maintained over several generations of the GM animal/trait combination under study (see section 3.2). While some potential long-terms effects might be revealed by such studies, questions will still remain, as to how much the confined system restricts the range of possible reactions or encourages untypical reactions. Information from such studies might be useful for defining the primary mechanisms by which the GM animal might interact with other organisms and

abiotic factors of the receiving environments, but would not be sufficient alone as a basis for assessment of long-term effects in a representative management/production systems.

Long-term effects of category II, by definition, cannot be investigated through an initial experimental phase of testing, as none of the possible experimental design can provide the range of complexity experienced after full commercial release. For example, it is likely to be difficult to mimic, with a confined experimental set up, all conditions occurring in the receiving environments in order to assess possible interactions of a GM animal with other animal species. Category II effects can only be investigated by reference to possible existing examples and case studies that provide evidence of rates and magnitudes of environmental impact due to change in production systems (e.g. intensive grazing) or external (e.g. climate change) factors.

Despite these uncertainties, there is information available in the published literature and reports that can be informative for the assessment of possible long-term effects of GM animals on the environment. Applicants should conduct appropriate desk-based studies to assess possible long-term environmental effects of the GM animal in relation to both categories of long-term effects. It is not the intention here to give precise instruction to applicants on which data, processes and indicators should be considered, since they will vary on a case-by-case basis. However, examples of the type of information that could be used in assessment are:

- Publications on similar GM animals as well as on non-GM and wild animals with similar characteristics and occupying a similar ecological niche (e.g. non-GM surrogates) related to various issues such as changes in management/production systems;
- Long-term ecological or environmental datasets applicable to the receiving environments; e.g. ecological surveys showing change in organisms range or abundance, diseases treatments;
- The results of confined experiments with GM animals or similar organisms;
- The results of 'large-scale' experiment with non-GM surrogates;
- The results of meta-analyses, if available, drawing together data from different sources;
- The use of models of ecological processes in combination with experimental data to explore or test scenarios: mathematical models of ecological processes are unlikely to be considered justification on their own, but may be used to argument or interpret data or to demonstrate that possibilities have been explored; descriptions would be necessary of the model, its verification using existing data, the input variables, etc;
- The use of pedigree data could support applicants in understanding the genetic structure of the GM population. The pedigree analysis can help derive the long-term dynamic development of the GM population. If possible, applicants should provide information about the level of inbreeding, relatedness, effective population size, generation interval, effective number of founders and ancestors (Boichard et al., 1997; Gutierrez et al., 2003, 2005). The DNA analysis (e.g. SNP, microsatellites, genome mapping) of biological samples of GM pedigree animals could also be used instead of or in conjunction with the quantitative analysis. If possible, a comparison with the non-GM pedigree population is recommended;
- Foreknowledge of relevant change in the management/production systems and wider environment that can be expected in the years following the deliberate release into the environment.

For chapter 4 on specific areas of risk applicants should conclude the risk assessment of long-term effects by summarising:

- The methods and approaches used to reach the conclusions, including the published long-term or large-scale experiments, reference datasets, analysis and models used directly in the assessment;

- The basis of and justification for a conclusion specific to the GM animal or its management/production (whether a conclusion is for or against the likelihood of a long-term effect);
- Identification of parts of the post-market environmental monitoring plan that are designed to detect possible long-term effects identified in the desk study (further details in Chapter 5).

3.7. Uncertainty analysis

3.7.1. Introduction

Directive 2001/18/EC and the Guidance Notes supplementing Annex II to Directive 2001/18/EC (EC, 2001, 2002) define risk as the product of the magnitude of the adverse consequences of the hazard and the likelihood of the effect. The identification of hazard, the likelihood, and the consequences are all terms characterized by, described with, and measured with various types and degrees of uncertainty. For example, limitations in the availability, relevance and quality of data used introduce uncertainties into the assessment and its outcome. According to EFSA opinion on transparency (EFSA, 2009a): although it may be impossible to identify all the uncertainties, each scientific output should describe the types of uncertainties encountered and considered during the different risk assessment steps, and indicate their relative importance and their influence on the assessment outcome. The scientific credibility, accuracy, and 'integrity' of a risk assessment hinges on the quality of its uncertainty analysis (Burgman, 2005).

Applicants should assess the overall uncertainty for each identified risk, possibly including consideration of:

- Assumptions and extrapolations made at various levels in the ERA;
- Any conflicting scientific literature and viewpoints;
- Specified uncertainties.

Applicants should therefore conduct and communicate an explicit and transparent uncertainty analysis as part of the risk assessment. Consistency among stakeholders in both the understanding of uncertainty and the use of terms describing uncertainty can also be developed through multi-stakeholder elicitation and deliberation methods (Carey and Burgman, 2008).

The analysis should use reproducible methods to identify and treat (i.e. analyse, eliminate or propagate) the sources of uncertainty identified. Examples of a variety of suitable approaches are given in Burgman (2005), Kapuscinski et al. (2007a) and Hayes (2011). A formal uncertainty analysis can recognize and treat different sources of uncertainty and help risk decision makers appropriately interpret the results of the ERA. The formal analysis should address three broad types of uncertainty:

Linguistic uncertainty – caused by different understanding of language used to describe environments, events and processes leading to ambiguous, context-dependent, underspecified or vague expressions (e.g. "moderate", "unlikely", "rare"), differences in interpretations, and arbitrary disagreement. It can be reduced by careful definition of terms and sensitivity to recognition of differences in interpretation.

Variability – caused by fluctuations or differences in a quantity or process, occurring over time (e.g. seasonal changes in prey species), with location (e.g. different species composition of prey across locations), or within a group (e.g. birth rates within a metapopulation of animals). The use of mathematical, statistical or other quantitative methods can help to quantify, understand and possibly reduce such uncertainty, both in relation to the receiving environments and the introduced GMO (see section 3.5 on experimental design and statistics).

Incertitude – caused by limitations of scientific knowledge and knowledge production systems such as motivational and systematic bias, censoring, measurement error, missing data, lack of suitable comparators or surrogates, and other causes of incomplete awareness, understanding and descriptions

of a mechanism, process or system (i.e. model and scenario uncertainty). Incertitude is sometimes called epistemic uncertainty (or subjective or type 1 uncertainty). It can be reduced using qualitative, semi-quantitative and quantitative modelling methods (see also sections 3.2, 3.4 and 3.6 on experimental environment, non-GM surrogates, and long-term effects respectively).

Applicants should apply appropriate methods to identify, describe and subsequently address these three types of uncertainty throughout the ERA. Guidance and selection of the most appropriate methods can be found, for example, in extensive reviews made by Hayes et al. (2007a), Beven (2009) and Hayes (2011). Uncertainty analysis software is available at various websites [e.g. see list of websites provided in Hayes et al. (2007a: 204) and Hayes (2011:99)].

It is pointed out that methodology for uncertainty analysis is evolving, especially to improve analysis in data poor situations [(Beven, 2009 and Hayes (2011:106-107)]. In all cases, applicants' uncertainty analysis should be conducted and presented in a reproducible manner, enabling EFSA or a third party to replicate the analysis by applying the same methods to the body of information presented by applicants. This is particularly important where extensive subjective experts' judgements have been applied. Subjective judgements can introduce uncertainty in model structure and parameter values, particularly in data-poor situations.

Depending on the hazard identification, the specific risk characterization approach and the statistical nature of the communicated outcome, the ERA, can be classified as qualitative, semi-quantitative, or quantitative.

Qualitative risk assessments are based on expert judgements and stakeholder opinions. Assessment outcomes are communicated on nominal scales used to categorize variables (e.g. sorting non-target insects into different species categories), or ordinal scales (e.g. rank order of categories of insects increasingly sensitive to a pesticide, but with no precise measurement of differences between ranks). Nominal or ordinal scales can classify and order variables but do not provide distance measures between ranks that enable understanding of risk levels.

Semi-quantitative assessments draw on the outcomes of qualitative assessments to construct discrete ranges of interval variables that are useful to construct and communicate a risk estimate (e.g. on a scale of one to ten). For instance, drawing on the ordinal scale used to describe the rank order of variables; insects can be ordered into different pesticide-sensitive and tolerant categories on an assigned numeric scale. However, the semi-quantitative assessments remain dependent on the rank-order and lack distance measures between rank variables (e.g. lacks understanding of the actual distance between sensitive and tolerant categories). Due to the reliance on assigned scales, semi-quantitative assessments are vulnerable to subjective bias. Moreover, they do not produce a numeric understanding of risk that allows them to be combined with other assessments to produce an overall quantitative risk estimate.

Quantitative risk assessments communicate outputs on continuous scales relevant to assessment endpoints. Interval or ratio scales are used that draw on a range of statistical and modelling techniques. Interval scales express values independent of their location on the scale (e.g. risk assessments based on the LD50 dose for different target and non-target insect species). Ratio scales includes a fixed location of the zero value (e.g. risk assessments of insecticide concentration remnants on plant food and feed in relation to NOAEL). Quantitative assessments rely on the assumptions underlying the approaches taken to reduce complexity in the biological system studied and the model structures proposed. These assumptions, and reasons for exclusion of alternative plausible model structures, must be made explicit when presenting the outcome of such models.

Probabilistic approaches may be useful to quantify some of the uncertainties. When such approaches are used, the outcome of the risk assessment should be characterized by reporting a distribution of the risk estimates. However, use of quantitative methods does not take away the need for a qualitative evaluation of the remaining uncertainties (EFSA, 2009a). In fact, it is recognized that most

characterizations of specific risks within the overall ERA will contain elements for which description and treatment of sources of uncertainty is both of a qualitative and quantitative nature.

Whenever possible, applicants should strive to conduct a quantitative risk assessment (Burgman, 2005; Hayes et al., 2007a) as this is less affected by linguistic uncertainty, and can explicitly carry uncertainties through chains of calculations and judgments in a transparent manner.

3.7.2. Guidance to identify and treat uncertainty

Applicants seeking to identify and treat uncertainty in their ERA should observe the guidance below, which draws on the synthesis provided by Hayes et al. (2007b):

- ✓ **Clearly define predictive terms** related to the description of risk (e.g. high, medium or low likelihood and consequence);

- ✓ **Identify critical uncertainty** at early stages of the ERA and propose treatment that is scientifically justified and recognizable among stakeholders.

- ✓ **Ensure appropriate endpoint selection** to minimize complexity in data collection. This may be done by establishing a careful balance between reality, complexity and stakeholder concerns. Assessment endpoints should be chosen that are clearly relevant to these concerns, but occur earlier (rather than later) in event chains that link exposure (e.g. release of GM animal) to effect (e.g. decline of a protected native animal population).

- ✓ **Use qualitative modelling** to ensure that conceptual models of environmental systems are valid representations, to test for internal consistency and robustness to complexity and to identify critical interactions within the system (Dambacher et al., 2003a,b).

- ✓ **Avoid predictive bias** caused by limited, subjective expert judgements due to insensitivity to sample size, overconfidence, judgemental bias and anchoring. This might be aided by the use of structured elicitation and aggregation techniques (Burgman, 2001, 2005, Hayes et al., 2004, add new Pub. Anne). Formal prioritization procedures, such as the analytical hierarchy process (Saaty, 2001) can also be helpful when prioritizing hazards or combining the predictions of different stakeholders.

- ✓ **Maintain transparency** in the identification and treatment of sources of uncertainty throughout the ERA.

- ✓ **Test risk estimates** against independent data sets where data permit. Predictions of semi-quantitative risk assessment should be tested against as many known high- and low-risk situations as possible (e.g. instances of harmful and benign non-native fish introductions into the same environment being considered for GM fish). A common approach when designing retrospective risk assessments is to divide a data set into two halves. The first half is used to design the risk assessment, including amending the total risk factor scores to maximize the correct number of predictions and minimize the number of incorrect predictions. The second half is used to test the accuracy of the risk assessment (e.g. Pheloung et al., 1999; Virtue et al., 2001; Copp et al., 2008). This technique is known as cross-validation in the statistical literature [e.g. http://en.wikipedia.org/wiki/Cross-validation_(statistics)].

- ✓ **Capture diversity of expert opinion** by ensuring several experts complete the assessment of specific components of the ERA simultaneously and independently, and through consultation processes. Their parametric scores can be aggregated in a variety of ways (e.g. a simple average or by interval arithmetic) (Aspinall, 2010) to maintain the minimum and maximum scores throughout the risk assessment process. Applicants should consider the degree of consensus among experts on understanding of and interpretation of various biological

processes and data availability (e.g. see Moss, 2011). This captures uncertainty and helps determine the range of plausible risk estimates.

- ✓ **Specify interval rather than point risk scores**. A deterministic risk estimate should be avoided by specifying interval, rather than point estimates for the risk factor scores (EFSA Journal 2011;9(9):2372 [17 pp.]. doi:10.2903/j.efsa.2011.2372) (Hayes et al., 2005). Examples of more complex approaches to uncertainty analysis for risk factor procedures are available in the literature (Hughes and Madden, 2003; Caley et al., 2006). Again, this captures uncertainty and helps determine the range of plausible risk estimates.

- ✓ **Choose mathematical models carefully**. Mathematical representations should generally be chosen well-corroborated (i.e. well-reviewed, widely-used, reliable and well-accepted by management agencies and/or the scientific community). There is a need to balance model realism and relevance against complexity and ease of use. Whenever possible, the model should be calibrated using site- or species-level data that are specific to the risk assessment problem at hand. The model should also be validated by comparing its predictions with independent laboratory and field observations (Devlin et al., 2007, Senanan et al., 2007). The steps in the final choice of model should be discussed and justified explicitly (see section 3.5 on experimental and statistical design).

- ✓ **Investigate alternative model structure effects**. Sensitivity analysis should be used to consider the effect of model structure by alternative risk factors, alternative ways of calculating the final risk score or alternative ways of grouping the final risk scores into high, medium or low risk. In this way, risk estimates, conclusions and risk management plans, can be challenged to test whether they might be substantially altered with alternative, plausible, model structures. This addresses uncertainty due to incertitude, and helps provide confidence in the validity of the chosen models.

- ✓ **Identify and assess model uncertainty and variability**. Investigate the effects of the abovementioned different types of uncertainty on the results of the risk characterization. The effects of these sources of types of uncertainties on the final risk estimate should be reported.

The applicant should in relevant sections of the ERA clearly communicate results and conclusions from the uncertainty analyses, as well as communicate how each type of uncertainty was treated, eliminated at a specific step, or further assessed and carried throughout the ERA.

Overall, the results of the ERA will be subject to varying levels of uncertainty associated with factors such as the availability of data and use of non-GM surrogates to inform the ERA, the range of receiving environments in the EU where the GMPs are likely to be cultivated, the diversity of production and management systems across EU regions as well. As far as it is possible, the overall conclusions of the ERA should (i) specify under which conditions (e.g. receiving environments, management systems) the risks/uncertainties identified are most likely to occur and (ii) clearly identify the factors/processes which might affect the conclusions of the ERA in order to make explicit the robustness of the conclusions of the ERA.

The management of an identified risk will depend on a shared understanding of the uncertainties, the assumptions made, the probabilities and ranges of outcomes, not only a single best risk estimate.

3.7.3. Interplay between ERA conclusions and PMEM

The concept of post-market environmental monitoring (PMEM) is built into EU regulations as an approach to deal with the uncertainties that are inherent in all risk assessments. The risks and uncertainties described in the overall conclusions of the ERA (see section 2.1.6) provide the basis for the PMEM plans proposed by applicants. The plan should address the specific risks and uncertainties identified in the ERA and also the general uncertainties inherent in the nature of the ERA (e.g. effects of spatial and temporal scales).

As discussed earlier, the ERA is often constrained/restricted by the available knowledge and experience of the GM animal and it can be difficult to predict and consider all potential future applications, production systems and receiving environments of the GM animal. Thus large-scale and long-term use of a GM animal could result in some effects which were not predictable at the time of the ERA or consent. Therefore, according to Directive 2001/18/EC (EC, 2001), applicants are required to conduct general surveillance (GS) to detect unanticipated adverse effects on the environment (for further details, see Chapter 5).

If risks and/or significant levels of critical uncertainty linked to the GM animal and its management have been identified in the ERA, then Case Specific Monitoring should be carried out after placing on the market, in order to further inform the ERA (for further details, see Chapter 5).

3.8. Aspects of GM animal health and welfare

One of the aims of the European Union legislation on animal welfare is to ensure that animals are not caused avoidable pain and distress and obliges the owner/keeper of animals to respect minimum welfare requirements. Community legislation concerning the welfare conditions of farm animals lays down minimum standards (EC, 1998).

Since the second half of the 20th century, genetic selection of animals has led to major changes in anatomy and physiology of different animals and this in turn has led to various welfare problems (SCAHAW, 2000; Bessei, 2006). Welfare implications of housing and feeding are also important issues (Decuypere et al., 2006; Renema et al., 2007). It is generally accepted that most of the welfare problems are caused by genetic factors, environmental factors and interactions between them. Recently the EFSA AHAW Panel has published Guidance Documents on risk assessment methodology for animal welfare (EFSA, 2012a) and indicators of poor welfare in various species (EFSA, 2012b, c,d). Genetically modified animals can be seen as an addition tool for animal breeders.

Applicants should pay particular attention to health and welfare of GM animals during the different production stages, transport and release into the environment. For assessing the health and welfare aspects related to GM animal itself, applicants should follow the strategy described in details in the EFSA Guidance document on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects (see Chapter D of EFSA, 2012a). As this Guidance Document also covers GM animals for non-food/feed uses, applicants might be expected to supply data, generated in the same way, showing that the health and welfare of these GM animals are not materially/significantly affected, compared with the appropriate comparators. Where no comparator can be identified, an assessment of health and welfare of the GM animal itself is considered (EFSA, 2012a).

In the case where data need to be generated by applicants through experimental studies with GM and non-GM animals (e.g. for studying environmental effects), such experiments have to be carried out in compliance with the EU legislation currently in place concerning the use of animals for scientific purposes. From 1st of January 2013, Directive 2010/63/EU (EC, 2010) on the protection of animals used for scientific purposes will enter into force in order to strengthen legislation, and improve the welfare of those animals still needed to be used. Applicants should consider and justify the trade-off between the welfare aspects of animals testing and the need and extend (e.g. number of replicates) of such tests for a comprehensive ERA.

3.8.1. Health and welfare aspects for GM mammals and birds

In the case of animals reared for food or feed uses, a comparison with the non-GM line has been proposed (EFSA, 2012a). However, for non-food and -feed animals, a comparator group may not be the best yardstick as the genetic load already carried by the non-GM line itself may be considerable. For example in dogs, it may even be advisable not to breed from some breeds or line e.g. brachycephalic and neotenic. Moreover, there are such variations in a breed due to decades of line/in-breeding (giving strains or lines associated with particular kennels for specified cosmetic traits and

also working traits but to a lesser extent). If the aim of the GM trait is to improve the health and welfare of a kennel line or strain then there might be more than one comparator reflecting: the chosen line to improve; the breed average or median; and the best line in that breed.

When determining the health and welfare of these GM animals for non-food/feed uses, certain aspects known to be present in the non-GM line may need special attention as they may not be easy to diagnose because of delayed onset (e.g. progressive blindness, progressive dysplasia, predisposition to cancerous growths) or needing specific environmental triggers and circumstances, such as for aggressive behaviours. Applicants would have to provide evidence that full consideration has been given to such issues and what criteria have been used select the line to modify.

It is highly likely that an applicant may aim to produce a GM line that improves the welfare of the animals through removing a deleterious trait (e.g. oncogenes), or which promotes health through making a line resistant to a disease or less susceptible to a disease or infection (see sections 4.1.4 and 4.3.3). In which case, that claim would have to be evidence-based.

Some genetic modifications have the objective of increasing the growth rate of animals so that the animals may have increased demands for nutrients and water at certain time intervals compared to non-GM animals, and they may also be larger requiring further feed and possibly husbandry modification. Increased growth thus requires changes in management compared with the non-GM animal and failure to implement appropriate nutritional and spatial management for these animals can result in increased stress for the animals. Overcrowding and nutritional stressors can increase the likelihood of damage to the animal through physical contact and disease. Applicants should therefore consider the specific management requirements of GM animals in order to optimise its health and welfare.

3.8.2. Health and welfare aspects for GM fish

The health and welfare aspects are also relevant for GM fish released into the environment, ranging from e.g. confined fish farm to a confined or free aquatic environment (e.g. stream, river, ocean). The same principles as laid down in the EFSA Guidance document on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects (see Chapter D of EFSA, 2012a) also apply for the assessment of health and welfare of GM fish released into the environment during different developmental stages, different production stages, and for different receiving environments.

The health and welfare assessment of a GM fish also relies on the comparative approach considering the appropriate comparators. In some cases, no appropriate comparator is available. Hence, for example, a clinical microbiological parameter obtained from a GM cold-resistant fish cannot easily be compared with the same parameter obtained from a non-GM fish under normal physiological temperatures, or a non-GM fish inhabiting the same water system at an abnormally cold temperature. In such cases, according to the aforementioned EFSA Guidance Document (EFSA, 2012a), an assessment of health and welfare of the GM animal itself is considered. Applicants should strive to assess the health and welfare aspects of these GM fish after their release into the environment: considering clinical signs (behaviour or physical changes, e.g. sudden death, overproduction of gill and/or skin mucus); measuring water for its physical and chemical parameters (e.g. ammonia, nitrite, nitrate, oxygen, pH, water temperature, salinity); and bacteriological indices. These should be carried out during regular monitoring and inspection and used as surrogate health and welfare indicators.

3.8.3. Health and welfare aspects for GM insects

So far, the European legislation related to health and welfare aspects of animals mostly focuses on farmed animals and, only in exceptional cases, on wild animals. Therefore, the EFSA GMO Panel considers that no additional welfare risk assessment is needed for GM insects, except for honey bees (EFSA, 2012a).

4. Specific areas of risk to be addressed in the ERA

An overview of the structure of the specific areas of risk addressed in this Guidance Document is presented in Figure 5.

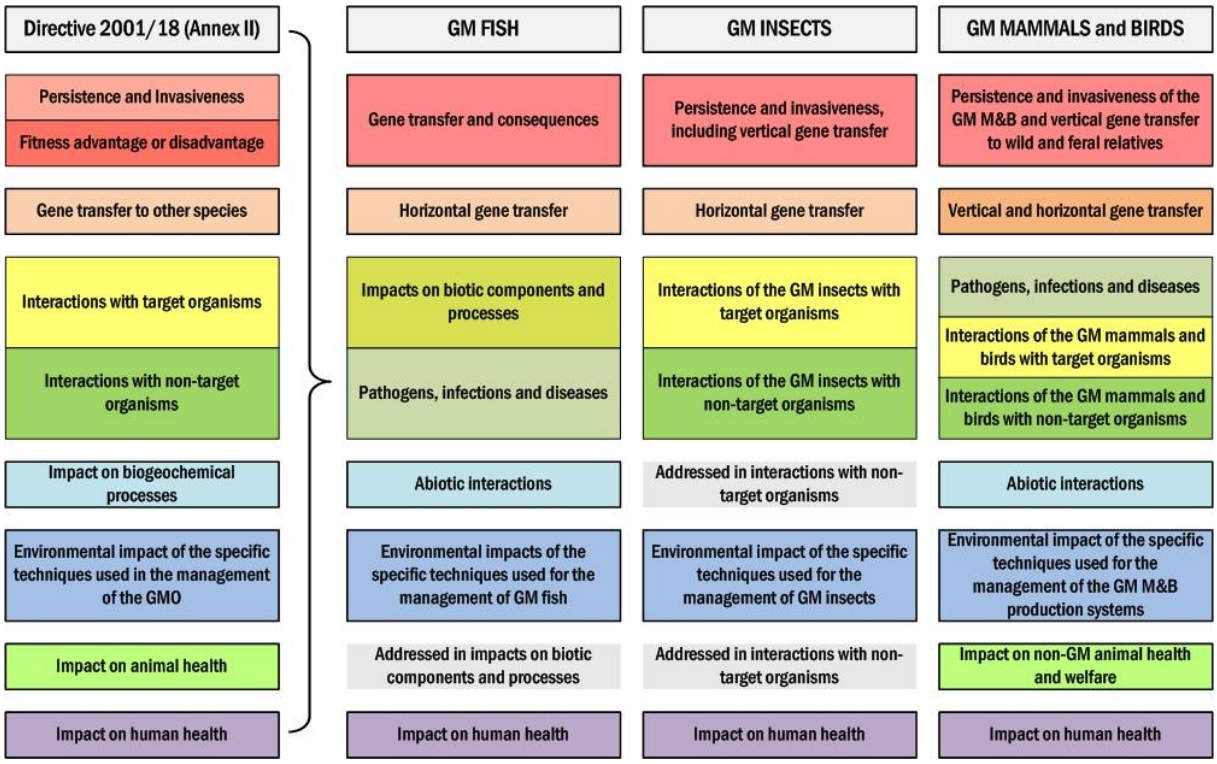


Figure 5: Relationship between the different areas of risk for GM fish, GM insects and GM mammals & birds, in comparison also with the points listed in Annex II, section D1 of Directive 2001/18/EC. The link between the different headings of the Directive and the respective areas of risk in the three groups of GM animals is identified by colours and blocks.

Although this Guidance Document follows the structure and ERA principles set by Directive 2001/18/EC (EC, 2001), the terminology used for specific areas of risk (see Chapter 4) was, when deemed necessary, slightly adapted to take into account the specificities of the ERA of the different groups of GM animals (GM fish, insects, mammals & birds) and the potential traits covered by this Guidance Document. For example, in the ERA of GM fish, the section 4.1.3 on biotic interactions includes the assessment of the interactions of the GM fish with target and non-target organisms as in Directive 2001/18/EC (EC, 2001). The target organisms are those which the GM animal is specifically designed to act on and manage their population as indicated by applicants (e.g. parasites, pathogens or other species which are displaced or consumed by the GM animal). All other organisms that might interact with and be affected by the GM animal would be considered as non-target organisms. Notwithstanding the flexibility in terminology, this Guidance Document covers all areas of risk as described in Annex II, section D1 of Directive 2001/18/EC.

The following sections 4.1, 4.2 and 4.3 should be read in conjunction with the cross-cutting sections in chapter 3, above. In particular, wherever the singular term ‘comparator’ is used in these sections, note that it refers also to the plural case where more than one comparator is appropriate and used for the ERA.

4.1. Specific areas of risk for the ERA of GM fish

Taxonomically, the fish considered in this Guidance Document belong to the vertebrates (see Chapter 1). The most primitive fish species, like hagfish and lampreys are classified as jawless vertebrates (A*gnatha*). The majority of fish species, however, belong to jawed vertebrates (*Gnathostomata*),

which again are divided into cartilaginous fish (sharks, rays, skates) and bony fish. Classified under bony fish are the teleosts, which comprise the majority of the about thirty thousand different fish species that so far have been described.[11] Almost all the fish species that are currently farmed belong to the teleosts, for which extensive knowledge exists.

This Guidance Document addresses the commercial and any associated unintended or accidental release of GM fish into the environment (see Chapter 1). This Guidance Document covers GM fish to be released into the environment and placed on the market for production or non-food/feed uses (like GM 'ornamental' fish). Deliberate release includes fish bred and reared within controlled aquaculture and aquaria facilities and considers their environmental impacts, both within the confinement facilities and if they are released or escape from these facilities.

4.1.1. Gene transfer and consequences

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

In this section, applicants shall address the consequences of escape, establishment, gene transfer and changes in the fitness of the GM fish and any recipients of the recombinant DNA. This may result in changes in persistence, competitiveness and invasiveness of the GM fish itself, and/or of its wild relatives outside production systems, and lead to environmental harm. The transfer of the recombinant DNA from the GM fish into wild species is not an environmental risk in itself; the risk is associated with the phenotypic and biotic effects arising from the gene transfer, and how these effects may influence the survival and reproductive capability of the GM fish and the resources it uses and provides to the ecosystem. The biotic interactions are considered in section 4.1.3 and abiotic interactions in section 4.1.5 while this section focuses on the genetic and population effects of the GM fish and any recipients of the recombinant DNA.

The potential consequences of gene transfer are of two main types:

1) Enhanced fitness of the GM fish or introgressed wild relatives may create feral GM populations, or hybrid populations in different habitats, which may change the diversity/abundance of flora and fauna. For instance, native fish species may be displaced by GM fish, which in turn might affect food chains and have consequences for other species in the food chain.

2) Decreased fitness of hybrid offspring and wild relatives, causing decline or local extinction of wild fish populations.

A staged approach describing how the presence of an recombinant DNA may change fish biology in and outside production systems, and lead to environmental harm in the wider environment is proposed as outlined in Figure 6. The purpose of the staged approach is to ensure that detailed case-specific information on assessment endpoints is supplied to test relevant hypotheses formulated in the problem formulation process, and that information requirements remain proportionate to the potential risk. The questions in Figure 6 outline the issues to be addressed to estimate the likelihood of occurrence of the two types of effect. The questions are divided into different stages. Depending upon the GM trait(s), fish species, the intended use and receiving environments under consideration, information may not be required at all stages.

In considering the questions in Figure 6, the mechanisms and routes by which GM fish and the recombinant DNA are exposed to the environment should be considered. The principle route will be through the escape or release, and the consequent dispersal into the wider environment. For GM fish applications with the scope of import and processing for food and feed uses only of dead wet GM fish, the ERA is concerned mainly with the accidental release of viable GM eggs or the spread of fish diseases during import, transportation, storage, handling and processing, and the environmental consequences thereof. Therefore, the ERA needs to consider the scale of environmental exposure, and

[11] To find out if a specific species is considered a fish, consult www.fishbase.org

if this could ultimately lead to GM fish becoming established in the environment or the development of novel disease epidemics in fish populations.

Initially basic information is required that enables characterising the GM fish and identifying biological differences between it and conventional counterpart. Information provided should be used to establish whether: (i) the GM fish will escape and survive outside fish farms, or be released and therefore have the potential to contribute to feral populations; (ii) the fish can produce fertile offspring; (iii) the GM fish can hybridise with wild types of the same species that may occur in the receiving environments and produce viable and fertile offspring or (iv) the GM fish can hybridise with other species of fish and produce fertile interspecific hybrids.

Once the extent and nature of the environmental exposure is determined then information is required to establish if recombinant DNA will change the biology of the GM fish or of hybrids, backcrossed and interspecific hybrids, in these receiving environments. If GM fish or hybrids exhibit changes in their phenology or environmental interactions in comparison with their conspecifics, then an assessment should be made of whether they will invade a wider range of environments to occupy larger ecological niches than their conventional counterparts. It is anticipated that certain GM traits (e.g. cold tolerance, salt tolerance) may enable a GM fish to expand its geographical range, and to survive in new areas possibly close to wild relatives from which it was previously isolated. In addition if the food preferences, food consumption or diet of the GM fish changes then this will have consequences for the food chains in receiving environments. If food chains are disrupted then this may remove food of other species that normally coexist with the wild type or these species may become prey. However it should be noted that fish behaviour, performance and fitness is itself also influenced by a range of ecological factors and factors such as competition are likely to modulate the rate at which fish populations recover from predation, competition or disease epidemics, and so possession of fitness or resistance genes may be more valuable when competition is high. Conversely some GM traits may result in changes in behaviour that decrease ability to survive in the wild, where competition for food and space may be different from that in fish farms. For example some GM fish may change their foraging behaviour and thus change their exposure to predators.

Finally, if enhanced fitness or the ability to occupy new niches are demonstrated, information is needed to establish whether this will allow populations to increase and invade new communities or, alternatively if this will lead to populations of wild relatives being displaced and becoming locally extinct, and to assess the potential environmental consequences of this.

Information required for testing the hypotheses formulated in the problem formulation process can be extracted from data generated by applicants and/or from the scientific literature. Some fish with the same traits or similar transformation events may have been grown for a number of years at a large scale such that field-generated data on persistence, competitiveness and invasiveness are available in and/or outside the EU. If applicants use data from outside the EU, they should justify why these data are relevant for the range of potential receiving environments in the EU.

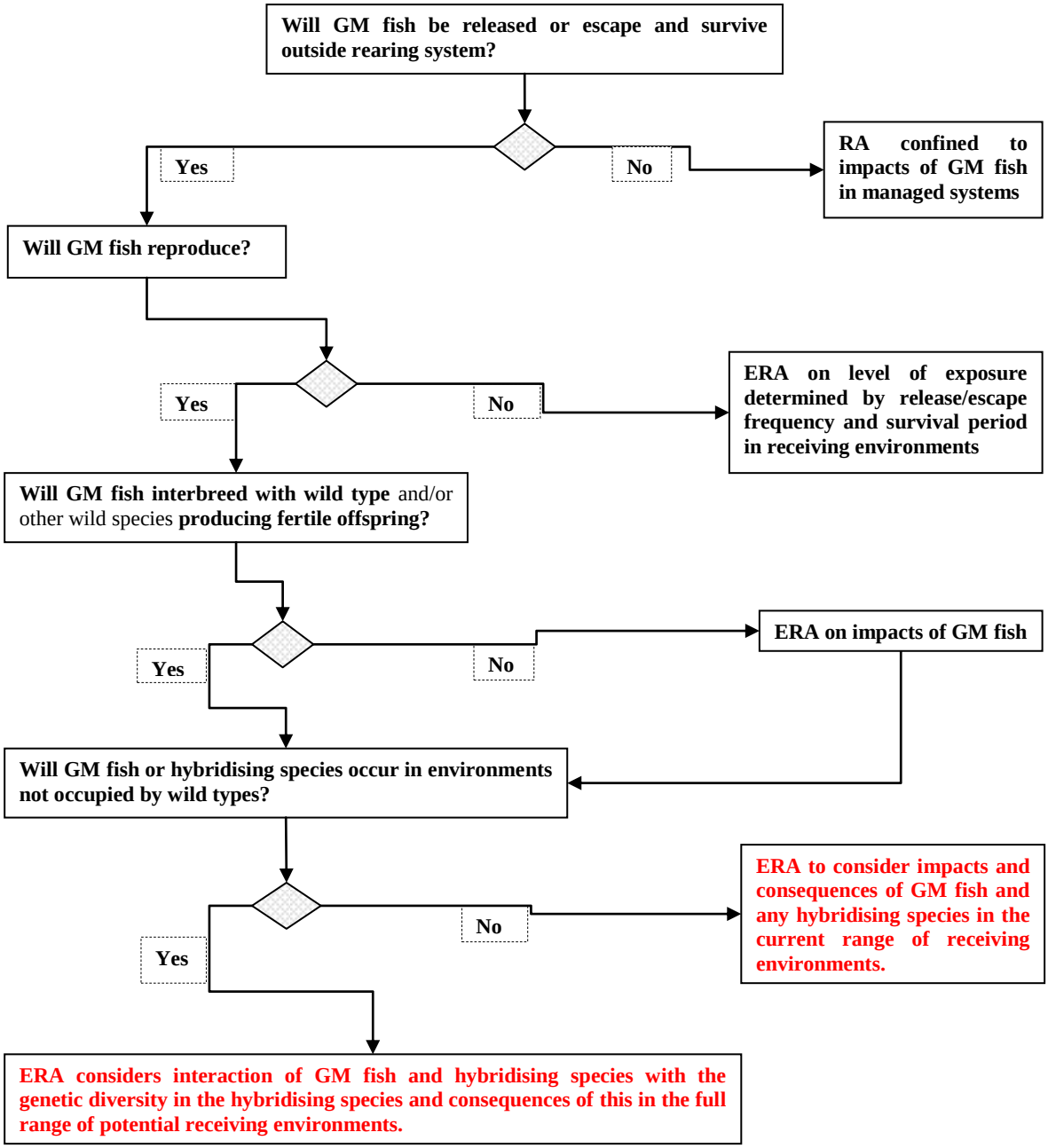


Figure 6: Problem Formulation questions on the extent of gene introgression and environmental exposure which determine the extent of the scope of the ERA required for GM fish.

Step 2: Hazard characterisation

Step 2 of the ERA consists of characterising any hazards, identified during the problem formulation process, which might lead to adverse effects, as a consequence of altered survival and reproductive success, in GM fish at the production site and in GM fish hybridising wild species in the wider environment. In GM fish carrying more than a single event (e.g. stacked GM fish events), applicants 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 traits.

Applicants should provide information on fitness and gene transfer differences between GM fish and appropriate comparators. The fitness of the GM fish and any hybrids arising from gene transfer should be assessed in the different receiving environments of the GM fish and the hybrids.

A major concern relating to the spread of transgenes into wild populations is the issue of 'Trojan' genes (Muir and Howard, 1999). This is where transgenes have the opposite effects on fitness components. Muir and Howard (1999, 2001) found that antagonistic pleiotropic effects of transgenes on different net fitness components resulted in unexpected harm, such as the local extinction of GM-invaded conspecific populations (eliminating both native and GM individuals). Thus, a Trojan gene may be defined as any gene that drives a population extinct as it spreads because of destructive self-reinforcing cycles of natural selection. For example, if a transgene enhances mating success of GM males while reducing the viability of their young, the mating advantage of the GM males quickly spreads the transgene into an invaded population but the low viability of their offspring (both male and female) results in a population decline. Thus, the downward spiralling of the population continues until both wild type and GM genotypes become locally extinct (Muir and Howard, 2002; Hedrick, 2001). This may then also provoke additional harm on the ecosystem through a possible cascading, negative effect on the biological community.

Information on the following characteristics which influence reproduction and survival should be assessed in relation to an appropriate non-GM comparator (see section 3.4).

a) ***Reproductive biology*.** The differences in reproductive biology of the GM fish including its breeding behaviour, fertility, fecundity and development to sexual maturity should be assessed. Since events can move into different genetic backgrounds this study should consider changes in other relevant fish which are recipients of the event, compared with their wild types.

b) ***Growth and morphology characteristics.*** Aquarium experiments and information collected during trials in semi artificial environments are required to determine whether these characteristics of the GM fish have changed and the extent of these changes in the different life stages.

c) ***Other phenotypic changes which affect interactions with receiving environments (e.g. tolerance of abiotic and biotic conditions):***

- *Changes in tolerance of GM fish to abiotic conditions*

  Applicants should determine whether the GM fish and any hybridising relatives show changes in tolerance to a range of abiotic factors such as temperature, pH, oxygen, turbidity, salinity, etc. The net fitness of the GM fish compared to non-GM comparators should be assessed under these different stress conditions. The ability to survive and reproduce in different receiving environments, due to these adaptive changes should be considered.

- *Changes in disease and parasite resistance/tolerance*

  If a wild type fish suffers reductions in fecundity through the action of pathogens, then introgression of fitness or resistance GM traits could result in enhanced reproduction and fitness. Applicants should assess changes in levels of disease and parasite resistance and/or tolerance and how these might affect the survival and reproduction of GM fish and hybridised species in different environments. Applicants should consider the interaction between resistance, fitness and competition in order to interpret the biological significance of their results.

  In addition applicants should assess whether any increased resistance/tolerance is likely to affect the epidemiology and distribution of the disease or parasite in non-GM fish and other species. Section 4.1.4 describes in more detail the approaches to disease resistance that should be considered by applicants.

d) ***Behavioural changes***. Changes in food consumption, diet, foraging behaviour, territorial behaviour, aggressiveness, mobility (including dispersal and migration), shoaling, predator parasite and disease avoidance etc, should be assessed.

Step 3: Exposure characterisation

The environmental exposure should be related to the intended uses of the GM fish and the potential of the GM fish to move and/or escape into other environments. Environmental exposure should be related to the whole production and life cycle of the GM fish and potential recipients of the recombinant DNA, considering the habitats of different stages and migration routes and interactions between the GM fish and compatible wild types in these different environments. In addition any mitigation measures to reduce gene transfer (e.g. reduced fertility) and environmental exposure (e.g. confinement strategies) should be considered (see step 5).

Gene transfer: The rate of reproduction depends on a range of sexual characteristics that may be altered in GM fish. Muir and Howard (1999) and Howard et al. (2004) identified that age at sexual maturity has the greatest influence on the frequency of gene transfer through a population of wild relatives, followed by juvenile viability, mating advantage, female fecundity and male fertility in the fish species they studied. Using their hypothesis that the likelihood of spread is a function of reproductive and life-history traits of the GM fish, applicants should assess the extent to which the phenotypic and biological changes identified in the hazard characterisation will affect the ability and frequency of GM fish to reproduce and hybridise with wild conspecifics and other related fish. This will in turn indicate the rate and the extent of the spread of the recombinant DNA into the wild gene pool and the range of environments likely to be exposed to the GM fish and hybridised species.

Receiving environments: Changes in the biotic and abiotic stress tolerance and other phenotypic and biological characters identified in the hazard characterisation will indicate the potential geographical range of the GM fish and the hybridised species. Applicants should describe the range of environments occupied by different life stages of these GM fish, particularly noting any changes in range (see section 3.1).

For GM fish with changed disease or parasite resistance, applicants should also describe the current geographic range of the pathogens. Where the GM fish are likely to change their geographic range, applicants should assess whether this will alter the distribution range of the pathogens (see section 4.1.4).

Step 4: Risk characterisation

Applicants should first assess whether the gene(s) inserted modify the potential for gene transfer due to altered male or female fertility and capacity to produce viable spawn. In addition applicants should assess whether the intra and inter-specific fertility changes so that the GM fish has a different potential to hybridise with the same or other species. Applicants should assess whether any changes in the behaviour of the GM fish influence success and competition in the breeding grounds. For example changes in fitness or competitiveness may mean that they can displace wild fish from spawning grounds in order to lay eggs or fertilise them. In addition they may be more cannibalistic, consuming spawn or fry.

Applicants should also assess whether reproduction between GM fish and wild conspecifics can change local genetic diversity and adaptation. There may be differences in how the modified gene is expressed between the hatchery and the wild environment, and hemizygous GM fish can produce non-GM as well as GM offspring. Thus where rates of gene transfer are high, applicants should assess whether this may cause wild relatives to decline locally or to become extinct (e.g. swarm effect, outbreeding depression, Trojan gene effect).

The consequences of the changes in reproductive potential and ability to transfer genes to wild relatives should be assessed in terms of population changes and impacts on these populations in the relevant receiving environments.

Applicants should assess whether the changes in survival, reproduction and gene transfer in the GM fish and hybridised relatives will result in population changes for the GM fish or conspecifics in the range of receiving environments. As well as considering the gene transfer consequences applications should consider whether there is enhanced competition for resources with wild conspecifics and thus the potential for suppression of the life-history traits of non-GM fish. This may lead to a cascade of consequences that culminate in temporal shifts in fish community structure and the ecology of the wild populations.

Where increased tolerance of abiotic and biotic stresses (including diseases) is identified, the consequences of any increased tolerance should be assessed for the relevant receiving environments of the GM fish and hybridised relatives. Applicants should assess whether GM fish or hybridised species will develop invasive populations, invade new habitats, and/or expand the range of wild fish and so result in competitive interactions with fishes with no previous experience of encountering the species. Where population changes are likely to occur, the ecological effects and consequences will need to be assessed as described in section 4.1.3.

Where increased disease resistance has been identified the impacts on the distribution of pathogens and the presence of native infectious agents should also be assessed for all potential receiving environments (see also section 3.1). The consequences of these pathogen population changes for native, reared and GM fish populations should be assessed and factors such as facilitating non-native species invasions (e.g. Ondračková et al., 2005) should be considered.

Step 5: Risk management strategies

If the ERA identifies risks related to reproduction, survival and invasion, strategies to manage these risks may be required and should be defined by applicants. These strategies might focus on reducing recombinant DNA movement by improved confinement, lowering sexual fertility, or be directed at controlling the progeny of GM fish resulting from gene transfer. If measures for controlling feral or wild relatives are proposed, the associated impacts should be considered by reference to section 4.1.6. 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: Overall risk evaluation and conclusions

The risk assessment should conclude on: i) the extent to which the recombinant DNA can move from the GM fish into other hybridising fish in both production systems and the wild; ii) the extent to which the GM fish and hybridised species are more successful in the relevant receiving environments; iii) whether any changes in fitness of the GM fish result in changes in population size of non-GM fish in the receiving environments; iv) the extent to which changes in population size are likely to result in other environmental impacts, including the consequences for food chains and other biota in the different receiving environments; v) risk management measures required to mitigate any identified environmental harm. This information should be taken forward where the full biotic and abiotic interactions and consequences of the changes in populations and biology of the GM fish can be considered.

Uncertainties associated with the ERA conclusions of this section should be identified and assessed (see section 3.7), 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 risks and uncertainties described in the overall conclusions of the ERA (see section 2.1.6) provide the basis for the PMEM plan to be proposed by applicants (see Chapter 5).

4.1.2. Horizontal gene transfer

Horizontal gene transfer (HGT) is here defined as any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. The evaluation of the impact of HGT from GM fish includes analysis of the potential of transfer of recombinant DNA 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 must be evaluated. Although the extent of environmental exposure is likely to differ between applications for environmental release and import for processing, the issues to be considered in the ERA are expected to be similar.

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

HGT from GM fish is expected to be rare. However it remains largely unexplored. Rare events may have consequences for human and animal health and the environment and are therefore considered in the ERA. This ERA will depend on the exposure routes, the potential for horizontal transfer, the trait conferred by the recombinant DNA, the prevalence of similar traits in exposed environments and the nature and range of potential consequences (EFSA, 2009b). The problem formulation needs to consider assessment endpoints being representative of the aspects/parts of the environment(s) that need to be protected from adverse effects.

HGT processes between higher eukaryotes are only infrequently observed and over long timescales and usually involve mobile genetic elements. Heritable HGT between multicellular eukaryotes would be limited by the need for transformation of germline cells. As such HGT events are considered to be rare, the initial problem formulation should focus on characteristics of the recombinant DNA that can lead to changed mobility (e.g. presence of mobile genetic elements). If changes in the potential for mobility of the recombinant DNA have been identified, a further detailed ERA is necessary. The problem formulation focusing on the potential for horizontal transfer of a recombinant DNA with a potential for altered mobility should consider: (1) the presence of plausible mechanism that could facilitate transfer, uptake and integration of the recombinant DNA fraction of fish DNA in new hosts, at biologically relevant frequencies; and (2) the potential of horizontal transfer relying on the understanding of the factors defining and limiting the current species distribution of the mobile genetic elements used, as well as of the mechanistic aspects of the replication/transposition of mobile elements in their current hosts (including absence or presence of factors in the GM fish that might influence the mobility of the recombinant DNA).

In contrast to the low proportion of germline cells in multicellular organisms that can act as recipients of heritable HGT events, all single celled organisms can, in principle, act as recipient cells of heritable HGT events (Keeling, 2009; Dunning Hotopp, 2011; Richards et al., 2011). However, of the known mechanisms of HGT in single-celled organisms, only natural transformation is known to facilitate uptake and genomic integration of DNA fragments.

Microorganisms, especially bacteria, are capable of acquiring genetic material both from related and unrelated species (Anderson and Seifert, 2011). The probability and frequency of horizontal transfer of fish DNA (including the recombinant DNA fraction) to exposed microorganisms is determined by the following factors: (1) the amount and quality of fish 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 fish DNA can be incorporated and thus stabilized in the microbial genome (including chromosomes or plasmids).

In bacteria, natural transformation with linear DNA fragments usually requires nucleotide sequence similarity to facilitate stable integration by homologous recombination. For this reason, it is considered that the presence of sequences with high similarity to bacterial DNA in the fish DNA would increase the probability of HGT (Bensasson et al., 2004; EFSA, 2009b). Due to the homology-based recombination mechanisms active in bacteria, the likelihood of HGT from GM fish DNA into

microorganisms should therefore be considered also in the absence of mobile genetic elements in the recombinant DNA. Differences in transcription regulation and the presence of introns and requirements for intron splicing represent a functional constraint to efficient expression of many eukaryotic genes in bacteria. The presence of intron-free coding sequences in GM fish genome with high similarity to microbial DNA would increase the probability of transfer and expression after transfer (EFSA, 2009b).

The range of microbial species identified as potential recipients for unintended HGT events will depend on the ability of the micro-organisms to develop competence and on the characteristics of the insert and to what extent homology-based recombination can be expected. Positive selection is usually considered a necessity for rare HGT events occurring into large microbial populations to be biological meaningful.

The presence and impact of inserted sequences that could affect the mobility of recombinant DNA should be assessed. Horizontal transfer of DNA from fish can be facilitated by the presence of mobile genetic elements in the inserted DNA or by the uptake of cell-free DNA. Therefore the problem formulation should focus on:

- A detailed molecular characterisation of the DNA sequences inserted in the GM fish to inform the assessment on the potential for horizontal mobility, stabilization, and expression of the inserted DNA.
    - The presence and source of mobile elements or recombinant DNA sequences showing similarities with DNA sequences from relevant recipients enhancing the probability of homology-based recombination and subsequent stabilisation; these characteristics will determine the host range of potential recipients.
    - Information on the functionality of the regulatory sequences of the recombinant DNA if horizontally transferred and on the presence of introns and requirements for intron splicing of the recombinant DNA.
- The release, stability and degradation routes of GM fish DNA, and the presence of relevant recipient organisms that could potentially acquire such DNA in the receiving environments.
- The presence of other sources of DNA that is similar to the recombinant DNA construct (with equal or higher recombination potential), in the exposed environments.
- The identification of environmental conditions in the receiving environments that could drive directional selection and long-term establishment of HGT events. Positive selection is usually considered necessary for rare HGT events to represent biological meaningful scenarios in the risk assessment.
- The identification of consequences of identified HGT scenarios from GM fish, should they occur.
- Assessment and measurement endpoints should address established protection goals.

In cases where the introduced genetic modification does not lead to changes in the horizontal mobility of the recombinant DNA at a higher probability than is likely for any other chromosomal fish DNA (non-mobile), applicants are expected to provide a short statement that substantiates this.

Step 2: Hazard characterization

If a hazard has been identified in step 1 of the ERA, the hazard should be further characterised. Hazard characterisation 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 taken into account.

Step 3: Exposure characterisation

If a hazard has been identified, the exposure characterisation should consider the characteristics of the insert(s), the copy number of the recombinant DNA, the levels and routes of exposure related to the hazard and the scope of the application. For instance, recombinant DNA containing cells will be released from shed epithelial cells inside the gut of fish and be present in faeces.

Applicants should take into account the methodological constraints to the quantification of DNA exposure levels in complex environments. In most cases, a numeric threshold level for a HGT event to be significant cannot be established. Other methodological limitations that warrant explicit considerations include the representativeness of the sampling strategy, the detection limit, and the tempo-spatial relationship between exposure levels and an observed impact of rare HGT events (EFSA, 2009b). Quantitative modelling approaches should be considered in cases where concerns over exposure levels have been identified. Modelling approaches may also be useful when representative data for environmental parameters cannot be obtained, for instance to address natural variability in exposure (see section 3.7).

Applicants are requested to provide an exposure characterisation, of the hazards characterised under step 2, considering the various routes and sources of exposure in the receiving environments (Rizzi et al., 2011):

- GM fish production systems. E.g. DNA from GM fish will be exposed to the microbiota of the fish itself during its lifespan (including the gastrointestinal system) and exposed to other organisms in the environment (e.g. faeces).
- GM fish harvesting and processing systems. E.g. GM fish material will be exposed to a number of environments during processing and storage; including processing of by-products.
- GM fish in the food chain.12 E.g. GM fish products will be exposed to the microbiota of the gastrointestinal tract of the consumer; and depending on the storage and type and level of processing; e.g. sushi. DNA may be a part of the consumed product. GM fish by-products may also be utilized as a feed source.
- Alternative sources of the relevant gene(s) should be identified and considered in the exposure assessment.

Step 4: Risk characterisation

Applicants should focus the risk characterisation on the identified hazards and its impacts that may potentially occur in the various receiving environments (as outlined above in steps one to three). Any identified risk should be characterised 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); taking into account the characteristics of the recipient species.

Step 5: Risk management strategies

Based on the outcome of the risk characterisation, applicants may need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions allowing DNA exposure or positive selection.

12 It is documented that DNA present in food and feed becomes substantially degraded during most processing and through digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA to the microbiota is expected. Several bacterial species with the potential to develop competence for natural transformation (take up and recombine with extracellular DNA) belong to the common gut microbial community (See EFSA, 2009 and Rizzi et al., 2011).

Step 6: Overall risk evaluation and conclusions

Identified knowledge gaps should be briefly summarized and a clear statement on the absence/presence of selective conditions should be provided. Applicants are required to conclude on the overall risk, i.e. a clear statement on the potential for HGT to occur and its consequences, taking into account the efficacy of any proposed risk management strategies.

4.1.3. Impacts on biotic components and processes

From an ecological point of view, a main issue with GM fish is to determine whether they have different biotic interactions when they have been released or escaped into the environment, compared with appropriate comparator (see section 3.3). Biotic interactions include those defined as target and non-target effects in EC2001/18. Target organisms are those which the GM fish is specifically designed to interact with and manage their population as indicated by applicants. These could include parasites, pathogens or organisms which are intended to be displaced or consumed by the GM fish (e.g. control of specific aquatic weeds). Pathogen interactions are dealt with specifically in section 4.1.4. All other organisms that might interact with and be affected by the GM fish would be considered as non-target organisms. These biotic interactions can be divided into direct and indirect effects.

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

Direct effects are those effects that the fish itself generates, e.g. through various means, such as predation, competition, habitat alteration, inter- and intraspecific hybridization, and introduction of new parasites and diseases that influence behaviour and/or survival of the wild biota. In some cases of GM fish, it may also be necessary to examine whether the GM fish actively or passively secretes substances or if such can be released to the environment upon death of the GM fish or as metabolites should the GM fish be consumed by a predator. Direct effects can have consequences that are considered harmful, such as a reduction in the population of species used for human consumption, or species that have conservational or functional roles in ecosystems, e.g. by maintaining water quality.

Direct effects can lead to secondary effects in which the GM fish is not in contact with the individual being affected. Such indirect effect can give rise to trophic cascades where an initially small direct effect by the GM fish can lead to larger ecosystem effects by shifting the balance in the system. These effects typically occur through a limited number of species, so called focal species,[13] and it is therefore especially important to identify such species in the receiving environments and to assess to what extent escaped GM fish affect such focal species. By their nature, indirect effects are more difficult to study and document than direct effects. The time perspective is also longer as direct effects first need to be transferred to the secondary recipient. Examination of indirect effects therefore requires careful planning of experiments and sufficient time to allow transfer of direct effects to secondary effects. Necessarily, experimental conditions must be complex enough for effects to mimic those that may exist in nature.

The examination of the biotic effects of the GM fish is necessarily done in perspective of the environment to which they may escape. If GM fish escape to an environment where wild conspecifics are present, the assessment of effects needs to be relative to the wild conspecifics, i.e. how does the biological interaction of a GM individual differ from its wild conspecific? If the number of GM fish escaping is large compared to the wild population, the increase in population of the species may also have to be considered even if the effect of the genetic modification is not great. If there are no wild conspecifics in the receiving environments, the impact of the GM fish will need to be assessed against the range of biota present in that environment. These aspects of environmental exposure and population effects are also considered in section 3.1.

[13] Focal species are those that have a disproportionate impact on their environment in relation to their biomass in an environment.

Step 2: Hazard characterisation

Applicants should examine whether the GM fish has changed foraging behaviour (for example the amount and nature of food), but also the effects on available food and prey in the system exposed to the GM fish, taking into consideration that GM fish may feed on food and prey types which wild types do not feed on. It is also possible that a GM fish would compete with other ecosystem members, either by competing for similar foods, diet space, breeding area or by consuming them. However, identifying whether increased competition for food is occurring in a natural system is often a difficult task and competition or predation may only occur when a shared diet is limited in supply or when alternatives are not available. Hence it is important to understand the factors limiting food availability and the factors controlling relevant species in receiving environments.

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 utilize the same range of resources (e.g. temperature, food particle size, spawning area). However, quantitative measures of resource use do not provide specific information about the mechanisms or effects of competitive interactions. Therefore, when possibilities to study the target ecosystem in situ are limited, competition experiments in the laboratory under semi-natural conditions or in the wild using surrogate models should be considered (see section 3.4).

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 can include physical competition for some habitat requirements (e.g.: shelter, refuge, breeding sites, warm water, still water) and territorial behaviour, with the same or other species; which may change, leading to increased stress to potentially affected species and ultimately their decline. Applicants should take into consideration that the GM fish will also add additional numbers to an environment, as well as novel GM trait(s). A good way of testing for these effects under confined conditions is through experiments in mesocosms. Only by comparing aquatic systems possessing either GM fish or similar numbers of conspecifics, can one infer that the GM fish is associated with changes in key ecological indicators.

The changes in behaviour, competition, dominance, feeding behaviour and predation will lead to food chain effects that will in turn have ecological consequences. Certain resources could be depleted, thus depriving these resources (e.g. food, shelter) for other biota and hence driving down their populations. Conversely changes in resource use could increase the supply of a resource allowing certain biota to flourish.

Symbiotic associations also occur within and between species. Examples are shoaling for both feeding and predator avoidance, and cleaning or pilot fish which remove parasites and/or provide food. Beneficial and commensal associations also occur with microbia (e.g. gut flora). Applicants should determine whether these associations are likely to be affected by changes in fish characteristics.

These types of chain effects are sometimes difficult to predict and assess, and applicants should consider using models and scenario testing to determine possible environmental consequences (see section 3.5).

An assessment is also required of whether the production of the GM fish and/or its by-products presents a new hazard for the health of other animals. Such assessment is particularly important for GM fish which are not destined for human or animal consumption (e.g. ornamental fish in aquarium) and where impacts on animal health may not have been so meticulously studied. Therefore applicants should estimate, on a case-by-case basis, all possible hazards (that have not been specifically addressed in other sections of Chapter 4) which may cause adverse effects on the health of other animals.

Step 3: Exposure characterisation

In section 4.1.1, applicants will have assessed any changes in the ability or propensity of GM fish to exploit various means of dispersal, establish in a range of potential receiving environments (see section 3.1) and become adapted to new environments. In addition, the assessments in section 4.1.1 will also indicate the extent that the recombinant DNA will introgress into conspecifics and other species. However it is also important not to assume that a physiological capacity to migrate will necessarily lead to a behavioural decision to actually migrate. Dispersal can also be through involuntary transportation by birds or animals, capture fishing or other human activities that facilitate dispersal (e.g. through water ballast, by purposeful introduction of pet species or as escaped food species). Thus full consideration of the management, transport and handling of the GM fish needs to be considered (see also section 4.1.6).

However, in determining the full geographic spread of the recombinant DNA, the GM fish and its influences, applicants should also consider the nature of the different receiving environments and determine whether these environments will actually sustain and support the GM fish. For example, GM fish may be able to survive for only limited time but they may have effects 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 therefore frequency of invasions and the numbers of fish invading may need to be taken into consideration. Changes in the ecology of the environments invaded by GM fish may expose them to novel biotic conditions. These can include by-products of metabolism (e.g. carbon dioxide, ammonia), faecal waste and products from decaying plants and animals. In some cases, micro-organisms (primarily algae) 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 (see section 4.1.1), but it also becomes important to examine 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 also consider testing different dispersal and migration scenarios in order to assess the full geographic range of the GM fish and hybridising species, and the niches they are likely to influence.

Step4: 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. Applicants should consider using the methods and approaches described by Devlin et al. (2006), Kapuscinski et al. (2007a) and in section 3.2.

Applicants should consider whether focal species and/or key ecological functions within ecosystems are being affected, the reversibility of these effects and the level of harm associated with them. Because of the complex nature of ecological interactions, applicants should clearly identify assumptions made in their ERA and any levels of uncertainty associated with conclusions on risks.

Step 5: Risk management strategies

Applicants should propose methods to reverse or reduce adverse impacts on biota and key ecological functions identified in the risk assessment. The practicality and efficacy of the methods should be evaluated and methods for their implementation described. Uncertainties associated with the efficiency or implementation of mitigation measures should be described and considered in relation to PMEM plans (see Chapter 5).

Step 6: Overall risk evaluation and conclusions

Applicants should conclude on the overall risks arising from the conclusions of both 4.1.1 and this section and considering the proposed risk management measures. 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. Applicants should describe identified risks or critical uncertainties that require further information from post market monitoring studies. In addition applicants should explain why identified environmental impacts are considered acceptable and do not present risks.

4.1.4. Pathogens, infections and diseases

Normally, fish live in an environment together with virus, bacteria, fungi, protozoa, helminthes, nematodes, copepods and other lower organisms. Some of these organisms may be harmless or even beneficial to their hosts (mutualism or commensalism). Examples are microorganisms of the commensal flora that help the digestive system to acquire necessary nutrients and microbes that protect fish by occupying niches on the fish and thereby keep the infectious agents away. Stimulation by the commensal flora seems to be crucial for the general development of the immune system during the larval stages (Gatesoupe, 2008; Gómez and Balcázar, 2008; Nayak, 2010). However, others may cause diseases (parasitism or amensalism) by their presence either interior or exterior of the fish body, or more indirectly exhibit negative effects like depriving the water for oxygen (alga blooming). The term 'pathogen' in this section refers to an agent that can cause disease.

Some pathogens have a broad range of host species, whereas others are specifically associated with one or only a few host species. Often, pathogens have preferences for specific organs or tissues (tissue tropism). Moreover, the virulence of a pathogen may differ considerably among susceptible species. Even within a single host species heterogeneity in the manifestation of infection can be seen from one individual to the other, depending for example on behaviour (stress) (Mustafa and Piasecki, 2005), physiological state (sexual maturation) (Magnadóttir, 2010), living environment (water temperature) (Bly and Clem, 1991), as well as the developmental stage of the host (Samuelsen et al., 2006). Under certain circumstances pathogens might persist in a host without causing clinical symptoms (host carrier state), but can be activated to cause disease or be further transmitted. Microorganisms and parasites may be primary pathogens, able to infect and cause disease themselves, or secondary pathogens (opportunists) that need a preceding infection by a primary pathogen, a malfunctioning immune system, or another kind of trigger to facilitate infection. A few fish pathogens might also cause infectious diseases of humans (zoonoses). Examples are bacteria like *Mycobacterium marinum* and *Aeromonas hydrophila*, and the nematodes *Anisakis simplex* and *Diphyllobothrium latum* (tapeworm). More guidance on the impact to human health by GM fish can be found in section 4.1.7.Viruses are usually much more species specific, and have not been reported to be zoonotic.

The mechanisms employed by fish for protection against pathogens can be divided into physical barriers, innate immunity and adaptive (acquired) immunity. Immune organs functionally homologous to those of the mammalian immune system are present in fish but vary by type of fish (reviewed in Zapata et al., 1996). Fish stand at the evolutionary crossroad between innate and adaptive immune responses (reviewed in Tort et al., 2003).

The physical barriers consist of tight layers of epithelial cells covering both exterior and interior body surfaces. Normally fish also have a thick mucus layer covering the epithelial cells, where the infectious agents may be trapped and subsequently excluded as the mucus is continuously excreted from the body (Cameron and Endean, 1973). Skin and mucus also contain enzymes and anti-microbial peptides (AMPs) that can degrade, inhibit growth or kill pathogens (Falco et al., 2009).

If the pathogens manage to enter the body of the fish, they will encounter the immune system where the first line of defence will be the innate immune system, consisting of macromolecules (like interferons and complement factors) and effector cells (like granulocytes, monocytes) that will interfere, inhibit or kill the pathogen.

The second line of defence is the adaptive immune system consisting of B-cells and T-cells, which due to somatic recombination are able to respond to any foreign component invading the body and to elicit an immunological memory to that component. There is, however, a close collaboration between the innate and adaptive immune system, as signals provided by the innate system often are crucial for activating the adaptive system (Magnadottir, 2006).

In addition, recent findings indicate that fish use RNA interference for protection against some intracellular pathogens, where the mechanism is to synthesize some RNA that interfere with the pathogens RNA and thereby its ability to multiply (Fenner et al., 2007). Similar to adaptive immunity, RNA interference will normally be very pathogen specific. This kind of resistance will be heritable, and therefore can be regarded as an adaptive immunity working at the population level.

Infectious diseases are among the major obstacles in aquaculture, causing losses and poor animal welfare. Resistance or tolerance to disease is therefore a desired trait in the development of GM fish to mitigate aggregated production and welfare losses in fish populations.

Fish can be genetically modified with the primary goal to make 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). All GM fish not in group 1 belong to group 2 according to this guideline.

Group 1 GM fish are created with the intention to increase resistance against pathogenic organisms, either by interacting with the life cycle of the pathogen (infection resistance) or by negating its pathogenic effect for example by having altered the target for a toxin produced by the pathogen (disease resistance). This group can be divided into two subgroups: a) GM fish with increased resistance against a specific pathogen (or a specific group of pathogens), and b) GM fish with a more generalized resistance against several pathogens. Specific resistance can be achieved for example by inserting sequences of viral origin into the genome of the host genome, causing RNA interference and thereby inhibit the replication of the virus in question. Another example of specific resistance will be by removing or altering the receptor where a specific pathogen or its toxin binds to host cells or tissues. If a pathogen requires that specific receptor to attach to its host and the GM fish no longer has that receptor, colonization and infection cannot take place. However, one can also imagine a situation where colonization still can take place, virulence of the pathogen remains unchanged, but the GM fish with enhanced disease tolerance (e.g. with altered receptor not binding a disease causing toxin) could serve as a reservoir/carrier for that pathogen and thus may increase levels of longer term exposure of other, more susceptible aquatic organisms including their non-GM comparators and other susceptible fish species. More generalized resistance can, for example, be achieved by making a GM fish over-express important components of the innate immune system, such as natural antibodies or antimicrobial peptides (Falco et al., 2009). GM fish of subgroup 1b may also, as a side effect, become more hostile to mutualistic or commensalistic organisms, and the altered trait may therefore have both advantageous and disadvantageous effects for the GM fish itself.

Group 2 GM fish are created with the primary intention not to increase resistance against pathogens, but a consequence of the genetic modification is an affect on the susceptibility of the GM fish to infection. This may be due to an interaction between immune system and the genetic modification in question. For example, fish that are genetically modified to increase productivity could have reduced immunity because too little resources in the body are allocated to the immune system. However, modifications that do not influence the immune system may also alter interactions with pathogens and microbiota. For example modifications in digestion or metabolism may alter excretion of compounds in body fluid (e.g. mucus), digestive tracts, urine and faeces, which can serve as substrate for microorganisms or parasites. Such changes in substrate could result in a change in the distribution of opportunistic microorganisms, and some, otherwise harmless microorganisms might become harmful if they multiply to high levels (Stephani, 2011). On the other hand, more substrate for symbiotic bacteria might become available, which could have a beneficial effect if they act as probiotics (Nayak, 2010). GM fish may also influence the transmission of pathogens if they have altered behaviour as

predators, prey or other means of contact with other species. GM fish that can invade and/or establish novel environments (see section 4.1.1) can be exposed to novel microflora, pathogens and parasites, as well as interacting with different fish species or populations. This may allow novel pathogen interactions to occur and new patterns of disease.

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. This section deals with risk assessment related to interactions between pathogens and GM fish, and the consequences for non-GM fish, other biota and their associated environments and ecosystems. This includes intended and unintended changes of interactions between the GM fish and pathogens. The assessment of welfare and health in GM fish itself is discussed in the Guidance on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects (EFSA, 2012) and in section 3.8. Whereas impacts on human health by GM fish is addressed in section 4.1.7.

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 develop the risk assessment by comparing GM fish to its conventional counterpart under representative environmental conditions.

The key question is: might the GM fish differently influence pathogens in the environment, in comparison to its comparator, in its captive environments and all other potential receiving environments?

In case the answer is 'Yes', applicants should further consider for example:

- Can the GM fish change virulence of the pathogens?
- Can the GM fish alter transmission range and frequency of pathogens?
- Can the GM fish become a silent carrier for pathogens?
- Can the GM fish release compounds that alter the pathogen population?
- If the GM fish can enter other environments or living conditions, would the GM fish introduce pathogens to this environment, and would the GM fish become pathogen reservoir?
- Would the aquatic management practices (see step 5) alter the interaction between the GM fish and pathogens?
- Will the change in the interaction with pathogens result in altered phenotype of GM fish (e.g. dispersal, migration, colonisation, fitness or behaviour (see sections 4.1.1 and 4.1.3)?

Step 2: Hazard characterisation

Factors influencing disease resistance and immune response of fish includes 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) (Shoemaker et al., 2001). 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 disease to other fish.

If a disease tolerant GM fish acts as a carrier of a pathogen, applicants should consider the following: a) characterization of the pathogen including description of host range (including if it may be

zoonotic), transmission mechanisms and geographic range; b) pathogen load on the GM fish and the capacity of the GM fish to introduce and spread the pathogen in comparison to the non-GM counterpart; c) description of other organisms in the environment that are susceptible to the introduced pathogens.

Information is required on the infectivity of pathogens to the GM fish and the subsequent transmission from the infected GM fish to other GM fish and non-GM fish. Transmission studies should demonstrate whether GM fish can transmit the pathogen to non GM fish and therefore act as a carrier of infection.

Applicants should provide data on whether GM disease resistant fish can maintain and transmit the pathogen to non-GM fish, and, if so, whether the infection can be perpetuated and maintained by the GM fish population, in order to demonstrate whether the GM fish will become an ongoing source of infection. In both cases applicants should determine whether multiplication ratio of the pathogen exceeds or is smaller than one.

Applicants should determine whether the genetic modification result in any change in the production of metabolites by a fish that can be used as a substrate by fish pathogens. Metabolites secreted both externally and internally of the fish should be considered.

Applicants should consider whether altered immunity of the GM animal itself compared to the non-GM counterpart could be transferred to other sexually compatible types and species (see section 4.1.1) and the consequences of both enhanced and reduced immunity should be considered for these fish and their associated biota.

For both group1 and group2 GM fish, there is a potential that the GM fish may exhibit a selective pressure on pathogenic organisms leading to more virulent forms. Applicants should discuss the risk of adaptation of the pathogen to the immunity of its host(s) and the probability that they evolve with higher virulence. Mathematical modelling can be useful to study the interaction between a pathogen and the immune system, to estimate the evolution of a pathogen, and to estimate the epidemiological consequences. When modelling is used, applicants should document in detail the hypothesis, the choice of model, the parameters and assumptions used to construct the model, and the validity of the model for different populations, species and pathogenicity.

Applicants should also consider other microorganisms and parasites present in the receiving environments of the GM fish and determine the likelihood of any changes in the pathogenicity of these microorganisms and parasites.

Step 3: Exposure characterisation

The high stocking densities at which fish normally are kept in the production facilities enhance transmission of infections and specific infectious diseases can have considerable environmental and economic consequences because of loss of production, impact on public health or trade restrictions. 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 (unrestricted release to the wild, semi-captive or complete-captive), and the presence of non-GM fish in the receiving environments. For captive GM fish, the likelihood and frequency of escape needs to be estimated. For semi-captive GM fish, the time fraction and developmental stage for captive and non-captive periods should be estimated. Applicants should describe in detail the different steps of handling fishes in different stages of life and during transport (see also section 4.1.6). Other pathogen dispersal routes such as aerosols, urine, faeces, farm runoff, and disposal of fish carcasses shall also be considered.

In relation to the spatial and temporal pattern of exposure quantitative assessments of acute and chronic exposure levels for each characterised hazard should be made. Where it is not possible to

estimate exposure quantitatively (expressed as probability), applicants can express the likelihood of exposure qualitatively using a categorical description and provide a range for the indication about the likelihood of adverse effects.

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 estimating their probability of occurrence, any positive selection conferred by the horizontally transferred trait and the magnitude of the consequences of the adverse effect(s); taking into account 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 using the methods and approaches described by section 4.1.1.

Step 5: Risk management strategies

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 only cultivate 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 any particular practices that should be adopted for GM fish rearing that are additional to the normal range of general good hygiene, welfare and husbandry practices that should be implemented in fish production systems to minimise disease and stress levels. These could include specific requirements for isolation, treatment, stocking density, nutrition, etc.

The practicality and efficacy of the mitigation measures should be evaluated and methods for their implementation described. Uncertainties associated with the efficiency or implementation of mitigation measures should be described and considered in relation to PMEM plans (see Chapter 5).

Step 6: Overall risk evaluation and conclusions

Applicants should conclude on the overall risks arising from the conclusions of this section considering the proposed risk management measures. Uncertainties due to gaps in information, the limited scope of experimental studies and the need to extrapolate results to long term exposure of a wide range of receiving environments should be discussed. Applicants should describe identified risks or critical uncertainties that may have implications for other sections of the risk assessment, (e.g. for biotic interactions see section 4.1.3) and require further assessments in those sections. In addition applicants should describe identified risks or critical uncertainties that require further information from post-market monitoring studies, as well as an explanation of why identified environmental impacts are considered acceptable and do not present risks.

4.1.5. Abiotic interactions

There are two aspects of abiotic interactions that are relevant for GM fish. Firstly, the GM fish may have an altered (increased or decreased) tolerance to abiotic factors. This can be either as the desired consequence of the modification or a pleiotropic consequence of the modification. Secondly, GM fish may affect the abiotic environment in a different way compared to non-GM fish by for example

making different nests or altered digging behaviour by females. This second aspect can be divided into direct effects by the GM fish itself and secondary effects cascading from the direct effects (as described for biotic effects) which can be on both abiotic components or acting through biotic components.

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

Modification can alter the sensitivity and behavioural response to abiotic conditions, both physical characteristics (water depth, water flow, substrate and temperature) and chemical characteristics (dissolved oxygen, nitrate content, pH, and salinity). This will affect the ability of the GM fish to disperse and sustain in specific environments. For example some GM fish are modified for increased cold tolerance, and *coho* salmon modified to grow rapidly also appears to have a reduced tolerance to low oxygen levels (Sundt-Hansen et al., 2007).

Direct abiotic effects are those effects that the fish itself generates through various means, such as: physical parameters like nest digging, cave construction, grazing on coral, etc. Physical impacts would be most apparent for GM fish considered as 'ecosystem engineers' or that affect "ecosystem engineers" that create, modify and maintain habitat structures.

The same applies to chemical parameters such as oxygen consumption, ammonia excretion, etc. If the fish also releases some chemicals (including proteins as part of their modification) during its lifetime or after death, this could have effects on abiotic components, either directly by, for example, lowering pH or indirectly, influencing biota that in turn affect abiotic components.

Indirect effects can arise from the direct effects, acting either on biotic components of the ecosystem (similar to biotic interactions) or abiotic factors that influence other abiotic factors, e.g. digging behaviour can result in increased silt release which is transported downstream and settle in the estuary thereby altering the abiotic conditions for the biota in the estuary.

By affecting abiotic factors GM fish can alter the ecosystem's trophic structure (i.e. energy flow and food web relationships) which are likely to generate secondary effects. Biotic effects of the GM fish are also likely to give rise to secondary abiotic effects, for instance consumption of plankton is likely to affect water chemistry and nutrient availability and effects on top predators are likely to cascade down the food chain with implications for abiotic characteristics.

To examine consequences of GM fish on abiotic components it is important to identify the relevant comparator (see section 3.3).

Step 2: Hazard characterisation

Applicants should examine whether the GM fish has changed behaviour or physiology that can affect their tolerance and response to abiotic characters. Firstly, applicants should consider whether the GM fish has a different abiotic tolerance in relation to the relevant comparator, such as the ability to tolerate higher and/or lower temperatures or oxygen levels. Next, the GM fish response to these changes in the abiotic factor should be assessed not only to see if they can survive specific conditions but also if the GM fish will change their behaviour under them. For instance, applicants should assess whether the different development stages of GM fish can develop, grow and reproduce under these novel conditions (i.e. fitness assessment). This assessment must also include combinations of abiotic parameters to examine the presence of interactive effects, e.g. an enhanced tolerance to one abiotic factor may enhance or reduce the tolerance to another abiotic factor. This analysis is also relevant for the assessment of the health and welfare of the GM fish (see section 3.8).

Once the behavioural response has been documented for the GM fish, applicants need to examine whether this leads to changed abiotic interactions within the range of the comparator (wild specimen) and also outside this range if the GM fish venture beyond it. Such effects need to take into

consideration potential changes in the population size and density of the GM fish and whether this will affect the interactions with the abiotic component, e.g. impact of construction of gravel nests may increase with the number of GM fish but only to a certain level, after which, adding more fish will increase biotic interactions among fish and may reduce their ability or time for nest construction. Such interactions may also lead to GM fish spreading into areas not normally inhabited by the species and/or exposing other abiotic characters to the GM fish.

Secondary effects should also be assessed by looking at what other biota or abiotic factors are affected by the direct effect on the abiotic factors examined, e.g. parrot fish destroying a coral reef may reduce the production of the reef with a large impact on the ecosystem. This may also need to include abiotic effects in distant areas, such as downstream of a river or along an ocean current.

Step 3: Exposure characterisation

In section 4.1.1 applicants will have assessed any changes in the ability or propensity of GM fish to endure and exploit various abiotic resources and become adapted to new environments. However it is also important not to assume that a physiological capacity to endure a specific abiotic factor will necessarily lead to a behavioural decision to actually exploit it. However, dispersal into a new range of abiotic conditions can also be through involuntary transportation by birds or animals, capture fishing or other human activities that facilitate dispersal (e.g. through water ballast, by purposeful introduction of pet species or as escaped food species).

Thus, full consideration of the management, transport and handling of the GM fish needs to be considered, as well as the accidental release of GM fish, which may have other consequences than non-GM fish, due to differences in ability to endure and propensity to exploit abiotic components (see also section 4.1.6). In addition, the assessments in section 4.1.1 will also indicate the introgression extent of the recombinant DNA into conspecifics and other species, and its likely downstream effects on abiotic components (i.e. effect that the recombinant DNA may have after introgressing into other background genotypes).

However, in determining the full geographic spread of the recombinant DNA, the GM fish and its influences, applicants should also consider the nature of the different receiving environments and determine whether they will actually sustain and support the GM fish, taking into account both the abiotic and biotic characteristics of the receiving environments during the exposure time.

Changes in the ecology of the habitats invaded by GM fish may expose them to novel biotic and abiotic conditions. These can include by-products of metabolism (e.g. carbon dioxide, ammonia), faecal waste and products from decaying plants and animals. In some cases, micro-organisms (primarily algae) can influence GM 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 abiotic factors (see section 4.1.1), but it also becomes important to examine if this comes at a cost of enduring other factors in receiving environments. Changes induced by the GM fish on abiotic and biotic components may feed-back and change condition to either reduce or enhance further the fitness of the GM fish in the environment.

Step 4: Risk characterisation

Applicants should consider the abiotic conditions present in the receiving environments of the GM fish and determine the likely direct interactions that will occur. The indirect effects from these direct effects should then be considered in terms of secondary effects on other abiotic and biotic characteristics of these ecosystems in the medium term and hence the long term prospects for these environments. Applicants should consider the methods and approaches described by Devlin et al. (2006) and in section 3.2.

Applicants should consider whether key components of the environment are affected and the reversibility of these effects and the level of harm associated with them. Because of the complex nature of ecological interactions, applicants should clearly identify assumptions made in their ERA and any levels of uncertainty associated with conclusions on risks following the steps outline in section 3.7.

Step 5: Risk management strategies

Applicants should propose methods to reverse or reduce adverse impacts on abiotic parameters and key ecological functions identified in the risk assessment. The practicality and efficacy of the methods should be evaluated and methods for their implementation described. Uncertainties associated with the efficiency or implementation of mitigation measures should be described and considered in relation to post market environmental monitoring plans (see Chapter 5).

Step 6: Overall risk evaluation and conclusions

Applicants should conclude on the overall risks arising from the conclusions of both section 4.1.1 and this section and considering the proposed risk management measures. Uncertainties, due to gaps in information, the limited scope of experimental studies and the need to extrapolate results to long term exposure of a wide range of receiving environments should be discussed. Applicants should describe identified risks or critical uncertainties that require further information from post market monitoring studies. In addition applicants should explain why identified environmental impacts are considered acceptable and do not present risks.

4.1.6. Environmental impacts of the specific techniques used for the management of GM fish

GM fish may require or be adapted to changes in the productions systems used for their breeding, rearing and production. There is a requirement in Directive 2001/18/EC (EC, 2001) to assess the environmental impact of the specific management and production systems associated with the GM animal compared with non-GM animal. GM fish characteristics may be changed so that they need modifications to their confinement, welfare, health and feeding regimes or they may be adapted to environments (e.g. lower temperature) which could result in production units being located in novel locations. An important aspect of the production system is also to prevent the escape of the GM fish and so the efficacy and impacts of the facilities should be considered including the breeding, rearing and production units and any transport between them.

Production systems also produce waste products and can harbour pathogens. Any differences in effluents, pollutants and pathogen release from production systems should also be considered (see also sections 4.1.4 and 4.1.5).

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

Applicants should consider all the novel characteristics of the GM fish both intended and unintended and determine whether these will allow or be associated with changes to the production systems. Any changes identified should then be studied to determine their immediate consequences and also any downstream, knock on, cumulative or long term effects. For example if changes in diet and/or feed consumption are a consequence then the impacts of this on use of natural resources and emissions of waste effluents from production units should be considered.

If cold, anoxia or salt tolerant GM fish are produced this may allow production units to be located in areas where they do not presently exist. The environmental impacts and consequences of the presence of production units in new areas needs to be considered and potential hazards identified. This should include both direct effects of the production unit, emissions of GM fish and production unit waste, water usage, reduction of water quality and also indirect effects associated with the introduction of new pathogens and parasites into the area (see also section 4.1.4 on fish disease aspects).

If the phenotype or behavioural characteristics of GM fish indicate a requirement for increased size of fish cages which will increase the overall scale and size of the production facility, then the environmental impacts of this need to be considered.

In summary, the ERA should:

1. describe the potential range of GM-based management and production systems likely to occur across receiving environments including new receiving environments and how they differ from current management systems;
2. describe the potential adverse environmental impacts associated with the differences in management systems of the GM animal compared to the non-GM comparator;
3. determine which differences in management and production systems are related to potential higher adverse effects than current systems.

Step 2: Hazard characterization

The hazards associated with the changes to the production systems identified in the step 1 problem formulation and any consequences of these changes, need to be characterised for their environmental impacts and the potential severity of harm associated with these impacts. Knock-on, indirect and downstream effects should be considered.

Step 3: Exposure characterisation

The scale and frequency of occurrence of the hazards should be determined, particularly in relation to any knock-on or downstream effects identified in the hazard assessment. The efficacy of any mitigation measures (e.g. for treating waste products or controlling diseases) in reducing levels of environmental exposure should be considered. In addition the efficacy of captivity measures for restricting or preventing escape of GM fish should be assessed, as well as the efficacy of retrieving escaped individuals.

The ERA should also account for the animal production system and accessible ecosystems as a whole and in particular should account for spatial effects at the regional scale and temporal effects at the long-term, multi-generational scale. For ERA, upscaling, modelling, simulation and analysis of production systems and accessible ecosystems may be required, in addition to the analysis of smaller-scale experiments (EFSA, 2008).

Step 4: Risk characterisation

The risks posed by any changes in management and production system should be assessed for their severity and likelihood to cause environmental harm. These risks should be related to the risks identified in other parts of the ERA. The likelihood and frequency of GM fish escapes will determine levels of exposure for other parts of the ERA described in Chapter 4. This risk should therefore be considered in relation to all the other parts of the ERA.

Step 5: Risk management strategies

If environmental risks and a potential for environmental harm are identified in step 4, then applicants should consider management measures to reduce risks. These could be measures to reduce numbers of escaped GM fish or retrieve them or reduce the release of by-products from the production unit. Measures could be taken to restrict the size or the location of the production unit. Applicants should describe these measures and quantify the reduction in exposure or environmental impact associated with them.

Step 6: Overall risk evaluation and conclusions

Applicants should assess the overall environmental impacts of changes in management and production systems associated to GM fish considering both direct impacts and also indirect consequences of these impacts. Applicants should indicate the levels of uncertainty associated with both individual and overall impacts. The environmental harm associated with these should be assessed and quantified where possible. Applicants should conclude on the relative significance and acceptability of any associated environmental harm.

4.1.7. Impact on human health

An assessment of impacts on human health, prior to the deliberate release of GM fish, should be conducted. Applicants should provide information, specified in the Annex III of the Directive 2001/18/EC (EC, 2001), to evaluate whether the GM fish present 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. This should cover for example any increased risk of disease to people in contact with GM fish and fish products. Applicants shall follow the step-by-step approach as described in section 2.1.

Food and feed safety evaluation of GM fish is considered in the EFSA Guidance Document on the risk assessment of food and feed derived from GM animals and on animal health and welfare aspects (EFSA, 2012a). In this document, potential impacts of GM fish on human health through other routes of exposure, than ingestion, are considered. These include exposure through ocular, oral, nasal and dermal contact, and inhalation. Fish are capable of carrying pathogens and parasites that can infect humans and these may be present in the water as well as the fish. Increased risks to human health from all sources and routes of infection, including oral, are considered in this section.

In addition fish possess morphological characteristics e.g. spines, teeth, generation of electric fields, that can cause physical harm if live fish are handled, as well as producing proteins and other compounds that might cause irritations or allergenic responses to exposed humans working with dead fish. Moreover, if live fish are handled, then consideration may need to be given to any increased size or behavioural changes that might result in increased hazards to humans. Applicants should assess whether phenotypic or morphological characteristics are changed in GM fish to the extent that they may cause additional harm to people during handling of live or dead fish and their products.

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 and cases of human bacterial infections are reported through contact with infected fish while handling them, with water or other components of the fish environment (e.g. excreta and waste products). Human infections caused by pathogens transmitted from fish or the aquatic environment vary depending on the season, human contact with fish and related environment, dietary habits and the immune system status of the exposed individual (reviewed in Novotny et al., 2004).

Examples of major pathogens reported to be causative of human diseases include:

<u>*Gram negative bacteria*</u>:

- *Vibrio vulnificus* can cause severe human infections, i.e. epidermic lesions and septicaemia through an open wound and/or contact (Blake et al., 1980; Veenstra et al., 1992; Bisharat et al., 1999).
- *Edwardsiella tarda* is a possible source of human diarrhoea in tropical and sub-tropical zones (Van Damme and Vandepitte, 1980). The bacterium can cause myonecrosis, pericarditis and ulcers to hands and feet.
- *Aeromonas hydrophila* is both opportunistic and true pathogen for fish, but some strains can cause disease in man where two forms exist: 1) gastroenteric type causing diarrhoea and 2) septicaemic form associated with meningo-encephalitis, endocarditis and pericarditis.

Gram positive bacteria:

- *Enterococcus seriolicida* (synonym of *Lactococcus garvieae*) is reported to cause septicaemia and liver abscesses, infective endocarditis, acute cerebral infarction and intestinal disorders in man (Mofredj et al., 2000; Wang et al., 2007; Li et al., 2008).
- *Streptococcus agalactiae* is sometimes isolated as the cause of "streptococcosis" in diseased fish, though it is more commonly associated with bovine mastitis and is currently the most common cause of sepsis (blood infection) and meningitis in newborn babies and also a frequent cause of pneumonia in newborns.
- *Streptococcus iniae* has been reported as causing soft-tissue infections and discitis in fresh whole fish handlers, for examples: fish farmers, fish processors and people preparing fish feed (Weinstein et al., 1997; Fuller et al., 2001; Koh et al., 2004).

Acid-alcohol resistant bacteria:

Some atypical Mycobacteria, particularly *Mycobacterium marinum*, but also *M. chelonae*, *M. fortuitum*, and others can cause lesions in humans mainly associated with cutaneous abrasions and exposure to contaminated water (including public swimming-pools). The disease can progress in man to the internal organs, particularly lungs, but slower than typical mycobacteriosis. Professionals in aquarium shops have been infected through lesions caused by bites or by infected fish fins (Giavenni et al., 1980; Kullavanijaya et al., 1993).

Parasites:

Some fish parasitic Nematoda (such as *Anisakis* sp., *Contracaecum* sp., *Pseudoterranova* sp., etc.), Trichuridae (*Capillaria philippinensis*); Cestoda (such as *Diphyllobotrium* sp., etc.), and Trematoda (such as *Heterophyes* sp., *Opistorchis* sp., *Chlonorchis*, sp., *Clinostomum* sp., etc.) have larval stages which can parasitize humans causing diseases.

Protozoa and viruses:

In addition some protozoa, such as *Cryptosporidium* spp. and *Giardia* spp. which are mainly transmitted by raw or poorly cooked molluscs, can cause zoonoses in humans; whereas there do not appear to be any fish viruses infecting humans.

Applicants should assess whether there is an increased capacity to cause human disease from GM fish that may be a more efficient reservoir for that pathogen and consequently be a more efficient disease vector for human disease. Applicants should also assess whether the GM fish may become a carrier to different pathogens that can cause human disease (see also section 4.1.4). Applicants should determine whether changes in management could alter the pathogen load associated with the GM fish, and the consequent hazard to human health, e.g. the use of antibiotics in the fish farm.

Other concerns for human health from GM fish should consider:

1) Potential toxicity and allergenicity resulting from the exposure to the GM fish

*Toxicity: t*he potential toxicity of the changed or new expression of proteins or their derivatives should be considered.

Sensitization and allergenicity: applicants should assess whether the GM fish has altered allergenic characteristics as a result of the genetic modification. To this end, both the direct and known indirect effects of the genetic modification to the physiology of the GM fish should be taken into account. The potential allergenicity due to changes in metabolism and expression of novel proteins should be assessed.

2) Changes in phenotype of the GM fish

Applicants should assess whether changes in behaviour, phenotype or morphology (e.g. longer spines, sharper teeth, altered electric field) are likely to increase hazards to human health (e.g. to fish handlers).

Step 2: Hazard characterisation

The hazards identified in step 1 should be characterised considering the following:

A. Altered disease transmission capacity to humans

Applicants should determine whether the pathogen load for a specific pathogenic agent will reach levels that can cause human diseases. Where a potential hazard is identified laboratory animal experiments may be required in order to determine infectivity and transmission capacity.

B. Emergence/selection of new pathogens and/or strains with the potential to cause human diseases

Applicants should examine the pathogen profile to determine whether or not a pathogen that can cause human diseases is likely to emerge. Pathogen genotyping can be a useful method (reviewed in Sintchenko et al., 2007).

C. Potentially altered allergenicity

If a new protein is expressed or there is altered composition or expression of components known to be associated with allergenicity, applicants should provide an up-to-date search for homology of the amino acid sequence of the proteins and altered constituents, to known allergenic substances. A search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be performed to identify potential IgE cross-reactivity between the newly expressed protein and known allergens. The alignment-based criterion involving 35% sequence identity to a known allergen over a window of at least 80 amino acids is considered a minimal requirement (EFSA, 2010d). All sequence alignment parameters used in the analysis should be provided including calculation of percent identity (PID). It is recommended that the calculation of PID is performed on a window of 80 amino acids with gaps so that inserted gaps are treated as mismatches. The database(s) and the methodology used to carry out the search should be specified. If any indications of potential allergenicity are found, additional studies may be required; this will need to be assessed on a case-by-case basis.

In addition, applicants should conduct an assessment of possible allergenicity with respect to potential differences between the GM fish and its non-GM comparator, bearing in mind that: (i) materials from fish represent complex matrices in which interactions between proteins and other constituents may occur and that such interactions might alter the allergenicity of the fish in an unpredictable manner; (ii) there is a great variability in the intensity and specificity of human allergic responses (see also section 2.2).

D. Phenotypical changes in the GM fish

Applicants should determine whether phenotypic, behavioural or morphological changes to the GM fish present an increased hazard to humans in contact with the GM fish.

E. Changes in specific management practices for GM fish

Applicants should evaluate whether changes associated with the breeding, rearing, transport and processing of the GM fish present greater hazards to humans. These can include changes in husbandry and disease management, e.g. the use of antibiotics may increase the pathogen load or the frequencies of antibiotic resistance in those pathogens that can cause human diseases (see also section 4.1.6).

Step 3: Exposure characterisation

The possible impacts of GM fish on human health may happen at different stages in the development and processing of the GM fish, in different intended uses for the GM fish and in a range of different receiving environments.

Applicants should assess the conditions of breeding, rearing, transport, storage and release and processing of the GM fish in order to assess the different levels of occupational exposure in relation to the characterised hazards associated with the GM fish. In this respect all human exposure routes should be taken into account. Applicants should also assess potential dermal, nasal, oral, ocular and inhalation exposure as applicable for each characterised hazard.

The procedures applied during production, management, care, killing, transport and storage of the GM fish or of their parts will differ between different management systems. Therefore, as a prerequisite for the exposure assessment, a detailed study of these procedures should be conducted in order to identify the critical points where exposure occurs as well as the level, frequency and duration of exposure during the different stages of the production systems. Levels of exposure should be assessed at the critical points at all stages of the fish life cycle in order to identify when exposure to human is likely to be highest.

If qualitative terms are used to express relative likelihoods of exposure, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of zero to one, to which the term is intended to refer. For example, “the likelihood of exposure of workers by dermal contact during fish cleaning was estimated to be moderate, where 'moderate’ in this context means within the range 0.1 to 0.4".

The risk to workers managing and handling any GM fish whose behaviour may have been changed as a result of the modification shall be assessed. Changed behaviour may change the contact rate between the GM fish and humans (see section 4.1.3).

Step 4: Risk characterisation

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects to human health should be made for each characterised hazard based on levels of human exposure through all exposure routes at all stages in the GM fish production, but particularly at critical points identified in the exposure analysis. The evaluation for each risk should consider the magnitude of the consequences of the hazard and the likelihood of its occurrence. Where precise quantitative evaluation of risk is not possible, qualitative terms should be defined where possible. The uncertainty associated with each identified risk should be described (see section 3.7).

Step 5: Risk management strategies

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). The risk management measures themselves should be assessed to determine whether they are effective in reducing occupational exposure and handling risks.

Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM fish to human health should be made taking into account the risks identified in step 4, the associated levels of uncertainty, and the efficacy of the proposed risk management strategies in reducing these risks at different points in the production cycle and in the range of the relevant receiving environments.

4.2. Specific areas of risk for the ERA of GM insects

Developments and scientific activities in the area of GM animals indicate that future applications on GM insects may include the following uses:

(1) managing agricultural pests

Several agricultural pest species (e.g. pests of cotton, olive and other fruit plants) might be controlled by using Sterile Insect Technique (SIT), mating/orientation disruption methods, or by using insecticides. New control methods are being developed by suppressing the wild population of these pest species with GM insects.

(2) controlling insects vector of human diseases

Insect-borne diseases impose a significant burden on public health worldwide, with approximately half of the world's population is at risk of infection by e.g. Malaria, Dengue fever, Chagas diseases and the human African trypanosomiasis.[14] There are currently no vaccines against most of these diseases and in some cases drug-resistance is making existing treatment less effective. Chemical insecticides are the current primary means of controlling insects causing public health concerns. GM insects are being developed to combat insect-born diseases by means of population suppression or population replacement (Umweltbundesamt, 2010).

(3) contributing to the enhancement of production systems

GM insects might be released in the future to contribute to the enhancement of production systems (e.g. silk production, improved pollination).

4.2.1. Persistence and invasiveness, including vertical gene transfer

According to Directive 2001/18/EC (EC, 2001), applicants should consider potential adverse effects that GM insects might have on human and animal health and the environment. Adverse effects may occur directly or indirectly through mechanisms such as the transfer of the inserted genetic material to other organisms. Further distinction should be made regarding possible gene transfer to the same, closely related, distantly related and unrelated species (EC, 2002).

This section focuses on transfer of genetically modified DNA and GM traits by vertical gene transfer through normal sexual reproduction to populations of the same or related species while horizontal gene transfer is covered in section 4.2.2.

In this section, applicants shall address the consequences of release, escape, establishment, vertical gene transfer and changes in the fitness or other characteristics (e.g. gene drive systems) of the GM insect and any recipients of the event which may result in changes in persistence, competitiveness and invasiveness of the GM insect itself, and/or of its relatives and lead to environmental harm. The flow of an event from the GM insect into relative species is not an environmental risk in itself; the risk is associated with the phenotypic and biotic effects arising from the action of the event, and how these effects may influence the survival and reproductive capability of the GM insect and the resources it uses and provides to the ecosystems in different receiving environments.

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

Up to date, mass releases of GM insects may be linked to four types of applications:

(1) the suppression of a wild insect population acting as agricultural pest or disease vector,

(2) the permanent or temporary replacement of a wild insect population acting as agricultural pest or disease vector,

[14] http://www.who.int/whr/1996/media_centre/executive_summary1/en/index9.html

(3) the prevention of establishment of a non-native insect population acting as agricultural pest or disease vector,

(4) the establishment of more efficient or adapted pollinators to enhance pollination.

The primary applications of GM insects are likely to be either induced sterility or lethality in target species progeny for suppression or prevention that could reduce the negative effects of the target species. Sterile insect programmes require continued augmentative release of sufficient numbers to ensure sterile males are likely to mate with the majority of wild females. Replacement strategies could include properties such as diminished disease transmission capability in vectors, or disease resistance or productivity enhancement in pollinators. While a permanent replacement of vector populations with more benign forms could offer disease control, it may require a fitness benefit that could pose new problems in the future. Shorter term replacement with declining levels of gene expression may also be contemplated to overcome the potential risk of adverse effects that could arise from new populations that possess fitness benefits. Declining gene frequency over a number of generations from an initial release may be part of a planned strategy. This could provide a mechanism for resistance management by substituting different traits, or for allowing the released population to die out if a programme is to be stopped.

In this section, applicants should therefore consider, among others, the following hazards:

1. Transfer of a novel gene controlling physiological trait(s) to the same or related species might trigger an enhanced fitness (e.g. increased pollination function ensured by related pollinators, increased competition with other pollinators) or the ability (e.g. increased mobility) to occupy new niches and invade new insects' communities.

2. Cross-mating of a GM insect with non-target species or subspecies complexes. If mating between a GM insect expressing a dominant mortality/lethality gene and non-target insects should occur, it could disrupt the population dynamics of these other species or subspecies, leading to harm (e.g. additional resources become available to other pests) or loss of valued ecological species.

3. In sterile GM insect production systems, unanticipated impacts could occur from unintended or accidental releases of untransformed fertile reared individuals or significant proportions of females when male-only releases are intended. Such programme failures can have adverse effects (see section 4.2.3) as the problems already posed by the wild target pests/vectors populations could be aggravated by the additional release of viable untransformed or female insects. The latter might cause harm to plant stands or cause harm through biting and/or disease transmission.

An important factor for persistence and spread of either a GM insect or of hybrids harbouring a certain GM construct in the environment is the fitness effect associated with the respective genetic modification. Dependent on the effects of the specific GM construct in the respective genetic background, the GM insect could demonstrate either increased fitness favouring persistence and spread or exert a fitness load on the organism. Hybrids formed between the released GM insects and cross-compatible relatives could also suffer from outbreeding depression.

Assessment of fitness is specifically relevant for applications of GM insects expressing novel gene controlling physiological trait(s) (e.g. drought tolerance). Genetic modifications of fitness traits, for instance: increased temperature or drought tolerance, may enable the GM insects to expand their geographical range, and to reach and colonise new habitats close to relatives from which it was previously isolated. Additionally, increased fitness of the GM insect itself, or of hybrid progeny, due to vertical gene transfer to cross compatible relatives, via geographical range alterations may influence the host range of the species considered. Finally, enhanced fitness or the ability to occupy new niches would allow such populations to increase and invade new communities. This may cause a population

decline or extinction of relatives through hybridisation or through competition, which may trigger a cascade of environmental consequences.

The spread to other habitats can be due to movement of the released GM insect itself. In case of accidental or intentional releases (e.g. in the case of preventative release), GM insects could be released directly into receiving environments other than those currently inhabited by the species of interest, provided that the environmental conditions permit survival and reproduction.

With applications employing genetic drive systems, further propagation of the genetic construct in the environment is expected. Such an approach could be used to spread different GM traits, which induce refractoriness against infection by disease-promoting parasites or viruses. The impact on persistence and invasiveness would then depend on the overall effects of these modifications on fitness and reproduction.

Step 2: Hazard characterisation

Step 2 of the ERA consists of characterising any hazards, identified during the problem formulation process, which might lead to adverse effects as a consequence of enhanced fitness in GM insect and/or hybridising relatives.

Applicants should provide information on fitness and gene transfer differences between GM insects and appropriate comparators. The fitness of the GM insect and any hybrids arising from gene transfer should be assessed for the relevant receiving environments. The frequency for vertical gene transfer from the GM insect to cross-compatible relatives is an important indicator in assessment of potential effects from transmission of GM traits. Different publications such as: Benedict et al. (2008) and Scolari et al. (2011), identify specific questions to be considered for relevance of the ERA of GM insects, with particular regard to the persistence and invasiveness linked to the GM trait.

Even though GM insect applications based on sterility or inherited lethality would suggest a very low incidence of vertical gene transfer, the novel sterility or lethality trait could be partially expressed, with the consequent effect that some offspring would survive (Handler et al., 2004). As this could facilitate further propagation of the novel traits into wild populations, applicants should provide evidence of the frequency of surviving progeny (e.g. via PCR, genetic markers).

In the case of GM insects kept in captivity (e.g. GM pollinators confined in greenhouses), applicants should also consider escape and movement into other environments where cross-compatible relatives occur and the consequences of the recombinant DNAs introgressing into these populations.

In GM insects with stacked events, the combination of the GM traits, as well as their single properties, should be considered in relation to enhanced persistence or invasiveness in existing or new receiving environments.

Step 3: Exposure characterisation

Under Step 3, applicants should describe the environments that will be exposed to the GM insect taking into consideration its intended uses and the conditions of the mass release (e.g. continued augmentative release of sufficient numbers to ensure sterile males are likely to mate with the majority of wild females). The numbers of GM insects released, the frequency of releases, the proportion that may be fertile, female or other off-types should be assessed taking into consideration all aspects of the production system and any mitigation measures. The level of hybridisation with wild types and likely levels of fertile offspring production should be calculated.

In the case of GM insects kept in captivity (e.g. GM pollinators confined in greenhouses), applicants should also consider the likely levels of escape and movement into other environments. Applicants

should focus on receiving environments where cross-compatible relatives occur and where the likelihood of hybridisation is the greatest.

In addition, any mitigation measures to reduce gene flow (e.g. reduced fertility) and environmental exposure (e.g. confinement strategies) should be considered (see Step 5).

Step 4: Risk characterisation

On the basis of the conclusions reached in Steps 2 and 3, an estimate of the risk of adverse effects should be made. Since there may be more than one potential adverse effect, the magnitude and likelihood of each individual adverse effect should be assessed. If a quantitative evaluation of risk is not possible, terms used in qualitative evaluation should be defined clearly.

Applicants should consider and conclude whether:

1. GM insects will be released into receiving environments where cross-compatible relatives occur,
2. Interbreeding may occur,
3. The offspring will express a fitness change,
4. Populations of the GM insect and/or hybridising relative will persist in current and new receiving environments and/or will change,
5. The consequences for the receiving environments of the persistence or change in populations of GM insects or hybridising relatives,
6. The consequences for human, animal and plant health of the persistence or change in populations of GM insects or hybridising relatives.

In addition, the uncertainty for each identified risk should be described as outlined in section 3.7.

Step 5: Risk management strategies

When a risk has been identified under Step 4, applicants should propose mitigation measures to reduce the exposure (e.g. GM pollinators kept in confined facilities) and limit the risk. Applicants should also assess the efficacy of the proposed mitigation measures in reducing the identified risk.

Step 6: Overall risk evaluation and conclusions

Applicants should conclude on possible harm due to populations of the GM insect and/or hybridising relatives persisting in current and new receiving environments, taking into account the efficacy of proposed risk management strategies to mitigate the identified harm. The consequences for human, animal and plant health of the persistence or change in populations of GM insects or hybridising relatives should also be considered in line with section 4.2.6 and effects on target and non-target species (see sections 4.2.3 and 4.2.4).

Applicants should assess the possible uncertainties associated to the risk evaluation and conclusions as described in section 3.7.

4.2.2. Horizontal gene transfer

Horizontal gene transfer (HGT) is here defined as any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. The evaluation of the impact of HGT in the ERA includes analysis of the potential transfer of recombinant DNA from GM insects and dissemination to other organisms. Furthermore, the consequences of such transfer for human and animal health and the environment must be evaluated. Although the extent of environmental exposure is likely to differ between applications for import and processing and for environmental release, the HGT issues to be considered are expected to be similar.

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

HGT from GM insects is expected to be rare. However it remains largely unexplored. The outcome of rare HGT events may have consequences for human and animal health and the environment and are therefore considered in the ERA. The hazard identification will depend on the genetic modification(s) and the biology of exposed organisms present in various environments [e.g. other arthropods, animal species, bacteria (endosymbionts[15]) or fungi]. The problem formulation therefore needs to consider assessment endpoints being representative of the receiving environments that need to be protected from adverse effects, if a hazard has been identified. Adverse effects could include harm to specific species, populations or ecosystem services. The ERA will depend on the exposure routes, the horizontal mobility of the recombinant DNA, the characteristics of the phenotypic trait resulting from the genetic modification, the prevalence and transferability of similar traits in exposed environments, and the nature and range of potential consequences of HGT events (EFSA, 2009b).

Horizontal dissemination of recombinant DNA present within the insects can potentially occur both to related species, such as other types of insects as well as to unrelated organisms, such as microorganisms. These two pathways are considered in more detail below (section A, B). The potential for horizontal transfer will, however, be case-specific and depend on the molecular characteristics of the introduced genetic modification. The potential for HGT may therefore not be limited to these species categories. Conversely, the potential may also not be relevant for species in section A, B, depending on the case in question. The probability, frequency and heritability of HGT events of insect DNA (including the recombinant DNA fraction) are broadly determined by the following factors:

(1) the amount and size of insect DNA exposed to the various organisms present in relevant receiving environments (e.g. insect predators and decomposers**,** ecto-, endoparasites, parasitoids, symbionts and pathogens);

(2) the presence of germline cells in multicellular organisms or single cell organisms that are directly or indirectly susceptible to direct DNA or DNA vector exposure in those environments;

(3) the presence of mechanisms enabling such cells to take up the recombinant insect DNA, for instance through the action of biological vectors or the presence of single celled organisms that are naturally competent for uptake of extracellular DNA;

(4) the existence of genetic recombination/integration processes by which the translocated DNA can be incorporated, and therefore heritably stabilized, in the germline cells of multicellular organisms, or into replicating units (chromosome, plasmids) in single celled microorganisms.

The biological relevance of HGT events occurring at low frequencies will depend on their likelihood of further vertical transmission in the larger populations, as previously discussed (see section 4.1), it is largely determined by:

(5) the presence of conditions leading to positive selection of the recipient of the HGT event so they will increase in relative numbers in the population; and/or

(6) the presence of gene drive systems in the recombinant DNA leading to the possibility that a HGT event (of the gene drive system and linked additional recombinant DNA) will increase in frequency during subsequent vertical transmission.

[15] For guidance of GM endosymbionts please also consult EFSA (2011d).

A. HGT between insects. HGT processes between multicellular eukaryotes, such as insects, are only infrequently inferred (Silva et al., 2004). Most reported cases are considered to have taken place over evolutionary timescales and have depended on the action of autonomous elements. Moreover, only those HGT events occurring into or between germline cells of insects would be heritable and hence observable over generations. Some genetic modification strategies of insects intentionally introduce DNA elements with mobile functions to confer the intended effects. For instance, gene drive systems are considered as tools for vertical dissemination of DNA inserts above the expected mendelian segregation rate (Sinkins and Gould, 2006, Marshall et al., 2008, Hay et al., 2010). Understanding of the stability, mobility and host ranges of such elements is therefore essential to assess the potential for wider horizontal dissemination.

The problem formulation step, focusing on the potential adverse effects arising from horizontal transfer of recombinant DNA, with intact mobile functions, should consider:

- The molecular description of the recombinant DNA:
  - Intended phenotypic trait conferring sequences, selection markers (including insecticide resistance) and vectors remnants.
  - DNA regions affecting stability and mobility of recombinant DNA including the potential for mobilization (Li et al., 2001). Any autonomous or non-autonomous elements used must be fully described.
- The biological factors governing distribution, mobility, and functionality of the recombinant DNA, if horizontally transferred to new hosts:
  - The presence of plausible mechanism that could facilitate uptake and integration of the recombinant fraction of insect DNA in new hosts, at biologically relevant frequencies.
  - The host range, specificity and activity of the promoter elements present in the recombinant DNA, including those, when present, affecting the mobility of autonomous elements (e.g. transposases).
  - The potential of horizontal transfer relying on the understanding of the factors defining and limiting the current species distribution of the used mobile genetic elements, as well as of the mechanistic aspects of the replication/transposition of mobile elements in their current hosts.
  - The characteristics, natural occurrence of and host range of the gene drive system, when used (e.g. Sinkins and Gould, 2006).
- In case a clear HGT potential has been established, the presence or absence of organisms in the receiving environments that can potentially receive recombinant DNA taking the above limiting or promoting factors into account.
  - The biological characteristics of any identified recipient species, for which a plausible HGT scenario has been established.
- In case the plausible HGT recipients has been identified in the receiving environments, conditions that would favour the growth dynamics of HGT recipients in comparison to non-GM comparators:
  - The fitness changes conferred by the recombinant DNA uptake to the new host that could lead to positive selection and long-term establishment of the HGT events.
  - Gene drive systems may not depend on positive selection for vertical or horizontal dissemination. Knowledge of the functional characteristics of drive systems is necessary to consider the potential for unintentional dissemination.
- In case the above conditions are met, the possible adverse effects of plausible HGT scenarios from GM insects, should they materialize.

- Assessment and measurement endpoints should address established protection goals.

In cases the introduced genetic modification in the insert does not lead to changes in the horizontal mobility of the recombinant DNA fraction beyond any other chromosomal insect DNA (non-mobile), applicants are expected to provide a short conclusion that substantiates the absence of a changed HGT potential.

B. HGT to microorganisms. In contrast to the low proportion of germline cells in multicellular insects that can act as recipients of heritable HGT events, all single celled organisms can, in principle, act as recipient cells of heritable HGT events (Keeling, 2009; Dunning Hotopp, 2011; Richards et al., 2011). However, of the known mechanisms of HGT in single-celled organisms, only natural transformation is known to facilitate uptake and genomic integration of DNA fragments.

Microorganisms, especially bacteria, are capable of acquiring genetic material both from related and unrelated species (Anderson and Seifert, 2011). In bacteria, natural transformation with linear DNA fragments usually requires nucleotide sequence similarity to facilitate stable integration by homologous recombination. For this reason, it is considered that the presence of sequences with high similarity to bacterial DNA in the insect DNA would increase the probability of HGT (Bensasson et al., 2004, EFSA, 2009b). Due to the homology-based recombination mechanisms active in bacteria, the likelihood of HGT from GM insect DNA into bacteria should therefore be considered also in the absence of mobile genetic elements in the recombinant DNA. Differences in transcription and regulation, the presence of introns and requirements for intron splicing represent a functional constraint to efficient expression of many eukaryotic genes in bacteria. On the other hand the introduction of intron-free coding sequences in the GM insect genome with high similarity to microbial DNA would increase the probability of recombination and expression, if transferred (EFSA, 2009b).

The range of microbial species identified as potential recipients for unintended HGT events will depend on the ability of the micro-organisms to develop competence, on the characteristics of the recombinant DNA and to what extent homology-based genetic recombination can be expected. Positive selection is usually considered a necessity for rare HGT events occurring into large microbial populations to be biological meaningful. The problem formulation step focusing on the potential adverse effects arising from horizontal transfer of recombinant DNA to microbial recipients should consider:

- A detailed molecular characterisation of the recombinant DNA sequences.
    - The presence and source of prokaryotic mobile elements or other recombinant DNA sequences (e.g. cloning vector remnants and selection markers) showing similarities with DNA sequences present in exposed microbes, (i.e. enhancing the probability of homology-based recombination with recipient genomes); these characteristics will determine the host range of potential recipients.
    - Information on the functionality of the regulatory sequences of protein coding sequences in the recombinant DNA if horizontally transferred and on the presence of introns and requirements for intron splicing in such sequences.
- If a microbial recombination potential has been identified; the release, stability and degradation routes of GM insect DNA, in the receiving environments where such microorganisms are present.
    - The presence of other sources of DNA that is similar to the recombinant DNA (with equal or higher recombination potential), in the exposed environments.
- The identification of environmental conditions and biotic/abiotic factors in the receiving environments and if they could affect directional selection and long-term establishment of recipients of HGT events. Positive selection is usually considered necessary for rare HGT events to represent biological meaningful scenarios in larger populations, and therefore to be considered

relevant in the ERA.

- In case the above conditions are met, the possible adverse effects of plausible HGT scenarios from GM insects to microorganisms, should they materialize.

- Assessment and measurement endpoints should address established protection goals.

In cases the introduced genetic modification does not lead to changes in the horizontal mobility of the recombinant DNA into microbial populations beyond any other chromosomal insect DNA (non-mobile), applicants are expected to provide a short statement that substantiates the absence of an altered HGT potential.

Step 2: Hazard characterisation

If a hazard has been identified in Step 1 of the ERA, the hazard should be further characterised. Hazard characterisation 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 the GM insect in relevant receiving environments should be taken into account.

Step 3: Exposure characterisation

If a hazard has been identified, the exposure characterisation should consider characteristics of the recombinant DNA, the number of insertions or modifications, the levels and routes of exposure related to the hazard, and the scope of the application. The latter is also important as exposure levels will differ, e.g. between insect population replacement strategies versus insect population reduction strategies.

Applicants should take into account the methodological constraints to the quantification of DNA exposure levels in complex environments. In most cases, a numeric threshold level for a HGT event to be significant cannot be established. Other methodological limitations that warrant explicit considerations include the representativeness of the sampling strategy, the detection limit, and the tempo-spatial relationship between exposure levels and an observed impact of rare HGT events (EFSA, 2009b). Quantitative modelling approaches should be considered in cases where concerns over exposure levels have been identified. Modelling approaches may also be useful when representative data for environmental parameters cannot be obtained, for instance to address natural variability in exposure (see section 3.7).

Alternative sources of the relevant gene(s) should be identified and considered in the exposure assessment.

Step 4: Risk characterisation

Applicants should focus the risk characterisation on the identified hazards and its impacts that may potentially occur in the various receiving environments (as outlined above in steps 1 to 3). Any identified risk should be characterised by estimating the probability of occurrence, any positive selection conferred by the horizontally transferred recombinant DNA and resulting phenotypic trait and the magnitude of the consequences of the adverse effect(s); taking into account the characteristics of the recipient species.

Step 5: Application of risk management strategies

Based on the outcome of the risk characterisation, applicants may need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions allowing DNA exposure or positive selection.

Step 6: Overall risk evaluation and conclusions

Identified knowledge gaps should be briefly summarized and a clear statement on the absence/presence of selective conditions should be provided. Applicants are required to conclude on the overall risk, i.e. a clear statement on the potential for HGT to occur and its consequences, taking into account any risk management strategies.

The extent of the assessment will depend on the characteristics of the recombinant DNA and the receiving environments. For the consideration of HGT between insects, the presence or absence of autonomous elements (biological vectors) will determine the extent of risk assessment necessary. For microorganisms, the opportunity for homologous recombination will determine the extent of risk assessment necessary.

Applicants are expected to provide a clear conclusion on risk related to the potential outcomes of unintended horizontal mobility of recombinant DNA containing autonomous, gene drive systems, or relevant sequence similarity to microbes. If plausible HGT events have been identified to represent a potential hazard, remaining knowledge gaps and areas of uncertainty should be summarized and information on the absence/presence of selective conditions in the receiving environments provided.

4.2.3. Interactions of the GM insects with target organisms

Currently the principal aims for releasing GM insects focus on control of insect pests and/or improving human health through the suppression or replacement of insect vectors (Umweltbundesamt, 2010). As such, the interactions of GM insects with target organisms (TO) are a particular area to be addressed, because many potential applications of GM insects will be intentionally directed at suppression, elimination or prevention of wild populations of the same species.

It is to be expected that the release of GM insects for pest and disease vector control will result in significant interactions with their TOs. According to Directive 2001/18/EC, in Annex II, paragraph D.1.4 this specific area of risk assessment for GM insects should be considered. Target organisms are organisms on which the GM insect is intended to act. In many cases, with GM insects, the TO will be the wild population of the same species as the GM insect being released. Applicants should define the TOs relevant to their application. Other organisms not specifically targeted should be considered as non-target organisms. Both immediate and delayed effects on TOs should be considered.

The environmental impacts of GM insects on target species will be determined by the intended use of the GM releases. Most releases are likely to be aimed at the suppression, permanent or temporary replacement, or prevention of establishment of pest/disease vector species, many of which are likely to be non-native species. Suppression (including eradication) and preventative release of non-GM insects are commonly applied for the control of agricultural and livestock pests using radiation-induced sterility in mass release programmes, in Europe and other continents. Replacement of populations through release of altered populations occurs in conventional breeding programmes for domestic animals and plants, but is a more novel concept for GM insects.

The primary applications of GM insects are likely to be either induced sterility or lethality in target species progeny for suppression or prevention that could reduce the negative effects of the target species. Sterile insect programmes require continued augmentative release of sufficient numbers to ensure sterile males are likely to mate with the majority of wild females. Replacement strategies could include properties such as diminished disease transmission capability in vectors, or disease resistance or productivity enhancement in pollinators. While a permanent replacement of vector populations with more benign forms could offer disease control, it may require a fitness benefit that could pose new problems in the future. Shorter term replacement with declining levels of gene expression may also be contemplated to overcome the potential risk of adverse effects that could arise from new populations that possess fitness benefits. Declining gene frequency over a number of generations from an initial release may be part of a planned strategy. This could provide a mechanism for resistance management

by substituting different traits, or for allowing the released population to die out if a programme is to be stopped.

The diversity of impacts, even for specific GM insect applications, calls for a broad range of risk assessment methods to predict likelihoods and consequences and of systematic analysis to determine relevant, effective and efficient risk management (WHO, 2010).

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

The applications of GM insects for pest and disease vector control, through large-scale release programmes would be expected to have significant effects on the size, distribution and age structure of target populations through the release of very large numbers of reared insects (relative to particular target populations). The mechanism of control, most likely sterility or inherited lethality, depends on behavioural and physiological compatibility of the mass reared GM insects and the wild population, which could change over time. Management processes that affect the consistency and efficacy of the mass reared GM insects and the release systems would also be important to achieve the intended outcomes, and these should also be considered in determining risks. Some potential applications involve vectors of human disease, which pose particular concerns in the event of programme failures, but also may induce significant changes in social systems as people adjust to reduced disease threats.

Effects of suppression releases and preventative releases

a) Changes in TO populations caused by the GM component of the releases (size, age structure, sex ratio, fertility, mortality) that may result in adverse effects leading to environmental harm.

Direct assessment endpoints are focussed on the wild population(s) of the TOs, for example GM induced sterility or inherited lethality which would reduce, eliminate or prevent establishment of a wild population in the release area. In the short term, populations in this area may be increased by releases of significant numbers of GM insects, but in the longer term populations would be expected to fall. Sterile insect programmes usually reduce populations significantly within several generations. Many sterile insect control strategies would be based on male only (or nearly male only) releases at a specific growth stage, so the releases would affect the numbers, age structure and sex ratio of the population. Much of the direct negative effect of the TOs, such as oviposition damage or biting, is generally only caused by females. Mixed sex releases of GM sterile insects could increase the number of damage-causing females in the short term. There could be indirect effects as a result of reducing the population of TOs, through reduced competition with other species, or altered predator or parasitic species interactions, which may feed back to affect the target population itself. Where the TO is a non-native pest, the reduction in population size should help to restore the environment to the state prior to the establishment of the non-native pest. For native species the implications of reducing one component of a complex ecological web, including feedback to the TO population itself, may be difficult to predict (see section 4.2.4). In the case of preventative release, the target is by definition non-native, so prevention should maintain the current ecological balance (see section 4.2.4).

Measurement endpoints should address the size, age structure and sex ratio of the target population. In the case of suppression, the measurement endpoint would be the population density of the wild population of the species. In the case of preventative release the measurement endpoint would be the frequency and extent of outbreaks of the target species. These endpoints are already commonly measured for many species in sterile insect suppression and preventative release programmes.

Applicants should describe appropriate comparisons and comparators for these endpoints, and indicate how GM insects are discriminated from wild populations in the field. Applicants should also describe how these population endpoints are measured and specify the appropriate time period (see sections 3.6 and Chapter 5).

b) Reduction in efficacy or resistance development in the TOs against the GM insect mediated effect.

Adverse environmental effects could occur either directly due to the reduction in efficacy of the GM insect trait in the target population, or due to management responses to such a breakdown in efficacy in the target population. The stability of the GM insect trait in mass released insects and/or the efficacy of the intended effects in suppressing the target population should be considered.

It is also possible that resistance to the GM insect releases could develop in a population, for instance if wild populations were able to discriminate between wild and released GM individuals and select wild mates instead of the GM releases. In radiation-induced sterile insect release programmes continuous quality control measures are part of programme procedures to ensure that mating compatibility is maintained, for example by testing mating frequency of wild and reared populations and renewing colonies with local wild genotypes.

Stand-by control measures and risk management strategies needed to deal with any such failure of suppression, such as area-wide pesticide application, could affect the TO population and have broader environmental consequences. Reliance on continued positive effects of suppression may induce human behaviour that increases economic, environmental or social vulnerability to the TO, particularly in the event of any control failure. For example, people may become complacent about environmental hygiene for mosquito management once a successful GM based suppression or prevention programme is underway, making the impact of any failure in a GM insect campaign more serious than it may have been. Such self-induced vulnerability could also apply to other forms of insect control as well.

Measurement endpoints should address the efficacy of releases in terms of their intended effects. Applicants should describe how resistance to the GM insect applications, or any other reduction in efficacy, could occur.

c) Changes in interactions with the TOs arising from a narrow diversity of a reared GM insect population that may result in adverse effects.

Reared colonies of transformed insects may be based on introduced or selective sub-populations of the target species, which may result in behavioural impacts on wild target populations. A narrow genetic base may, for example, limit the host range of a population or its mating potential. On the other hand, a too broad genetic base may mean that many released individuals do not have sufficient behavioural similarity to interact in the way that is intended for the application. This could be a short-term effect in sterile releases until populations are substantially reduced. In preventive release programs, it is more difficult to establish a mating compatibility between GM insects that are released and the wider range of target populations that may subsequently invade, since there is no specific target population initially present. There may also be physiological impacts of introducing large numbers of novel individuals in a release programme, for example allergic reactions to mosquito biting may be greater when people are exposed to a new population of a mosquito species derived from another location (see section 4.2.6).

Measurement endpoints should address changes in interactions between released GM insects and wild populations over time.

Applicants should describe the origin, diversity and initial population size of the reared colonies and how this relates to the wild target populations and the mode of action of the intended GM applications. Applicants should describe how the compatibility of reared colonies and wild target populations would be maintained over time.

d) Effects on TOs due to release of low quality GM insects or non-GM insects that may result in adverse effects.

Unanticipated impacts could occur from unintended or accidental releases of untransformed fertile reared individuals or significant proportions of females when male-only releases are intended. Mass release of reared insects could accidentally include secondary species not intended in the programme,

such as parasites or pathogens. These unintended releases could result from imperfections or failures in the production and rearing process (see section 4.2.5). These releases could enhance the active population of the target pest or other species leading to more severe adverse effects, or in case of a preventive release, could lead to the establishment of a novel active population.

Measurement endpoints may include: the proportion of transformed individuals, the proportion of males, the average weight, flight activity and levels of contamination by other species in colonies.

Applicants should describe the standards to be used to ensure consistency in performance of the intended releases. Applicants should describe the expected effects of permanent and temporary replacement releases, when relevant, and how and for what period these will be monitored.

Effects of permanent and temporary replacement releases

Replacement releases involve drive mechanisms, competitive substitution and interbreeding. These strategies rely on non-Mendelian segregation of gene drive systems or provide relative fitness benefits to the GM insect strains.

a) Change in TO population parameters, fitness and behaviour that may result in adverse effects.

Replacement strategies may not be intended to change the size of wild populations. Over time they would become proportionately more numerous within a wild population of more or less similar size to the original wild population. However, the reproductive advantage may also result in an increase in the abundance and geographical distribution of the population, while reducing its primary impact (on disease transmission, for example). In the case of mosquitoes, this could result in biting rates increasing despite disease transmission decreasing. The subject of some replacement strategies may be very specific, for example the reduced transmission of a primary disease, so the replaced populations may have similar, or possibly increased capacity for some other negative property, such as transmission of a secondary pathogen or alternative strain of a primary pathogen.

Measurement endpoints may be adult longevity (e.g. in the context of dengue prevention), sexual maturation rate, or other factors that affect the objective of the release (e.g. disease transmission or pollination capacity). The transmissibility of significant secondary diseases should also be measured, if relevant.

Applicants should describe the intended rate and extent of spread of GM traits to the wild population. Applicants should describe appropriate comparisons and comparators for these endpoints, and indicate how GM insects will be discriminated from wild populations in the field. Applicants should also describe how and for what time period these population endpoints will be measured.

b) Reduction in efficacy of the GM insect mediated trait that may result in adverse effects.

Adverse environmental effects could occur either directly due to the reduction in efficacy of the GM insect trait in the target population, or due to responses to such a breakdown in efficacy in the target population.

For temporary replacement releases, the breakdown would be intended to occur at a particular rate, in which the GM insect portion of the population decreases over a predictable timeframe. A possible adverse effect could therefore be the failure of the programmed reduction of the GM insect population leading to either permanent establishment of the GM insect or to a faster decrease of the GM insect population, either of which could result in target populations with properties that result in environmental harm. For example, a permanently established GM mosquito population may transmit a non-target disease more efficiently, or become a nuisance biter. On the other hand, the subpopulation consisting of a GM pollinator insect that fades out more quickly could temporarily lead to reduced

pollination. Appropriate monitoring and management measures should be introduced to address the identified adverse effects, where relevant (see Chapter 5).

Stand-by control measures needed to deal with any failure of replacement, such as area-wide pesticide application, could have environmental consequences. Reliance on continued positive effects of replacement may induce behaviour in human populations that increases economic, environmental or social vulnerability in the event of any failure. For example, people may become complacent about environmental hygiene for mosquito management once a successful GM reduced disease transmission programme is underway, making the impact of any failure in a GM insect campaign more serious than it may have been. Such self-induced vulnerability could also apply to other forms of insect control as well (see section 4.2.6).

Measurement endpoints may include the prevalence of a phenotypic marker linked to the GM trait in the population, or the expression of a GM trait in a specified proportion of the population.

Applicants should describe the intended dynamics of GM traits in the target population after release. Applicants should indicate how and for what time period this would be monitored and what response would be made if deviations from the intended dispersal rates, geographic extent or penetration of trait occur.

c) Changes in interactions with the TOs arising from a narrow diversity of a reared GM insect population that may result in adverse effects.

Reared colonies of transformed insects may be based on introduced or selective sub-populations of the target species, which may result in behavioural impacts on target populations. This may result in long-term changes in behaviour in population replacement strategies. There may also be physiological impacts of introducing large numbers of novel individuals in a release programme, for example allergic reactions to mosquito biting may be greater when people are exposed to a new population of a target mosquito species derived from another location (see section 4.2.6).

Measurement endpoints should address changes over time in interactions between released GM insects and wild target populations.

Applicants should describe the origin, diversity and initial population size of the reared colonies and how this relates to the wild target populations and the mode of action of the intended GM applications. Applicants should consider how the compatibility of reared colonies and wild target populations would be maintained, when multiple releases are proposed and the consequences of this in terms of variations in compatibility and effects on subsequent target and GM insect populations.

d) Effects on TOs due to release of low quality GM insects or non-GM insects that may result in adverse effects.

Unanticipated impacts could occur from unintended or accidental releases of untransformed fertile reared individuals or significant proportions of females when male-only releases are intended. Mass release of reared insects could accidentally include secondary species not intended in the programme, such as parasites or pathogens. These unintended releases could result from imperfections or failures in the production and rearing process (see section 4.2.5). These releases could enhance the active population of the target pest or other species leading to more severe adverse effects, or in case of a preventive release, could lead to the establishment of a novel active population.

Measurement endpoints may include: the proportion of off-types, untransformed/wild type individuals, the proportion of males and females, the average weight, flight activity and levels of contamination by other species in colonies.

Applicants should describe the standards to be used to ensure consistency in performance of the intended releases. Applicants should describe the expected effects of permanent and temporary replacement releases, when relevant, and how and for what period these will be monitored.

Step 2: Hazard characterisation

Endpoints depend on the ability to measure populations of both released and wild types over time and to determine relative proportions of the released type and the wild types. The principal TO related hazard is that wild populations are not suppressed, prevented or replaced as expected over time. In addition, changes in alternative or complementary pest or vector management may occur due to-reliance on control conferred by the GM insect release and this may also contribute to control failures (see section 4.2.5).

Applicants should specify expected outcomes of releases in terms of density and proportions of both GM and wild type insects. A specific requirement of GM release programmes would be a means of marking released individuals so that they could be distinguished from wild individuals by a practical test with sufficient speed and accuracy to feed into responsive management actions.

Step 3: Exposure characterisation

To quantify the likelihood of occurrence of an adverse effect on TOs, it is important to understand the efficacy of the GM insects, and the temporal and spatial characteristics of a proposed release. The quantification of the likelihood of occurrence should consider:

a) The number, frequency, life stage and spatial pattern (point, linear, uniform, etc) of GM insects released, which should be considered in the context of the wild target population with which they will interact.

b) The genetic stability of the GM trait in the released target population, and where relevant in subsequent hybrid generations. Unexpected variation of the GM trait expression could occur over time, either in rearing facilities prior to releases or, for replacement strategies, in populations after release. Note that in some applications there may be a planned decline in the frequency of the GM trait in the population over time. The time period over which exposure occurs should be specified.

c) The mating frequency and behaviour of GM and wild insects, and the fertility of hybrid offspring.

d) The frequency and extent of the anticipated deviation from the release protocol of the GM insects, for example numbers, distribution, duration, etc, which could affect expected performance. Noting the size of the proposed release, its mechanism (for example, localised ground release or area-wide release from aircraft), the temporal frequency (how often and how many) and the spatial extent are all important aspects of the likelihood of failure (and require quantitative assessment).

e) Frequency and the extent of poor quality control procedures resulting in releases of unintended secondary organisms along with the target species, such as parasites or pathogens that may affect their performance.

f) The frequency, scale and effectiveness of monitoring of GM and wild target insects in the field after release could affect feedback for continued effective management of releases. The frequency of failure to discriminate between GM and wild target insects may contribute to the scale of ineffective monitoring.

g) The rate and extent of the changes in the wild population being targeted could make it less susceptible to releases of GM insects, by mutation or immigration of other genotypes.

In the context of exposure, applicants should describe the quantitative spatial and temporal parameters (for examples: distribution, heterogeneity and density of population) of the wild target population and

the intended interaction with the GM insects released. Quantitative measures of quality control procedures in rearing, PMEM plan and management responses to deviations from planned procedures and outcomes should be described.

Effects of suppression releases and preventative releases on exposure assessment

In suppression releases it is possible, in theory, to plan ratios of released to wild insects through sampling. The ratio of released GM insects to wild target insects is expected to increase over the course of the releases, increasing the relative interaction of GM insects with the declining wild target population. In the case of preventative releases it is not possible to sample wild target populations to plan release ratios. Interaction of GM insects with wild target populations only occurs when incursions of wild target insects enter the area, so any exposure to TOs would be intermittent, if at all, and the ratio would depend on the scale of the incursion.

Effects of permanent and temporary replacement releases on exposure assessment

Exposure in replacement releases would be expected to increase as the genetic trait spreads through successive generations of the target populations. The rate of increased interaction would depend on the drive mechanism or relative fitness benefits associated with the GM insects. In temporary replacement strategies, the exposure would be expected to decline over successive generations of the target population, as the expression of the GM traits reduces.

Step 4: Risk characterisation

Magnitude and likelihood of risks would be affected by the events described in 4.1.2 and 4.1.3. The characterization of risk should use some form of (semi) quantitative assessment, such as that used to assess the release of *Wolbachia* in *Aedes aegypti* (Murphy et al., 2010) or risk posed by invasive non-native species (Mumford et al., 2010).

In the case of GM insect releases the overall program should be assessed for risk (see also section 4.2.5), not just the GM technology component. Likelihoods and consequences of risks will be affected by a number of factors, such as the numbers of insects released, the rearing quality, the spatial distribution of releases, the GM technology employed, and the ability to differentiate GM and wild insects in the field. Key considerations include:

Programme design

Knowledge of the ecology (dynamics of temporal patterns of distribution and abundance) of principal pests/vectors in the area of GM insect releases should consider characterizing the spatial pattern and the scale of the risk. Site size and release rates should be predicated on the focused aims and endpoints of the type of release. Immigration or evolution of diverse incompatible wild strains of the TO in the release area should be assessed over an appropriate time period.

Key risks related directly to the GM technology are the likelihood of sterile releases becoming self-sustaining and the magnitude of any additional adverse effect in the resulting self-sustaining populations compared to the original wild target population. For a replacement strategy, the risks are the likelihood that a new population will have an adverse effect and the magnitude of these adverse effects of the replaced population compared to the original wild target population. In principle, non-replacement strategies, based on sterility or inherited lethality, are likely to revert to the original status soon after they are stopped. An exception would be if released individuals were not effectively sterile (or inherited lethality was incomplete) to the extent specified. If they do not successfully prevent reproduction of further generations, some replacement in the wild target population could result. In contrast, replacement strategies that fail to replace would revert to an original wild target population. In the case of vector control strategies, reversion to the wild type may restore adverse impacts to their original level so that effective post release monitoring is an important component of release strategies.

Failure in discriminating the GM insects from the wild type will result in ineffective post-release monitoring of the TO so that risks could be restored. Applicants should consider this as part of the risk assessment.

Programme management

In their risk assessments, applicants should consider key production and management issues such as the efficacy and consistency of released individuals, genetic stability of released target populations, continued compatibility between released and wild target populations, and the quality of post-release monitoring and management responses to feedback.

Ecological dynamics

In suppression releases the ratio of released GM insects to wild target insects is expected to increase over the course of the releases so the likelihood of interactions between released GM insects and wild target populations will increase, but the consequences of the individual interactions should remain the same, unless the quality of the released insects or the wild target populations change. In the case of preventative releases exposure of GM insects to wild target populations only occurs when incursions of wild target insects enter the area, so any exposure of TOs would be intermittent, if at all. An important issue in all continuous release programmes is the ability to practically discriminate between GM insects and wild target insects, across the expected range of ratios of GM to wild target insects. The likelihood and consequences of immigration of incompatible strains of wild target insects should also be considered.

In replacement releases uncertainty about the risk from interactions between GM insects and wild target insects is expected to increase as the genetic trait spreads through successive generations of the target populations. The likelihood and consequences of emigration of GM insects from the release area should be considered. The temporal pattern of these dynamic interactions should also be considered.

Step 5: Risk management strategies

Various risk management issues have been highlighted in the description of hazards above and essentially require the adopting of standard operating procedures (SOPs). Risk management strategies should place specific emphasis on the failure of SOPs:

Key issues are:

a) Relevant programme design specifications are developed.

b) Rearing procedures can be followed as specified.

c) Rearing quality can be monitored.

d) Release procedures can be followed as specified.

e) Release quality (numbers, distribution, duration, survival, etc) can be monitored.

f) Post-release monitoring procedures can be followed as specified (wild population numbers, distribution, behaviour; released population numbers, distribution, behaviour, survival (no survival for sterile strategy, established replacement for replacement strategy).

g) Operational responses to quality control and monitoring feedback can be carried out as specified.

h) Management of any potential resistance can be carried out.

Applicants should propose how responsibility for implementing these risk management strategies and managing unpredicted outcomes that arise from the release of GM insects could be ensured.

For continuous release strategies, applicants should indicate how protocols, product efficacy and consistency will be maintained, how and for what time period monitoring will be carried out in the field, and what responses would be taken in the event of adverse effects occurring. Applicants should particularly indicate how any self-sustaining target populations with adverse traits would be detected and managed.

For permanent replacement strategies, applicants should describe recall strategies or other measures to respond to any adverse effects. For temporary replacement strategies, applicants should indicate the intended rate of decline in the frequency of GM traits, the monitoring plan, and the response plan in the event the rate is not as intended.

Post-release risk management would depend on an ability to discriminate between GM and wild type insects with sufficient speed and accuracy to feed into responsive management actions.

Step 6: Overall risk evaluation and conclusions

It is recognised that a principal aim for releasing GM insects is to control or prevent establishment of populations of the TOs that have significant negative impacts as pests of human health or agriculture, and that in many cases these target pest populations are not native in the release area. In such cases negative impacts on TO populations are the intended endpoints. Other applications aim to replace the original TO population with a new population with more favourable genetic traits. Interactions with the wild populations of the TOs are crucial to both these aims.

Applicants should describe the intended purpose of GM insect releases and provide a description of the genetic traits involved and the release protocols. Risk assessment should determine (1) the possible mechanisms of impact of the GM insect on wild populations of the TO; (2) the likelihood and impact of the GM insect and/or sustained hybrid populations of the TO in managed and natural ecosystems, through a change in fitness, physiology or behaviour, or through interactions with social systems; (3) the levels of uncertainty associated with the effects and their consequences (4) what risk management measures may be required to mitigate any harm or uncertainty associated with changes to TO populations, (5) why the impacts of the management measures and any anticipated or unintended changes to TO populations, together with their uncertainty, are considered acceptable.

4.2.4. Interactions of the GM insect with non-target organisms

According to Annex II of Directive 2001/18/EC, an ERA should consider the potential immediate and/or delayed environmental impact of the direct and indirect interactions of GMO with non-target organisms (NTOs) (EC, 2001). NTOs are defined as all those species that are directly and/or indirectly interacting with GM insects and that are not the organisms on which specifically designed characteristics of the GM insect are intended to act. Thus, the ERA as described in this EFSA Guidance Document should address the potential environmental impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens (EC, 2001). Impacts of GM insects on human health are addressed in section 4.2.6. Furthermore, the potential impact on ecosystem services and ecological functions provided by NTOs, as well as species of conservation concern should be considered. Therefore, the range of functional groups of NTOs, including pollinators and decomposers, should be considered in an ERA of GM insects. For a general overview and description of protection goals relevant to the ERA of GM insects see section 2.1.1.

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

When considering potential adverse effects of GM insects on NTOs, the ERA should distinguish between effects on biodiversity and those on ecosystem services provided by NTOs. Considering the aim and type of GM insect releases, potential impacts on NTO that may cause adverse effects, include:

(a) a change in the abundance or species composition of natural enemies and the pest regulation service they provide;

(b) a change in abundance or species composition of competitors (e.g. insects occupying the same ecological niches) of GM insects and the ecological functions they provide;

(c) a change in abundance or species composition of pollinators and the pollination service they provide;

(d) a change in biodiversity concerning species of conservation value;

(e) a change to other ecosystem services such as decomposition of organic matter, soil nutrient cycling, water regulation and purification;

(f) a change in abundance or species composition of host plants or host animals and the ecosystem services they provide.

Note that it is impossible to list in the guidance all possible interactions between GM insects and the NTOs and their environmental consequences. The guidance therefore provides a non-exhaustive list of examples of potential adverse effects on NTOs so that applicants can consider possible interactions on a case-by-case basis, based on the particularities of the GM insect, traits, receiving environments, intended uses, and the combination of these characteristics.

(a) Effects on abundance or species composition of natural enemies and the pest regulation service they provide

Ecological interactions of any type of released GM insect may involve natural enemies such as predators, parasitoids and pathogens. Adverse effects could be associated with the unnatural fluctuation in abundance of the target species. Resource pulses in nature (the periodic super-abundance of resources in terms of insects as a food source, which may occur when large numbers of GM insects are released at once for suppression strategies) can have large scale, complex direct and indirect effects that are transmitted through trophic levels of food webs and can have quite profound impacts on the community structure of many taxa. These effects will be dependent on the timing, frequency and size of GM insect releases, which may significantly differ between various GM insect applications. For example, the release of sterile GM insects to suppress the target population implies that the number of released insects is around 10-100 times the number of individuals living naturally in the target area. The many individuals added to the ecosystem, which only live for a few days, provide available food for species of higher tropic levels. Depending on the release characteristics, this artificially increased amount of food is available during the time GM individuals are present in the environment, but will decline sharply when the target species is successfully eradicated. These changes in abundance may have consequences on predators or other natural enemies.

More generally, the loss of available prey through suppression or disappearance of the target species could have an adverse effect for predators, especially if the specificity of the predator is high and no sufficient alternative food sources exist.

Following suppression and preventative releases, GM insects will be present only for a limited time and in a limited area. It is therefore expected that long-term effects on natural enemies would be limited, except if very host specific endemic or threatened species are likely to be affected. In such situations, the applicants should demonstrate that no such species are affected by the GM insect release.

It is most likely that the release of GM insects in a replacement strategy will affect natural enemies in a different and more permanent manner. Released GM insects may be more or less susceptible to natural enemies than non-GM individuals, which may affect natural enemy populations and their role as natural control agents. If the presence of GM insects is meant to be permanent, and released GM insects may spread over wider areas, potential effects on natural enemies and the pest regulation service they provide are likely to be permanent and widespread. Therefore, even abundant and widely distributed natural enemies may be affected.

Further potential adverse effects on natural enemies could be due to direct effects of the phenotype and expression of GM traits upon predation/parasitism. Thus, predators or parasitoids feeding on GM insects containing novel metabolite expression or phenotype (e.g. behaviour) may be affected.

(b) Effects on abundance or species composition of competitors of GM insects and the ecological functions they provide.

Different species compete for the same food resources, habitat and reproduction sites. It is possible that the release of GM insects will alter competition with species occupying a similar ecological niche. For example GM mosquitoes, plant pests or pollinators may become more (or less) abundant or more (or less) competitive than their non-GM equivalent, affecting negatively or positively other mosquitoes, herbivores or pollinators. Changes in competitors' abundance may, in turn, have consequences on disease prevalence and transmission, on secondary pest outbreaks, pollination, food chain, etc.

These changes might cause undesired effects in community structures sharing similar ecological niches. Many pathogenic diseases are transmitted by a range of different vectors. The control of one species of this guild due to the release of GM insects might increase the density of other disease transmitting species. This might lead to the transmission of the target disease on a similar level or to more efficient dispersal of non-target diseases. Additionally these other species might become nuisance biters. Similar effects might be observed, if single pest species were suppressed by the release of GM insects. Other pest species (e.g. secondary pests) might employ the available resource and build up high populations which might result in crop damage. These effects are also addressed in sections 4.2.5 and 4.2.6.

(c) Effects on abundance or species composition of pollinators and the pollination service they provide

Given their importance for floral biodiversity and food/feed production, pollination services provided by pollinators are recognised important ecosystem services. Impact on plant pollination due to the release of GM insects could be direct, if they are involved in pollination or indirect if they impact on other insect species that are pollinators. In the first case, the genetic modification would need to interfere with characteristics of the parental species such as the abundance at the time of pollination, mobility of the animals, preference for certain plants, which are important for the pollination behaviour. In the latter case, pollination loss could be caused by decrease of pollinator abundance in the environment. For agricultural crops, this could lead to yield reductions; in case of other plants, this may lead to a decrease in abundance of the considered species, thus to a decrease in floral biodiversity.

The fact that GM insects, e.g. mosquitoes and plant pests, likely to be used in suppression and preventative releases will not be important pollinators, these types of releases are unlikely to have a direct effect on pollination of wild plants or crops. In contrast, with the possible development and use of GM honeybees or bumble bees, it will be important to investigate carefully whether the release of these GM insects adversely impacts populations of indigenous pollinators, the pollination of wild and agricultural plants or alters interactions within the pollinator community.

(d) Effects on biodiversity, concerning species of conservation value (rare or threatened species), or of cultural value (aesthetic value) and food chain effects

The release of GM insects may have adverse effects on natural enemies, or pollinators, as described in the sections above. These adverse effects might have implications on the wider biodiversity. In contrast, specific species could be affected without adversely influencing ecosystem services and ecological functions.

Conservation of biodiversity is a general protection goal (see section 2.1.1). Therefore directly affected species such as rare, endemic or threatened or species of cultural value need to be considered. Furthermore, by a cascading effect, other species, linked to the primarily impacted species via the food web, could be indirectly affected by its disappearance or its decreasing population size. In

suppression/preventative releases, this effect is potentially limited in space and time, the effect on biodiversity may be temporary and, therefore, of concern only if rare and threatened species are affected. However the magnitude of such effects might also depend on the time span of the release which range from single years to continuously over longer periods as in the case of preventative releases. In replacement strategies, the effects may spread to wider areas and last for a longer period, which may affect even widespread and abundant species and their ecological functions. Impacts on biodiversity are likely to be more important when the TO is native in the release area than when it is non-native, since in the latter case interactions with the native biodiversity may be reduced.

(e) Effects on other ecosystem services such as decomposition of organic matter, soil nutrient cycling, water regulation and purification

Other relevant ecosystem services such as decomposition of organic matter, soil nutrient cycling, water regulation and purification may potentially also be affected by the GM insect release, depending on the organism released and the release strategy chosen. For example, the use of sterile GM insects to suppress or eliminate or prevent a target population may lead at least to a temporary increase in the number of a particular species. These will die and will be available temporarily to the scavenger community and it may locally alter decomposition functions. The potential adverse impact on soil and water environment is likely to be greater with replacement releases for which specific characteristics of the target population are changed permanently.

Hence, according to Annex II of Directive 2001/18/EC (EC, 2001), possible adverse effects of mass release of GM insects onto biogeochemical processes and ecosystem functions (e.g. soil decomposition, food web structure, diversity of soil or water ecosystems) should be considered by applicants as GM-insects material may enter soil and water bodies (Umweltbundesamt, 2010).

(f) Effects on abundance or species composition of host plants or host animals and the ecosystem services they provide

When a GM insect is released in the environment, it is also likely to affect species of the lower trophic level, i.e. plants for GM herbivores or animals for GM mosquitoes. Mass releases of sterile male plant pests are meant to protect cultivated plants but may also, secondarily, protect other host plants, favouring their populations. Mass releases of sterile male mosquitoes in suppression or preventive releases are unlikely to severely affect host animals, unless a significant number of females is released with the males, or if non-GM males and/or females are unintentionally released. Mosquito larval predation arising from some types of GM release may need to be considered. In contrast, in replacement strategies, it is possible that the GM mosquito may become more (or less) abundant, more (or less) aggressive or more (or less) prone to carry other diseases and, consequently have adverse effects on non-target animals and, by cascading effects, on other components of the local biodiversity and ecosystem services they provide. GM mosquitoes may also have some adverse unintended effects on human health (e.g. allergic reaction), which are covered in section 4.2.6.

As indicated above, the potential adverse effects of GM insects on NTOs and the ecosystem services and ecological functions they provide depend upon various factors, which are summarised below:

- ☐ The type of organisms, intended use and the applied strategy of the GM releases. Mosquitoes, agricultural pests and bees all have different roles in ecosystems and are likely to affect NTOs, and the ecological functions provided by those NTOs in a different manner. Of particular importance for identifying the effects and their magnitude on NTO organisms will be the duration of the presence of the GM insect in the environment, i.e. whether the presence will be temporary (as for suppression or prevention releases) or permanent (as usually the case for replacement releases). In situations where the presence intends to be temporary, the likelihood of permanent establishment (e.g. via the failure of the "fading out/decay" mechanisms) should be assessed and, if this likelihood is not negligible the case should be assessed as permanent establishment.

- The characteristics of the receiving environments (see section 3.1). The receiving environments (i.e. where the GM animal is likely to occur) of the different life-stages of the GM insect should be considered. Also the environmental impacts are likely to be more varied and important if natural or semi-natural habitats are also exposed than if the GM organism is released temporarily in purely human-made habitats.
- The origin and distribution of the TOs. A particularly important factor is whether the organism is native or non-native in the region of release. An environmental impact on NTOs may be higher when the target species is native to the area of releases since this will play a more important role in local ecosystem service and, in particular, have stronger trophic interactions with native natural enemies and plants. By contrast, the introduction of alien GM species may have additional effects of displacing native species or through novel interactions with native flora and fauna.
- The life-stage released and its interactions with NTOs. The fact that GM insects may be present, whether in fluctuating or stable numbers, throughout their entire life-cycle or only during specific life-stages, is an important element to consider in the ERA. It should also be considered that different life-stages of the TOs may be related to different habitat requirements (e.g. different feeding habits, larvae and adults living in different habitats) and, therefore, interactions with NTOs will differ in these different environments (e.g. interactions with different predators and parasitoids).

Impacts on NTOs may also theoretically occur through HGT which are addressed in section 4.2.2.

Step 2: Hazard characterisation

4.2.4.1. General background information for the hazard characterisation

To assess whether GM insects may cause harm to NTOs and the ecological functions they provide, it is important to identify assessment endpoints, being representative of environmental resources that need to be protected from harm, according to protection goals set out by EU legislation, based on GM insect characteristics capable of causing environmental harm. In this section, all information considered relevant to the characterization of the identified hazards in the ERA of GM insects is listed in the form of data requirements. Whether information is required for all points listed or only for specific points will depend upon the insect species, trait(s), the intended use, and the receiving environments under consideration.

Some NTO species contribute to ecosystem services and ecological functions in ways that are unique and hence their addition or loss from a community would cause detectable changes in functioning. In this situation, the population abundance of these focal species should be assessed. Most ecosystems, however, exhibit some redundancy, where several species are able to perform the same critical function. These species are at least partly substitutable and their loss can be compensated for by other species.

It is possible that some adverse effects on ecosystem services and ecological functions such as pest regulation or pollination will be difficult to quantify directly. Therefore, assessment of the population abundance of focal species contributing to the same ecosystem function (e.g. predation or pollination) will be appropriate.

Basic information to assess the potential hazard to NTOs comprises a description of the biology and ecology of the species, which will be genetically modified and released. This includes:

- Data on the origin of the species i.e. if the modified species is native or non-native in the region of release (see above).
- Description of the ecological relationships including the involvement of the species in basic ecosystem services and ecological functions. This will help to identify potentially affected non-target species interacting with GM insects and implications on ecological functions. For

example, natural enemies, competitors and hosts of the wild type of target species might be affected by a GM-application with the aim of population suppression and replacement. Since it will be difficult to estimate the magnitude of non-target species loss or decrease in abundance of natural enemies, a recommendable approach is to define key natural enemy, competitor and host species on the basis of pre-release habitat analyses and to investigate their abundance during meaningful periods before release. The selection of these focal species is generally based on several criteria, including the ecological relevance of the species, sensitivity to known or potential stressors, anthropocentric value, testability, exposure pathways (e.g. predators and parasitoids through preys and hosts). The number and type of species to be considered will depend upon the hypotheses generated in the problem formulation. Therefore, NTO testing shall start with a clear problem formulation to enable the development of decision trees for species selection. Potential hazards for the abundance of the non-target species and the related ecosystem services and ecological functions could be assessed using different approaches, e.g. modelling, lab, semi-field, and/or field tests.

- ☐ Population dynamics of the target species, including the ability to react to environmental factors influencing the population dynamics (environmental stress, disturbance, etc.). In particular, for suppression releases, information is needed on the ability of the species to recover after the release of GM insects in order to determine the time scale of ecological responses.

Further information is needed on the characteristics of the phenotype of the GM compared to the unmodified insect. It is important to describe how such changes may have an adverse impact on the NTO. The following information should be provided by the applicants:

- ☐ Fitness, effectiveness and behaviour of the modified strain. It is important to consider if the fitness, effectiveness and behaviour of the GM individuals of a species are altered compared to the non GM individuals. For example, changes in fecundity, longevity, resistance to natural enemies, preference for a host plant or animal, or any other characteristics might result in changes in species communities leading possibly to altered ecosystem services and ecological functions.
- ☐ Changed susceptibility of the GM insect to control and management measures e.g. it should be shown that GM species have the same susceptibility against conventional management measures such as e.g. pesticides or biological control to ensure the availability of alternative management measures (see section 4.2.5). This is mainly relevant for replacement strategies.
- ☐ Factors altering the spatial distribution of the GM species are also of importance. In particular the range of distribution should be described. This is helpful to assess the dispersal ability of the species. Furthermore the distribution of the identified interacting species e.g. predators or parasitoids is also of importance. Potential effects on threatened and protected non target species should be considered which occur in the area of release of the GM insect, or in the area where in the GM insect can spread.

4.2.4.2. Potential assessment and measurement endpoints

Once hazards have been identified, focal (NTO) species should be identified that interact with the GM insects in the receiving environments where those insects are likely to be released or where those insects could spread.

Assessment and measurement endpoints will depend of the nature of the GM application and the expected impacts. For example, natural enemies, competitors or plant or animal hosts of the target species may be affected by a GM insect application with the aim of population suppression and, especially, replacement. Focal non target species could be defined as assessment endpoints, either on the basis of literature reviews or on the basis of pre-release habitat analyses. It must be noted that focal non-target species are likely to differ, for the same GM insect, between suppression and replacement releases because suppression releases generally involve only high numbers of adults (usually sterile

males) while, in replacement releases, all development stages will be present in the environment, for an undefined period of time. Measurements endpoints would then be, e.g. the absolute abundance of the non-target species or, for natural enemies, parasitism/predation rates. In suppression strategies involving the release of sterile GM insects, the impact on focal non-target species should be assessed through experimental releases. Measuring the impact of replacement releases is more difficult because experimental releases cannot be made in the open field. Impact assessment should then be based on releases carried out in other regions in similar conditions or in contained conditions.

Laboratory feeding/parasitism or competition experiments can also be carried out to test whether modified products expressed in the GM insect harms the natural enemy or the competitor in any way. Questions concerning horizontal gene transfer with natural enemies are also addressed in section 4.2.2.

If other species will be indirectly affected by population suppressions or replacement is difficult to assess, albeit the procedure should be similar. Focal interacting species should be identified in the released environment, which can be made on the basis of literature reviews or pre-release habitat analyses. An example of assessment and measurement endpoints may be an alternate host or prey of a potentially affected parasitoid or predator, and its abundance in the field.

Step 3: Exposure characterisation

To assess whether GM insects may cause harm to NTOs and the ecological functions they provide, the ERA should identify exposure pathways through which GM insects may harm the environment.

If potential adverse effects on NTO or ecosystem services and ecological functions are identified in the problem formulation, an exposure characterisation should be conducted. Therefore a detailed description of the temporal and spatial characteristics is needed.

Releases can comprise single or repeated (in one or consecutive seasons) releases (suppression strategy) or an establishment of GM insect populations (replacement strategy) in a certain area. To describe the temporal dimension of the release information on the expected number of released GM individuals, the sex ratio of released GM insects, the expected duration of presence of the GM insect after release, the number of releases per vegetation period and, whether continuous releases are planned over longer time periods in the same area are essential.

The spatial characteristics of a release are related to the release area and factors which might influence the potential dispersal of GM insects. Applicants should describe in detail the habitat types, the climatic conditions of the expected release areas and provide information on the mobility of the TOs. Furthermore the applicants should consider whether the dispersal of the GM insect is restricted by geographical or climatic barriers. Applicants should assess whether these factors might isolate the release area from other habitats or whether the GM insect will move into areas outside the intended release areas.

The intensity of interaction between TO and NTO might impact the exposure of NTO e.g. importance as prey or host of a released GM insect species for a predatory or parasitic natural enemies. However this aspect will be closely related to the number of released GM insects, the range of prey/hosts of the natural enemy, and the availability of alternative hosts or prey.

In particular for the assessment of potential long-term effects, applicants should consider factors in the exposure characterisation which might change over longer time periods such as climate (see section 3.6).

This is of particular importance for releases following a replacement strategy. Local climatic differences such as temperature, precipitation or seasonality in different receiving environments might impact life history traits of GM insects such as survivorship or growth rates. Furthermore the consequences of climate change and its impact both on the suitable “climate envelopes” of a particular

species as well as suitable habitats should be considered in the ERA. Evidence suggests that there has been significant latitudinal and altitudinal range expansion or retraction within the EU across a wide variety of species due to climate change. Such responses to longer term abiotic changes may affect the conditions necessary for establishment and spread of the GM insect.

Step 4: Risk characterisation

Based on the assessments in Steps 2 and 3, applicants should estimate each identified risk that a GM insect will cause to NTOs. They should consider both the magnitude of the effects detected and the likelihood of their occurrence. Applicants should summarise the outcomes of the ERA and consider adverse effects on NTOs, as outlined in Step 2 Hence, applicants should conclude on the risk for intended and unintended effects on NTOs taking into account the focal species affected as well as any impact of this effect on ecosystem services provision in the environment. The impact of the identified risks will be contingent on a combination of the specifics of the GM insect and its life history traits relative to NTOs, the receiving environments and, in particular, its intended uses. Therefore, applicants should provide an assessment of the range of effects based on collected data and other relevant information that describes the GM insect's possible spatial-temporal interactions with NTOs and the environment (see section 4.2.4).

Considering the range of 'receiving environments - GM insects' combinations, applicants should characterise the risk (a) in the immediate receiving environments and (b) potential habitats where the GM insect could spread and where relevant exposure of NTOs may occur. Quantification of risk and, in particular, its uncertainty shall be provided in relation to each selected assessment endpoint. Additionally, up-scaling of data from modelling, lab, semi-field and field trials to landscapes needs to consider the expected adoption rate of the GM insect. The conclusions of each risk characterisation and associated uncertainties should be described.

The ERA of potential adverse effects on NTO is linked to significant level of uncertainties from different sources. Besides the sources of uncertainty described in section 3.7, particular aspects regarding the ERA of potential adverse effects on NTO should be considered: (i) Ecological functions of specific species and their complex biotic or abiotic interactions are not understood in all details. Therefore it is uncertain if every potential effect or exposure pathway was considered in problem formulation. (ii) The methodologies for testing potential effects on NTO are limited. The implementation of field trials will not be acceptable in all cases, because it might be impossible to eradicate the released GM insect population, if an adverse effect is identified related to releases in particular applying replacement strategies. (iii) It is not feasible to simulate the complexity of the receiving environments in laboratory test, semi field test or modelling. It is possible that factors, which were not considered in such test systems, potentiate or elicit potential adverse effects on NTO. Consequences of the decrease or eradication in population size of a certain species or the replacement of wild population by GM insect populations might not be exactly predictable. Therefore the ERA should be completed by a comprehensive uncertainties analysis (see section 3.7).

The environmental consequences of the combined impacts on NTOs by the GM insect should be considered in the different receiving environments. The conclusions of the overall risk characterisation of NTOs and associated uncertainties should be described. Applicants should fully consider the consequences of the identified adverse effects on NTOs when considering risk management measures.

Step 5: Risk management strategies

In cases where risks due to the release of GM insects on NTO or ecosystem services and ecological functions have been identified and characterised in the ERA, applicants should propose appropriate risk management strategies. These strategies should be designed to minimise undesired interactions between GM insects and NTO to a level considered acceptable by risk managers. Applicants should indicate the efficacy, reliability and expected reduction in risk associated with these strategies.

Essential tools for risk management include successful implementation of standard operating procedures (SOP) and quality control systems. These should prevent uncontrolled releases in receiving environments which were not adequately assessed in the ERA and might result in adverse effects on NTOs. Additionally applicants have to provide appropriate mitigation plans (such as stand-by control capacity) in case unintended or unanticipated adverse effects on NTO or ecosystem services and ecological functions are identified after the release of GM insects. This is of particular importance when applying replacement strategies. Specific mitigation measures will depend on the biology and ecology of the released GM insect as well as the receiving environments. Potential measures might be the use of traps including pheromones where appropriate, control and destruction of reproduction sites or the area wide use of insecticides to decrease or eradicate the population of GM insects. The environmental consequences of mitigation measures should be evaluated and be proportionate with the identified risk of the GM release.

Comprehensive/intensive monitoring is essential to evaluate the efficacy of the mitigation measures as well as being of high importance in minimising risk due to a lack of experience and the problems in conducting large scale releases which will not have been fully assessed before approval.

Step 6: Overall risk evaluation and conclusions

Applicants should provide an assessment of the range of effects on NTOs likely to occur in relevant EU receiving environments based on the collected data and other relevant information. Risk assessment should determine (1) the possible mechanisms of impact of the GM insect on populations of other, non-target, organisms; (2) the likelihood impact and environmental consequences of adverse effects arising from the GM insect and/or sustained hybrid populations in managed and natural ecosystems on ecosystem services and ecological functions; (3) the levels of uncertainty associated with the effects and their consequences (4) what risk management measures may be required to mitigate any harm or uncertainty associated with changes to NTO populations, (5) why the impacts of the management measures and any anticipated or unintended changes to NTO populations, together with their uncertainty, are considered acceptable.

4.2.5. Environmental impact of the specific techniques used for the management of GM insects

There is a requirement in Directive 2001/18/EC (EC, 2001) to assess the environmental impact of the specific management and production systems associated with a GM animal compared with a non-GM animal.

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

Directive 2001/18 (EC, 2001) requires an assessment of the possible immediate and/or delayed, direct and indirect environmental impacts of the management practices used in the different receiving environments in which the GM insect may be produced and released, and whether these techniques and practices are different from those used previously in the comparable non-GM insect system. It is likely that the management practices will differ in their production and following the release of GM insects, so that the potential harm associated to the specific differences in management should be appropriately assessed. For example, suppression of the population of an insect disease vector or agricultural pest may result in the establishment/increase of previously absent (or present at a lower density) disease vector/agricultural pest species that may play a significant role in disease transmission, endanger human health or agricultural production and therefore require additional controls (e.g. different use of pesticides with different environmental load).

The assessment will involve comparing the production, management, control, pesticide treatments, etc.

Examples of possible scenarios, related to the production and release of GM insects in agricultural and vector management systems that may lead to a hazard are:

- GM insect release management may require exploitation of different environmental resources and use of different management and control/recovery systems which have novel environmental impacts. For example, additional applications of pesticides may be needed to manage programme failures and to control the untransformed insects. These may cause novel environmental loads and/or decrease of sustainability of the system;
- GM insect production units may be newly established in areas where they do not currently occur and thus have novel impacts in terms of resource usage and waste production;
- GM insect production units may be increased in scale, in use of resources or in outputs of effluents;
- GM insect production units may have new confinement requirements and measures to prevent or respond to escapes (e.g. GM pollinators in glasshouse);
- Introduction of GM insects into an existing system may alter the natural regulating mechanisms (e.g. predation, parasitism) and change previous management practices;
- Altered management and control measures of other (secondary) vector or pest species, such as increased pesticide use or other disruptive controls, that arise as a consequence of the control of the primary target vector or pest species.

Applicants should identify the relevant assessment endpoints (see section 2.1.1), associated with the aspects of the environment that need to be protected from adverse effects due to changes in management practices (e.g. biodiversity, natural regulating mechanisms).

Therefore, any relative potential adverse effect to the environment resulting from these changes in management and production practices associated with the GM-insect should be identified, taking into account the various protection goals, such as the sustainability of production systems (e.g. insectariums, crops, orchards, glasshouses), impacts on other biota and/or protection of human and animal health (e.g. vector borne diseases).

Experiences from current agricultural (e.g. mass release of parasitoids and predators, bumblebees for glasshouse pollination, integrated pest management according to the principles of sustainable use of pesticides) and vector management practices (i.e. integrated vector control) provide useful information and serve as comparators in the management practices assessment. Alteration to management practices might provide both environmental benefits as well as harm so that the net environmental impact of the overall production system needs to be considered.

Step 2: Hazard characterization

Hazard characterization is defined as the qualitative and/or quantitative evaluation of environmental harm associated with the hazard as set out in one or more hypotheses derived from problem formulation. Based on the identified hazards in Step 1, applicants are requested to characterise the potential changes to current production and release management practices which are foreseeable in the near future, through the definition of measurement endpoints and the description of appropriate methods and associated criteria of analysis.

Specifically, hazard characterization for production systems and release management may be supported by:

- literature review;
- selection of relevant case-studies;
- scenario analysis, modelling and related approaches;
- where appropriate and feasible, experimental studies at appropriate scale and statistical power.

Since GM insect production and release management are defined by the specific practices and tools used, and they may change over time, applicants should also consider any reasonable expected difference in management practices for a relevant time horizon.

Step 3: Exposure characterisation

The aim of the exposure characterisation is the quantitative estimation of the level of exposure of other biota and the environment to the changes in production and release management of the GM insect.

The exposure to production and release management should be interpreted in a range of spatial and temporal scales (e.g. the size and location of the area of release, duration and timing of the release). Depending on the management modifications, their location, the duration and scale of the changes, these production and release management changes may expose:

- natural or semi-natural environments and their associated biodiversity (e.g. protected crop systems, open agricultural fields, forests, water courses);
- populated urban and rural areas at various scales.

Applicants may consider a scenario analysis for the range of cases and should justify that the selected scenarios cover the range of production and release management practices which may occur in various receiving environments. Validated models (e.g. the use of mathematical models on mosquito vector control dynamics, see Yakob et al., 2008; White et al., 2010 and Alphey et al., 2011) may be used to support the scenario analysis and complement applicants statements on exposure characterization.

Step 4: Risk characterization

Risk is characterized by combining the magnitude of the consequences of each hazard and the likelihood of the consequences (EC, 2002). Applicants should characterize the relative risks for each hazard, related to changes in GM insect production and release management practices. The scenario approach, covering representative situations that may be encountered, should indicate the circumstances that may lead to specific GM insect-related management practices causing greater, similar or lower adverse environmental effects than the current agricultural and vector management practices they are likely to replace. The conclusions should take into account any uncertainties identified during the risk characterisation.

Step 5: Risk management strategies

When the risk characterization (Step 4) identifies risks in production and release management practices, compared to non-GM related outcomes, then applicants should propose measures to mitigate them. These risk management strategies should aim to reduce the risk associated with the management of GM insects to an appropriate level of protection and should consider identified areas of uncertainty (see section 3.7). The efficacy and feasibility of each proposed management strategy in the relevant receiving environments should be evaluated by applicants and, where appropriate, the consequent reduction in risk should be quantified.

Where GM insect management and production systems have been refined to minimise adverse environmental impacts identified in previous sections of the ERA, the efficacy as well as the impact of the measures should be determined. For example, if management systems are implemented to minimise or prevent production of fertile male and female mosquitoes, their environmental impacts should be considered as well as the efficacy of these systems to manage risks identified in other sections of the ERA (e.g. see section 4.2.6).

Step 6: Overall risk evaluation and conclusions

Applicants should assess the overall environmental impact of the production and release management systems for GM insects, considering both direct impacts and also indirect consequences of these impacts in various receiving environments. The efficacy of proposed risk management strategies and all associated uncertainties should also be taken into account.

4.2.6. Impact on Human Health

GM insects to be released in the environment in the near future are likely to cover the following uses (for further details, see the introduction to section 4.2.3):

- managing agricultural pests,
- controlling insects vector of human diseases,
- contributing to the enhancement of production systems.

An assessment of impact on human health, prior to the deliberate release of GM insects, should be conducted. Applicants should provide information, specified in Annex III of Directive 2001/18/EC, to evaluate whether the GM insects present a new hazard for human health. Applicants should consider both immediate and delayed effects on human health resulting from potential direct and indirect interactions with GM insects. This includes the risks for workers working with, and members of the public coming into contact with GM insects. Applicants shall follow the six-step comparative approach promulgated throughout this document on a case-by-case basis (see section 2.1). It should be recognized that impact on human health caused by accidental ingestion of GM insects is not considered by this section.

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

As a general principle, a baseline of public health concerns caused by a specific insect species should be established before any harmful characteristics of the GM insects can be identified. The baseline serves as a point of reference, against which changes due to the genetic modification can be compared.

It is recognized that the public health relevance of GM insects can be complex: varying from species to species, resulted by intended as well as unintended changes due to the genetic modification; varies by receiving environments, depending on the presence of specific local sources and climatic influences. The concerns of human health by releasing GM insects may include:

a) Introduction of toxic effects of the GM insects and/or their metabolic products into the receiving environments, e.g. qualitative or quantitative change in the production of toxins by the GM insects when compared to their conventional counterparts.

With respect to the potential toxicity of newly introduced proteins, the potential toxicity of the newly introduced proteins should be discussed as part of the general safety assessment. Specific toxicity testing of the newly introduced proteins as such will not be required within the framework of this Guidance Document, but the introduction of proteins known to be detrimental to consumers should be discussed by the applicants and the intake should be avoided.

It should be verified whether the GM insect in question produces toxins which can cause harm to human. For a toxin-producing GM insect, any change in the toxin production profile compared with non-GM comparator should be determined.

b) Introduction of allergenic effects of the GM insects and/or their metabolic products into the receiving environments, e.g. qualitative or quantitative change in the production of allergens by the GM insects when compared to their conventional counterparts.

With respect to the potential of sensitisation and allergenicity as a result of occupational and accidental exposure to the GM insects, it should be assessed whether the GM insects have altered

allergenic characteristics as a result of the genetic modification. To this end both the direct and known indirect effects of the genetic modification to the physiology of the GM insect should be taken into account. In addition, foreseen shifts in insect populations as a result of the introduction of the GM insect, resulting in an increased number of insects that can cause allergenic reaction to humans, should be regarded in this respect as well.

c) Altered disease transmission capacity to humans due to the physiological and/or behavioural changes as a result of the genetic modification, e.g. GM insects with altered vector competence for some pathogens or biting host-preference.

Applicants should assess whether there is an increased capacity to cause human disease from the GM insect that may be a more efficient disease vector.

Possible physiological and behavioural changes induced by the genetic modifications should be considered and discussed as part of problem formulation.

d) Emergence/selection of new pathogen strains with different virulence to humans as a result of genetic manipulation and/or mass rearing procedures of GM vector species.

Pathogens or parasites may find favourable condition to infect the GM colony. This aspect should be discussed, as well as whether this change would make GM insects becoming pathogenic to humans.

GM vector species developed for refractoriness to a human pathogen may induce a selection in the pathogen itself which may results in higher virulence (i.e. dengue).

e) Hazards deriving from possible malfunctioning of the GM release technology:

- Possible adverse effects due to unintended/accidental field release of females in male-only SIT. Malfunction of the male-only SIT strategy can enhance significantly the proportion of females and therefore the biting activity of the vector population. In case of a preventive release strategy the release of fertile females could lead to the establishment of a novel active population. Both situations would impact negatively on human health.

- Possible adverse effects due to release of “low quality GM insects” or non-GM insects (e.g. increased human biting rate or disease transmission capacity).

f) Loss of immunity in the human population and reliance on continued long-term positive effects of suppression of a vector species.

Long-term suppression of vector population may induce human behaviour that increases economic, environmental or social vulnerability to the TO, particularly in the event of any withdrawal or failure of the SIT strategy. For example, people may become complacent about environmental measures to prevent mosquito development once a successful GM based suppression programme is functioning, making the impact on human health of any failure more serious than it may have been. Such self-induced vulnerability could also apply to other forms of insect control as well.

g) Unintended/accidental release/escape of GM insects during stocking or transportation into the environment.

Applicants should consider the scenario of escape happening out of the authorized/intended target area or in an area where the target species is not present.

Step 2: Hazard characterisation

In line with the Directive 2001/18/EC (EC, 2001), information, required in Annexes III A and IV, shall take into account the diversity of sites of use of the GMO as or in a product, and shall include

information on data and results obtained from research and developmental releases concerning their impact on human health and the environment.

Hazards identified in Step 1 are further discussed as examples of hazard characterisation:

a) Introduction of toxic effects of the GM insects and/or their metabolic products into the receiving environments.

For the toxin identified in Step 1, whenever feasible, a dose-response should be established between the quantity of toxin and the degree of harm.

b) Introduction of allergenic effects of the GM insects and/or their metabolic products into the receiving environments.

On the basis of the hazard identification potential altered allergenic characteristics of the GM species will be established in this phase. This assessment of allergenic characteristics will be performed for the newly introduced proteins, but also for any known indirect effects of the genetic modification that may lead to altered constituents that may alter the allergenic profile of the GM insect.

It should be verified whether the source of the GM trait is allergenic. To demonstrate the safety of the newly expressed proteins and known altered constituents in the GM insect's physiology, applicants should provide an up-to-date search for homology of the amino acid sequence of the introduced proteins and known indirect effects, i.e. altered constituents, to known allergenic substances (see also EFSA, 2010d). The database(s) and the methodology used to carry out the search should be specified. If any indications of potential allergenicity are found, additional studies may be required, this will need to be assessed on a case-by-case basis.

c) The potential alteration of the disease transmission capacity to humans due to the possible physiological and/or behavioural changes.

Applicants should determine whether vector competence of a GM insect for specific pathogenic agents is changed versus the non-GM comparator species, thus increasing the risk to cause human disease(s), or alterations of biological parameters influencing disease transmission capacity (e.g. biting behaviour, longevity) have been produced in the GM species. To establish the dose-response relationship between the pathogen load and the degree of health concern, medical incident report on human should be systematically review at the first place, sometimes it may be needed to conduct laboratory animal experiments.

Specific tests on vector competence, host-feeding preference might be required within the framework of this Guidance Document.

In case a replacement strategy is proposed, applicants should assess precisely possible physiological and/or behavioural changes induced by the genetic modifications and verify their relevance for human health. Specific comparative tests should be conducted on the vector competence, on the host-feeding preference and on the longevity of the new GM insect versus the non-GM comparator species. Vector competence tests should consider the most important human pathogens already present or considered at risk of introduction in the target area. Behavioural tests should be focusing on human versus other animal preference in blood-feeding. Both, vector competence and behavioural tests should include the vector longevity parameter.

d) Emergence of pathogens transmissible to humans as a result of genetic manipulation and/or mass rearing procedures.

Pathogens or parasites may enter the mass rearing facility, find favourable condition to infect the GM colony and enter in contact with humans when GM insects are released. Possible ways of GM insect infection (e.g. blood used to feed them) should be identified and measures to prevent the

possible GM colony infection adopted. These include SOPs to check for the presence of pathogens in the rearing primary products and in the GM insect itself. Such risk of infection could also apply to the mass rearing of non-GM insect as well.

Applicants should consider this hazard and propose methods to quantify the impact of genetic modifications on the risk of pathogen infection.

e) Hazards deriving from possible malfunctioning of the GM release technology.

When male-only SIT strategy is used, data obtained during the development of GM insect should allow the estimation of the proportion of male and female in the GM insect released stocks. The degree of harm should be discussed in relation to the possible presence of biting females in the released stocks. The same principle should be applied to evaluate the degree of harm due to the release of "low quality GM insects" or non-GM insects.

f) Loss of immunity in the human population and reliance on continued long-term positive effects of suppression of a vector species.

Such hazard is a clear example of delayed effects, and should be considered by the applicants. Mathematical modelling can be useful in this case, using insect distribution maps and epidemiological data.

g) Unintended/accidental release/escape of GM insects during stocking or transportation into the environment.

This hazard is more an exposure issue. When realistic scenario is not possible to establish, applicants are recommended to evaluate the hazard under worst-case scenario.

Step 3: Exposure characterisation

For the exposure assessment generally a tiered approach will be followed: if any hazard is identified, the exposure to this hazard will need to be determined.

In the case of identified toxins or potential allergens the exposure to the respective toxins or allergens will need to be determined. In this case the expressed levels of the toxins or potential allergens in the GM insect (or other insects with known allergenic traits that have increased as a result of the introduction of the GM insect) will need to be determined as well as the exposure of relevant exposed groups to the insects of interest in this perspective (producers, transporters, field technicians, etc.). In this case data may be required from applicants with respect to the different identified exposure routes (e.g. via dermal or inhalatory exposure).

The possible impacts of GM insects on human health may happen in different receiving environments and under conditions of different intended uses for the GM insects. Basically, agricultural pest and human disease insect vector control could be achieved by large scale and temporally repeated releases of GM insects (population suppression and preventative as well as permanent and temporary replacement releases). Production enhancing GM insect modifications and production (mass rearing) of GM insects to be released are likely to be used under contained/captive conditions. These differences in spatial scale of the releases will result in different exposure pattern. The implications of these exposure patterns should be considered (see section 4.2.3). Exposed human subjects includes operators handling GM species (i.e. rearing and delivering operators), general population such as residence following an intended release of GM insects into the environment, or general population throughout an unintended/accidental release outside of the target releasing area. For example, applicants should compare the aforesaid workers and general population with those producing, processing or coming into contact with non-GM insects. The comparisons should be made under similar working conditions, typical for those workers. A conclusion is required of the overall risk on human health.

Applicants shall assess the conditions of transport, storage and field release of the GM insects in order to assess the occupational exposure with respect to potential altered allergenic characteristics of the GM insect. In this respect both allergenicity via dermal exposure or via the inhalatory route of the GM insects should be taken into account.

The risk from dermal contact during handling GM insects should be assessed by operators and members of the public passing by or in the vicinity of those insects. Such a risk should be considered particularly for GM insects developed for public health purposes, e.g. refractor to pathogens infection.

Step 4: Risk characterisation

On the basis of hazards identified and characterised and considering appropriate exposure routes and patterns, applicants should characterise the risks of the release of GM insects on human health. Since the type of genetic modification and the release scale will be different according to the intended use (population suppression, preventative releases, permanent and temporary replacement releases)), the exposure and subsequent risk will be case-specific. For certain risks (e.g. allergenic response by humans, pathogen transmission) the "magnitude" may range from some individuals (operators in mass rearing, handling) to higher number of citizens (e.g. disease vector release). Where precise quantitative assessment of risk is not possible, terms should be defined where possible. The evaluation for each risk should consider the magnitude of the consequences of the hazard and the likelihood of its occurrence. The uncertainty for each identified risk should be described (see section 3.7).

In general, this process can be iterative: if the risk characterisation results in new questions or in the identification of new hazards, additional questions may need to be asked and additional data may need to be provided by the applicants, until all relevant questions with relation to human health issues are satisfactorily dealt with to conclude this part of the risk assessment.

Step 5: Risk management strategies

Applicants shall develop proposals for measures intended to minimise the exposure of workers and the general public to the possible risks caused by GM insects and the expected impact of these measures should be assessed:

- operators monitoring pre-exposure (baseline data useful to answer the question whether there are problems in the health and welfare of operators),
- operators questionnaire, check up periodicity, external independent health evaluation (comparative assessment approach with same operators category working with non-GM insects),
- possible use of worker protection clothing and equipment known to be used in insect rearing facilities, pesticide application (etc) should be considered by the applicants,
- methods to reduce adverse effects on human health due to large scale release and exposure should be detailed by the applicants,
- when the risk of emerging pathogen(s) is identified, or when in the case of malfunctioning of the GM release technology, implementation of specific standard operative procedures (SOP) to prevent the possible hazard caused by these agents might be required.

Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM insects should be made taking into account the results of the ERA and associated levels of uncertainty, the weight of evidence and the risk management strategies proposed in the receiving environments.

4.3. Specific areas of risk for the ERA of GM mammals and birds

Description of the case studies

At present a relatively small number of mammal and bird species have been genetically modified, due to the high level of sophistication and the low success rate of the biotechnological techniques being adopted (FERA, 2010). Consequently the current range of mammal and bird species commercially or near-commercially available is quite narrow; therefore four case studies were chosen in order to provide sufficiently credible environmental risk scenarios that would help the applicant in identifying a large range of possible risk assessment criteria. According to a report issued by the Food and Environment Research Agency (FERA, 2010), two of the case studies, i.e. Enviropig and the flu resistant chicken, are in an advanced stage of development.

1. The "Enviropig" (*Sus scrofa*)

The Enviropig (Golovan et al., 2001a, 2001b; Golovan, 2002) has been modified to produce the phytase enzyme in its saliva, allowing the pigs to digest the normally indigestible plant phytate in their feed. This leads to a reduction in phosphorous supplementation of feed, and consequently, manure with a much lower phosphate content than that from conventional swine. Despite the possible improved food conversion and environmental effects of effluents of the Enviropig, it may require new management and production strategies that might impact adversely on the environment. An additional issue to be considered is the fact that pigs are usually farm animals but they can form feral populations (Nogueira-Filho et al., 2009) and have the added feature to cross breed with wild boars found in several areas of Europe (FERA, 2010).

2. The growth enhanced cat (*Felis sylvestris*)

Cats have been used as experimental animals for genetic modification (Gomez et al., 2007). Since cats are companion animals this case study could allow applicants to explore the environmental issues related to the animals held as companion animals. The increased capacity for growth, although not yet developed as a GM trait in cats, could be a possible future goal for breeders of companion animals and an appreciable characteristic for owners. An important issue to be considered is the cat's ability to breed with wild cat populations (*Felis sylvestris*) (Daniels et al., 2002).

3. The sterile rabbit (*Oryctolagus cuniculus*)

Most of the GM rabbits developed so far are human disease models and live bioreactors for producing human therapeutic proteins (Fan and Watanabe, 2003).Because of their intended uses they are kept in controlled and confined environments. Nevertheless, since rabbits are both an important farm species and a wild/companion animal, their release to the wider environment can be foreseen in the near future. A sterile rabbit is chosen as an example for a theoretical GM rabbit: Growth-enhanced males with early maturity and increased size would have a mating advantage compared to the wild types. These GM rabbits pass genes for sterility to their female offspring (FERA 2010). This could be seen as a useful tool to manage pest populations and a suitable case study for assessing long term effects of such a trait in rabbits.

4. The "avian flu resistant chicken" (*Gallus gallus*)

The "avian flu resistant chicken" (Lyall et al., 2011) has been modified to inhibit the replication of the influenza virus rand its packaging. When such GM chickens are exposed to the virus they still may get infected but the transmission of the virus is limited. The avian influenza resistant chicken can be a suitable model to assess potential environmental effects of pathogens.

General differences among captive, semi-captive, and non-captive GMOs relevant for ERA

Above in Chapter 1, in the section on the Scope of the Guidance Document, a classification of GM animals according to their intended uses and possible releases was introduced, with the three groups: (1) captive, (2) semi-captive, and (3) non-captive GM animals (Table 1). Captive GM animals are those that are intended to be permanently held in captivity (i.e. in containment, fenced areas, or other

4415 areas from which they are not normally expected to escape). Examples of captive GM animals include
4416 domesticated animals and companion animals held indoors, or animals in a fenced area or zoological
4417 gardens. Semi-captive GM animals are those that are under human control, but can sometimes browse
4418 freely, e.g. cattle browsing on an unfenced pasture or cats exploring their owners' neighbourhood.
4419 Finally, non-captive GM animals are intended to be directly released into the environment without
4420 human control, e.g. sterile rabbits that are released to control wild rabbit populations.

4421 Environmental risk assessment differs for these three groups of organisms, as they will be found in
4422 different receiving environments. In addition, their routes and methods of release or possible escape
4423 differ (Table 1). Specifically, captive GM animals will predominantly affect their captive
4424 environment. Only those GM animals that escape will have effects in the wild. Semi-captive GM
4425 animals will affect their captive environment during captive periods (e.g. a growth-enhanced GM cat
4426 will have effects in its owner's house while being held captive there) and will have effects in the wild
4427 during non-captive periods (e.g. when the cat is allowed to explore the neighbourhood). If the cat
4428 escapes or is released by its owner, it will also have effects in the wild. It is furthermore possible that
4429 the escaped cat enters other captive environments, e.g. other houses, and may cause effects there.
4430 Finally, non-captive GM animals have effects in the wild (but may enter captive and non-captive
4431 environments as above).

4432 Although the most dramatic effects may typically be those caused by GM animals in the wild, effects
4433 of GM animals in captivity can be important as well. It should be considered that effects in captivity
4434 also include effects on organisms that are able to move in and out of the area where the GM animal is
4435 being held captive. For example, a GM goat held captive in a fenced area will interact with wild
4436 organisms entering the fenced area. Also, effects of the GM animal on chemical substances or the
4437 geological structure of its fenced area may affect the chemistry and/or geology of areas outside of the
4438 fenced area, e.g. nutrient fluxes might be affected.

4439 The likelihood that captive or semi-captive GM animals escape into the wild differs among species.
4440 Hence, effects on the environment may be expected to differ among captive, semi-captive, and non-
4441 captive GM animals. Consequently, the ERA, including the possible risk management strategies (e.g.
4442 containment fences) (see section 2.1.5), should take into consideration these three groups of GM
4443 animals. In each of the following sections about specific ERA considerations, differences among
4444 captive, semi-captive, and non-captive GM animals should be allowed for where relevant.

4445 **Table 5:** Three groups of GM animals and associated differences in environmental effects.

	Captive GM animals	**Semi-captive** GM animals	**Non-captive** GM animals
Definition	GM animals intended to be kept in captivity	GM animals under human control but not always in captivity	GM animals directly released into the environment without human control
Examples for GM mammals or birds	Chicken (except free-range); pigs; fenced mammals; caged birds; pets held indoors	Cats; cattle or goats sometimes browsing on an unfenced pasture	Rabbits released to control wild populations
Environmental effects	Environmental effects of captive GM animals	Environmental effects during captive periods Environmental effects during non-captive periods	
	Environmental effects of escaped GM animals	Environmental effects of escaped GM animals	Environmental effects of released GM animals

4.3.1. Persistence and invasiveness of GM mammals and birds and vertical gene transfer to wild and feral relatives

The production and keeping of GM mammals and birds provides substantial opportunity for species to persist and invade in the environment. The impacts of biological invasions generally, are small initially, often latent and then increase over time. However, these impacts have the potential to continue indefinitely if remedial action is not taken. Concerns relating to the potential persistence or invasiveness of GM mammals and birds in the environment and their potential to hybridise with non-GM relatives need to be directly addressed. In particular, the potential risks of GM species to environmental safety include (i) detrimental effects on the environment, (ii) adverse ecological interactions with other organisms, (iii) disruption of biotic and abiotic processes, and (iv) environmental impact caused by the recapturing of individuals and/or ameliorating invasiveness. In this regard, it is vital that the characteristics that could influence the ability of GM mammals and birds to persist and become invasive are investigated and addressed sufficiently.

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

In this section, the applicant shall address the potential for GM mammals and birds to escape, persist, and become invasive. A GM trait may provide individuals with specific advantages to persist and invade; particular attention should be paid to how the GM traits may modify these abilities in populations of species other than their non-GM comparators. In the wild, mammals and birds fulfil key ecological functions (e.g. as browsers, predators, pollinators, seed dispersers, pathogen reservoirs, and generalist habitat modifiers). In cases where non-GM taxa have been released outside their native range (i.e. as 'exotic species') and have persisted and become invasive, there are numerous examples of detrimental changes/disturbances to habitats and food webs resulting in reductions to native biodiversity and disruption of ecosystem functioning (Keller et al., 2011).

It is highly desirable that GM taxa are not allowed to persist and become invasive pests in the wild. Therefore, problem formulation should focus on the potential of a GM bird or mammal to be more persistent or invasive than the non-GM comparator, and on the potential for hybridisation (i.e. vertical gene transfer) with compatible wild and domestic relatives whose offspring may contribute to the decline of native genetic diversity and, e.g. through increased hybrid vigour, make them more persistent or invasive than comparable native species.

In order to cover all relevant receiving environments of the GM species and its compatible relatives, risk assessment should consider not only the place in which the species is housed but also all the proximal environments (both undisturbed and disturbed) to which the species could move. Risk assessment should also consider all aspects of housing, transportation, storage, handling and processing that could lead to the persistence and invasion of the species outside of its intended location.

To date, most GM animals have been produced for improvements in productivity, disease resistance, feed conversion, prolificacy, and production of pharmaceuticals (Melo et al., 2007), as well as for research into development and disease. The FERA report (2010) identified only 15 species of mammals and birds that have been the subject of genetic modifications, and the majority of these were for proof-of-concept experiments and/or used for method optimisation. However, it is likely, that in the future, selection for environmental tolerance, as well as companion animals and sentinel species (i.e. environmental indicator species) and GM animals for pest management will also be a consideration. Application of recombinant DNA to produce GM companion animals is hypothetical at present, but may become more widespread when more efficient gene-transfer technologies are developed and specific genetic traits can be targeted. These species are often commensal with humans (as are genetically modified model rodent species), and there are many examples of how their non-GM comparators have become global exotic pests throughout human-modified environments (Long, 2003).

Identification of the criteria that may influence the persistence and invasiveness of a GM mammal or bird species requires consideration of both the species intrinsic traits and the potential influence of the specific genetic modification on these traits. In addition to the species' inherent life history and ecological traits, phenotypic plasticity (behavioural, morphological and physiological flexibility), the characteristics of the receiving environments and the potential rate of introduction (see also definition of propagule pressure in the glossary) can be important (Lockwood et al., 2005; Jeschke and Strayer 2006; Lockwood et al., 2009; Keller et al., 2011). The nature and consequences of any gene transfer will vary depending on the opportunities for breeding and the nature of the genetic modification.

Basic information is required that enables characterisation of the GM mammals and birds and the identification of biological and ecological differences between them and their non-GM comparators. The following questions should be answered by all applicants, including those for the import of GM species. These questions will be used to perform an evaluation of species persistence, invasiveness, and potential for vertical gene transfer. Information required for answering these questions, and testing the specific hypotheses formulated in them can be extracted from both direct data generated by the applicants during the development of the GM animal and/or from the scientific literature. Despite the acknowledged difficulty of experimentation with mobile mammals and birds to study persistence and invasiveness, applicants should consider the feasibility of experimentation to supply estimates of particular important parameters. If applicants use data from outside the EU, they should justify why these data are relevant for the range of potential receiving environments in the EU.

Question 1: Persistence in the EU

A relatively high percentage of introduced animal species are able to establish themselves (persist in novel environments) and spread (become invasive). The exact proportion varies between species (taxonomy) and the receiving environments (ecosystems and habitats), but the values for non-GM mammals and birds generally scatter around 50% (Jeschke and Strayer, 2005; Jeschke, 2008). These values should only be considered as rough estimates. Among non-GM species, the establishment of exotic animals can exceed that of exotic plants (Jeschke and Strayer, 2005) and persistence of exotic mammals has been found to exceed that of exotic birds (Forsyth and Duncan, 2001; Jeschke, 2008), so between these classes different predictive rules are likely to apply.

Question 1: Can the species persist under EU conditions?

The main source of data is expected to be provided from both literature sources, modelling, where applicable and any experiments conducted during the development of the GM trait.

Species-specific background information is required describing the biology of the parental species including traits specific to its (i) reproductive biology, (ii) survival, (iii) dispersal, and (iv) any characteristics specific to different receiving environments. Information should be provided on the dietary range of the species and its ability to overwinter in the EU. Any experimental data that is available confirming physiological and ecological tolerances should be included here.

In cases where sufficient data is available bioclimatic models (or species distribution models; e.g. Jeschke and Strayer 2008 and references therein) can be used to describe the 'ecological niche' of a species and to provide a probabilistic estimate of whether a given region has a suitable climate (or additional abiotic and biotic factors) for a species to persist and become invasive. Only in the case where direct information on the GM parental species is not available, is it permitted to fulfil information requirements using taxonomic and ecological niche-surrogate non-GM species (FERA, 2010). Taxonomic surrogates are directly equivalent in terms of the taxonomic origins of the GM species. Ecological-niche surrogates, though taxonomically different, would potentially fill similar ecological niches and manifest many of the same potential trophic interactions in the environments to which they have been released as GM-species of taxonomic equivalence (see section 3.4). Using ecological surrogates that have some taxonomic proximity to the GM species in question means that some taxonomic issues can still be evaluated, such as likelihood of persistence (where the taxonomy of

a species plays an important role). Information from the native geographic range of the parent species should always be included. This is especially important where the GM species is produced outside the EU but may experience similar climatic conditions when transferred to the EU. Ideally, models need to consider the important biotic factors (presence/absence of competitors, predators, parasites, prey/food species, and mutualists) as well as abiotic factors (temperature, rainfall, seasonality).

In all applications, a Population Viability Analysis (PVA) should be provided to assess persistence and invasion potential, and the ability to control the species if it were to become a pest (Boyce, 1992). Further guidance on modelling is provided below in step 2 Hazard characterisation.

Question 2: Hybridisation

Question 2: To what extent can the GM mammal or bird species reproduce and hybridise with non-GM animals of the same or different species under EU conditions?

The main source of data is expected to be provided from both literature sources, modelling, where applicable and any experiments conducted during the development of the GM trait. The ability to hybridise with other domesticated or wild species occurring in the EU and the biology and ecology of these relatives should be considered. The presence of escaped GM-conspecifics, feral non-GM conspecifics, existing domestic animals, or wild ancestral parent species provides opportunities for the vertical transfer of recombinant DNA into offspring. The likelihood of reproduction resulting in sterile or reproductive offspring should be considered, as this will result in different risks. The applicant should indicate whether and which relevant recipient organisms that could potentially acquire the recombinant DNA by hybridisation are present in the receiving environments. For each recipient organism, the applicant should indentify and describe the environmental conditions in the receiving environments that could affect selection and the long-term establishment of populations arising from such hybridisation.

The applicant should also consider the biological and ecological consequences of potential heterosis or hybrid vigour carried by the hybrids in relation to the non-GM domestic animals, feral or wild animals.

Questions 3 and 4: Relation to non-GM comparators

Focal species-level characteristics that are linked to the persistence and invasiveness of species are its: (i) rate of population growth; (ii) ability to exceed a positive density-dependence threshold, or allele effect; and (iii) broad environmental tolerances (Blackburn et al., 2009). In the case of GM mammals and birds, there will always be the initial characteristics of the parent species, which may then be influenced by the specific genetic modification. Where this modification increases any of the components of the fitness of the organism (e.g. fecundity, survival, competitiveness), this will increase the risk.

Question 3: Will the GM trait confer increased fitness to the resulting population that could allow it to persist or invade more than that of their non-GM comparator?

The main source of data is expected to be provided from both literature sources and any experiments conducted during the development of the GM trait.

It is noted that in GM animals, a number of traits directly related to the characteristics of persistence and invasiveness may be actively selected for. Applicants must evaluate whether feral GM animals or compatible relatives containing the GM trait will exhibit changed fitness outside of any relevant production system. Applicants should also carefully consider the effect of heterosis or hybrid vigour which might provide hybrids (offspring) with a genetic advantage that could affect fitness. If fitness is enhanced, populations may increase; if fitness is reduced, outbreeding depression may occur. Enhanced fitness of GM offspring, and their succeeding generations, or of introgressed wild relatives,

may create feral GM populations, or hybrid populations in different habitats. These populations may change the diversity, abundance, and composition of a range of fauna and flora. Potential environmental effects from such changes are further considered in step 2.

Examples for traits related to persistence and invasiveness are provided below and should be considered further for the GM animal and compatible relatives containing the GM trait.

Growth - Individuals that can grow faster than their wild type comparators may have a competitive advantage in foraging and mating due to their larger size and earlier maturation. Growth hormone over-expression can cause significant enhancement of growth rate, which can result in large differences in size at a particular age and a compression of the species' life history. The growth-enhanced cat is a case study example of a species that may, in the wild, have increased foraging ability and mating opportunities over non-GM comparators.

Dispersal - Dispersal is one of the underlying requirements for a species to be invasive. Natural dispersal ability will influence how quickly a species can spread and the subsequent extent of its impact; genetic modification may have the effect of increasing or decreasing the organism's natural rate of spread. A high dispersal ability will increase the magnitude of impact. Dispersal ability will also influence how readily a species can be contained or removed from the environment. Species that are highly mobile (e.g. pigeons), able to utilise a variety of habitats, and are suited to the environment are more likely to have a high dispersal rate. Dispersal behaviour can be directly affected by transgenesis, which is particularly relevant if GM animals are more likely to explore novel habitats compared with genetically wild comparators (Sundström et al., 2007). Increased herding behaviour, as a GM trait, while perhaps reducing dispersal per se, may also lead to a greater propensity for persistence if mating success and survival is influenced by positive density dependence.

Reproduction - If mating preference for the GM individuals is increased over that of wild type comparators, or if GM traits are related to increased fecundity, then this will confer considerable advantages for the hybridisation and persistence of a transgene within a wild type population (Aikio et al., 2008). Species with a larger potential pool of mates from extant feral or wild populations are more likely to persist in the environment. This may be enhanced by behavioural traits such as social herding. Parental species with environmentally co-occurring wild populations also increases the opportunities for hybridisation (see Question 2). Additionally, some species with wild or feral populations (e.g. pigs) are known to break into fields to breed with domesticated animals. Some domesticated species (e.g. cats) which interact freely can easily find mates in both domesticated and feral populations.

Development and survival - If GM traits confer individuals with improved survival, they may be able to persist in greater frequency and in a wider range of environmental conditions than their wild comparators. Transgenic expression of a bovine lactalbumin construct in sow's (*Sus scrofa*) milk resulted in higher lactose contents and greater milk yields, which correlated with a better survival and development of the piglets (Wheeler et al., 2001). Disease tolerance and/or resistance (e.g. avian flu resistant chicken case study) may increase survival in the wild. Disease resistance may also provide increased opportunity for persistence of small (escaped/released) populations, allowing them to escape some of the environmental and demographic vagaries of a positive density-dependent threshold. Companion GM species produced for longevity and or neoteny (e.g. dogs and cats) will provide extended opportunities for escape and vertical gene transfer with non GM relatives.

Question4: Will the GM trait alter the habitat and/or the range of the GM species or hybrid populations?

The main source of data is expected to be provided from both literature sources, modelling, where applicable, and any experiments conducted during the development of the GM trait.

It is important to consider the environmental matching of the GM species (see questions 1 and 2). If the GM trait confers an advantage such that the species can exceed climatic (or other abiotic factors)

limits, then the species may be able to extend its geographic range and persist at environmental extremes beyond that of its wild type comparators. This has been observed in GM fish (Shears et al., 1991). Alterations to the diet and/or habitat of a GM species may allow it greater opportunity for persistence and invasion. The Enviropig case study is an example of an increase in dietary range. Species that are produced for increased human commensality should also be noted, as non-GM commensal mammal and bird species are global invasive pests (Jeschke and Strayer, 2006).

Further requirements for modelling

Some of the major sources of information used to answer questions 1 to 4 above may concern models. In this section guidance is given for the modelling process. The population dynamics of a species can vary considerably between different environments and thus care is required to determine with any certainty that the introduction of a certain recombinant DNA will make a species invasive or not. The provision of all comparative data to address the above four questions and the accompanying model estimates for assessing climatic suitability, population growth rates, and extrapolating probabilistic risk assessments must be accompanied with suitable quality assurance and full explanation of the methodology used (see below). In particular, we highlight the following five steps to be performed (see also FERA, 2010):

i. Parameter estimation: parameters obtained from both wild and production populations should be assessed with an indication of their variability or uncertainty. Parameters that are specifically affected by the GM trait should always be presented with an indication of their uncertainty in comparison with the wild type values.

ii. Comparative data and model verification: where comparative data are presented and/or a model has been constructed, verification of the model code and algorithms should be provided.

iii. Model validation: climatic suitability and population growth models should wherever possible be validated against real data. This could involve validation against reported population growth and expansion in their native area, as an invasive non-GM species or against available field data on the GM population. If suitable data for validation are lacking, the credibility of the model behaviour and outputs should be assessed carefully in relation to any other relevant sources of evidence (e.g. qualitative evidence, expert knowledge, general principles).

iv. Sensitivity analysis: a thorough sensitivity analysis should be performed. This should account for the known uncertainty and variability in parameter estimates, and any uncertainty in which parameters the GM trait affects (see section 3.7).

v. Evaluation of unquantified uncertainties: in the case of any uncertainties that remain unquantified, it is essential to identify these and evaluate their potential impact on the outcome of the assessment (i.e. how different the true risk might be and how likely that is), so that this can be taken into account by risk managers. Practical approaches for doing this are presented in section 3.7 of this Guidance.

If either the sensitivity or uncertainty analysis has identified key parameters, the values of which have not been sufficiently well established, applicants should consider the feasibility of experimentation to supply improved parameter estimates.

Step 2: Hazard characterisation

In step 1, considerable information relevant to hazard characterisation has already been provided, to directly compare the fitness, persistence and invasiveness of the GM animal compared to its non-GM comparator. Answers to the previous four questions will have been provided through data obtained from both existing scientific literature sources and any experiments conducted during the development of the GM animal. Species distribution modelling and population viability analysis should also have been used.

In this step 2 applicants should provide any additional information relevant to an assessment of whether identified changes of the GM animals compared to the non-GM comparator which relate to persistence and invasiveness pose actual environmental harm. Despite the acknowledged difficulty of experimentation with mobile mammals and birds to study the environmental harm caused by an increased persistence and invasiveness, applicants should consider the feasibility of experimentation to supply estimates of particular important parameters.

The frequency with which the non-GM parent species has established persisting exotic populations outside its native range may provide the simplest proxy for characterising the likelihood of the risk of persistence. For example, rabbits, cats, pigs, and pigeons with global exotic pest populations (Long, 1981; Long, 2003) can be considered to pose a higher risk of becoming invasive than cattle or chickens. In addition, parental species with a longer, and more intense, history of domestication and captivity may pose a lower risk of becoming invasive than species derived from wild type parents. For example, among non-GM birds, captive bred species have a lower risk of persistence than wild caught species (Carrete and Tella 2008). Native or endangered mammal and bird species may be displaced by GM mammal and bird species, which in turn might affect trophic interactions and have consequences for other species up and down the food chain. Potential adverse environmental effects should be assessed both in production systems and in the wild. Effects of changes in fitness, persistence and invasiveness of the GM animal compared to its non-GM comparators on non-target organisms are assessed in section 4.3.5.

Step 3: Exposure characterisation

In the environmental exposure characterisation, the applicant should describe the conditions in which the GM animals are kept and what are the possibilities to move and/or escape into other environments. The three terms (captive, semi-captive, non-captive; see Table 5 in the beginning of this section on GM mammals and birds) may be used to describe different categories of captivity. For all species, the category of captivity will affect the opportunities for escape. The keeping of domestic and recreational species is largely controlled by private individuals and societies, and there are considerably greater opportunities for these species to occur/escape outside captivity.

GM species that are subject to higher levels of production are more likely to be introduced by means of escape, criminal activity (e.g. livestock rustling, semen/egg theft), or unauthorised translocations (e.g. game species such as pheasant or rabbit). Similarly, species that are more frequently transported afford greater opportunities for escape – including transport during importation as well as between different housing/rearing facilities. There is a higher risk of escape associated with species raised in multiple environments/locations at different stages of its lifecycle, e.g. pigs are often 'finished' at different premises to that at which they are farrowed. Consideration should be given to whether, compared to the parent species, the transport of the GM animal is changed (e.g. movement at a different or less manageable stage of development) in a way that may increase opportunities for escape.

It is necessary to ask whether the GM trait would alter accessibility to the environment. The risk of sabotage, resulting in the release of GM animals from containment, should also be considered here.

As well as the potential release of GM species from production facilities to the environment, species with increased commercial value (e.g. sterile rabbit or growth-enhanced cat) may be subject to the possibility of kidnapping and theft. In all cases, GM animals may be at risk from property theft if the technology is patented or not publicly available.

In addition, the exposure assessment should focus attention on a worst-case scenario (see Chapter 2, step 4 risk characterisation).

For birds and mammals, the most consistent predictor of establishment success (persistence) in a novel receiving environment is propagule pressure (Lockwood et al., 2005, 2009). Even if populations are

short-lived, a high propagule pressure would increase the likelihood of the occurrence of transient populations. For captive GM animals, potential releases and/or escapes of GM mammals and birds will likely be rare events comprising low numbers of individuals. High propagule pressure would be more likely for species kept together in large numbers, for example, domesticated livestock (e.g. cattle, sheep, horses) and game species (e.g. rabbits, pheasants) than for companion animals (e.g. cats, dogs). Non-captive GM animals, might also have a high propagule pressure. For them, optimal numbers of individuals to be released per release event, and number of release events, should be theoretically assessed before release, so that propagule pressure remains at a level that does not lead to an increased risk of persistence and invasiveness.

Step 4: Risk characterisation

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects should be made. Since there may be more than one potential adverse effect, the magnitude and likelihood of each individual adverse effect should be assessed. If a quantitative evaluation of risk is not possible, terms used in qualitative evaluation should be defined clearly. In addition, the uncertainty for each identified risk should be described as outlined in section 3.7.

The weakest link in the chain of the successive events which lead to persistence (escape, survival, reproduction and inserted gene spread) and invasion (spread, population increase, ferality) must be identified. This will help to identify the area of greatest risk.

Step 5: Risk management strategies

All GM bird and mammal species will vary in their propensity to be controlled or managed. Management strategies can be implemented to reduce the risk of persistence and invasion of the environment and the degree to which a species can be managed will influence the risk posed by that species.

The risks of persistence and invasion posed by GM species can be minimized at the outset through the careful design of GM species (e.g. form and expression of genetic alteration), proper planning and regulation for the containment of a population. In particular, full consideration must be given to reducing the propagule pressure available for escape into the wild at any one time and/or place. In addition, any mitigation measures to reduce vertical gene transfer and environmental exposure should be considered. The risk of release/escape can be reduced through appropriate levels of physical containment and fully licensed and monitored transport movements. In all situations where GM releases can be sterile, this should be considered (e.g. the proof-of-concept ‘sterile rabbit’ case study). The likelihood of persistence can be reduced through maximising the ability to detect and recover escaped individuals and reducing their ability to reproduce in the wild. This may be achieved through measures such as the tagging or marking of individuals or the maintenance of single-sex herds. The latter will also remove the risk of vertical gene transfer. The likelihood of dispersal can be reduced through improving the detection of animals through marking, or phenotypic changes including adjusting feather or coat characteristics. Further measures include the reduction of their mobility through, for example, wing-clipping. Reducing the opportunities for interactions between GM and non-GM animals will reduce the risk of hybridisation and vertical gene transfer.

The applicant should describe whether possible recombinant DNA approaches to containment of a population below a certain level (e.g. sterility, phenotypic or diagnostic marking, or impairment to movement or survival) have been considered. Diagnostic genetic markers (e.g. single nucleotide polymorphisms (SNPs), sequence information, or microsatellites) available for the GM locus and rest of genome for a GM animal could be used, so that any escape, hybridisation or introgression can be tracked quantitatively through genetic means in the wild.

Step 6: Overall risk evaluation and conclusions

It is important that applicants ensure that their risk assessment concludes on all of the following: (1) the likelihood of the GM bird or mammal persisting outside of the production environment; (2) the likelihood of the GMO invading semi-natural and natural habitats, through changes in traits specifically linked to persistence and/or invasiveness; (3) the risks of hybridisation and/or reduction of biodiversity or ecological function outside of the production environment; (4) why any anticipated harm may be considered acceptable; and (5) what risk management measures may be required to mitigate any harm.

A summary of the risk and associated uncertainty and confidence levels should be provided for all of the answers to the questions provided above and then collated to produce an overall assessment for the GM species.

The outcome of this assessment can impact the other aspects of the ERA (biotic and abiotic interactions) and should be assessed by applicants in the following sections of the ERA.

4.3.2. Vertical and horizontal gene transfer

4.3.2.1. Vertical gene transfer

Vertical gene transfer (VGT) is here defined as any process in which a gene is passed to offspring. The most common form of VGT is sexual reproduction. VGT of a recombinant DNA from a GM mammal or bird 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. The ERA should cover the full range of outcomes from VGT; these include, but are not necessarily restricted to: the offspring of animals of the same species with which the GM mammal or bird can reproduce; the offspring of feral relatives with which it can hybridise; and the offspring of wild relatives (including other (sub-)species) with which it can hybridise. Most of the potential consequences of VGT are dealt with elsewhere in this document. Hence, the impact on persistence and invasiveness and the effects of hybridisation with feral and wild relatives is discussed in section 4.3.1, above; interactions with non-target organisms are considered in section 4.3.5 and abiotic interactions in section 4.3.6, below. Applicants should consider any effects of VGT on the reproductive and the survival capacity of the GM animal itself, if not previously assessed, in section 4.3.1; similarly any effects on resources used in or provided to production systems in section 4.3.7. However, the possible effects of any loss in genetic diversity are addressed below.

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

According to Annex II of Directive 2001/18/EC, ERA should consider not only effects 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 within the EU. The proposed EU Biodiversity Strategy to 2020 (EC, 2011e) stresses the need to support genetic diversity in agriculture and forestry and the fair and equitable sharing of benefits of genetic resources. Specifically, the European Council resolved to encourage the conservation and sustainable use of genetic resources for food, agriculture, aquaculture, fishing and forestry (The European Council, 2011).

The breeding of mammals or birds with genes that are considered advantageous can result in artificial selection. This artificial selection may change the frequency with which the recombinant DNA is passed from one generation to the next. Under artificial selection, individuals with the advantageous recombinant DNA may contribute to more offspring in the succeeding generation than do others without it. Selection can therefore increase the prevalence of the recombinant DNA, and consequently the genetic variability within the population/species/group may change. Commercial breeding programs that are normally applied to non-GM mammals and birds may be applied equally to GM animals. For example, Yang et al. (2011) described the process of artificial selection of GM embryos

in the production of human lysozyme in the milk of cows. Since breeding can increase the prevalence of specific desired gene(s), it may impact on the genetic variability within populations. Loss of genetic diversity has been shown to contribute to the risk of extinction of some species (Frankham, 2005). Infectious disease is often crucial to the survival and adaptation of animal populations; the importance of maintaining genetic diversity with respect to disease defence genes is well-known (O'Brien and Evermann, 1988).

The ERA should consider the effects of loss of genetic diversity due to the introduction of a GM mammal or bird into the environment; this applies both to animals in production systems and to companion animals. The applicant should assess the extent to which loss of genetic diversity is likely to represent a hazard in principle, and should use the next two steps to characterize 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

In line with the requirements of the Guidance Document on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects (EFSA, 2012), the applicant should clearly describe the breeding strategy for both the development of the GM animal prior to commercialization and that planned post-commercialization (see also the recommendation under step 5). If there is insufficient information on the latter to enable a conclusion to be drawn then estimates may have to be derived from modelling. Applicants should indicate which generation of the GM animal will be marketed, including its zygosity with respect to the sequence actually inserted. Applicants should give details of the number of genetic backgrounds into which it is planned to breed the GM animal event post-commercialization.

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 ERA should focus on differences in breeding strategies and/or in their likely outcomes between the GM animal and its appropriate non-GM comparator. For the latter, the strategies should represent current conventional practice. It should be stressed that if there are no such differences then there is no need for the ERA to proceed to the next step.

Where possible, quantitative estimates should be made of: the likely strength of the selection pressure, the prevalence of the recombinant DNA and how this may vary through time and, similarly, the resulting genetic variability. Applicants should evaluate the magnitude of the potential harm caused by a loss in genetic diversity to human and animal health and the environment.

Step 3: Exposure characterisation

The exposure assessment should focus attention on a worst-case scenario (see Chapter 2, step 4 risk characterisation). In order to determine the likelihood of the potential loss of genetic diversity, the exposure assessment needs to take into account a potentially large uptake of the GM animal and the number of genetic backgrounds (see above) into which the trait is likely to be bred pre- and post commercialisation. 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 (Brown, 1984). Applicants should consider the potential effects of environmental stress, including disease, on selection and genetic diversity. Applicants should consider the effects of these aspects of exposure on the ability of populations to withstand further stress and to avoid local and/or global extinction.

For GM companion animals, it should also be considered that shows (e.g. dog or cat shows) are traditionally important venues where owners select companion animals for breeding purposes. If owners of GM companion animals attend such shows, their animals may mate with non-GM animals, with a long-term consequence that a specific GM trait will gain high frequencies and eventually dominate a particular breed.

Step 4: Risk characterisation

On the basis of the conclusions reached in steps 2 and 3, 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. Since precise quantitative evaluation of risk may not be possible for this hazard, terms used in qualitative evaluation should be defined clearly. In addition, the uncertainty for each identified risk should be described as outlined in section 3.7.

Step 5: Risk management strategies

Based on the outcome of the risk characterisation, 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. For example, applicants might ensure that GM trait is bred into a sufficiently high number of genetic backgrounds pre- and post-commercialization to avoid loss of genetic diversity.

Step 6: Overall risk evaluation and conclusions

Identified knowledge gaps should be briefly summarized. The applicant is required to conclude on the overall risk and provide a clear statement on the presence or absence of conditions facilitating the loss of genetic diversity and its likely consequences, taking into account any risk management strategies.

4.3.2.2. Horizontal gene transfer

Horizontal gene transfer (HGT) is here defined as any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. The evaluation of the impact of HGT from GM mammals/birds includes analysis of the potential of transfer of recombinant DNA 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 must be evaluated. Although the extent of environmental exposure is likely to differ between applications for environmental release and import for processing, the issues to be considered in the ERA are expected to be similar.

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

HGT from GM mammals and birds is expected to be rare. However it remains largely unexplored. Rare events may have consequences for human and animal health and the environment and are therefore considered in the ERA. This ERA will depend on the exposure routes, the potential for horizontal transfer, the trait conferred by the recombinant DNA, the prevalence of similar traits in exposed environments and the nature and range of potential consequences (EFSA, 2009b). The problem formulation needs to consider assessment endpoints being representative of the aspects/parts of the receiving environments that need to be protected from adverse effects. Both multicellular eukaryotes (a) and microorganisms (b) should be considered as potential recipients.

(a) HGT from GM mammals and birds to other mammals and birds and to other multicellular eukaryotes (e.g. parasites) can occur by the direct uptake of cell-free DNA and can be facilitated by the presence of mobile genetic elements (e.g. viral and transposable DNA elements) in the recombinant DNA.

HGT processes between multicellular eukaryotes are only infrequently observed and usually materialize over long, evolutionary timescales (Richardson and Palmer, 2007, Dunning Hotopp, 2011). Heritable HGT between multicellular eukaryotes is also physically limited by the need for transformation of segregating germline cells. Mobile genetic elements have been implicated in cases of HGT between eukaryotes (Gladyshev et al., 2008; Feschotte and Wessler, 2002; Danchin, 2011) but is not always a prerequisite.

As such HGT events are considered to be rare, the initial problem formulation should focus on characteristics of the recombinant DNA that can lead to changed mobility. If changes in the potential for mobility of the recombinant DNA have been identified, a further detailed ERA is necessary. This problem formulation step focusing on the potential for horizontal transfer of a recombinant DNA with a potential for altered mobility should consider: (1) the presence of plausible mechanism that could facilitate transfer, uptake and integration of the recombinant DNA fraction of mammal and bird DNA in new hosts, at biologically relevant frequencies; and (2) the potential of horizontal transfer relying on the understanding of the factors defining and limiting the current species distribution of the used mobile genetic elements, as well as of the mechanistic aspects of the replication/transposition of mobile elements in their current hosts (including absence or presence of factors in the GM mammal/bird that might influence the mobility of the recombinant DNA).

(b) In contrast to the low proportion of germline cells in multicellular organisms that can act as recipients of heritable HGT events, all single celled organisms can, in principle, act as recipient cells of heritable HGT events (Keeling, 2009, Dunning Hotopp, 2011, Richards et al., 2011). However, of the known mechanisms of HGT in single-celled organisms, only natural transformation is known to facilitate uptake and genomic integration of DNA fragments.

Microorganisms, especially bacteria, are capable of acquiring genetic material both from related and unrelated species (Anderson and Seifert, 2011). The probability and frequency of HGT from mammals and birds (including the recombinant DNA fraction) 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 mammals and birds DNA can be incorporated and thus stabilized in the microbial genome (including chromosomes or plasmids).

In bacteria, natural transformation with linear DNA fragments usually requires nucleotide sequence similarity to facilitate stable integration by homologous recombination. For this reason, it is considered that the presence of sequences with high similarity to bacterial DNA in the mammal/bird DNA would increase the probability of HGT (Bensasson et al., 2004; EFSA, 2009b). Due to the homology-based recombination mechanisms active in bacteria, the likelihood of HGT from GM mammals and birds into microorganisms should therefore be considered also in the absence of mobile genetic elements in the recombinant DNA. Differences in transcription regulation and the presence of introns and requirements for intron splicing represent a functional constraint to efficient expression of many eukaryotic genes in bacteria. The presence of intron-free recombinant DNAs in the GM mammal or bird with high similarity to microbial DNA would increase the probability of transfer and expression after transfer (EFSA, 2009b).

The range of microbial species identified as potential recipients for unintended HGT events will depend on the ability of the micro-organisms to develop competence and on the characteristics of the insert and to what extent homology-based recombination can be expected. Positive selection is usually considered a necessity for rare HGT events occurring into large microbial populations to be biological meaningful.

Therefore the problem formulation should focus on:

- A detailed molecular characterisation of the DNA sequences inserted in the mammals and birds to inform the assessment on the potential for horizontal mobility, stabilization, and expression of the inserted DNA, including:
    - The presence and source of i) mobile elements in the recombinant DNA that could facilitate horizontal movements (e.g. viral and transposable DNA elements) and factors required for such movement; or ii) the presence of DNA sequence similarities in the recombinant DNA with DNA sequences from relevant recipients (i.e. enhancing

the probability of homology-based recombination with recipient genomes); these characteristics will determine the host range of potential recipients.

- Information on the functionality of the regulatory sequences of the recombinant DNA, if horizontally transferred, and on the presence of introns and requirements for intron splicing of the recombinant DNA.

- The release, stability and degradation routes of GM mammals and birds DNA, and the presence of identified recipient organisms that could potentially acquire such DNA in the receiving environments.

- The presence of other sources of DNA that is similar to the inserted DNA construct (with equal or higher recombination potential), in the receiving environments;

- The environmental conditions in the receiving environments and if they could effect directional selection and long-term establishment of recipients of HGT events. Positive selection is usually considered necessary for rare HGT events to represent biological meaningful scenarios in larger populations, and therefore to be considered relevant in the ERA.

- The identification of consequences of identified HGT scenarios from GM mammals and birds should they occur.

- Assessment and measurement endpoints should address established protection goals.

In cases the introduced genetic modification does not lead to changes in the horizontal mobility of the recombinant DNA into microbial populations beyond any other chromosomal mammals and birds DNA (non-mobile), the applicant is expected to provide a short statement that substantiates the absence of a HGT potential beyond other non-mobile mammals and birds genes.

Step 2: Hazard characterisation

If a hazard has been identified in step 1 of the ERA, the hazard should be further characterised. Hazard characterisation should establish the nature and range of potential consequences (short and long term). Information on the prevalence and distribution of genes similar to the those introduced in the GM mammals and birds in all receiving environments should be taken into account.

Step 3: Exposure characterisation

If a hazard has been identified, the exposure characterisation should consider the characteristics of the insert(s), the copy number of the recombinant DNA, the levels and routes of exposure related to the hazard, the stability of the released DNA in the relevant environment(s) and the scope of the application. For instance, recombinant DNA containing cells will be released from shed epithelial cells inside the gut of mammals and birds and be present in faeces.

The applicant should take into account the methodological constraints to the quantification of DNA exposure levels in complex environments. In most cases, a numeric threshold level for a HGT event to be significant cannot be established. Other methodological limitations that warrant explicit considerations include the representativeness of the sampling strategy, the detection limit, and the tempo-spatial relationship between exposure levels and an observed impact of rare HGT events (EFSA, 2009b). Quantitative modelling approaches should be considered in cases where concerns over exposure levels have been identified. Modelling approaches may also be useful when representative data for environmental parameters cannot be obtained, for instance to address natural variability in exposure (see section 3.7).

Applicants are requested to provide an exposure characterisation, of the hazards characterised under step 2, considering the various routes and sources of exposure in the receiving environments:

- GM mammals and birds production systems. E.g. DNA from GM mammals and birds will be exposed to microorganisms and pathogens of the mammals and birds itself during its lifespan (including the gastrointestinal system) and exposed to other organisms in the environment (e.g. faeces) (Rizzi et al., 2012).
- GM mammals and birds processing systems. E.g. GM mammals and birds material will be exposed to a number of environments during processing and storage; including processing of by products.
- GM mammals and birds in the food chain. E.g. GM mammals and birds products will be exposed to the microbiota of the gastrointestinal tract of the consumer. Depending on storage and the type and level of processing (EFSA, 2009g), DNA may be a part of the consumed product. GM mammals and birds by-products may also be utilized as a feed source.
- Alternative sources of the relevant gene(s) should be identified and considered in the exposure assessment.

Step 4: Risk characterisation

The applicant should focus the risk characterisation on the identified hazards and its impacts that may potentially occur in the various receiving environments (as outlined above in steps 1-3). Any identified risk should be characterised by estimating the probability of occurrence, any positive selection conferred by the horizontally transferred trait and the magnitude of the consequences of any adverse effects, taking into account the characteristics of the recipient species.

Step 5: Risk management strategies

Based on the outcome of the risk characterisation, applicants may need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions allowing DNA exposure or positive selection.

Step 6: Overall risk evaluation and conclusions

Identified knowledge gaps should be briefly summarized and a clear statement on the absence/presence of selective conditions should be provided. The applicant is required to conclude on the overall risk i.e. a clear statement on the potential for HGT to occur and its consequences, taking into account any risk management strategies.

4.3.3. Pathogens, infections and diseases

This guideline addresses the deliberate release and placing on the market of mammals and birds kept for either production purposes or as companion animals. Although infectious diseases play a role in both groups of animals, the impact is usually higher in production animals. The high stocking densities at which e.g. poultry and pigs are kept in the production facilities enhance transmission of infections and specific infectious diseases can have considerable environmental and economic consequences because of loss of production, impact on public health or trade restrictions. This Chapter deals with the risk assessment of changes in susceptibility or interactions of the GM animal with pathogens, infections and disease compared to the non-GM comparator. GM mammals and birds may pose a potential risk to the environment after being infected with pathogens.

Resistance or tolerance to disease is a much desired trait in the development pipeline for GM mammals and birds. A disease-resistant animal is not infected by a particular pathogen, whereas a disease-tolerant animal can be infected by that pathogen but does not manifest disease. Animals can be genetically modified with the primary goal to make them disease-resistant or tolerant (direct effects), either to a specific disease or more generally to many diseases (Donovan et al., 2005; Lyall et al., 2011), but they may also be genetically modified to express other traits which may change their susceptibility to diseases. The existence of disease-resistant or disease-tolerant GM animals could

have impacts for the GM animal itself, for the animal populations of which the GM animal is a part, and for human health. GM mammals and birds with enhanced resistance could increase production efficiency and protect welfare. The assessment of animal health and welfare of the GM animal itself is discussed in the Guidance on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects of the EFSA GMO Panel (EFSA, 2012). This section gives guidance for an environmental risk assessment for pathogens, infection and disease for GM mammals and birds, which includes the impacts on non-GM domestic and wild animals and their surrounding ecosystems. More guidance on the impacts of GM mammals and birds on human health can be found in section 4.3.9. The impact on non-GM animal health is summarised in this section and in 4.3.8.

Applicants should consider micro-organisms and parasites present in the receiving environments of the GM mammals and birds and determine the likely direct interactions that will occur in terms of infection and disease. The indirect effects from these direct effects should then be considered in relevant sections. Time scales should be quantified when characterizing direct or indirect effects to be manifested as immediate and delayed effects for organisms other than the GM animals and birds present in the receiving environments. Applicants should consider the methods and approaches described by sections 3.1 and 3.2 on experimental environments. The applicant should consider through this section also the risk associated with disposal of animal carcasses in the contest of their intended uses.

Mammals as well as birds live in an environment with many viruses, bacteria, protozoa, helminths and other lower organisms. Some of these organisms may be harmless or even beneficial to their hosts (mutualism or commensalism); others may cause disease (parasitism or amensalism). The term 'pathogen' in this section refers to an agent that can cause disease. Some pathogens have a broad range of host species, whereas others are specifically associated with one or only a few host species. Moreover, the virulence of a pathogen may differ considerably among susceptible species. Even within a single host species heterogeneity in the manifestation of infection can be seen from one individual to the other, depending for example on behaviour (resulting in variation in exposure to the pathogen), physiological state (Hoye et al., 2011) as well as the developmental stage of the host (Mast and Goddeeris, 1999). Microorganisms and parasites may be primary pathogens, being able to infect and cause disease themselves, or secondary pathogens that need a preceding infection by a primary pathogen, a malfunctioning immune system, or another kind of trigger facilitating infection. Certain ecological shifts in the microbiome allow pathogens to manifest and cause disease (Round and Mazmanian, 2009; Sansonetti, 2011).

To prevent and limit the spread of disease birds and mammals have a variety of defence mechanisms that correspond to three categories: 1) barriers such as skin, mucosa and mucus aiming to prevent entry of microorganisms; 2) innate immunity, the immune reaction responsible for defence where there has been no preceding contact with the pathogen (the first line of defence upon incursion of a pathogen) and 3) adaptive immunity that arises after contact with a specific pathogen (Tizard, 2009).

GM mammals and birds that may pose a potential risk to the environment after becoming infected with pathogens can be categorized into two groups:

Group 1 GM animals are created with the intention to increase resistance against pathogenic organisms, either by interacting with the life cycle of the pathogen (infection resistance) or by negating its pathogenic effect, for example, by decreasing the growth rate of the pathogen within the host (disease resistance). This group can be divided into two subgroups: a) GM animals with increased resistance against a specific pathogen (or a specific group of pathogens), and b) GM animals with a more generalised resistance against several pathogens. Specific resistance can be achieved for example by removing or altering the receptor for a specific pathogen or toxin. If a pathogen requires that specific receptor to attach to its host, for example, F4+ receptors for the attachment of certain pathogenic strains of E. coli in the pig (Geenen et al., 2007), and the GM animal no longer has that receptor, colonization and infection cannot take place. However, one can also imagine a situation

where colonization still could take place, virulence of the pathogen remains unchanged, but the GM animals with enhanced disease tolerance could serve as a reservoir/carrier for that pathogen and may thereby in the long term increase the exposure of other, more susceptible animals (their non-GM comparators or other susceptible species).

The Avian Influenza resistant chicken (Lyall et al., 2011) is an example of a GM animal with increased resistance against a specific pathogen (subgroup 1a). These chickens can still be infected experimentally and replicate the virus. Although transmission among these chickens seems to be reduced, it is yet unknown whether this reduction is sufficient to stop transmission to AIV or indeed influence susceptibility to other microorganisms. More generalised resistance can, for example, be achieved by making a GM animal over-express important components of the innate immune system, such as natural antibodies or antimicrobial peptides (Star et al., 2007).

Group 2 GM animals are created where the primary intention has not been to increase resistance against pathogens, but, for example, to alter the growth or productivity or reproduction of the animals, and where such changes also affect the susceptibility to infection. For example modification of digestion and metabolism in dairy cows may have an effect on immunity (Goff, 2006; van Knegsel et al., 2007) and subsequently result in changes in susceptibility of the animal to infection, thus creating chances for secondary pathogens to invade the body. The latter may be enhanced if modifications in digestion or metabolism are associated with altered digestion or excretion of compounds in body fluid (e.g. sweat), respiratory tracts, digestive tracts, urine and faeces that can serve as substrate for microorganisms or parasites. Such changes in substrate could result in a change in the distribution of opportunistic microorganisms, and some, otherwise harmless microorganisms might become harmful if they multiply to high levels (Stephani et al., 2011). On the other hand, more substrate for symbiotic bacteria might become available, which could have a beneficial effect if they act as prebiotics (Gaggia et al., 2010). In addition, animals genetically modified to increase productivity could have reduced immunity because too few resources in the body are allocated to the immune system (Roth et al., 2011). However, other modifications might affect the immune system in different ways. All GM animals not in group 1 belong to group 2 according to this guideline. The Enviropig (Golovan et al., 2001) is used as an example of group 2 GM animals in this section.

The ERA of a GM animal involves generating, collecting and assessing information on that GM animal in order to determine its potential adverse impacts relative to a non-GM animal comparator, and thus assessing its comparative safety (see section 3.3). In this section, the applicants should develop the risk assessment by comparing the GM animal to its conventional counterpart, where possible, or to other appropriate non-GM comparators, under representative environmental conditions.

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

The focus in the problem formulation for these two groups of GM animals is to determine the likelihood that the GM animals may become a (silent) reservoir of pathogens. The hazard is caused by the interaction between pathogen and GM host that can result in transmission to non-GM domestic and wild animals. The consequences of such transmission can be manifested immediately, but can also be delayed.

The following subsections describe possible examples for hazard identification, but are not meant as an exhaustive list of hazards to be identified for groups 1 and 2 GM animals.

Group 1 GM mammals and birds:

1) Hazard: Disease-tolerant GM animals might become silent carriers of pathogens.

Risk pathway: Disease-tolerant GM animals become infected, can transmit the infection, but do not show clinical signs. The disease could spread unnoticed among GM animals and cause severe problems when transmitted to non-GM animals. This is an immediate effect.

Example: The avian influenza resistant chicken could act as a silent carrier of HPAIv and transmit HPAI virus to susceptible non-GM poultry.

2) Hazard: Evolution and emergence of increased resistance.

Risk pathway: A population of GM animals with increased resistance compared to populations of its non-GM comparators may select for pathogen strains with increased virulence, causing more severe disease in non-GM animals than did the previously circulating strains.

As an example, the selection of more virulent pathogens may be induced by vaccination. The resulting pathogens may be more harmful to the non-vaccinated population (Schat and Baranowski, 2007). A similar thing could happen in group 1 of GM animals when their primary disease resistance phenotype becomes a driver in the evolution of pathogen virulence. This hazard could be delayed; the selection process of more virulent pathogens will normally take a considerable amount of time (see section 3.6 on long-term effects of GM animals).

Example: The avian influenza resistant chicken could select for more virulent HPAIv strains, causing more severe morbidity and mortality in non-GM animals.

Note that this section deals with the hazard identified for non-GM animals through the selection of pathogen strains with increased virulence amongst populations of GM animals, and the consequent increased severity of disease in non-GM animals. Whereas the risks to the GM animals themselves and non-GM animals are covered in this section, risks to the environment, for example through the need to use larger doses of medication against this increased pathogenicity or other medications not currently used, will be addressed in section 4.3.4 (target organisms).

Group 2 GM mammals and birds

1) Hazard: Increase of pathogens causing diseases with a long incubation period due to increased longevity of the GM animal compared to the non-GM comparator.

Risk pathway: Longevity could be a desirable trait for GM animals. The extension of life span may change the incidence and transmission pattern of age-associated diseases, such as tuberculosis, Johne's disease or BSE. Consequently, the pathogen load in the environment could increase. This is a delayed effect, since the animals first have to live long enough to increase the pathogen load in the environment.

Example: Longevity is not currently known to be under development as a commercial GM trait. Nevertheless, this can be postulated as a possible goal for pet breeders (FERA, 2010).

2) Hazard: Increased transmission of infection due to changed behaviour compared to the non-GM animal.

Risk pathway: Changed behaviour may change contact rates between animals within the same species, but also with animals of other species. This could alter the population dynamics of infection. Increased contact rates may increase transmission.

Example: Growth-enhanced GM cats might hunt for different prey than a non-GM cat. This altered predation behaviour not only can enlarge the transmission arena of cat pathogens, but can also increase the possibility to introduce new pathogens into the cat population.

3) Hazard: Change in distribution of specific microorganisms in the environment.

Risk pathway: Altered efficiency of processes in the digestive tract could lead to a different distribution of microorganisms in the digestive tract and thus in the environment.

Background: The alteration of microflora is not necessarily a hazard by itself, but becomes a hazard if this alteration causes a change in the host immunity which subsequently affects the ecosystem more widely. The intestinal tract represents the largest mucosal surface and is a major site of multifaceted interactions between host mucosal immunity system and components of the intestinal microbiota (Nicholson et al., 2005). For example Mazmanian et al. (2005) demonstrated that Bacteroides fragilis polysaccharide directs the cellular and physical maturation of the developing immune system of the mouse host. Altered metabolism may also lead to change in composition of body fluids resulting in changes of the associated microflora. This is an immediate effect.

Example: The Enviropig synthesizes phytase in the salivary glands and secretes active enzyme in the saliva (Golovan et al., 2001). The amount of phosphate in the content of the gut changes and this could be associated with a change in the gut microflora and subsequently microflora deposited in the environment.

Note that the aspects regarding pathogen and disease of this hazard is dealt in this section, whilst the aspect regarding the change of microflora of this hazard will be further dealt with in section 4.3.5.

4) Hazard: Increased pathogen load due to reduced immunity of the GM animals compared to the non-GM comparator.

Risk pathway: GM animals modified to maximize production could allocate too few resources to their immune system, which might result in an increased susceptibility to infection or reactivation of infection when compared to their non-GM comparator, consequently an increased pathogen load in the GM animal. This could be an immediate effect. If the GM animal can spread the pathogen into the environment, this would be a delayed effect.

Example: Bovine Herpes virus (BHV) could be reactivated more easily in cows under stress of a negative energy balance, bringing this virus in the environment (Hage et al., 1996).

Note that before this hazard impacts on the environment, the reduced immunity will first affect the health and welfare of the GM animal itself. The impact on the environment is addressed in this section, whilst the impact on the health and welfare of the GM animal itself is dealt with in section 3.8.

Step 2: Hazard characterisation

If a hazard has been identified, the applicants are requested to further characterise it and to assess the magnitude of harm it might cause to the environment or animal and human health. To exemplify this process, data requirements are presented below for those hazards identified in section 4.3.1.

Group 1 GM mammals and birds

1) Hazard: Disease-tolerant GM animals act as silent carriers of pathogens.

Hazard Characterisation: The applicants should consider how specific the introduced trait is for the pathogen, or group of pathogens. Data generated during the product development phase may help to elucidate these issues. The applicant should provide relevant information on: a) characterisation of the population of the pathogen that the trait is intended to affect, such as host range (including if the pathogens are zoonotic), transmission mechanisms and geographic range (population structure); b) description of the mechanism of the introduced resistance at a molecular level (patho-physiological); c) description of other organisms (relative to the pathogens) in the environment (non-target organisms, NTOs) that may be affected by the introduced resistance mechanism (for characteristics of NTOs refer to section 4.3.5); and d) the level of resistance against the intended pathogen of the GM animal compared to the non-GM comparator. Detailed information should be given regarding the effect of the modification on infection, disease and transmission. The applicant should carry out experiments examining infection upon challenge and subsequent transmission to GM animals with the trait as well

as to their non-GM comparators. The designs of such experiments have been described by Velthuis et al. (2007). The applicant could then estimate transmission rates to GM and non-GM animals.

Example: The avian influenza resistant chicken: Transmission experiments should demonstrate whether GM chickens can transmit the virus to non-GM chickens. If this is possible, they should quantify experimentally whether the infection can be perpetuated among GM chickens (van der Goot et al., 2005) to demonstrate whether the basic viral reproduction ratio exceeds or is smaller than 1 (in that case the infection will fade out).

2) Hazard: Evolution and emergence of increased resistance.

Hazard characterisation of the hazard "evolution and emergence of increased resistance" is dealt with in section 4.3.4.

Group 2 GM mammals and birds

1) Hazard: Increase of pathogens causing diseases with a long incubation period.

Hazard characterisation: The applicant should assess whether the life span of the GM animal is altered when compared to the non-GM comparator and discuss the influence of longevity on the population dynamics of pathogens with a long incubation period associated with the GM animal species. Preferably this discussion is based on the results of mathematical modelling.

Example: Johne's disease has a long incubation period in cattle; as infected animals get older, pathogen excretion increases with age in infected animals. Increased longevity might affect the population dynamics of this disease. Mathematical models have been developed to estimate such an effect, for example modelled herd dynamics and the infection process.

2) Hazard: Increased transmission of infection due to changed behaviour compared to the non-GM comparator.

Hazard characterisation: The applicant should estimate relevant contact rates both for the GM animals and its comparators. The applicant should estimate how any alterations in contact rates will affect intra and inter species transmission of pathogens.

Example: In case of the hypoallergenic companion animal (FERA, 2010) the applicant should discuss possible changes in contact rate with other animals and how this might affect transmission of (zoonotic) pathogens (e.g. *Bartonella henselae*).

3) Hazard: Change in distribution of specific microorganisms in the environment.

Hazard characterisation of the hazard: Changes in distribution of specific microorganisms in the environment are dealt with in section 4.3.5.

4) Hazard: Increased pathogen load due to reduced immunity of the GM compared to the non-GM comparator.

Hazard characterisation: The applicant should use information on the degree of reduction in immunity of GM compared to non-GM to discuss whether the altered immunity of the GM animal itself could cascade into the other animals or organisms. Both enhanced and reduced immunity should be considered.

Example: GM cows producing a large amount of milk may have a negative energy balance and a reduced immunity to e.g. BHV (Goff, 2006). The applicant should assess whether this reduced immunity of the GM cow could impact on other organisms via pathogen and disease (e.g. the

previously given example of BHV or high fat lactating cattle that become more susceptible to tick infestation).

Step 3: Exposure characterisation

This step is to evaluate the likelihood of occurrence for each identified hazard and the potential environmental harm caused by it. It is important that the applicant considers the specific trait of the GM animal itself (e.g. group 1 or group 2), its receiving environments (captive, semi-captive, non-captive), and the presence or absence of non-GM animals in the receiving environments. For captive or semi-captive GM animals, the likelihood of escape needs to be estimated. For semi-captive GM animals, the proportion of time spent in captive and non-captive periods should be estimated. Regarding the exposure routes of disease-resistant GM animals, the applicants should describe in detail the different steps of handling the animals at different stages of their life and during transport. Concerning the infection transmission routes, aerosols, urine, faeces etc, shall be considered. Regarding the temporal pattern of exposure to a hazard, acute or chronic exposure should be addressed separately. The applicant should also consider the exposure of pathogens via farm effluents.

It is recognized that it may not be possible to estimate the exposure (likelihood) quantitatively (expressed as probability) for each hazard identified and characterised. In those cases the applicant can express the likelihood of exposure qualitatively using a categorical description and provide a range for the indication about the likelihood of adverse effects (see section 2.1). The following subsections provide examples of how to perform exposure characterisation for hazard scenarios described above. They are meant as examples but not as an exhaustive list of exposure endpoints to be identified for groups 1 and 2 GM animals.

Group 1 hazard scenarios

1) Hazard: Disease-resistant GM animals act as silent carriers of pathogens.

Exposure characterisation: Applicants should quantify the exposure to non-GM animals in the same farm, to non-GM animals on other farms and to wild life. The exposure of animals can be estimated from the results of the experimental transmission from GM to non-GM animals. Applicants should demonstrate that the experimental conditions remain relevant to the receiving environments. In case such transmission does not occur in an experiment, exposure of non-GM animals within a farm is unlikely and exposure of non-GM animals on other farms or in the wild is even more unlikely. In this case, no further experimentation is necessary. However, in case such experimental transmission does occur, exposure of non-GM animals in a farm must be quantified by modelling, using the transmission rate of infection from GM to non-GM animal. Moreover, the transmission kernel (e.g. Boender et al., 2007) from an infected GM farm should be estimated in order to quantify the exposure of surrounding farms and wildlife. The comparator here is modelling the infection in a non-GM population.

Example: The avian influenza resistant chicken: In case of chickens it is not very likely that GM and non-GM animals will be kept on the same farm. So here in particular, exposure of other farms and wildlife must be quantified by a mathematical model (e.g. Boender et al., 2007) to investigate if and to what extent transmission among GM animals is still possible.

Group 2 hazard scenarios

1) Hazard: Increase of pathogens causing diseases with a long incubation period.

Exposure characterisation: Applicant should discuss the exposure of pathogens with a long incubation period (such as *Mycobacteria*) to non-GM animals in the same farm, to non-GM animals on other farms and to wild life. To this extent mathematical models can be helpful.

Example: In case of longevity of cattle, transmission of *Mycobacterium avium* subs paratuberculosis could be enhanced. While adjusting the cattle replacement rate in the model for GM animals,

transmission within and between farms can be evaluated by mathematical modelling (e.g. Weber et al., 2004; Marce et al., 2011).

2) Hazard: Increased transmission of infection due to changed behaviour.

Exposure characterisation: Applicants should discuss the effect of the changed behaviour on the rate of interactions within the species and between species and discuss whether this has an effect on the transmission of pathogens.

Example: In case of a hypoallergenic cat the applicant should discuss possible changes in contact rate with other cats and humans and how this might affect transmission of (zoonotic) pathogens (*e.g. Bartonella henselae*).

3) Hazard: Increased pathogen load due to reduced immunity.

Exposure characterisation: Applicants should discuss the effect of the genetic modification on immunity and discuss whether this will make them more susceptible to infection and disease. If so, the applicant should then discuss whether this will affect primarily the GM animal itself (see also section 3.8), or whether it will also affect non-GM populations. In the latter case, the same approach as in step 3, group 1 should be followed.

Example: GM cows producing large amounts of milk may have a negative energy balance and a reduced immunity (Goff, 2006). The applicant should assess whether this reduced immunity of the GM cow could impact reactivation of BHV and lead to exposure of non-GM animals. The comparator here is the probability of BHV reactivation in non-GM cattle.

Step 4: Risk characterisation

The risk characterisation should focus on the identified impacts that may potentially occur in the various receiving environments (as outlined above in step 3). Any identified risk should be characterised by estimating the probability of occurrence and the magnitude of the consequences of any adverse effects; taking into account the characteristics of the recipient species.

Step 5: Risk management strategies

Applicants should propose methods to reverse or reduce identified risks, e.g. pathogenicity and disease parameters and key ecological functions identified in the risk assessment. For example, to reduce the transmission of pathogens from GM to non-GM animals within a farm, an obvious risk management strategy is to allow only GM animals in a farm (that is not a mixed population of GM and non-GM animals). Moreover, to reduce the transmission of pathogens from a farm housing GM animals to other farms and wild life population, stringent biosecurity measures should be implemented on the farm, to minimise release of pathogens, for example, sufficient levels of containment to prevent animal escape, adequate waste treatment to minimise release of GM materials through farm runoff, appropriate disposal of carcasses from diseased animals, etc. The practicality and efficacy of the methods should be evaluated and methods for their implementation described. Uncertainties associated with the efficiency or implementation of mitigation measures should be described and considered in relation to post market environmental monitoring plans (see Chapter 5). For GM animals that fall into the hazard 2 scenario, in cases of remaining uncertainty, post market monitoring of specific pathogen strains should be implemented. For disease-resistant or tolerant GM animals, the applicant should implement strategies of handling carcasses to minimise the further spread of pathogens and disease.

Step 6: Overall risk evaluation and conclusions

Applicants should conclude on the overall risks arising from the conclusions of this section considering the proposed risk management measures. Uncertainties due to gaps in information, the

limited scope of experimental studies and the need to extrapolate results to long term exposure of a wide range of receiving environments should be discussed. Applicants should describe identified risks or critical uncertainties that require further information from post-market monitoring studies. In addition applicants should explain why identified environmental impacts are considered acceptable and do not present risks.

4.3.4. Interactions of the GM mammals and birds with target organisms

Target organisms (TOs) are organisms on which specifically designed characteristics of a GM animal are intended to act. In cases, such as the sterile rabbit or the growth-enhanced cat, where there is no target organism other than the animal itself, this definition specifically excludes the GM animal itself.

Target organisms should be defined by applicants. All other organisms should be considered as 'non-target organisms'. Target organisms are typically pathogens (e.g. micro-organisms, parasites), but may also be pests. Hence, this chapter should be read in conjunction with section 4.3.3 dealing with interactions with pathogens, infection and disease. GM animals may be created with the ability to increase resistance or tolerance against pathogenic organisms. Increased resistance may be obtained either by interacting with the life cycle of the pathogen (infection resistance) or by negating its pathogenic effect, for example by decreasing the growth rate of the pathogen within the host (disease resistance). In this document, for GM mammals and birds, the term resistance is used to indicate that the animal is not colonized by the pathogen or pest. The term tolerance is used to indicate that colonization takes place but that the clinical manifestation of the resulting disease or infestation through the presence of symptoms is considerably reduced, relative to that which is expected in the non-GM animal.

One example for pathogens is the Avian Influenza resistant chicken (Lyall et al., 2011), which is resistant to clinical disease, but not to infection. A further example for pathogens is disease-(mastitis)-resistant cattle (*Bos primigenius*) (Donovan et al., 2005). GM pathogen-resistant animals might be developed in several ways. Animals might be modified to express specific proteins, peptides or antimicrobial compounds that are directly toxic to pathogens or influence their growth in situ; or that produce products that destroy or neutralise a component of the pathogen, or that express resistance gene products involved in hypersensitive response and interaction with its virulence; or express recombinant antibodies that inactivate pathogens or pathogen proteins.

An example for parasites might be tick (Ixodes spp.)–resistant GM sheep (*Ovis aries*) (Brossard, 1998). GM pest-resistant animals might also be developed in the future using a variety of mechanisms. These could possibly involve animals that express insecticidal substances, repellent substances, anti-feedants, or altered volatiles to influence the host-finding process.

In addition, whilst there are no reports yet within the scientific literature, it is possible to foresee that GM animals might be produced with the ability to consume food items that are toxic or non-palatable to their non-GM comparator. Alternatively, GM animals might be produced with altered specificity of preferences so that they consume disproportionately more of a food resource. The food items could be plants (in which case the GM animal would be a herbivore or omnivore) or prey (in which case the GM animal would be an omnivore or carnivore). One motivation for such production might be to facilitate biocontrol of pest organisms, whether the pest is a plant (e.g. bracken, *Pteridium aquilinum*) or animal (e.g. the rat, *Rattus norvegicus*); another motivation might be to increase animal production, by giving improved access to some otherwise unusable food resource.

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

The focus in the problem formulation is to determine the likelihood that the TO will evolve mechanisms to reduce the efficacy of the modification, usually by overcoming the resistance to the pest or pathogen and allowing colonization to take place, or by reducing the tolerance to a significant degree. In the latter case there could evolve a continuum of efficacy in populations of GM animals

which might range, in the worst case, from that which is expected in a non-GM animal to, in the best case, that which is expected in a GM animal in which the TO is controlled with maximal efficacy.

Recall that in section 4.3.3 (step 1) a hazard was identified for non-GM animals through the selection of pathogen strains with increased virulence amongst populations of GM animals, and the consequent increased severity of disease in non-GM animals. Whereas the risk to the GM animals themselves is covered in section 4.3.3 above, there may be an additional risk to the environment through the need to use larger doses of medication against this increased pathogenicity or alternative medication not currently used. Applicants should address that risk to the environment separately, according to the guidance notes in this section.

Reduction in efficacy may prove an environmental concern because alternative methods of protection against pathogens or pests would then have to be found. In the case of pathogens this might, for example, lead to the increased use of antibiotics, with a risk of contributing to an enhancement of antibiotic resistance. In the case of pests this might, for example, lead to an increase in pesticide use, or compromise other existing pest control products and/or destabilise integrated pest management strategies. As a further consequence, it might lead to the need for isolation of the GM animal, leading to changes of husbandry practices which might necessitate waste disposal systems having potentially adverse environmental effects (see section 4.3.7).

Both herbicide-tolerance in genetically modified herbicide-tolerant plants and insect-resistance in genetically modified insect-resistant plants (EFSA, 2010) might be considered paradigms for such decreases in the efficacy of GMOs. Because of the levels of exposure, reductions in the efficacy of the modification for resistance is only relevant for applications with the scope of release of GM animals into the environment, and not for applications restricted to the import and processing of GM animals and their products.

It is important to identify the TO of the GM animal in the receiving environments (see section 3.1). For pathogens and pests, the potential of these TOs to develop mechanisms to reduce efficacy should be evaluated based on any history of development of resistance to veterinary medication and conventional pesticides. Appropriate data should be provided by applicants to characterise this potential, depending on the TO and the genetic modification. These should include: data on biology, life cycle, ecology and/or behaviour of the TO; data on mechanisms to reduce efficacy that may develop in the TO and their genetic control, heritability and linkages to virulence, fitness and selective advantage; distribution of the TO and its populations in the relevant receiving environments; host range of the TO; information on the population genetics; mode of action of the active GM animal product towards the TO and GM animal characteristics related to this trait; and data on baseline susceptibility of the TO to GM products either from the literature or from laboratory tests. In addition, applicants should provide if possible: data on the epidemiology of TOs both susceptible and less-susceptible to effects of the intended modification; and on the frequency of individuals or alleles among such less-susceptible TO populations. When addressing the environmental effects of hazard 2 of group 1 GM animals as specified in section 4.3.3 (step 1), the applicant might use, as a starting point, models similar to those referred to in this section. These might describe interactions between the virus and the immune systems of appropriate non-GM animals. The applicant should gather suitable data to parameterize and test such models.

Since data may be incomplete, applicants should consider various scenarios, including a worst-case scenario (see section 2.1, step 4), to estimate the potential for efficacy reduction within Europe.

Where the TO is a food item, similar information is required, but in this case focussing on the ability of the TO to develop mechanisms to increase its toxicity or reduce its palatability.

Step 3: Exposure characterisation

For pathogens and pests, the applicant should gather data characterising the exposure of TOs to the GM animal which should include: expression levels of the GM products in the animal tissues attacked

by the TO; estimation of the levels of intake of any GM products by various developmental stages of the TO; the influence of the expression level and its variability on the interaction between GM animal and the TO; the proportion of population of the TO exposed to the GM animal in the receiving environments; the baseline frequency of less-susceptible individuals or alleles in TO populations. Applicants should allow for any changes either in the longevity of the GM animal or on the period over which a GM animal is susceptible to the pathogen or pest, and how this might affect the population dynamics of the interactions between the GM animal and the pathogen/pest. Applicants might also consider data from the deployment of other GM animals expressing similar traits. Similar data are required where the TO is a food item.

Initially, baseline data for applications may have to come from F1 screening or other screening methods. Once information has accumulated, relevant data for Europe may be obtained from the scientific literature, but note that data from outside the EU can be considered only if they can be shown to be relevant to receiving environments within the EU.

Applicants should evaluate the exposure under the worst case scenario as referred to above in section 2.1.

Step 4: Risk characterisation

After assessing all these data, the risk should be characterised for pests, pathogens and food item TOs for (a) the evolution of mechanisms to reduce efficacy and/or (b) the development of undesirable changes in the interaction between the target pests and/or pathogens and/or food items with conventional animals in the receiving environments. The risk characterisation should focus on the 'worst-case' scenario described above.

The applicant should attempt to estimate the degree to which medication and/or pesticide would be increased as a result of any reduction in efficacy. The applicant should attempt to quantify the environmental impact of any use of alternative control products. The applicant should estimate any effect on existing management strategies, particularly within the context of sustainable use of pesticide as outlined in Directive 2009/128/EC[16]).

Step 5: Risk management strategies

Based on the outcome of the risk characterisation, applicants should propose management strategies, although it is recognized that in the case of pathogens these might be limited. Applicants should evaluate the likely effectiveness of targeted risk management strategies which could minimise undesired interactions between GM animals and TOs in the European receiving environments. Applicants should indicate the efficacy, reliability and expected reductions in risk associated with the strategies. In addition, the risk of a reduction in efficacy may change when taking into account newly available information or changes in husbandry systems (see section 4.3.7). Therefore, production management measures need to be able to respond to these changes and appropriate monitoring measures might be required as part of case-specific monitoring within PMEM.

Step 6: Overall risk evaluation and conclusions

A conclusion is required of the overall risk considering the development of mechanisms to reduce efficacy in the TO or in undesired changes in the interaction between the GM animals and the TO. The risk characterisation and conclusions will determine the resistance management measures and requirements for the PMEM plan.

[16] Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. Official Journal of the European Union L 309:71-86.

4.3.5. Interactions of the GM mammals and birds with non-target organisms

Non-target organisms (NTOs) are defined as all species that are directly and/or indirectly exposed to the GM animal except target organisms (TOs) (see section 4.3.4 for TOs). NTOs may also include wild type relatives of the GM animal. According to Annex II of Directive 2001/18/EC, the ERA should consider potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and non-target organisms, including the impacts on populations of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

This section provides guidance to applicants and risk assessors for assessing potential adverse effects of GM animals on NTOs, together with a rationale for data requirements in order to complete a comprehensive ERA for NTOs.

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

Several environmental concerns may be raised, and testable hypotheses formulated, that are associated with the release of GM animals into the environment. One of these concerns is that the GM animals will have an adverse effect on the biodiversity, ecological functions, and services of accessible ecosystems (see section 3.1) through interactions with the species or populations of species referred to as non-target organisms (NTOs). In defining biodiversity, we follow the Convention on Biological Diversity (United Nations, 1992): "Biological diversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems".

In this section, ecosystems both inside (gut microflora and -fauna) and outside of a GM animal's body are of relevance, as a genetic modification can e.g. also affect gut microorganisms in the GM animal itself. For example, environmental aspects of changes in distributions of gut microorganisms and therefore in the manure of GM animals are considered here, e.g. if manure of a GM animal goes into the field that contains a different composition of microorganisms than manure of the non-GM comparator.

Accessible ecosystems and their NTOs should be considered in the problem formulation step. Since the environment (including biodiversity) is to be protected from harm according to protection goals set by EU legislation, the protection of biological diversity and ecological functions should be considered in the ERA (see Table 1 in Chapter 2). Certain protection goals not only apply to natural ecosystems but also to human managed (production and farming) systems, since sustainable production in these systems is also an important protection goal.

A crucial step in problem formulation is the identification of aspects of the environment (e.g. valued entity, ecosystem services/functions) that need to be protected from harm according to protection goals set out in existing EU legislation, and to translate those into concrete measurable phenomena (i.e. assessment endpoints). An assessment endpoint is therefore defined as an explicit expression of the environmental value to be protected as set out by existing legal frameworks (see Chapter 2). It is important to note that an assessment endpoint is not an indicator of environmental conditions but is the ecological resource that is to be protected (Sanvido et al., 2012). For example, typical assessment endpoints can be the distribution, abundance, and species richness of certain groups of organisms (e.g. pollinators) 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. These data and information may originate from other parts of the ERA, from literature, as well as from laboratory and field experiments. The use of data from outside the EU may be important and informative, but applicants must justify why these data are relevant to the receiving environments in the EU where the GM animal will be released. The sources of data should be properly justified and described.

Hazard identification

Problem formulation starts with the identification of potential hazards. In case of effects on NTOs, two different scenarios can be discriminated:

Scenario 1) For accessible ecosystems where the conventional counterpart or an ecologically similar comparator species is present, potential hazards are genetically modified traits (i.e. differences as compared to the comparator) that have the potential to cause adverse effects on NTOs in the environment. An example would be the larger size of a growth-enhanced cat as compared to a non-GM cat which potentially causes a diet shift of the GM cat as compared to a non-GM cat. Many other morphological changes represent potential hazards when compared to the non-GM comparator. Other examples are behavioural, biochemical, physiological, developmental, and reproductive changes; modified responses to husbandry and dietary regimes; and bioactivity (endocrine, pharmacological, or immunological activity) and toxicity of newly expressed substances. Accessible ecosystems where the non-GM comparator is present also include production systems where the non-GM comparator could be totally replaced by the GM animal, so that the non-GM comparator is not simultaneously present with the GM animal. For accessible ecosystems in the wild, GM animals and comparators will typically be simultaneously present. A special case of scenario 1 could be the release of a GM animal into the wild aiming at reducing the abundance of a target organism (e.g. a pest). In this case, the intended reduction of the target organism could cause unintended effects on NTOs. For instance, the target organism could be an important food source for an endangered species.

Scenario 2) For accessible ecosystems where neither the conventional counterpart nor an ecologically similar comparator species is present, the potential hazards include all traits of the GM animal (not only those that are different to the conventional counterpart or another appropriate comparator species) that potentially alter species interactions and can lead to adverse effects on NTOs (see also section 3.3 on choice of comparators, and section 4.3.1 on persistence and invasiveness).

The features of the GM animal that are different from the non-GM comparator may lead to potential hazards since environmental impacts can be a consequence of changes to the GM animal, to its management as well as the effects of the introduced traits. For example, growth-enhanced cats (and similar GM animals) might be able to prey upon species that are outside the diet of non-GM cats. As a result, in Europe where large mammalian predators are nowadays relatively rare, the presence of growth-enhanced cats will be a potential hazard to species living there in the wild. Such novel predators might cause serious harm. In addition, their novelty might increase the likelihood of them becoming invasive (novel weapons hypothesis in invasion biology; Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004) (see section 4.3.1).

Each genetic modification differs in its intended use, hence the resulting GM animal and its biology will differ from GM animals with other modifications, so a case-by-case-approach should be followed. To roughly categorize different types of intended uses and releases, it is useful to distinguish three classes of releases: captive, semi-captive, and non-captive GM animals. As indicated in the beginning of Chapter 4, these three groups differ in the type of environment in which they live:

1) Captive GM animals are intended to live in a production system or other captive environment, so these animals interact with the NTOs that are present in the captive environment. Those individuals that escape will additionally interact with NTOs in the wild, and in other captive environments if they enter there.

2) Semi-captive animals will interact with NTOs both in the captive environment and in the wild. For example, growth-enhanced cats and many other GM pets will interact with NTOs in their owners'

houses and (if applicable) gardens, and will also interact with NTOs in the wild when browsing and hunting in the neighbourhood. If such a GM companion animal escapes or is released into the environment by its owner, it will interact with NTOs in the wild; if it enters other captive environments (e.g. other houses), it will interact with NTOs in these other captive environments as well.

3) Non-captive animals interact with NTOs in the wild, and in captive and semi-captive environment if entered. These differences should be considered when identifying interactions of GM animals with NTOs.

NTOs belonging to different functional groups and their interactions

Non-target organisms may belong to different functional ecological groups (trophic levels), e.g. as primary producers, herbivores, predators, decomposers, parasites/pathogens, or mutualists/symbionts (Table 6). These NTOs will have different direct and indirect interactions with the GM animal. Direct effects can be positive, neutral, or negative for an NTO. In case of predator-prey and other consumer-resource interactions, one species (the predator or consumer) benefits from the interaction, whereas the other species (the prey or resource species) is negatively affected by the interaction. Host-parasite interactions concerning pathogens and disease are similar but should be considered under section 4.3.3. In case of mutualistic interactions, both species benefit from the interaction. In case of commensalism, one species benefits by the interactions, whereas the other species is not affected. In case of amensalism, one species is negatively affected by the interaction, whereas the other species is not affected (Begon et al., 1996).

Indirect effects can also be positive, neutral, or negative for an NTO, but are more complex in the way they are mediated. For example, in case of predator-prey and other consumer-resource interactions, density-mediated indirect effects are discriminated from trait-mediated indirect effects (Trussell et al., 2006). Density-mediated indirect effects result from two or more direct consumer-resource interactions. For example, competition between two consumers that share a common resource is an important indirect density-mediated interaction. The GM animals may not only compete with NTOs of other species but also with non-GM individuals of the same species; severe competition can lead to displacement or replacement of NTOs which should be assessed. Other important examples of density-mediated indirect effects are trophic cascades in food chains (Begon et al., 1996, 2005; Eisenberg, 2010; Terborgh and Estes, 2010). In case of trait-mediated indirect effects, the presence of a third species modifies the strength of interaction between two species by altering the behaviour, morphology, or physiology of one or both of the interacting species. For example, the presence of growth-enhanced GM cats in the environment might not only cause direct mortality in populations of bird, mammal, and other species, but potential prey species may reduce their activity in open habitats and spend more time in refuges in order to avoid encounters with growth-enhanced cats. This reduced activity may lead to reduced food consumption of the prey species, which may in turn affect both their own reproduction and population dynamics, and also those of NTO species they are consuming. There are many examples in the literature where the presence of a predator changes the behaviour of prey species and thus their biotic interactions (Nellis and Everard, 1983; Fenn and Macdonald, 1995; Brown et al., 1999; Ripple and Beschta, 2004; Eisenberg, 2010; Barun et al., 2011). The effects of such trait-mediated interactions can exceed those of density-mediated interactions (Trussell et al., 2006). As stated above, there are many more possible indirect effects (Begon et al., 1996, 2005). One-way indirect effects are discriminated from multitrophic effects. The former are indirect effects via one species, e.g. the GM animal indirectly affects a competing NTO via a resource species they are sharing (competition), or the GM animal is a predator and indirectly affects a plant species that is consumed by a herbivore which is in turn consumed by the GM animal (3-level food chain). Multitrophic effects are here defined as more complex indirect effects via two or more species, e.g. 4-level food chains (see further below in this section).

Table 6: Examples of functional groups (trophic levels) of NTO species (compare EFSA, 2010, for a similar table for GM plants).

Functional group	Examples of taxonomic groups
Plants and other primary producers	Angiosperms (*Magnoliophyta*,), conifers (*Coniferophyta*), ferns (*Pteridophyta*), mosses (*Bryophyta*), red algae (*Rhodophyta*), brown algae (*Phaeophyta*), green algae (*Chlorophyta*)
Herbivores and other primary consumers	Ungulates (*Mammalia: Artiodactyla*, e.g. *Bos*, and *Mammalia: Perissodactyla*, e.g. *Equus*), rodents (*Mammalia: Rodentia*), pigeons and doves (*Aves: Columbiformes*), aphids (*Hemiptera: Aphididae*), grasshoppers (*Orthoptera: Ensifera)*, gastropods (*Mollusca: Gastropoda*)
Predators	Carnivora (*Mammalia: Carnivora*), raptors (*Aves: Falconiformes*), owls (*Aves: Strigiformes*), piscivorous fishes (e.g. *Esox*, *Perca*)
Decomposers and scavengers	Diptera larvae (e.g. *Phoridae*, *Sciaridae*), Nematoda (e.g. *Rhabditidae*, *Dorylaimidae*), springtails (*Collembola*), mites (*Acarina*), earthworms (*Haplotaxida: Lumbricidae)*, *Isopoda*, microorganisms (including fungi)
Parasites & pathogens	See section 4.3.3
Mutualists & symbionts	Endosymbionts, e.g. bacteria living in the gut of animals (gut flora); mutualistic interactions between e.g. plants and pollinators (e.g. small birds and bats), plants and seed dispersers (e.g. birds, mammals), spore dispersers (typically mammals; Johnson, 1996)

Four steps for selecting focal NTOs

In any accessible ecosystem, specifically in non-captive environments, there is usually a high number of NTO species in each functional group that may be (directly or indirectly) exposed to GM animals. Considering that interactions of the GM animal with all of these species cannot be tested or assessed, applicants may need to select, on a case-by-case basis, a representative subset of NTO species for consideration in the risk assessment. This representative subset contains what are termed here focal NTO species. Depending on the species of the GM animal, the modified traits, the characteristics of the accessible ecosystems, and the intended use and conditions, the range of NTO species will differ. The selection of focal NTOs can be divided into four steps (Figure 7; this general approach follows Birch et al., 2004; Hilbeck et al., 2006; EFSA, 2010).

In order to decide whether or not to go through the four-step selection process, the applicant should first consider the GM animal itself (e.g. the genetic construct, the donor and recipient organism, the new/modified traits, and also the phenotypic and reproductive characteristics of the GM animal), identify the accessible ecosystems, and intended uses and releases of the GM animal. The four-step process needs to be followed either for scenario 1, scenario 2, both scenarios, or not at all.

For scenario 1 where a comparator species is present in the accessible ecosystems, the applicant needs to outline how the genetic modification may lead to different (direct or indirect) species interactions of the GM animal as compared to the comparator species (comparative assessment). In case of the growth-enhanced cat, for instance, it is likely that species interactions with NTOs differ from species interactions of non-GM cats with NTOs, as a result of the different size. Besides size differences and other morphological changes, behavioural differences between the GM animal and the comparator species can also lead to different species interactions. A change in the digestive tract might also lead to different species interactions, as consumption of food species might be altered due to changes in e.g. gut turnover rates. However, many GM animals that will be proposed to be placed on the market are expected to have species interactions that are not different from comparator species. No specific example is given here, as the comparison needs to be done on a case-by-case basis, yet many GM

domesticated species as part of the food production process might not have different species interactions than their comparator species. If the applicant clearly shows that the GM animal has no different species interactions than the comparator species under scenario 1, it will not be necessary to follow the four-step selection process for scenario 1. Otherwise, i.e. if differences in species interactions cannot be excluded, this will be necessary.

It should then be assessed if the GM animal will be released, or might escape, into accessible ecosystems where the comparator species is not present, i.e. if scenario 2 applies. If is the case, applicants should follow the four-step selection process outlined in Figure 7 for scenario 2; otherwise, this will not be necessary. Based on this reasoning, it will hence be necessary to follow the selection process either for scenario 1, scenario 2, both scenarios, or not at all.

For scenario 2, applicants should follow the four-step selection procedure for NTOs as follows:

Step A) Identification of functional groups

As a first step in species selection, it is necessary to identify the functional groups of NTOs that are directly exposed to or indirectly interacting with the GM animal, e.g. through food-web interactions, scale of release, and dispersal. The functional groups given in Table 6 may be used, but other functional groups should be considered.

Step B) Identification of NTO species from functional groups

In the second step, species corresponding to each functional group identified in the previous step should be listed, considering the accessible ecosystems. 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

From the list built in step B of species selection, applicants shall prioritise NTO species from each relevant functional group. The main criteria to be considered in this prioritisation process are:

- Known sensitivity of the species to the GM animal or its products (i.e. the genetically modified trait), taking also into consideration data from non-European countries and laboratory experiments (considerations made before entering the four-step selection process regarding differences in species interactions between the GM animal and a comparator species under scenario 1 might be particularly useful here).

- Biomass of the species in the accessible ecosystem (rough biomass estimates are sufficient; biomass is used as a rough indicator here for a species' importance in the accessible ecosystem).

- Species vulnerability (i.e. are certain populations already threatened and thus more vulnerable to additional pressures?).

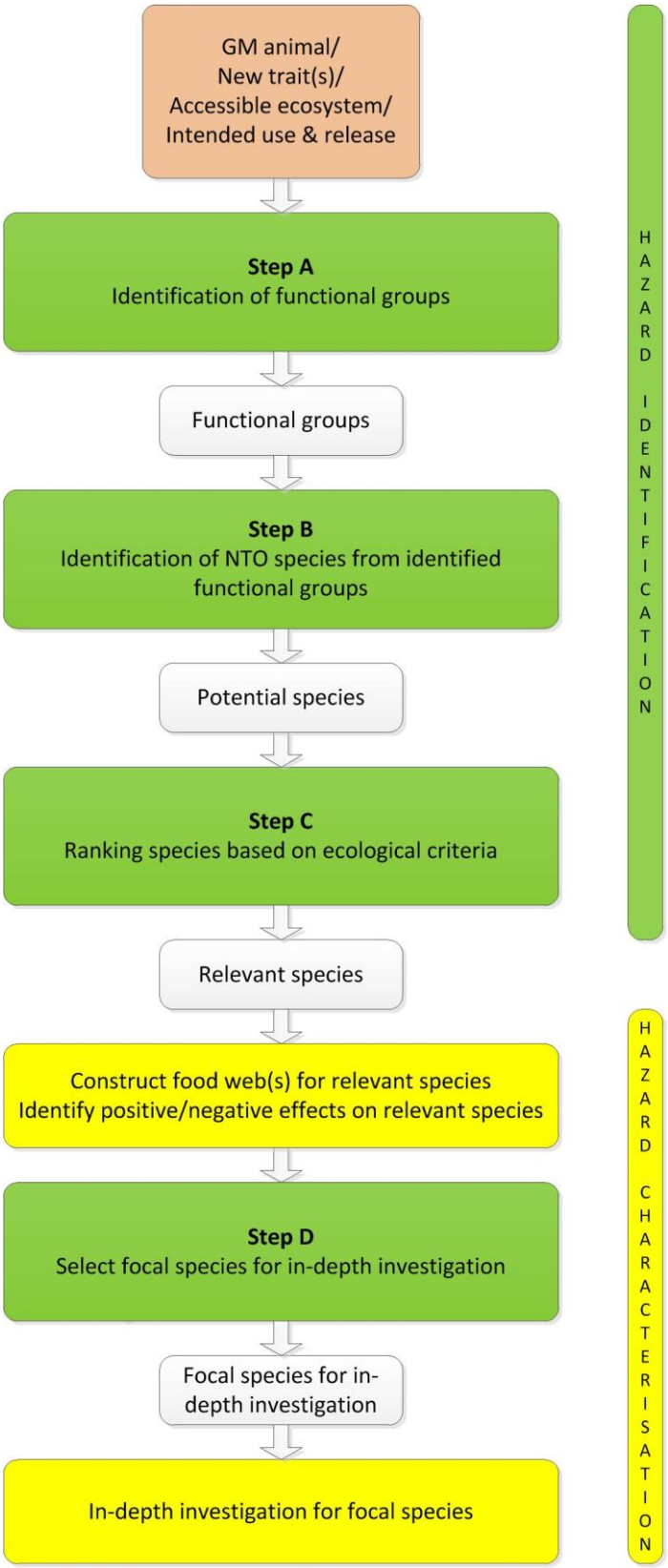
- Ecosystem services affected by the species, e.g. primary production; provisioning of food, wood, or fuel; water purification; regulation of climate, flood, or disease; aesthetic, spiritual, educational, or recreational value (Millenium Ecosystem Assessment, 2005).

A food web with all high-ranked species will be required in the next step of hazard characterisation, with all direct interactions between species (incl. the GM animal) being indicated. For scenario 1, an additional food web with the comparator species instead of the GM animal should be constructed (all NTO species remaining the same), in order to allow comparing the species interactions of the GM animal with NTOs versus species interactions of the comparator species with NTOs. Positive or negative effects on any relevant NTOs not included in the food web (e.g. endosymbionts) should be identified as well. As no specific experiments are conducted in this step C, it is possible that some

interactions are unknown. Such unknown interactions should be indicated, e.g. by question marks. Details and further information are provided in step 2, hazard characterisation, below. The number of relevant species that should be selected depends on the specific GM animal under consideration and the complexity of the accessible ecosystems. As a rough guide, 20-30 relevant species might usually be considered, but in cases where many interactions were expected then it might be necessary to consider considerably more species. Positive or negative effects on any relevant NTOs not included in the food web (e.g. endosymbionts) should be identified as well; relevant species should be identified for possible selection as focal species step D.

Step D) Selection of focal species for in-depth investigation

Due to practical considerations, a restricted number of focal species needs to be selected for in-depth investigations from the relevant species identified in step C. Criteria should include a high-ranking position in the ranking process performed for step C and number of direct and indirect food-web interactions with the GM animal, given the food webs constructed after step C. Practical criteria to be considered may include that some species can be tested more effectively, or legal constraints may limit testing of certain NTOs (e.g. protected species). It is expected that the applicant normally selects at least one focal species from each relevant functional group for an in-depth investigation. Depending on the GM animal under consideration, the required numbers of species may be lower (for certain captive GM animals), but can also be substantially higher (especially for non-captive GM animals). The applicant should explain the number, functional groups etc. in his species-selection process and justify that the selection leads to appropriate ERA conclusions. The details of the in-depth investigation are outlined in hazard characterisation below.



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5732 **Figure 7:** Four steps for selecting focal NTOs for an in-depth investigation (modified after Birch et
5733 al., 2004; Hilbeck et al., 2006; EFSA, 2010). This approach should be followed for each relevant
5734 accessible ecosystem (circumstances under which it is not necessary to follow the approach are
5735 outlined in the text). Applicants should justify that the selected ecosystems are relevant and, where
5736 appropriate, that the data gathered can be extrapolated to other accessible ecosystems.

Additional considerations

Evolutionary changes should be considered by applicants. Due to the potential changes in species interactions that GM animals may have on NTOs, the presence of GM animals may cause a selective pressure on NTOs and thus affect their evolution. For example, the presence of growth-enhanced cats that are able to prey upon NTOs not included in the diet of non-GM cats may cause selection of larger NTOs that are outside the diet of growth-enhanced cats. These NTOs could be predators, too, and their increased size may have similar effects on other organisms as the growth-enhanced cat has on them. NTOs that have evolutionarily changed as a result of changed selective pressures caused by GM animals might also be transported to other regions and, due to their novelty, change species interactions there as well. Again, such possible evolutionary consequences of changes in species interactions that GM animals may exhibit, compared to their non-GM comparator, should be assessed for GM animals of all ecological types, not just predators.

If gene transfer to cross-compatible relatives and feral animals after escape or within the offspring of the GM animal 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.

Other important points to consider are knowledge gaps and scientific uncertainties. They should be identified in the problem formulation. 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 animals with NTOs. Although a food web including direct and indirect interactions with selected NTOs (Figure 7) will capture important interactions, direct and indirect interactions with other NTOs will not be included. The uncertainty resulting from excluding non-focal NTOs and their direct and indirect interactions with GM animals should be discussed by applicants.

Step 2: Hazard characterisation

Hazard characterisation relates to relevant (step C) and focal (step D) species identified in the four-step selection process (Figure 7).

Step C) Construct food webs for relevant NTO species

The procedure to select relevant NTO species was described above and depicted in Figure 7. For all relevant NTO species, their interactions in the food web with the GM animal should be shown (based on available information; an example is provided in Figure 8). For scenario 1, an additional food web with all relevant NTO species and their interactions with the conventional counterpart or another comparator species should be drawn. The relevant NTO species are expected to be the same in the food web with the GM animal 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 experts. Applicants should report such information they acquired. If no specific information about the species is available, information about similar and related species may be used if applicable. It is possible that the strength and/or positivity of some identified interactions remain unknown; they should be indicated, e.g. by question marks.

In an additional table, it should be identified for each relevant species if it is directly or indirectly (via one other species), positively or negatively affected by the GM animal (see Table 2, however there it is only shown for three selected NTO species). For example, if the GM animal is a competitor of the NTO, then the NTO is indirectly negatively affected by the GM animal. Positive or negative effects on any relevant NTOs not included in the food web (e.g. endosymbionts) should be identified as well. An introduction to positive or negative effects of different types of interactions has been provided above, but more information can be found in ecological textbooks, e.g. Begon et al. (1996, 2005). Again, unknown interactions should be indicated, e.g. by question marks. Please note that Table 2 merely repeats the information given in the food webs. It is not required to provide additional information for

this table. However, if the applicant is aware of evidence or other information that support, or not support, the species-interaction effects provided in the table, such evidence should be provided.

These positive or negative effects of the GM animal on relevant NTOs should be compared to positive or negative effects of the comparator for those accessible ecosystems where a comparator species is present (scenario 1 above). The applicant should also, where possible, provide a quantitative estimate of the effect of the genetic modification on species interactions as compared to the comparator species. This additional information is necessary, as species interactions might be qualitatively unchanged but quantitatively different compared to the comparator species. In this circumstance, the two food webs look qualitatively identical (i.e. the arrows connecting the species are the same), but there are quantitative differences that should be outlined by the applicant. For example, imagine that growth-enhanced cats consume a certain bird species present in the accessible ecosystem, i.e. the bird species is negatively affected by the growth-enhanced cats. If the bird species is also consumed by non-GM cats, then it is also negatively affected by them. In other words, there is no qualitative difference in the effect on the bird species between the growth-enhanced cats and non-GM cats. However, there might well be a quantitative difference, e.g. because it might be easier (or harder) for the growth-enhanced cat to capture the bird species. If such information on quantitative differences is available, it should be provided by applicants.

For scenario 2 where no comparator species is present, the comparison is between a food web without the GM animal (the current situation) and a food web with the GM animal (the possible future situation). Hence, the positive or negative effects of the GM animal on relevant NTOs are indicating possible future changes, caused by the GM animal, in the populations of relevant NTOs.

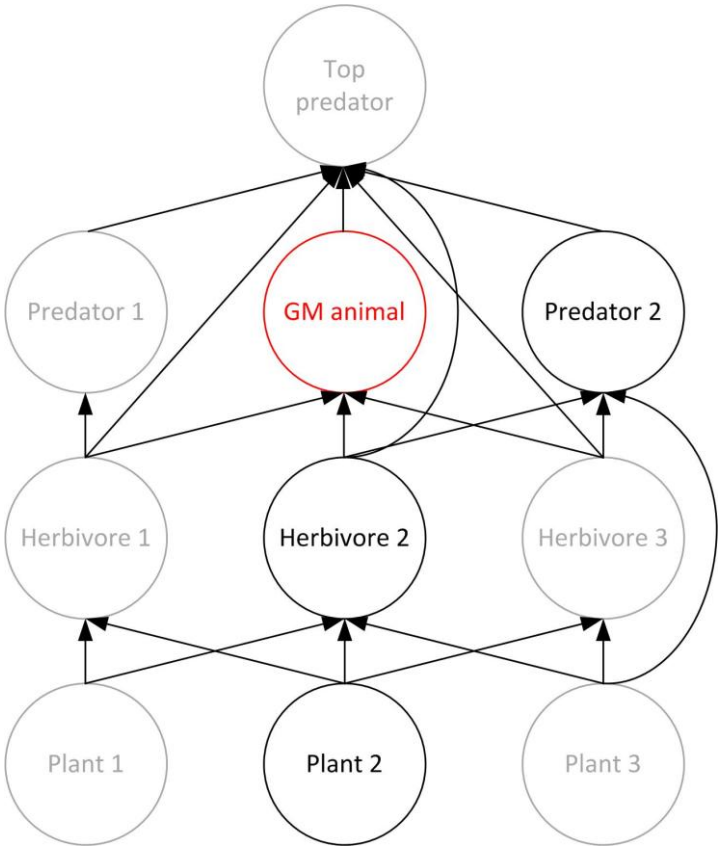

Figure 8: A hypothetical food web, consisting of the GM animal (in red), focal NTOs (in black), and relevant NTOs (in grey). For illustrative purposes, this food web only includes a few relevant and focal NTOs. Applicants are typically expected to construct food webs with a greater number of relevant and focal NTOs, as described in the main body text.

Table 7: Positive and negative effects of direct and one-way indirect interactions of the GM animal with focal NTOs from Figure 8.

Focal NTO	Effect of direct interaction with GM animal for NTO	Effects of one-way indirect int. with GM animal for NTO
Predator 2	No direct interaction	Via top predator: negative Via herbivore 2: negative Via herbivore 3: negative
Herbivore 2	Negative	No one-way indirect int.
Plant 2	No direct interaction	Via herbivore 1: positive Via herbivore 2: positive Via herbivore 3: positive

Step D) Focal NTO species for in-depth investigation

Based on problem formulation and hazard identification, focal NTO species for in-depth investigation were selected from relevant species in the four-step selection process (Figure 7). 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). From (1) to (3), the complexity of these effects increases, and it may not be practical to quantify effects for (3) or (2), depending on the GM animal in question; in such cases, qualitative effects should be identified, as further outlined below.

NTOs can have different direct, indirect, and multitrophic effects with GM animals, hence measurement endpoints differ among NTOs and the functional groups they belong to. In the following paragraphs, we give examples for measurement endpoints, focussing on the first three functional groups given in Table 1: plants, herbivores, and predators. Similar measurements could be done for the functional group formed by decomposers and scavengers. In case of mutualists and symbionts, many mutualistic interactions result from indirect consumer-resource interactions that are captured in the constructed food web. Such interactions can thus also be studied similarly. For endosymbionts and other NTO species living inside of the GM animal's body, the exact quantities to be measured will differ due to the internal environment. Even for those NTO species, however, the next paragraphs will be useful, as they provide general information about measurement endpoints to estimate or measure direct, indirect, and multitrophic effects.

Figure 8 depicts three hypothetical focal NTO species (a plant, a herbivore, and a predator) and their hypothetical direct and indirect interactions with the GM animal, partly via their interactions with non-focal NTO species. These interactions should already be known from step C above (if information was sufficiently available), including the directions (positive or negative) of their effects. For the focal species given in Figure 8, positive and negative effects of direct and one-way indirect interactions are listed in Table 2. The effects given there are those of the GM animal on focal NTO species. As outlined in step C above, the effects should be compared to those of the conventional counterpart or another comparator species if present in the accessible ecosystem (scenario 1). If no appropriate comparator species is present in the accessible ecosystem (scenario 2), then the comparison should be between absence and presence of the GM animal.

The aim of the in-depth investigation is to ensure that adequate data exist to assess the effects of interactions between the GM animal and each focal NTO. This may be done by performing experiments, where feasible. Applicants should use other available information to assess effects of those interactions where experimental investigation is not feasible (depending on the GM animal) (see Figure 9). Experiments should be performed for direct effects in every case and, if possible, also for multitrophic effects. For practical reasons, effects of one-way indirect interactions need not be quantified if multitrophic effects can be quantified. If multitrophic effects cannot be quantified

experimentally, one-way indirect interactions should be quantified by experiments if feasible, and multitrophic effects should be assessed on the basis of these experimental results and other available information. More detailed information on in-depth investigation of (1) direct effects, (2) one-way indirect effects, and (3) multitrophic effects is provided below.

The level of data generation for various measurement endpoints related to characteristics of the GM animal, conventional counterpart or other appropriate comparator species (e.g. morphology, physiology, behaviour, feeding, development, reproduction) and interactions among GM animals and NTOs may extend to:

- Laboratory studies: expected to be the main source of data for most GM animals;
- Enclosure studies: can deliver additional data – such studies should be done for those GM animals where they are feasible;
- Open field studies: where feasible (consider potential environmental risks of such studies);

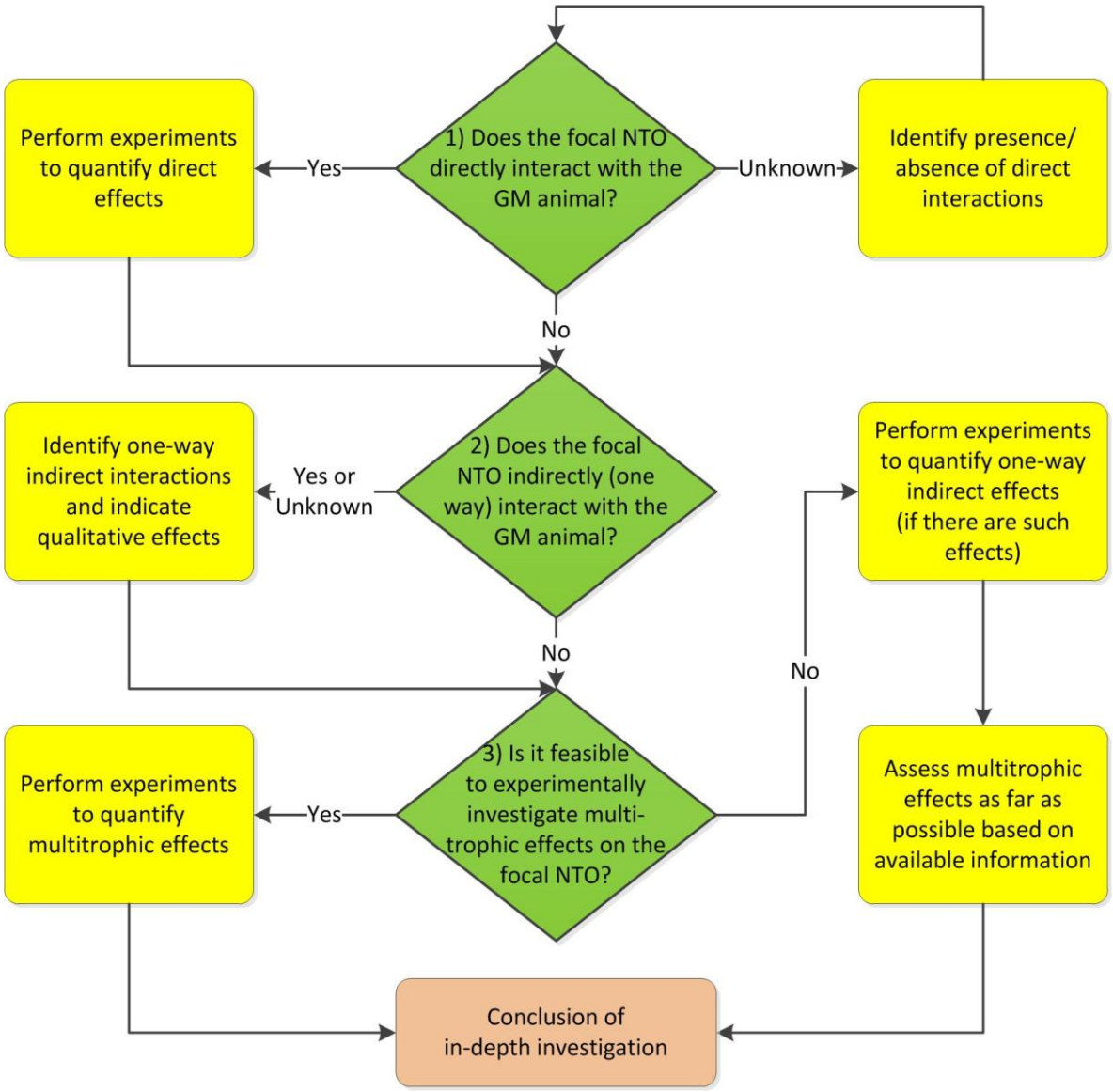

Figure 9: Flow chart, summarizing in-depth investigation for each focal NTO: (1) direct effects, (2) one-way indirect effects, and (3) multitrophic effects on NTOs.

1) Direct effects on NTOs

If direct interactions of the GM animal on focal NTOs are unknown from step C, as information was not sufficiently available, direct interactions need to be first identified. For scenario 1, where the comparator species (the conventional counterpart or some other appropriate comparator species) is present in the accessible ecosystem, information on its direct interactions can be used together with information how the genetic modification may affect these interactions (if the latter is known). If information on the comparator's direct interactions is not available, field studies should be done to identify them. For example, gut analyses can be performed to identify the diet of the comparator and/or its potential predators. Behavioural observations are possible as well.

For scenario 2 where no comparator species is present in the accessible ecosystem, the first step for identifying potential direct interactions with NTOs should be to identify species that are directly interacting with the conventional counterpart in all the ecosystems within its geographic range. The second step should be to compare these species with NTOs in the accessible ecosystem: ecologically similar species can be used as surrogate species that potentially directly interact with the GM animal in the accessible ecosystem. This will require consideration of the genetic modification and how it can affect species interactions, and of the differences between ecosystems within the conventional counterpart's geographic range and the GM animal's accessible ecosystem. The third step should be to test if the surrogate species are really directly interacting with the GM animal, e.g. using simple feeding experiments.

Once direct interactions are identified, their quantitative effects should be experimentally assessed (Figure 9). Let us take the focal herbivore species in Figure 8 as an example ("herbivore 2"). It is consumed by the GM animal. As the consumption rate of a given consumer (in this case: the GM animal) generally depends on the density of its food (in this case: herbivore 2), a useful measurement endpoint is the functional response (Holling, 1959, Jeschke et al., 2002). To measure the functional response, the number of food items consumed per unit time must be measured as a function of food density. In the example, different densities of herbivore 2 should be used to measure the GM animal's consumption rate. Of course, replicates are necessary for each herbivore density (see section 3.5 on experimental design and statistics). Functional responses can be measured either in the laboratory or in enclosure experiments, depending on the size and requirements of the involved organisms. If the comparator species is present in the accessible ecosystem (scenario 1) and also consumes the focal NTO, the functional response of the comparator species should be measured, too, and then compared to the GM animal's functional response. Higher consumption rates indicate stronger direct effects. It can also be helpful to parameterize population-dynamic models with the functional response data obtained in the experiments.

2) One-way indirect effects on NTOs

If one-way indirect interactions between the GM animal and focal NTOs are identified in step C, then their qualitative effects (positive or negative) should be indicated. If strength and/or positivity of these interactions are unknown from step C, then they need to be first identified. It may help to identify important direct interactions between the GM animal and other species in the food web, and then identify if/how these species interact with a given focal NTO. As for direct effects on NTOs, information about species interactions of the non-GM comparator can be used to assess species interactions of the GM animal, with necessary considerations as outlined above.

After identifying qualitative one-way indirect interactions between the GM animal and focal NTOs, applicants should compare them to those of the comparator species (scenario 1), and indicate if they are expected to be quantitatively similar or different. For example, it is possible that indirect interactions are qualitatively similar (e.g. they are both negative), but that the effects are quantitatively stronger or weaker for the GM animal. Such quantitative differences should be assessed as far as possible based on available information. Experiments to study one-way indirect effects should be performed if experiments are not feasible for the quantification of multitrophic effects (Figure 9). If experiments are carried out to assess multitrophic effects (3, see below), the data derived from those

experiments will typically result from all kinds of interactions, including one-way indirect effects (2). Although such data cannot be typically separated into effects of different interactions, applicants do not necessarily need to perform additional experiments to separately assess one-way indirect effects if multitrophic effects are experimentally assessed. Similarly for scenario 2, applicants should indicate qualitative effects of indirect interactions, and quantifying the effects should be done depending on the focal GM animal. Behavioural and feeding experiments (see functional response above) should normally be feasible to quantify effects of direct interactions, which can then be combined to assess effects of indirect interactions. Behavioural experiments might be used to identify trait-mediated indirect effects (see above). Longer-term experiments under semi-natural conditions (e.g. in enclosures) with the interacting species in question being present simultaneously will typically give more reliable data about indirect effects, but the feasibility of such experiments depends on the GM animal in question.

3) Multitrophic effects on NTOs

In a food web, all organisms are linked to each other in multiple ways, and via many other organisms. It is usually impossible to predict the combined effects of all multitrophic interactions by theoretical means, as it is unfeasible or even impossible to quantify all interactions in a food web (not to mention the complexity of adding further non-trophic ecological interactions, e.g. pollination networks, Pocock et al., 2012). As a result, multitrophic effects should usually be estimated by applicants empirically, under semi-natural conditions, e.g. in enclosures with as many important interacting species present as is feasible. Again, effects of the GM animal should be compared to those of the conventional counterpart or another appropriate comparator species, if present in the accessible ecosystem (scenario 1). A possibility would be to release GM animals in enclosures. The change in biodiversity, ecosystem functions, and services due to release of the GM animal can then be compared to changes due to the non-GM counterpart or other comparator species (scenario 1) or to the existing situation if the comparator species is not present (scenario 2). For example, the change in general species community composition can be measured by counting organisms found in plots of a given area or by trapping animals. Also, changes in population densities of focal NTO species can be measured. The literature includes many examples of relatively large-scale experiments (e.g. Niwa et al., 2011), but a specific experimental protocol is not recommended here, as this needs to be designed on a case-by-case basis. Experiments under semi-natural conditions should be scaled up to predict landscape-level effects.

Given the specific GM animal in question, it may not always be feasible to perform such semi-natural experiments for investigating multitrophic effects. In these cases, one-way indirect interactions should be experimentally investigated, and the results of these experiments be combined with other available information, e.g. via modelling, to assess multitrophic effects as far as possible (Figure 9).

Step 3: Exposure characterisation

To evaluate the likelihood that identified hazards will actually pose a risk, it is important that the applicant considers the specific characteristics of the GM animal, its intended use and extent of release (Table 5), and the accessible ecosystems including NTO species. Regarding the intended use of the GM animals, it is clear that NTOs will have a higher exposure to GM animals intended for direct release into the environment than to those that will be held in captivity. For the latter, the likelihood of escape needs to be estimated, based on the characteristics of the facilities where the GM animals will be held captive and on the characteristics of the GM animals themselves, e.g. their mobility. For semi-captive GM animals, the time fractions for captive and non-captive periods (where the animals are freely browsing in the wild) should be estimated. Additionally, for such animals, the likelihood of their escape requires estimation.

Accessible ecosystems were determined by the applicant in the previous step, hazard characterisation. Here, the likelihood has to be evaluated that the genetic modification affects the internal ecosystem of GM animals (e.g. gut microflora) in addition to external accessible ecosystems. Regarding external ecosystems, the exposure is related to the density of GM animals in these ecosystems. For GM animals that are able to reproduce, the density may far exceed the original density upon release. For such

animals, it will be required to estimate population dynamics and to determine whether interbreeding with non-GM species is expected (see section 4.1 on persistence and invasiveness). For GM animals that are not able to reproduce, estimates of maximum numbers of individuals to be released, or that can escape, respectively, should be provided by the applicant. The life expectancy of GM animals is important here as well, since GM animals could be released or could escape, at different points in time, and previously released or escaped individuals might still be alive, so the number of GM animals will vary over time, and may even accumulate.

The exposure assessment should also account for the worst case scenario outlined in section 2.1, step 4, which describes the effects of large-scale uptake of the GM animal. In addition, applicants need to ensure that estimates of exposure determined locally at small temporal and spatial scales are supplemented by considerations of how those estimates may vary when scaled up to regional and longer-term scales (EFSA, 2008, 2011).

Step 4: Risk characterisation

Based on the conclusions reached in the previous steps (hazard and exposure characterisation), the applicant should estimate for each hazard the risk posed by the GM animal for NTOs. The risks to focal NTOs (Figure 7) should be estimated quantitatively wherever possible. It may only be possible to estimate the risks posed for other NTOs that could not be specifically investigated qualitatively, but some estimation is still required. In addition, the risks posed in general for the biodiversity, functions, and services of accessible ecosystems, as specified above, should also be considered and estimates attempted, where possible. The different types of intended uses and releases of the GM animals should be discriminated here, e.g. for captive GM animals, the risk for NTOs in the captive environment should be separately stated from the risk posed by escaped GM animals for NTOs in the wild.

Step 5: Risk management strategies

If risks caused by the GM animals on NTOs have been identified and characterised, the applicant should propose appropriate risk management strategies for each risk. These strategies should be designed, under assumptions of high exposure scenarios, to reduce the risk to a level considered acceptable (criteria defining this acceptability should be explicitly discussed). The implementation of risk management measures should fit to common principles, e.g. the principles of good husbandry, good agricultural practice, and practices related to integrated production (Boller et al., 2004).

Possible risk management strategies include measures to reduce the probability that captive and semi-captive GM animals escape (cf. section 4.3.1 on possibilities to reduce GM animal persistence and invasiveness). For GM companion animals, the applicant should propose strategies to prevent owners from releasing their animals into the wild if they are not longer required (a common phenomenon for non-GM companion animals).

Step 6: Overall risk evaluation and conclusions

After combining the risks of the GM animals for NTOs (risk characterisation, see above) and the possibilities to mitigate them (risk management strategies, see above), the applicant should provide an overall conclusion about the expected level of risk for NTOs if management strategies are in place. This conclusion should also consider the issues of persistence and invasiveness assessed in section 4.3.1. Uncertainties of the conclusion (see section 3.7) should be explicitly mentioned and discussed.

The results of PMEM (see Chapter 5) may provide information which may be useful feedback to inform the ERA subsequently.

4.3.6. Abiotic interactions

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

Abiotic interactions include processes mediated by GM animals that are concerned with the movement, transformation and storage of energy, water, carbon, nitrogen and other elements in

ecosystems. Examples are intake and output of carbon dioxide from the atmosphere by GM animals, alterations by GM animals of plant or aquatic materials, of soil organic matter, and transformation of nitrogenous compounds. Such processes may affect the flux of greenhouse gases (CO2, CH4, N2O) and thereby impact on climate change. Effects of GM animals on soil organisms may be an important driver of abiotic processes since they determine soil structure, nutrient cycling, immobilisation and mobilisation of nutrients, degradation of soil organic matter and emission of greenhouse gases. Applicants should assess whether GM animals and their associated management have potential adverse effects on abiotic aspects of the environment compared to the effects of current production systems. If any factors have been identified that are likely to alter abiotic processes, then experimental work may be needed to characterise the hazard and its associated adverse environmental effects. In all cases the choice of comparator needs to be considered carefully and justified explicitly.

Problem formulation should cover principally two spatial scales: more immediate receiving environments such as production sites in which the GM animal may be kept; and the wider environment comprising land, water and air outside these sites with which the sites interact through exchanges of energy, elements and materials. Indirect impacts due to altered management and husbandry techniques could affect both of these scales and should be considered. In particular, assessment should take account of the import and export of materials (such as animal feed, fuel, pesticides, medication) and losses to the atmosphere and water as a result of human (e.g. agricultural) operations. When taking account of imports of materials, manufacture and procurement should be included, and assessment should not be restricted to application or turnover at the production site. Any negative impacts of abiotic processes on organisms at the sites and in the wider environment should be carefully evaluated on a case-by-case basis with particular reference to the characteristics of the introduced trait and the consequences of the genetic modification or alteration of the GM animal.

Information may be limited on many aspects of abiotic processes. Accordingly, the level of detail required in the ERA will depend upon the characteristics of the GM animal, the GM trait and the scope of the application. Problem formulation could start with a desk study comparing the management and husbandry system used for the GM animal with current conventional production systems. Such a desk study could refer to available data and apply published methods of assessing, for instance, greenhouse gas emissions, erosion, soil degradation and the potential to pollute watercourses. Desk studies should be supplemented by more specific experimental data if available.

With respect to abiotic processes at the production site, the evaluation should address the potential impact of GM animals through factors such as: release of recombinant gene products, GM specific metabolites or other compounds into the environment which may directly influence soil organic matter; excrement that decomposes differently from that of non-GM animals due to either the presence of specific compounds (e.g. toxic metabolites) or altered concentration of substances resistant to decomposition; abiotic habitat modification (e.g. undermining of physical structures due to digging of burrows); and alterations to nitrogen cycling. With respect to abiotic processes in the wider environment, the evaluation should address the potential impact of GM animals and their associated production systems through factors such as: losses from production sites systems to air, or water e.g. greenhouse gas emissions, including those that result from operations and processes that are essential to animal production but which occur outside the production site (e.g. manufacture and transport of animal feed); the capacity of production systems to store water, carbon, nitrogen, phosphorus and other elements essential for ecosystem functioning. Any indications in the desk study that the GM animal and its management have potential effects on abiotic processes should receive detailed attention in the following steps.

Step 3: Exposure characterisation

An assessment is required of the likelihood that abiotic processes in the receiving environments will be exposed to any hazards arising from the GM animal and its management. Exposure in this instance should be considered in terms of how the GM animal and its management may affect abiotic processes both at the production site and in the wider environment, as outlined above. Whilst the degree of

exposure may be higher at production sites than in the wider environment, the assessment should cover all scales and in particular should ensure that the full range of variability between possible receiving environments is accounted for. In most cases, there will be little or no exposure of biogeochemical processes to imported GM animals and their products; however, the assessment should consider whether there will be exposure to products of a GM animal through manure derived from the faeces of animals that are fed an imported GM animal product.

The exposure assessment should focus attention on the worst-case scenario outlined in section 2.1, step 4, which describes the effects of large-scale uptake of the GM animal.

Step 4: Risk characterisation

After assessing all this information, the risk should be characterised to establish the degree of risk from the characterisation of hazard and exposure. Risk characterisation should be carried out for both the production site and the wider environment by considering the potential impacts identified, as outlined above. Risk characterisation should compare existing data from current conventional production systems with that expected in the management and husbandry of the GM animal. The characterisation should demonstrate whether the GM animal and its management have adverse effects on abiotic processes that exceed any present conventional system.

Step 5: Risk management strategies

Based on the outcome of the risk characterisation, applicants should determine and evaluate targeted risk management strategies which could minimize undesired impacts of the GM animal on abiotic processes. Since abiotic processes are influenced by many operations in animal production systems, it may be possible to compensate for negative effects associated with the release of the GM animal by modifying other operations in the system.

Step 6: Overall risk evaluation and conclusions

A conclusion is required of the overall risk to abiotic processes in the environment caused by the GM animal. The risk characterisation and conclusions will determine management measures and requirements for the PMEM plan.

4.3.7. Environmental impact of the specific techniques used for the management of GM mammals and birds production systems

A GM mammal or bird will be introduced to the EU into various receiving environments and will be managed according to the requirements of the animal and the rearing and production systems into which it is introduced. There is a requirement in Directive 2001/18/EC to assess the environmental impact of the specific management and production systems associated with the GM animal.

The introduction into the EU of GM mammals and birds may require specific management practices and, therefore, require changes to the systems for their breeding, rearing and production. Procedures for the disposal of the animal, products derived from the animal, including treatment of all wastes and effluents from the production sites might need to be changed compared with non-GM animal species.

Changes in farming and management practices due to the introduction of GM animals need to be assessed in the context of the existing and evolving range of current management and production systems in the EU and their environmental impact. The applicant should evaluate under which circumstances any changes resulting from the specific GM management and production systems may lead to greater, similar or lower adverse environmental effects than the current system.

The possible environmental impact of the rearing and use of GM animals in non-EU countries is out of the scope of this present ERA. However, any such studies could provide useful information relevant to the management practices and environmental impact should an application be made to import, rear and use these GM animals and animal products (e.g. eggs/ova, semen, chicken egg products) in the EU.

The applicant should provide all relevant available information from outside the EU and explain why this data is relevant to this EU-specific ERA, or why such available data is not considered or is excluded.

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

The rearing, production and maintenance systems of the GM animal are defined by the intended uses of the GM mammal or bird. For example, the influenza resistant chicken and Enviropig may be reared principally indoors, but it is conceivable that some may be reared as free-range animals and, therefore, accessible to other animals and pathogens. This scenario represents a change of use from captive (non-GM animals kept in captivity) to semi-captive (GM animals under human control but not always in captivity). Consequently, the applicant should describe all novel characteristics of the GM animal and evaluate whether these can be accommodated within the management, rearing and production systems currently employed for non-GM animals, or will require changes to these (e.g. husbandry practices; procedures for disposal of animals, animal derived products and waste emissions). Changes in management practices should be seen and assessed in the context of the existing and evolving range of current production systems of non-GM and GM animals, and their environmental impacts.

Novel traits may be associated with increased adaptation to particular environments allowing management and production units to be located in a broader range of environments. Therefore, the problem formulation shall take into consideration receiving environments, including the various husbandry practices under which the GM animal would be kept (see section 3.1).

Changes in the dietary range or in the amount of feed consumed may be a consequence of the genetic modification and should be evaluated by the applicant. The impacts of this on use of natural resources and emission of waste (e.g. faeces, urine, gas emissions, waste water) from production units should be considered.

Due to the large diversity of management and production systems for different animal species and types of production across multiple receiving environments, the detailed requirements for ERA must be identified on a case-by-case basis. It may often be useful to base the ERA on a scenario analysis, which should consider scenarios representative of the diverse situations that may occur and assess their potential implications. The assessment of potential consequences should be carried out by reviewing the scientific literature from both peer-reviewed and technical publications (preferably systematic review where possible), and other methods such as performing meta-analyses, studying commercial uses in non-EU countries, modelling studies and conducting field trials. Applicants should identify and describe any practices that may impact on the environment; for example, management practices associated with altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors, those associated with increased size of animals that may require different waste and effluent disposal/treatment, disposal of on-farm dead GM animals (the cause of death should be properly determined) and disposal of GM animals at the end of, or during, their commercial life.

Where applicants have identified, in other sections of the ERA, management measures associated with the GM animal that mitigate environmental risks (e.g. the use of electrified rather than non electrified fences), the implications of these measures for environmental impacts should also be considered in this section. For example, applicants should describe measures, such as the design of the rearing and production systems, to prevent the escape of the GM animal unless the GM animal is non-captive. Similarly, applicants should describe what measures are required when the GM animal is released to a semi-captive environment, such as enclosed pasture that allows interaction and/or crossbreeding with wild species (wild boar/pigs; release of GM companion animals) or exposure to pathogens (wild birds/free-range chickens) (see also section 4.3.3). Some indication of the long-term risks should be an integral part of the assessment.

In summary, the environmental risk assessment should:

1 describe the potential range of GM-based management and production systems likely to occur across receiving environments including new receiving environments and how they differ from current management systems;

2 describe the potential adverse environmental impacts associated with the differences in management systems of the GM animal compared to the non-GM comparator;

3 determine which differences in management and production systems are related to potential higher adverse effects than current systems.

Step 2: Hazard characterization

Based on the hazards identified in step 1 problem formulation, applicants are requested to characterise further the proposed production and management systems for the GM animals with special reference to changes and associated hazards from already existing systems These should take into account the two scenarios described in detail in section 4.3.5, where a conventional counterpart or comparator species is present/absent in the accessible ecosystems.

The environmental impacts and the potential harms associated with such changes should be characterised.

Step 3: Exposure characterization

If management changes are required, the applicants should evaluate the scale and frequency of those changes on the receiving environments. Applicants need to consider and assess various levels of uptake of the GM animal. This procedure gives the following two or three alternative scenarios:

a) A 'small scale' scenario, which considers the local replacement of the non-GM comparator by the GM animal;

b) A 'wide scale adoption/uptake' scenario ('worst case' scenario);

c) If applicable, a scenario where the GM animal is introduced into an environment where the non-GM comparator is not present.

The ERA must account for the animal production system and accessible ecosystems as a whole and in particular should account for spatial effects at the regional scale and temporal effects at the long-term, multi-generational scale. For ERA, upscaling, modelling, simulation and analysis of production systems and accessible ecosystems may be required, in addition to the analysis of smaller-scale experiments (EFSA, 2008).

Step 4: Risk characterisation

Based on the information gathered in steps 2 and 3, the risks posed by any changes to the rearing, management and production systems should be assessed for their likelihood and degree to cause environmental harm. This risk characterisation should also consider the risk mitigation measures identified in other sections of the ERA.

Step 5: Risk management strategies

In situations where the ERA concludes that changes in management and production systems may have adverse environmental impacts compared with the non-GM management and production systems, applicants should present and assess risk management strategies to mitigate these adverse effects.

The efficacy of each proposed management strategy in the relevant receiving environments should be evaluated by applicants.

Step 6: Overall risk evaluation and conclusions

A conclusion is required of the overall risk presented in the different scenarios, considering the proposed mitigation methods to reduce the perceived risk.

4.3.8. Impact on non-GM animal health and welfare

An assessment is required of whether the production of the GM animal presents a hazard for its own health and welfare as explained in the EFSA on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects (EFSA, 2012a) and in section 3.8 of this document. This section covers the required assessment of whether the production of the GM animal and/or its products presents a new hazard for the health and welfare of other animals (see also sections 4.3.3 and 4.3.5 where this is also addressed). Such assessment is particularly important for GM animals, such as companion animals, which are not destined for human or animal consumption and where impacts on animal health may not have been so meticulously studied.

The risk for cross infection between animals has already been covered in section 4.3.3. For example, if a semi-captive GM companion animal was infected with rabies from a wild animal that was a reservoir host and the GM animal had a changed behaviour pattern so that it was more docile and friendly to other animals, then it might increase the risk of transferring the infection to other companion animals (or humans) in the household.

For the assessment of non-GM animal health and welfare, the applicant is asked to refer to the principles of animal health and welfare assessment as outlined in several scientific opinions of the EFSA AHAW Panel (EFSA, 2007, 2010, 2011f, 2012b, 2012c, 2012d). Further the applicant should refer to the principles in the Guidance Document on the risk assessment of food and feed from GM animals and on animal health and welfare aspects (EFSA, 2012a).

If considered necessary, the applicant should follow the six steps approach as outlined in section 2.1 on a case-by-case basis. A conclusion is required for the overall risk on non-GM animal health and welfare.

4.3.9. Impact on human health

In accordance with Directive 2001/18/EC, the protection of human health requires that due attention be given to controlling risks from the deliberate release into the environment of GM mammals and birds. This includes, in particular, the risks for farmers and other workers working with, and members of the public coming into contact with and/or being in the vicinity of GM mammals and birds. The applicant shall consider whether the modified mammal or bird presents a new hazard for human health. The applicant shall consider both immediate and delayed effects on human health resulting from potential direct and indirect interactions with GM mammals and birds.

This guidance considers effects of GM mammals and birds on human health through non-ingestion routes that includes exposure through ocular, oral, nasal and dermal contact, and inhalation. The impact on human health caused by accidental ingestion of GM mammals and birds is not considered explicitly in this section because such exposure is likely to be exceedingly small. However, if applicants are aware of any reasons why the accidental ingestion of GM mammals and birds might have a greater impact on human health than the accidental ingestion of their non-GM comparators then these reasons should be discussed fully in this section (see also EFSA, 2012a).

For GM animal applications for food and feed purposes, the applicant is requested to refer to the requirements detailed in the EFSA Guidance document on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects (EFSA, 2012a) and, where relevant, any EFSA Opinions dealing with e.g. allergenicity (EFSA, 2010d). However, the ERA in this section is required particularly for GM mammals and birds which are not destined for human or animal consumption and where impacts on human health may therefore not have been so meticulously studied. Three examples of such GM animals are (see introductory part of GM mammals and birds

section): (i) the sterile rabbit; (ii) companion animals such as the growth-enhanced cat; (iii) the Avian Influenza resistant chicken (Lyall et al., 2011) which may serve as pathogen reservoir and release pathogens of their host ranges overcoming the species barrier (see section 4.3.4 target organisms and section 4.3.2 on horizontal gene transfer). The avian influenza A virus H5N1 is one such example of a pathogen. The expected host range of H5N1 is avian species, but natural infections of H5N1 have been reported unexpectedly in humans (reviewed by WHO, 2005), as well as in other unrelated mammals (e.g. domestic cats (Songserm et al., 2006) and pigs (reviewed by Neumann and Kawaoka, 2010).

The applicant shall follow the six-step comparative approach promulgated throughout this document on a case-by-case basis (section 2.1). The applicant shall compare the aforesaid farmers, workers and members of the public with those producing, processing or coming into contact with non-GM mammals and birds. For farmers and workers, the comparisons shall be made under similar working conditions, typical for those workers. A conclusion is required of the overall risk to human health.

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

Depending on the characteristics of the GM mammals and birds, some but not all of these GM animals might cause undesired health effects to humans, ranging from itchiness and irritation to potentially serious diseases. The public health relevance of the effects of GM mammals and birds varies, not only from intended trait to intended trait, but also between receiving environments, depending on the presence of GM animals and climatic influences. The applicant should evaluate whether changes associated with the management of the GM mammals and birds present greater hazards to humans as compared to their non-GM comparators.

Zoonosis, any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa, have important impacts on public health[17]. Databases that systematically document infectious pathogens causing disease in domestic mammals and humans have been constructed; the reported infectious agents include bacteria, fungi, helminths, protozoa, viruses and prions. Of these, viruses, in particular RNA viruses, are the most likely to cause emerging diseases (Cleavelan et al., 2001).

Considerations of potential pathogenic impacts on human health through the deliberate release of GM mammals and birds should include but not be restricted to:

a) disease transmission capacity to humans due to the physiological and/or behavioural changes as a result of the genetic modification, e.g. disease-resistant GM mammals or birds, hypoallergenic GM companion animals;

b) capacity to cause new human diseases, e.g. mammals or birds that are genetically modified to be disease-resistant may become a reservoir for other pathogens that can consequently cause human disease (dynamics of the existing pathogens should also be taken into consideration by the applicants and detailed recommendations for this hazard scenario can be found in section 4.3.3);

c) emergence/selection of new pathogens and/or strains with altered host ranges that include humans (detailed recommendations for this hazard scenario can be found in section 4.3.3).

Other considerations of potential non-pathogenic impacts on human health by the deliberate release of GM mammals and birds should include but not be restricted to:

d) introduction of toxic or allergenic effects of the GM mammals and birds and/or their metabolic products into the receiving environments, e.g. as newly expressed products or changes in the production of toxins or allergens by the GM mammals and birds when compared to their conventional counterparts;

[17] WHO, www.who.int/zoonoses/en

e) any phenotypic changes in the GM mammals and birds identified during development which may increase the risk to human health;

f) any changes in specific management practices for GM mammals and birds.

The risk to workers managing and handling any GM animal whose behaviour may have been changed as a result of the modification should be assessed. Changed behaviour may change the contact rate or the nature of the contact between animals and humans (see section 4.3.3). In particular, the risk from modified companion animals to workers in the pet industry such as the growth-enhanced cat should be addressed. Such animals may pose a greater risk of harm through biting or scratching than appropriate non-GM comparators.

Step 2: Hazard characterisation

Hazards identified in step 1 should be characterised.

a) Disease transmission capacity to humans

Applicants should determine whether the pathogen load spread from the GM animal, for a specific pathogenic agent, will reach levels that can cause human diseases. Where a potential hazard is identified, laboratory animal experiments may be required in order to determine infectivity and transmission capacity.

b) Capacity to cause new human diseases

Applicants should determine the magnitude of the potential for mammals or birds (that are genetically modified to be disease-resistant) to act as reservoirs for other pathogens as outlined in section 4.3.3.

c) Emergence/selection of new pathogens and/or strains with the potential to cause human diseases

The applicant should examine the pathogen characteristics to determine whether or not a pathogen that can cause human diseases is likely to emerge. Genotyping can be a useful method in this aspect (for an example, see Xiao et al., 2006).

d) Introduction of toxic or allergenic effects of the newly expressed products in the GM mammals and birds and/or their metabolic products into the receiving environments

It should be verified whether the GM mammals and birds under consideration produce a toxin which might cause harm to humans. The applicant should therefore discuss potential toxic effects in the light of the intended effects of the newly introduced proteins and of any observed alterations in the GM mammals and birds compared to their conventional counterparts. The assessment endpoint will be to determine whether the GM mammals and birds have altered toxicological characteristics compared to their appropriate non-GM comparator that may lead to adverse impacts on human health.

With respect to the potential for sensitisation and allergenicity as a result of occupational and accidental exposure to the GM mammals and birds, it should be assessed whether the GM mammals and birds have altered allergenic characteristics as a result of the genetic modification. To this end both the direct and known indirect effects of the genetic modification on the physiology of the GM mammals and birds should be taken into account. In particular, applicants should distinguish between, and allow for the effects of both the allergenicity of any newly expressed proteins as a de-novo or cross-reactive allergens and any changes to the intrinsic allergenicity of the animal.

In addition, these risks should include an assessment of possible allergenicity with respect to potential differences between the GM animal and its non-GM comparator, bearing in mind: (i) that materials from animals represent complex matrices in which interactions between proteins and other constituents may occur and that such interactions might alter the allergenicity of the animal in an

unpredictable manner; and (ii) there is a great variability in the intensity and specificity of human allergic responses (and see section 2.1.5 of EFSA (2012a)). Applicants shall record carefully and analyze any adverse effects occurring in those people working with GM animals during their development and subject to occupational exposure and frequent contacts with them. Following this, potential allergenic effects may be assessed for the general population. Applicants shall take particular care over this allergenic assessment if any new (recombinant) protein is expressed in dander, saliva or urine.

e) phenotypic changes in the GM mammals and birds can increase risk to human health

The applicant should determine to what extent phenotypic changes to the GM mammals and birds present an increased hazard to handlers.

f) consequences of any change in specific management practices for GM mammals and birds

The applicant should evaluate to what extent changes associated with the breeding, caring, transport and processing of the GM mammals and birds present greater hazards to humans. These include changes in husbandry and disease management. For example for disease management, the applicant should determine to what extent the use of antibiotics may increase the pathogen load or increase frequencies of antibiotic resistance in those pathogens that can cause human diseases.

Step 3: Exposure characterisation

The possible impacts of GM mammals and birds on human health: (i) may occur at different stages in the development and processing of the GM mammals and birds; (ii) may vary with different intended uses for the GM mammals and birds; and (iii) may differ between different receiving environments. Applicants should assess management practices of the GM mammals and birds in order to assess the different levels of occupational exposure, in relation to the characterised hazards, associated with the GM mammals and birds. In this aspect all human exposure routes should be taken into account, including those to members of the public.

The applicant shall assess potential dermal, nasal, ocular and inhalatory exposure as applicable. The risk from dermal contact with, nasal or ocular discharge from, or contact with the dander, saliva, urine or faeces from GM animals should be assessed as a consequence of skin contact with or inhalation or ingestion of material from GM animals, by farmers, workers and members of the public passing by or in the vicinity of those animals. Such a risk should be considered particularly for GM animals resistant to pathogens (see section 4.3.3).

Contact rates may be quantified through behavioural experimentation (e.g. Mayberry et al., 2010). Such techniques may be used to estimate measurement endpoints such as daily percentage of time spent in activity, walking/flying/swimming speed, timidity or aggressivity (Dall et al., 2004) against humans, etc. Applicants should attempt to quantify behavioural endpoints wherever possible (and see Wouter et al., 2010). The importance of accounting for animal personality is increasingly recognized (e.g. Gosling and John, 1999; Wolf et al., 2007).

It is expected that the procedures applied during management, care, killing, transport and storage of the GM mammals and birds or of their parts or products will differ widely between different management systems. Therefore, as a prerequisite for the exposure assessment, a detailed description of these procedures is required (see also section 4.3.7). These descriptions should focus on the identification of critical steps where contact and/or inhalation could occur as well as the level, frequency and duration of exposure during the production systems. The exposure assessment should focus attention on the worst-case scenario outlined in section 2.1 and describe the effects of large-scale uptake of the GM animal.

If qualitative terms are used to express relative likelihoods of exposure, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication

should be given of the range, within a numeric scale of 0 to 1, to which the term is intended to refer. For example, “the likelihood of exposure of a worker to nasal discharge in housing units was estimated to be moderate, where 'moderate’ in this context means within the range 0.1 to 0.4".

Step 4: Risk characterisation

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects should be made for each hazard identified in step 1. Where precise quantitative evaluation of risk is not possible, terms should be defined where possible. The evaluation for each risk should consider the magnitude of the consequences of the hazard and the likelihood of its occurrence. The uncertainty for each identified risk should be described (section 3.7).

Step 5: Risk management strategies

The applicant shall develop proposals for measures intended to minimise the exposure of farmers, workers and passers-by to the GM animals, and the expected impact of these measures should be assessed.

Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM mammal or bird should be made taking into account the results of the ERA and associated levels of uncertainty, the weight of evidence and the risk management strategies proposed in the receiving environments.

5. Post-Market Environmental Monitoring plan

An objective of Directive 2001/18/EC (EC, 2001) and other environmentally-related legislation is to protect the environment, including natural resources and ecosystem services. The EFSA GMO Panel recognises that all human activities can have environmental impacts and the potential to affect ecological functions and processes, so that there is a general need to consider the impacts of any new product, development or process on environmental protection goals. In this respect, Directive 2004/35/EC (EC, 2004) on environmental liability with regard to the prevention and remedying of environmental damage defined environmental damage as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly.

Directive 2001/18/EC (EC, 2001) introduces an obligation for applicants to implement monitoring plans in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human and animal health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the aforementioned Directive.

According to Annex VII, the objectives of (an environmental) monitoring plan are:

- **Case-Specific Monitoring** (CSM) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct;

- **General Surveillance** (GS) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA.

Article 20(1) of Directive 2001/18/EC states that: '*Following the placing on the market of a GMO as or in a product, the notifier shall ensure that monitoring and reporting on it are carried out according to the conditions specified in the consent*'. Thus the final monitoring plan and implementation of the monitoring will be determined by risk managers in association with applicants.

When risks have been identified and characterised (see step 4 in section 2.1.4), applicants should then consider the need for post-market environmental risk management strategies and should describe how these are incorporated into the PMEM plan of the GM animal. Thus the overall conclusions of the ERA provide the basis for PMEM plans, which focus on risks to human and animal health and the environment (including domestic animal health) identified in the ERA and can be used to provide data on uncertainties identified in the ERA. When risks and/or significant levels of critical uncertainty linked to the GM animal and its management have been identified in the ERA, then Case-Specific Monitoring (CSM) should be carried out after placing on the market, in order to further inform the ERA. Thus the role of CSM in PMEM plans is to check the assumptions made during the ERA and to ensure that the ERA conclusions are valid as regards the authorised use of the GM animal. PMEM also includes General Surveillance (GS) and can provide data on any unanticipated adverse effects of the GM animal on the environment. PMEM information is also useful for confirming the results of the ERA.

In addition, EFSA advises that PMEM data are recorded in centralised national and/or EU-wide databases which would be accessible when required for analysis purposes. Such data could be used by both Member States and the European Commission to take decisions on the level of release of a GM animal. In order to reach these decisions, the appropriate data and analyses need to be available for scrutiny at both national and EU level.

The Guidance Document on the risk assessment of food and feed from GM animals including animal health and welfare aspects (EFSA, 2012a) also addresses the possible need for post-market monitoring and surveillance of health and welfare of GM animals as well as post-market monitoring of GM animals-derived food and feed. The adverse events reports will have to be labelled as urgent or

routine, in line with EU legislation on the reporting of adverse health effects of GM animal after their release on to the open market.

5.1. Case-Specific Monitoring (CSM)

When risks or important gaps in scientific information or significant levels of critical uncertainty linked to the GM animal and its management have been identified in the ERA, then CSM should be carried out after placing on the market, in order to confirm assumptions made in the ERA and to further inform the ERA. CSM is hypothesis-driven and should be targeted at the assessment endpoints and protection goals identified in the ERA conclusions as being at risk or where levels of critical uncertainty were identified in relation to potential risks associated with the GM animal.

Monitoring of potentially adverse cumulative long-term or large-scale effects and the resolution of areas of critical uncertainty, identified in the ERA, are important objectives of PMEM, which should be considered initially within CSM. When there is critical uncertainty concerning the impacts of time and scale and/or the acceptability of environmental risks of a GM animal compared to non-GM animal, then CSM is indicated. Applicants shall clearly explain their rationale for not adopting CSM where risks and critical uncertainty have been identified in the ERA, e.g. where applicants develop risk management strategies that reduce risks to levels where no environmental harm is occurring (see step 5 of section 2.2).

Monitoring is often an important component of the management and stewardship of a GM animal and so, where risk management strategies have been put in place due to identified risks or critical uncertainty, applicants should consider monitoring their efficacy in order to determine the actual reductions in exposure. In such cases, the monitoring results can be used to modify the risk management strategies, so that they are appropriate and proportional to the remaining levels of risk. Depending on the objectives of CSM, studies should be conducted at production or release sites with the GM animal under commercial conditions in order to determine effects at these scales of the release. Where identified environmental hazards trigger the need for specific confinement, the monitoring plan should also consider the need to monitor the effectiveness of the confinement measures. If there is uncertainty on the reliability or efficacy of confinement measures, then specific monitoring may be needed to assess the reliability and efficacy of the confinement measures. The results of this monitoring can be used to re-assess the risk management strategy and make appropriate modifications, as well as assessing the levels of exposure and risk that are occurring.

For each CSM study, all the relevant scientific questions that the study is designed to address shall be listed explicitly at the design stage of the study and, in addition, each of these questions shall be re-stated in formal terms, in the form of the null hypothesis that is to be tested to answer the question. Clear and explicit statements shall be made concerning the minimum levels of data acceptable for each variable being assessed, below which results would lack credibility (EFSA, 2010a). A minimum effect size shall be specified that the study is designed to detect. In addition, where appropriate, a statistical power analysis shall be done to estimate the power of the study to detect this effect, based on the stated effect size and assuming a 5% type I error rate. The power analysis shall use only information verifiable as available prior to the study; under no circumstances shall data from the study itself be used. For situations where many species are sampled, a power analysis should be done only for focal species expected to be the most abundant.

Applicants should provide the raw data and analysis of the CSM results to Member States and the European Commission at the agreed time intervals. Applicants should describe the methods used to analyse the data and a clear rationale for the statistical methods chosen. They should establish effective quality assurance and auditing schemes for the analysis and archiving of data. Applicants should discuss the biological significance of any impacts observed, discuss to what extent the results confirm or not the assumptions made during the original ERA and conclude on the implications of their results for confirming the conclusions of their original ERA. If CSM of the GM animal provides new information which could have consequences for the risks of the GM animal on the environment

and human health, then the conclusions of the ERA need to be re-addressed in order to (1) determine whether the initial risk characterisation has changed; and (2) determine whether it is necessary to change risk management strategies (including lifting some of them) as well as (3) to determine whether changes to the monitoring procedures are needed. Therefore the CSM plan should also indicate how it will be reviewed in order to consider results and experiences gained from the previous year(s) of CSM.

5.2. General Surveillance (GS)

The objectives of GS are to detect unanticipated adverse effects, to determine the harm to protection goals and to determine the causality between the detected unanticipated adverse effects and the production, release and/or escape of the GM animal. While it is considered the role of applicants to develop PMEM and GS plans, it is also clear that EU Member States have certain responsibilities for broader environmental protection monitoring, which could be used by applicants in GS. Thus GS planning and implementation will also involve Member States.

The major challenges in designing GS plans are:

- to detect a change (= an alteration that results in values that fall outside the normal range, given the variation due to changes in management practices, receiving environments and associated biota in the EU). This requires that comparisons and/or baselines are assessed so that deviations from current or normal values can be detected;

- to determine whether the change is causing an adverse effect (e.g. causing irreversible damage to a protection goal) and;

- to determine whether the adverse effect is associated with the production, release and/or escape of the GM animal.

Environmental damage can be determined by considering effects on certain relevant subjects of protection associated with environmental protection goals (see Table 1). The subject of protection is considered to be damaged if the adverse effect is considered biologically significant. The identification of a biologically significant adverse effect should consider its intensity (e.g. extent of loss), the value of the impaired subject of protection (e.g. high value of the populations of a species protected by law) and the reversibility of, or recovery from, the damage. A range of existing monitoring networks (e.g. for aquatic systems) can supply baseline data and provide the ability to compare data from a range of different sources in order to indicate whether an effect is unusual and potentially adverse. To determine whether an effect is harmful and linked to a GM animal, a specific study to evaluate the harm and determine the cause would then be required.

A crucial step in designing a GS plan is to identify the aspects of the environment that need to be protected from harm and to define the assessment endpoints and measurable indicators to be considered for monitoring. Defining assessment endpoints is necessary to focus GS on assessable/measurable aspects of the environment, i.e. a natural resource or natural resource service that could be adversely affected by the GM animal and that requires protection from harm. Defining the assessment endpoints should be done considering the receiving environments where the GM animal will be produced/released or can escape to and the EU standards implemented by Member States. The selected assessment endpoints need to be examined to determine how these endpoints can be monitored and whether they are already being surveyed by existing environmental monitoring networks. General environmental monitoring networks in EU Member States (e.g. national surveys on insects, birds) are an expression of the need to observe assessment endpoints systematically in order to detect or measure impacts on protection goals.

It is the task of applicants to identify the appropriate tools in the GS plan to cover the indicators and measurement endpoints defined for the protection goals. For example, existing monitoring networks and the review of ongoing research & development, including scientific literature, are likely to be

potentially useful sources of information. Applicants should then consider the use of these monitoring networks in GS plans for the GM animal. Furthermore, information on research & development activities on GM animals from empirical risk assessment research in laboratories and microcosms could be reviewed and the implications of the results considered. These data will also be helpful to set up baselines.

Applicants should consider the range of assessment endpoints that the identified tools for GS will cover and whether they are likely to detect unanticipated effects as well as their cost-effectiveness and proportionality.

Additional guidance on PMEM of GM plants, as in the Scientific Opinion providing guidance on PMEM of GM plants (EFSA, 2011b), might be considered, on a case-by-case basis, for PMEM of GM animals.

Following the placing on the market of a GM animal, applicants have a legal obligation to ensure that monitoring and reporting are carried out according to the conditions specified in the consent. Applicants are responsible for submitting the PMEM reports to the European Commission and Member States. The PMEM results of the deliberate release into the environment of GM animal should be presented in accordance with the standard reporting formats established by Commission Decision 2009/770/EC (EC, 2009).

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 13 February 2007, to the EFSA Executive Director requesting a guidance on GM animals, addressing both food/feed and environmental safety.

2. Acknowledgement letter, dated 17 August 2007, from the EFSA Executive Director to the European Commission.

3. Letter from EFSA to European Commission, dated 24 July 2008, updating the European Commission on the work carried out and requesting an extension of the deadline of the mandate.

4. Acknowledgement letter, dated 1 September 2008, from the European Commission to the EFSA Executive Director.

5. Letter from EFSA to European Commission, dated 24 November 2009, updating the European Commission on ongoing developments.

6. Letter from the European Commission, dated 25 March 2010, asking for a revision of the mandate.

7. Acknowledgement letter, dated 28 April 2010, from the EFSA Executive Director to the European Commission.

8. Letter from EFSA to European Commission, dated 10 January 2011, requesting an extension of the deadline.

9. Acknowledgement letter, dated 14 March 2011, from the European Commission to the EFSA Executive Director.

10. Letter from EFSA to European Commission, dated 20 July 2011, updating the European Commission on ongoing developments.

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GLOSSARY

Note: Specific terms may have a different meaning depending upon the context in which they are used. Therefore the definitions provided in this glossary are to be considered in the context of the present Guidance Document on ERA of GM animals.

Where applicable glossary terms have been taken from the IPPC standard ISPM 5, see: https://www.ippc.int/file_uploaded/1273490046_ISPM_05_2010_E.pdf

Accessible ecosystem: is defined as a biological system (where the system includes all the living organisms and abiotic factors occurring within it) within receiving environments to which the GM animals, including their by-products and the recombinant DNA, have access and may interact with.

Active population: the part of the population that causes an effect. For instance, female mosquitoes bite, so they are the part of the overall population that causes an effect. They are therefore considered the active population.

Adverse effects: are defined as undesired effects, leading to harm, and consisting of measurable changes in the environment (e.g. change in a natural resource or measurable impairment of a natural resource service) beyond accepted ranges.

Amensalism: a biotic interaction between two types of organisms (or species) where one organism (or species) negatively affects the second organism (or species, e.g. its population density), but the second has no effect on the first.

Artificial selection: more commonly known as selective breeding, where professionals study the genotype and phenotype of parent organisms in the hope of producing a hybrid that possesses many of the desirable characteristics found in their parents.

Assessment endpoint: an assessment endpoint can be defined as a specific natural resource or natural resource service studied in the ERA, that needs protection. It is the valued attribute of a natural resource worth of protection (Suter, 2000).

Autonomous elements: a defined region of DNA (genetic element) that is capable of independent movement within a genome; usually through the production of a transposase. Non-autonomous elements may be able to move within a genome if a transposase is provided in trans.

Captive GM animals: animals that are permanently held in captivity (i.e. in containment, fenced areas, or other areas from which they are not normally expected to escape). Examples for captive GM animals include domesticated animals and pets held indoors, or animals in a fenced area.

Case-by-case: is defined as the approach by which the required information may vary depending on the type of the GMOs concerned, their intended use and potential receiving environments, taking into account i.a. GMOs already in the environment (EC, 2001).

Censoring: term used to describe situations when the value of a measurement falls outside the measurable range, or the value can only partly be known due to limited experimental design or measurements.

Commensalism: a biotic interaction between two types of organisms (or species) where one organism (or species) positively affects the second organism (or species, e.g. its population density), but the second has no good or bad effect on the first (modified after Begon et al., 1996).

Competitive substitution: genetic traits that are substituted in a population through a process of competitive selection.

Conspecific: another organism of the same species.

Critical uncertainty: uncertainty that, once resolved, may result in a conclusion that an effect is likely to cause environmental harm (EFSA, 2011c).

Deliberate release: is defined as any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific measures are used to limit their contact with and to provide a high level of safety for the general population and the environment (EC, 2001).

Disease resistance: capability of an animal to prevent colonisation of a pathogen, or to prevent disease upon colonisation by a pathogen.

Disease tolerance: capability of an animal to allow pathogens entry, distribution and survival without any significant long term effects on animal health and survival.

Disease vector: an animal that transmits pathogens causing disease to other organisms.

Ecological niche: an ecological niche is defined as an *n*-dimensional hypervolume within which individuals of a species can survive, grow, and reproduce, with *n* being the number of environmental conditions and resources.

Ecosystem(s): are all recognizable self-contained entities with living beings (the species community) and non-living components within their boundaries.

Ecosystem services: include all services provided by ecosystems, e.g. production of food, fuel, fibre and medicines, regulation of water, air and climate, maintenance of soil fertility, cycling of nutrients. Ecosystems services are distinct from ecosystem functions by virtue of the fact that humans, rather than other species, benefit directly from these natural assets and processes (Millennium Ecosystem Assessment, 2005).

Elicitation process (and aggregation techniques) **for ERA:** is a methodology used to identify which possible environmental effects of a GMO matter the most to the different, potentially affected and interested parties, and building on these findings, to identify which sources of uncertainty in an ERA are the most important to address through an explicit uncertainty analysis (Dana G.V. et al, 2011).

Enhanced fitness: can be defined as a characteristic of an individual or sub-population of individuals that consistently contribute more offspring to the subsequent generation (Wilkinson and Tepfer, 2009).

Environmental harm: is defined as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly (EC, 2004).

Environmental risk assessment: is defined as the evaluation of risks to human health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose and carried out in accordance with Annex II (EC, 2001).

Establishment: the process in which a population becomes self-sustaining in a new environment; Perpetuation, for the foreseeable future, of a pest within an area after entry [ISPM 5]

Feral animals: are animals existing or having returned in a wild or untamed state, especially after being domesticated.

Fitness: the success of an individual in surviving and reproducing, measured by the individual's genetic contribution to the next generation and subsequent generations. The biological fitness of an organism depends on various factors, including its ability to proliferate, to resist disease to survive with limited resources, cope with difficult growth conditions, colonize new territory and outwit predators.

Fitness benefit: more effective reproduction (more effective fertility), for example because the genetic modification allows insects to reach maturity earlier, live longer, produce more eggs, increase larval survival, or otherwise causes greater population fertility.

G x E interactions: when different environments have a different effect on one genotype than on another. An interaction may change the relative ranking of genotypes when the same traits are measured under different environments.

Gene drive system: genetic elements that show non-mendelian inheritance and are known to spread within populations even in the absence of fitness advantage. They can be used to drive linked genes through populations (e.g. some transposons).

Genetically modified organism (GMO): is defined as an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (EC, 2001).

Hazard: (harmful characteristics): is defined as the characteristics of an organism that can cause harm to or adverse effects on human health and/or the environment (EC, 2002).

Horizontal gene transfer (HGT): is any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. HGT is usually unidirectional and transfer only a limited amount of DNA from the donor organism into the genome of the recipient organism.

Hybridization: is the genetic process of crossbreeding between genetically dissimilar parents to produce a hybrid.

Indicator: parameter/tool used to demonstrate, during monitoring activities, a shift from the current baseline, possibly indicating unintended/unexpected effects of the GM animal.

Inherited lethality: gene constructs that when inherited by offspring are fatal to survival.

Introgression: transfer of the gene(s) of one species into the gene pool of another through repeated backcrossing of an interspecific hybrid (bred by mating two species, normally from within the same genus) with one of its parents.

Limits of concern: are defined as the minimum ecological effects that are deemed biologically relevant and that are deemed of sufficient magnitude to cause harm. These limits of concern are set for each assessment endpoint in the problem formulation.

Measurement endpoint: a measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint and is a measure of biological effects (e.g. death, reproduction, growth) of particular species, and can include measures of exposure as well as measures of effects.

Microbes/microorganisms: any microbiological entity, cellular or non-cellular, capable of multiplication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture, archaea, bacteria, filamentous fungi, yeasts, protozoa and microalgae (EFSA, 2011d).

Modelling: is an attempt to describe the behaviour of a natural system or to predict the likelihood of an event occurring within a system, and may utilize mathematical formulas and computer simulations.

Mutualism: a biotic interaction between two types of organisms (or species) where both positively affect each other, e.g. their growth, growth rate, or population density (modified after Begon et al., 1996).

NOAEL: 'no-observed-adverse-effect-level' the maximum concentration of a substance that is found to have no adverse effects upon the test subject.

Non-captive GM animals: are animals that are intended to be directly released into the environment without human control, e.g. mosquitoes.

Non-Mendelian segregation: non-random separation of genetic traits during gamete formation that favour one allele over another.

Non-native: a species or population not ordinarily resident in a location.

Outbreak: the presence of detectable individuals of a non-native pest species in a new environment; A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area. [ISPM 5]

Parasites: an organism living on (exoparasites) or within (endoparasites) another organism (the host) and benefits from the association while harming the host. Parasites may be unicellular (like protozoa) or multicellular (like tapeworms or sea lice). Some parasites may have a very complex life cycle involving different host for the different life stages.

Parasitism: a parasitic relationship is one in which one member of the association benefits while the other is harmed. Parasitic symbioses take many forms, from endoparasites that live within the host's body to ectoparasites that live on its surface. In addition, parasites may be necrotrophic, which is to say they kill their host, or biotrophic, meaning they rely on their host's surviving.

Pathogen load: is the amount of pathogens.

Pathogens: are agents that can cause diseases.

Pests: the concept of pest organisms is anthropocentric and is defined as any organisms that is perceived by humans to interfere with their activities. Echologically there are no such organisms as pest. Organisms in several phyla are considered to be pests: arthropods, nematodes, molluscs, vertebrates, to name but a few.

Phenotype: is the whole of the observable characteristics of an organism, resulting from the interactions between genome, environmental and random factors.

(Phenotypic) Plasticity: is the general responsiveness of phenotypes to environmental conditions, or put differently, the ability of a single genotype to develop into either one of a range of phenotypes directed by prevailing environmental conditions.

Pleiotropy: a single gene controlling or influencing multiple (and possible unrelated) phenotypic effects.

Preventative release: in the sterile insect technique, the release of sterile male insects of a species that is not present in an environment with the intention of preventing establishment of an incipient outbreak.

Problem formulation: is defined as the process including the identification of characteristics of the GM animal capable of causing potential adverse effects to the environment (hazards) of the nature of these effects, and of pathways of exposure through which the GM animal may adversely affect the environment (hazard identification). It also includes defining the assessment endpoints and setting of specific hypothesis to guide the generation and evaluation of data in the next risk assessment steps (hazard and exposure characterisation).

Production system: is defined as the specific use of the GM animal, the context in which the GM animal is reared and related management systems.

Propagule pressure: (also termed introduction effort) is a composite measure of the number of individuals of a species released into a recipient region. It is the combined effect of the total number of individuals involved in any single release event (propagule size) and the number of separate release events (propagule number).

Protection goals: are defined as natural resources (e.g. arthropod natural enemies, bees) or natural resource services (e.g. regulation of arthropod pest populations, pollination) that are to be protected as set out by EU legislations.

Receiving environment: is defined as the environment into which the GM animal(s) will be released or escape to and into which the recombinant DNA(s) may spread.

Replacement: changing in the genetic composition of a population through the release of new genotypes with a fitness driver.

Reproduction: is the biological process by which offspring are produced from their parents. Reproduction is a fundamental feature of all known life, and the methods of reproduction are broadly grouped into two main types, the sexual and asexual. Sexual reproduction is the creation of a new organism by combining the genetic material of two organisms and asexual reproduction is a mode of reproduction by which offspring arises from a single parent, and inherits the genes of that parent only.

Resistance: a mechanism inherent in an individual or population that prevents management from occurring effectively or efficiently, for instance through physiological or behavioural change.

Risk: is defined as the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences occur (EC, 2002).

Semi-captive GM animals: are animals that are under human control, yet are not always in captivity but can freely browse sometimes, e.g. cattle browsing on an unfenced pasture or cats exploring their owners' neighbourhood.

Species vulnerability: the susceptibility of a threatened species to additional pressure.

Stacked events: are events that can be combined or "stacked" by conventional breeding or other approaches (e.g. re-transformation) to produce a GM animal containing stacked events.

Step-by-step approach: is used in this Guidance Document to describe the six steps (1.Problem formulation; 2.Hazard characterisation; 3.Exposure characterisation; 4.Risk characterisation; 5.Risk management strategies and 6.Overall risk evaluation and conclusions) for the ERA. This assessment approach is different from the Tiered approach defined below.

Sterile insect technique (SIT): the release of insects (usually males) in large numbers to mate with wild populations so that the majority of the resulting generation are infertile or carry lethal traits; SIT is widely practiced already with radiation induced sterility. It could be adapted to use GM induced sterility; Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species. [ISPM 5]

Suppression: a managed reduction of a population for a specified period of time; The application of phytosanitary measures in an infested area to reduce pest populations. [ISPM 5]

Surrogate: a surrogate animal is an individual that does not bear the genetic modification at issue but shares enough traits with the GM animal that can act as a substitute for the GM animal in risk assessment tests and experiments.

Target organism: is the organism on which the GM animal is specifically designed to act (e.g. parasites, pathogens or other species which are displaced or consumed by the GM animal).

> For GM insects the target organism will in many cases be the species itself, as the application aims to change the numbers or behaviour of that organism directly; in some cases, GM insect applications may be directed at another target species, such resistance to a pathogen affecting the wild population.

Tiered Approach: is defined as all the steps (used in the sense of 'confinement-level') beginning with experiments in the confined use system through temporarily and spatially restricted deliberate release up to placing on the market, where data should be collected stepwise as early as possible during the procedure.

Transposons (or *transposable elements*): are discrete pieces of DNA that can move from one location in the genome to another. This process is referred to as *transposition*.

Unintended effects: are defined as consistent differences between the GM animal and its conventional counterpart, which go beyond the intended effect(s) introducing the target gene(s).

Vector competence: is the genetic capability of an animal to serve as a host for the complete development and /or replication of a specific pathogen.

Vertical gene transfer (VGT): is any process in which a gene is passed to offspring.

Virulence: is the degree of pathogenicity of a disease-causing organism.