Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Report 20[XX]: [XX]

Environmental risk assessment of genetically modified sterile VIRGIN® salmon for use in research trials in marine aquaculture net-pens

VKM general comments to the application for approval of VIRGIN® salmon for use in research trials in marine aquaculture net-pens:

- The application does not provide sufficient information to perform a risk assessment.
- Throughout the application, the applicant must describe in detail all information required for the risk assessment and provide all relevant data, in accordance to the EFSA guidelines and the Norwegian "Forskrift om konsekvensutredning etter genteknologiloven".
- If parts of the EFSA guidance, referred to by VKM, are considered out of scope or irrelevant by the applicant, VKM requests that the applicant states the full rationale behind this. VKM is aware that not all parts of the EFSA guidance are suited for genome-edited organisms.
- The applicant must extract relevant information from cited literature to support all statements. VKM shall assess the information/data/statements made by the applicant, not interpret results, or make conclusions based on cited literature.
- In the attached document the VKM comments to the applicant are not meant to be exhaustive, but need to be addressed in the application.
- VKM would prefer the application to be written in English. This would ease the process, e.g., by avoiding possible misinterpretations due to translations of texts cited from literature.

1 Molecular characterisation

From the EFSAs Guidance for risk assessment of food and feed from genetically modified animals (EFSA 2012):

The applicant should provide sufficient information on the genetic modification to identify the nucleic acid intended for transformation and related vector sequences potentially delivered to the recipient animal, and to characterise the DNA actually inserted in the animal and expression and stability of the intended trait(s).

Forskrift om konsekvensutredning etter genteknologiloven:

II Opplysninger om den genmodifiserte organismen

A. Donor-, mottaker- eller (hvis relevant) foreldreorganismen(e)s egenskaper:

1. Vitenskapelig navn.

2. Taksonomi.

3. Andre navn (vanlig navn, sortnavn, osv.).

4. Fenotypiske og genetiske markører.

5. Grad av slektskap mellom donor- og mottakerorganismene eller mellom foreldreorganismene.

6. Beskrivelse av identifiserings- og påvisningsteknikker.

7. Påvisnings- og identifiseringsteknikkenes følsomhet, pålitelighet (kvantitativt) og spesifisitet.

8. Beskrivelse av organismens geografiske utbredelse og naturlige habitat, herunder opplysninger om naturlige predatorer, byttedyr, parasitter, konkurrenter, symbionter og verter.

9. Organismer som man vet kan overføre genmateriale under naturlige betingelser.

10. Verifikasjon av organismenes genetiske stabilitet og faktorer som påvirker denne.

11. Patologiske, økologiske og fysiologiske egenskaper:

a. Fareklassifisering i henhold til gjeldende fellesskapsregler om vern av menneskers helse og/eller miljøet,

b. generasjonstid i naturlige økosystemer, kjønnet og ukjønnet formeringssyklus,

c. opplysninger om overlevelse, herunder årstidsrytme og evne til å danne overlevelsesstrukturer,

d. sykdomsfremkallende evne: Smittsomhet, giftighet, virulens, allergiframkallende evne, bærere (vektorer) for patogener, mulige vektorer, vertsområde, herunder ikke-målorganismer; mulig aktivering av latente virus (provirus); evne til å kolonisere andre organismer,

e. antibiotikaresistens og mulig bruk av disse antibiotika hos mennesker og domestiserte organismer for forebyggende og terapeutiske formål,

f. medvirkning i miljøprosesser: Primærproduksjon, omsetning av næringsstoffer, nedbrytning av organiske stoffer, respirasjon osv.

12. Naturlig forekommende vektorers egenskaper:

a. Sekvens,

b. mobiliseringsfrekvens,

c. spesifisitet,

d. forekomst av gener som gir resistens.

13. Beskrivelse av tidligere genmodifikasjoner.

B. Vektorens egenskaper

1. Vektorens art og opprinnelse,

2. sekvens av transposoner, vektorer og andre ikke-kodende genetiske segmenter som brukes til å bygge opp den genmodifiserte organismen og til å få de innførte vektorer og geninsertet til å fungere i dem,

3. den innførte vektors mobiliseringshyppighet og/eller genetiske overføringsevne samt bestemmelsesmetoder og

4. opplysninger om i hvilken utstrekning vektoren er begrenset til det DNA som kreves for å utføre den planlagte funksjonen.

C. Den modifiserte organismens egenskaper

1. Opplysninger om genmodifiseringen:

a. Metoder som anvendes ved modifikasjonen,

b. metoder som anvendes ved oppbygging av geninsertene og innføring av dem i mottakeren eller til å fjerne en sekvens,

c. beskrivelse av geninsertets og/eller vektorens oppbygning,

d. geninsertets renhet med hensyn til enhver ukjent sekvens samt opplysninger om i hvilken utstrekning den innførte sekvensen er begrenset til det DNA som kreves for å utføre den planlagte funksjonen,

e. utvelgelsesmetoder og -kriterier,

f. sekvens, funksjonell identitet og plassering av det eller de aktuelle endrede/innførte/fjernede nukleinsyresegmenter, særlig opplysninger om enhver kjent skadelig sekvens.

2. Opplysninger om den endelige genmodifiserte organismen:

a. Beskrivelse av genetisk(e) egenskap(er) eller fenotypiske egenskaper, særlig av nye egenskaper og egenskaper som kan være uttrykt eller ikke lenger være uttrykt,

b. struktur i og mengde av enhver vektor- og/eller donornukleinsyre som er igjen i den endelige oppbygningen av den modifiserte organismen,

c. organismens stabilitet når det gjelder genetiske egenskaper,

d. uttrykksrate og -nivå for det nye genmaterialet. Målemetoder og deres følsomhet,

e. de uttrykte proteinenes aktivitet,

f. beskrivelse av identifiserings- og påvisningsteknikker, herunder identifiserings- og påvisningsteknikker for den innsatte sekvensen og vektoren,

g. påvisnings- og identifiseringsteknikkenes følsomhet, pålitelighet (kvantitativt) og spesifisitet,

h. beskrivelse av tidligere utsetting eller bruk av den genmodifiserte organismen,

i. vurderinger med hensyn til menneskers og dyrs helse såvel som plantehelse:

i. giftige eller allergiframkallende virkninger av de genmodifiserte organismene og/eller deres stoffskifteprodukter,

ii. sammenligning av den modifiserte organismens og donor-, mottaker- eller (eventuelt) foreldreorganismens sykdomsfremkallende evne,

iii. koloniseringsevne,

iv. organismens sykdomsfremkallende evne hos mennesker som ikke lider av svekket immunforsvar:

- fremkalte sykdommer og sykdomsfremkallende mekanismer, herunder spredningsmåte og virulens,
- overføringsevne,
- smittsom dose,
- vertsområde, mulighet for endring,
- mulighet for overlevelse utenfor menneskelig vert,
- forekomst av vektorer eller spredningsmidler,
- biologisk stabilitet,
- mønstre for antibiotikaresistens,
- allergiframkallende evne,
- tilgang til egnet sykdomsbehandling.

v. andre farer forbundet med produktene.

1.1 Information relating to the genetic modification

1.1.1 Description of the methods and vectors used for the genetic modification

The applicant should provide information on the following:

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

1.1.2 Comments to applicant regarding chapter 1.1.1.:

In the application, the applicant should describe in detail the methods used for the genetic modification/genome editing. All information relevant to the transformation process, i.e., method(s) used, target tissue/organism, life stage, mode of transfer (mRNA injection in eggs?) etc., should be described. All listed requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to the methods used for VIRGIN® salmon. If some of the listed requirements are deemed irrelevant, the applicant must justify the reason for this.

1.1.3 Source and characterisation of nucleic acid intended to be inserted

The applicant should provide information on the donor organism(s) and on the nucleic acid sequence(s) intended to be inserted in order to determine whether the nature of the donor organism(s) or the nucleic acid sequence(s) may trigger any safety issue. Information should be provided on the origin of the nucleotide sequence intended to be inserted, including information on any deliberate alteration(s) to the corresponding sequence(s) in the donor organism(s) and on the techniques used for producing these changes (site-directed mutagenesis, gene shuffling, production of synthetic nucleotide sequences). Information regarding each donor organism should comprise its taxonomic

classification and its history of use regarding food and feed safety. In case of synthetic nucleotide sequences with no gene counterpart in existing organisms, information should be provided on the design and the functional elements of the synthetic nucleotide sequences introduced. Information regarding the DNA region(s) intended for insertion should comprise the following elements:

- History of consumption of the gene product(s) arising from the regions intended for insertion;
- Data on the possible relationship of the gene products with known toxins, anti-nutrients, allergens and other compounds with potential adverse health effects;
- If viral vectors, transposons or known zoonotic organisms have been used, information on their natural hosts, target organs, transmission mode and stability, pathogenicity, and potential for recombination with endogenous or exogenous pathogens (e.g. viruses);
- Available information related to the occurrence of transposons or viruses in the recipient animals which are related to the construct used and which might be able to provide transacting transposase or act as helper virus

1.1.4 Comments to applicant regarding chapter 1.1.3:

In the application, the applicant should describe in detail the source and characterisation of nucleic acids intended to be inserted (used for the genome-editing). All listed requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to VIRGIN® salmon. If some of the listed requirements are deemed irrelevant, the applicant must justify the reason for this.

1.2 Information relating to the GM animal

1.2.1 General description of the trait(s) and characteristics introduced or modified

The introduced trait(s), its mode of action, and the resulting changes in the phenotype of the GM animal should be described. Information provided should also include a description of the generation of the GM animal to be marketed from the initial GM animals, including the breeding strategy, information on whether the initial GM animal was mosaic, whether the GM animals were to be marketed, are hemizygous or homozygous with regard to the sequence(s) actually inserted, and the ploidy of the GM animals to be marketed.

1.2.2 Comments to applicant regarding chapter 1.2.1:

In the application, the applicant should describe in detail the introduced trait(s), its mode of action, and the resulting changes in the phenotype of the genome edited animal. All requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to VIRGIN® salmon. If

some of the requirements are deemed irrelevant, the applicant must justify the reason for this.

1.2.3 Information on the sequences actually inserted/deleted or altered

The applicant should provide the following information:

a. The size and copy number of the inserts, both complete and partial. The analysis should cover sequences that could be inserted into the host animal, such as any parts of the plasmid/vector. The analysis should span the entire insert locus/loci as well as flanking sequences;

b. The organisation and sequence of the inserted genetic material at each insertion site;

c. Size and function of the deleted/modified region(s), in the case of intended deletion/modification(s);

d. Sub-cellular location(s) of insert(s) (integrated in the nuclear or mitochondrial genome, or maintained in a non-integrated form) and methods for ascertaining those sub-cellular location(s) of the insert(s);

e. Sequence information for both 5" and 3" flanking regions at each insertion site, with the aim of identifying interruptions of known genes and functional elements, presence of genes in the vicinity of the insert and possible deletions in the recipient DNA. Bioinformatics analysis should be conducted using up-to-date databases with the aim of performing both intraspecies and interspecies homology searches. The characteristics and versions of the databases must be provided;

f. Open reading frames (ORFs) present within the insert and spanning the junctions. The ORFs should be analysed between stop codons, not limiting their lengths. Bioinformatics analyses should be conducted to investigate possible similarities with known toxins or allergens using up-to-date databases. The characteristics and versions of the databases should be provided;

g. Depending on the information gathered, further analyses may be needed to complete the risk assessment.

1.2.4 Comments to applicant regarding chapter 1.2.3:

In the application, the applicant should describe in detail information on the sequences actually inserted/deleted or altered. All listed requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to VIRGIN® salmon. If some of the listed requirements are deemed irrelevant, the applicant must justify the reason for this.

1.2.5 Information on the expression of the inserted/modified sequence

The applicant should provide information to demonstrate whether the inserted/modified sequence results in the intended change(s) at the protein, RNA and/or metabolite level(s). In many cases the intended genetic modification will lead to the expression of new protein(s), therefore protein expression data will be the most relevant. In other cases (e.g. silencing approaches or where biochemical pathways have been intentionally modified) the analysis of specific RNA(s) or metabolite(s) may

be the most informative. The assessment of risk associated with a change of protein and metabolite level(s) is covered in Section C.2.1.4.

Data should be derived from animals bred, fed and reared under representative conditions. Information should be provided on tissues of the animal where the inserted/modified sequence is expressed and tissues where the expressed products are localised. Data on expression levels from those parts of the animal that are used for food/feed purposes and relevant to the scope of the application are considered necessary in all cases. Where tissue-specificity is intended, information on expression and presence of expression products in different tissues, fluids and other compartments relevant for the risk assessment should be provided. The requirement for information on developmental expression should be In cases such as silencing approaches or where the modification is intended to modify the levels of specific proteins or metabolites, the experimental design should include a non-GM comparator in order to compare the levels of relevant endogenous RNA(s), protein(s) and/or specific metabolite(s). If the genetic modification results in newly expressed protein(s) and where the analytical method has been shown to be specific, the comparative approach is not applicable.

The applicant should provide the following information:

a. Description of the method(s), including specificity and sensitivity, used for expression analyses;

b. The mean and range of concentrations of newly produced proteins or expression levels of endogenous animal proteins, deliberately modified in the GM food(s) and feed(s) to be placed on the market, together with the raw datasets;

c. When justified by the nature of the insert (e.g. gene silencing through RNA interference), information on the expression of targeted gene(s) and on possible effects to related endogenous genes (to be selected by in silico analysis) should be provided;

d. On a case-by-case basis, expression of genes situated near the inserted/modified sequence.

For applications which include the use of living organisms in the scope, the above requirements for food, feed, import and processing should be met (including trial design). Depending on the trait and scope of the application, information on the expression of the inserted/modified sequence may also be required for the assessment of impacts on other organisms (see ERA GD under preparation). In such cases, information on expression in various parts of the animal during development is required. Data should be related to the conditions in which the animals are bred, fed and reared in Europe.

1.2.6 Comments to applicant regarding chapter 1.2.5:

In the application, the applicant should describe in detail information to demonstrate whether the genome editing results in the intended change(s) at the protein, RNA and/or metabolite level(s). All listed requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to VIRGIN® salmon. If some of the listed requirements are deemed irrelevant, the applicant must justify the reason for this.

1.2.7 Inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the GM animal

Information should be provided to demonstrate the inheritance and genetic stability of the locus/loci altered by the genetic modification and the phenotypic stability and inheritance pattern(s) of the introduced/modified trait(s).

The applicant should provide data on the inheritance pattern and the stability of the introduced/modified nucleotide sequences and associated phenotypes in the offspring across multiple sexual generations, dependent on the animal species. The source of the material, the sampling design, the number of animals used for the analysis and the number of generations should be specified and clearly indicated on the breeding diagram.

1.2.8 Comments to applicant regarding chapter 1.2.7:

In the application, the applicant should describe in detail the inheritability and genetic stability of the introduced edits/changes. All listed requirements above should be answered, accompanied by all data required for risk assessment. The applicant must adapt their answers to VIRGIN® salmon. If some of the requirements are deemed irrelevant, the applicant must justify the reason for this.

As the experimental fish are offspring of VIRGIN rescue broodstock, an assessment of potentially increased homozygosity around the gene edits is necessary.

Can immunosuppression be an unwanted outcome of the gene edits?

1.3 Conclusions of the molecular characterisation

- The molecular characterisation should provide data on the structure of the genetic modification, expression and stability of the intended trait(s) and the applicant should indicate whether it raises safety issues.
- It should be specifically indicated whether the molecular characterisation of the genetic modification(s), raises safety issues with regard to production of unintended proteins/products, including new toxins or allergens, as well as the potential mobilisation of the insert.
- The potential unintended changes identified in this section should be addressed in the relevant complementary part(s) of the safety assessment.

1.4 Overall comments to applicant regarding the molecular characterisation:

- The application does not provide sufficient information to perform a risk assessment.

- For all parts of the molecular characterisation the applicant must describe in detail all information required for the risk assessment and provide all relevant data. The applicant must extract relevant information from cited literature to

support all statements. VKM shall assess the information/data/statements made by the applicant, not interpret results or make conclusions based on cited literature.

- VKM would prefer the application to be written in English. This would ease the process, e.g., by avoiding possible misinterpretations due to translations of texts cited from literature.

2 Cross-cutting considerations

Forskrift om konsekvensutredning etter genteknologiloven:

III Opplysninger om utsettingsforhold og mottakermiljø

A. Opplysninger om utsettingen

1. Beskrivelse av den planlagte utsettingen, herunder formålet eller formålene samt planlagte produkter.

2. Planlagte tidspunkter for utsettingen, fremdriftsplan for forsøket, herunder utsettingenes hyppighet og varighet.

3. Forberedelse av stedet før utsettingen.

4. Stedets størrelse.

5. Planlagt(e) utsettingsmetode(r).

6. Mengder genmodifiserte organismer som skal settes ut.

7. Forstyrrelser på stedet (dyrkingstype og -metode, gruvedrift, vanning eller annen virksomhet).

8. Vernetiltak for arbeidstakere under utsettingen.

9. Behandling av stedet etter utsettingen.

10. Planlagte teknikker for disponering eller inaktivering av de genmodifiserte organismene når forsøket er avsluttet.

11. Opplysninger om og resultater av tidligere utsetting av de genmodifiserte organismene, særlig i ulikt omfang og i forskjellige økosystemer.

B. Opplysninger om miljøet (både om selve stedet og om miljøet omkring)

1. Stedets eller stedenes geografiske beliggenhet samt nærmere opplysninger om denne (ved søknad om utsetting i form av omsetning iht. § 9 annet ledd bokstav f og § 10 i genteknologiloven vil utsettingsstedet eller -stedene være produktets planlagte bruksområder).

2. Fysisk eller biologisk nærhet til mennesker eller andre viktige biota.

3. Nærhet til viktige biotoper, verneområder eller drikkevannsforsyninger.

4. Klimatiske egenskaper ved den eller de regioner som kan bli berørt.

5. Geografiske, geologiske og pedologiske egenskaper.

6. Plante- og dyreliv, herunder avlinger, husdyr og trekkende arter.

7. Beskrivelse av måløkosystemer og ikke-måløkosystemer som kan bli berørt.

8. Sammenligning av mottakerorganismens naturlige habitat og planlagt(e) utsettingssted(er).

9. All planlagt utvikling av eller endring i arealbruken i området som kan ha innflytelse på utsettingens innvirkninger på miljøet.

From the EFSAs Guidance on the environmental risk assessment of genetically modified Animals (2013):

2.1 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 i.a. other GMOs already in the environment.‖ Further, this Directive provides details on required information relating to the conditions of placing on the market or 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. Section 3.1 provides guidance to applicants on the assessment of relevant receiving environments in which the GM animal is likely to be deliberately or accidentally released.

2.1.1 Definition of receiving environments

The range of environments into which the GM animal(s) and their effluents (e.g. faeces, urine) 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 services and human interventions occur in the EU. The receiving environments for GM animals will vary in spatial scale from a very limited number of enclosed areas to large regions within the EU. They will also vary in the extent of management, from those that are wild, through those that are subject to some level of management, to those that are completely synthetic (e.g. confined aquaculture facilities), 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).

2.1.2 Identification and characterisation of the receiving environments

The potential receiving environments for each GM animal will be identified by three components (Figure 3):

a) Factors related to the GM animals to be considered: e.g. wild/feral populations of the animal species, ecological requirement of the animal species, wild relatives, genetic modification(s) and intended uses(s).

b) 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 (where the system includes all the living organisms and

abiotic factors occurring within it) within a receiving environment to which the GM animal, including effluents and recombinant DNA, will be released or may escape or be distributed through active or passive spread and may interact with.

c) Management systems (i.e. management of the placing on the market, release and production units, including rearing, breeding, production, transport and processing, e.g. pest and disease management, nature conservation activities, release in confined environments).

The first component is defined by the factors related to the GM animal itself (see Figure 3). Both the animals and the GM trait(s) determine where the GM animals will most likely be released. Some GM animals (e.g. GM olive fly) can realistically be released in some geographical zones only, while others, such as GM pigs and GM salmon, may be released or become more widely established in the whole EU. The GM traits (e.g. disease resistance, cold tolerance) will determine which GM animals are likely to survive and where they could establish. Consideration should be given to the influence of the GM trait in determining the range of environments the GM animal may inhabit: traits that confer tolerance to, for example, heat, cold, dehydration, salinity or disease may allow the GM animal to be produced or establish in environments not occupied by its conventional counterpart. Therefore, all these elements should be taken into account when defining the receiving environments for the ERA of each GM animal. GM animals have widely different characteristics of reproduction, spread, invasiveness and survival. Also, they may be developed for different uses (e.g. food production in the case of mammals, birds and fish; suppression or replacement of plant pest species or disease vector populations in the case of insects). The intended use(s) and the characteristics of the GM animals will determine their behaviour and interactions with other biotic and abiotic factors in the receiving environments (see Table 2).

The accessible ecosystem(s) component (see Figure 3) may contain a wide range of varying habitats at various scales (e.g. marine, fresh water, cultivated agricultural habitats, natural and semi-natural habitats, rural and urban areas) and are characterised by specific conditions (e.g. physic-climatic conditions, altitude, water quality) where native and other biota including humans may interact with the GM animals. An accessible ecosystem is a subset of (and may be smaller than) a receiving environment; it follows that some parts of a receiving environment may not be accessible to the GM animal. For example, within the receiving environment defined by the Pannonian region, a GM fish might be found in a certain aquatic ecosystem but not in a particular terrestrial ecosystem. The former ecosystem is accessible to the fish, but not the latter. Certain animals migrate and some reproduce in different environments. Some may have different life stages in different environments. Therefore, the whole life cycle of the GM animal and the receiving environments of these different stages require consideration. Interactions between GM animals and non-GM animals such as herbivores, predators, parasitoids, decomposers, pollinators, pathogens and 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. through their organic waste products (see Table 2).

The management systems component (see Figure 3) should include consideration of factors such as land and water use and livestock husbandry or rearing facilities and their management, since the management of the placing on the market, release and production units 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 unit 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 and/or spread of waste products (i.e. effluents) of the GM animal and (2) the pests, pathogens and endosymbionts associated with the GM animal. Identifying the receiving environments of waste products of confined GM animals may be a more important factor than the distribution of the living GM animal itself for the risk assessment.

Therefore, interactions of such effluents with the biotic and abiotic factors (see Table 2) in receiving environments should be considered. Furthermore, GM animals with enhanced resistance may act 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 effluents 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).

2.1.3 Selection of relevant sites in receiving environments

The ERA should take into account 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 maximised and where it is anticipated that effects, where they exist, will be detected.

In order to select appropriate sites in which to study each potential hazard, applicants need to consider the full geographic range of the GM animal and the receiving environments in which these hazards could occur. For example, if a NTO is selected for a field study, then these studies should be conducted in environments where there will be exposure of the NTO to the GM animal and where there are measurable numbers of the NTO, in order to assess population effects (see section 3.2). Applicants should follow the steps shown in Table 3 in order to select these relevant sites.

Since not all receiving environments where the GM animal and its waste products (i.e. effluents) will be intentionally or might be accidentally released and spread can be considered in detail, applicants should discuss and justify the applicability of studies outcomes obtained in some relevant sites 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 the placing on the market, release and production units on the interactions between the GM animal and the environment). Some categorisations of regions or habitats into geographical, climatic or bio-geographical zones, which could

be used for this purpose, already 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/).

A baseline of the receiving environments, including production units, indigenous biota and their interactions, should be established to identify any potentially harmful characteristics of the GM animals (EC, 2002). Subsequently, the characterisation of the GM animal and its potential harmful characteristics should inform the decision of which parameters of the baseline(s) of the receiving environments are relevant. Relevant baseline(s) refer to current production units and associated management practices for which published literature is available, and serve as a point of reference against which future changes can be compared (see also 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, including potential long-term effects, for the presence of any other GMOs and other introduced species that have been placed on the market and released in the same receiving environments, considering the specific management practices 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 sites in receiving environments identified in step 3 of Table 3, applicants should describe:

The characteristics of those 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 animals‘ waste products (i.e. effluents) are likely to be spread.

The representative management practices (e.g. treatments against pests and diseases) associated with the rearing, breeding, production, transport and processing of the GM animals considering the presence of any other GMOs.

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 units.

Where appropriate, the presence of cross-compatible wild relatives and 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 (see also section 3.7). 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. In addition, 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 in 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.

2.1.4 Comments to applicant regarding chapter 2.1.1 to 2.1.3:

In the application, the applicant needs to describe in detail the receiving environments that the experimental fish may escape to, according to the EFSA guidelines in chapter 2.1.1 to 2.1.3. This includes factors related to the modified animal component, the accessible ecosystems component (in marine and fresh waters that experimental fish may escape to or enter), and the management systems component as outlined in the EFSA guidelines. The applicant must adapt their answers to the VIRGIN® salmon experiment. If some of the listed requirements are deemed irrelevant, the applicant must justify the reason for this.

In the application, the applicant needs to describe in detail the characteristics of those receiving environments where the animal is likely to occur if they escape from the research facility, both marine and in freshwater. This includes distances to other fish farms (experimental and commercial) and rivers holding salmon or other salmonids. VKM regards that freshwater should be included in the description of receiving environments, because it is known that non-mature wild and farmed Atlantic salmon can enter rivers. The range of relevant biotic and abiotic interactions likely to occur in the receiving environments, taking into consideration the range of environmental conditions, must be described, including which other animals and other organisms are present that may be impacted (e.g. ecologically or by pathogens, see other parts of the EFSA guidelines) by the VIRGIN® salmon, whether they are kept in the open net pen or escaped. If farmed Atlantic salmon have escaped or been released from the research facility at Matre before, a description of knowledge of their spread in nature is relevant. According to the EFSA guidelines described above, the presence of cross-compatible wild relatives and the ability of the animals to form feral populations, and hence the potential impacts on the receiving environments, should be better described.

2.1.5 Experimental environment

The complexity of the environmental concerns requiring study in any risk assessment is related to the complexity of the organism or substance 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 lifetime, and, whereas plants are usually at the bottom of the food chain, an animal may be either a predator or prey, or both. Hence, it might be expected, firstly, that the ERA of a GM animal would be more varied and complex, and encompass a wider range of issues than the ERA of a plant or a substance, and, secondly, that the mobility of animals would also focus the ERA on questions related to invasiveness and persistence and thus draw on the considerable scientific literature concerning alien species. Hence, the ERA of a substance, such as a pesticide, has traditionally been restricted largely to studies of its eco-toxicological effects, using a tiered approach (EC, 2009c). For a GM plant which is at the base of the food chain, toxicity remains important but the ERA 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 multitrophic hierarchy in which the animal exists. Therefore, the tiered eco-toxicological approach (e.g. Andow et al., 2006; Romeis et al., 2008) promulgated through standardised methodologies (developed by, for example, the Organisation for Economic Co-operation and Development (OECD), the International Organization for Standardization (ISO) and the European and Mediterranean Plant Protection Organization (EPPO); see also EFSA, 2010a), in which studies performed within laboratories may trigger further studies in wider environments, should not be regarded as the sole paradigm for the ERA of GM animals.

For any identified hazard, once the rationale and hypotheses of an experimental study on a GM animal, prior to being placed on the EU market, 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 the confinement measures to deploy to avoid accidental 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 confined laboratory, up to larger scales that may include, respectively, ocean mesocosms of many thousands of cubic metres (van der Meeren and Lønøy, 1998); screened enclosures of thousands of cubic metres (Gary et al., 2009; Miller et al., 2010); and 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 confinement measures, applicants should consider the mobility of the GM animal within the experimental environment, the likelihood of escape, the feasibility of recapture and 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 also consider the intended use(s) of the GM animal. 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-confined environment (e.g. mosquito) (see also chapter 1).

Applicants should discuss and justify explicitly the choice and scale of experimental environment and of confinement measures. Applicants should consider the arguments for and against small- and largescale 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 their generality. However, there could be a need to incorporate realistic evaluation of certain factors that can be addressed only at the large scale, such as animal mobility, multitrophic 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 sections 3.7 and 3.8) and should consider to what extent this may be facilitated by limited experimentation within semi-natural environments. Applicants should consider the use of surveys of potential receiving environments to provide relevant data where there is no experimental imposition of treatments.

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 the rainy season and dry season.

2.1.6 Comments to applicant regarding chapter 2.1.5

In the application, the applicant needs to describe in detail the experimental set-up and confinement measures during according to the EFSA guidelines in chapter 2.1.4. This includes how the fish are transferred to the net pen in the sea, how sea-lice or other treatments during the experiment are planned, and an evaluation of the risk of VIRGIN® salmon escaping during these procedures.

The experimental net pens (consisting of four smaller pens within 12 x 12 m pens) need to be described in more detail with respect to the mesh sizes and material used, their shape and dimensions, the attachment of small net pens within a larger, their anchoring and their performance during bad weather conditions and potential attacks by predators.

The density of fish within each pen, the wall area to volume ratio for these pens compared with commercial net pens, and how the fish will be fed, need description.

It would aid the description if there is experience from experiments in similar net pens at Matre with data on potential stress to the fish, fin damage and risk of disease outbreaks in these facilities and with the planned density of fish. Also, information should be presented on how these pens are constructed and anchored compared with the standard for fish farms (NS 9415 Flytende akvakulturanlegg).

2.2 Choice of comparators

The ERA of a GM animal is based on the comparative approach (see chapter 2) as prescribed by Directive 2001/18/EC (EC, 2001). 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, of the same species, with a genetic background that is as close as possible to that of the GM animal. 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 and 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, 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 (see chapter 1); 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 any other non-GM animals (including other (sub-)species) with which it can hybridise (see chapter 1). Each of these types may require a different comparator(s) to determine environmental effects. 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, and therefore subject to national and European legislation (e.g. 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 with its 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 characterisation of the aggregate of all adverse environmental effects as a consequence of the placing on the market or release of the GM animal into the receiving environments and the comparison must be with the state of the receiving environments prior to marketing or release (additional comparators may be required in some cases; see section 3.3.2). In cases where the conventional counterpart is not present, a possible comparator might be a non-GM animal from the same species as the GM animal, and which already occurs in the receiving environments (e.g. wild types of the GM animal). Again, the selection of appropriate comparators may be aided by considering genetic distance and/or pedigree. An alternative choice might be a non-GM animal from a different species, but one that exploits the same (failing that, a similar) ecological niche and that has similar biotic and abiotic 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. 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 indigenous non-GM surrogate animals with similar characteristics or traits to those of the GM animal being considered in the wild, 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 placing on the market or 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 with those of the non-modified organisms should be highlighted (see sections on management, 4.1.6, 4.2.6 and 4.3.7). Here, _use' includes the functions of companion animals and _management' covers all aspects of the rearing, breeding, production, transport and processing (e.g. confined aquaculture facilities, livestock husbandry). 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 documented clearly; practices should conform to the latest EU regulations and guidance concerning sustainability, e.g. aquaculture,12 husbandry13 and pesticides.14

2.2.1 Choice of comparators for ERA of GM fish

The ERA of GM fish should compare the GM fish to (1) its non-GM source progenitor line; (2) one or more populations of wild fish within the same species originating from the location or locations into which it is proposed to release the GM fish; (3) one or more populations of wild fish species exploiting a similar ecological niche as the GM fish in accessible ecosystems, as explained below; and (4) aquaculture lines of the same species as the GM fish, whenever an aquaculture line is currently produced in aquaculture in the accessible ecosystems. In addition to the non-GM line, applicants should use at least one wild population as comparator where the risk assessment has predicted that escape into environments occupied by wild types is a possibility. For each comparator used, the risk assessment should apply appropriate statistical methods to test for differences between the GM fish line and the comparator line (EFSA, 2010b). i. For initial characterisation of the GM fish line The comparator for characterisation of the gene construct, gene expression and whole-organism phenotype of the GM line should be the non-GM line, that is the line used to produce the GM fish (EFSA, 2012a). Applicants should use this comparator to characterise, in a statistically sound manner, all the intended and unintended phenotypic changes in the GM fish line (see Devlin et al., 2007; Gong et al., 2007; Kapuscinski et al., 2007a). The non-GM line provides an initial but not a sufficient comparison for a reliable environmental risk assessment. Applicants should compare the GM fish line with one or more additional fish populations, as outlined below. ii. For assessing ecological effects, including genetic effects, of GM fish that might enter accessible ecosystems It is necessary to assess ecological differences and similarities between the GM fish line and wild fish populations that exploit a similar ecological niche in the accessible ecosystems (Devlin et al., 2007, and references therein). Following this fundamental ecological principle, and depending on the wild species composition in the accessible ecosystems (Moreau et al., 2010, 2011), appropriate comparator specimens include one or more of the following types: 1. wild population of the same species as GM fish, and which occurs in possible accessible ecosystems; 2. wild population of species closely related to the GM fish, and which occurs in possible accessible ecosystems; For example, if the GM line is a rainbow trout and the accessible ecosystem contains wild brown trout, the comparisons could be made with the wild brown trout population or populations from the accessible ecosystem.

wild populations of other fish species in the accessible ecosystems exploiting a similar ecological niche and, thus, with which the GM fish could compete. Applicants should support the choice of the wild population they use with relevant information on differences in quantitative traits and local adaptation. If this information is missing, applicants can either provide that information or consider this in the uncertainty analysis (see section 3.8). Applicants 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 Devlin et al., 2007, and Kapuscinski et al., 2007a, b, for detailed guidance on selection

of appropriate comparators). iii. When accessible ecosystems also involve aquaculture of non-GM line of same species It is also appropriate to compare the GM fish with 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 from 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 in knowledge exist regarding the ecological effects of non-GM farmed species and lines within species that are in current use (Devlin et al., 2007; Kapuscinski et al., 2007b; 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 of (1) their impact over multiple generations following an incident of a single escape and (2) the impact of recurrent escape incidences. In most cases, conventionally bred strains will express altered phenotypes as a result of changes in a range of genes with additive effects, whereas in GM strains a single recombinant DNA 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 recombinant DNA largely maintains its phenotypic expression across generations. This means that, if the recombinant DNA enhances fitness, it will spread in the population and will soon be present in all individuals, and if it decreases fitness it will be purged from the population. However, during this purging process, the wild population can also suffer (e.g. the Trojan gene (Muir and Howard, 1999)). When selection acts on the recombinant DNA, other parts of the genome may also be affected and reduce or enhance the phenotypic effects of the recombinant DNA. This can lead to changes in the background genetics, i.e. the rest of the genome that is not the recombinant DNA molecule, and these effects can even carry over to those individuals that do not carry the recombinant DNA since hemizygous transgenic animals can produce wild genotype offspring (Ahrens and Devlin, 2011). With a domesticated genotype, the genetic contribution is on average halved at each reproductive occasion, so that the pure domesticated phenotype will eventually disappear from the wild population even though the domesticated genes will be present. Domesticated genotypes cannot produce wild genotypes, so over time the population will consists of individuals with mixed wild–domesticated genotypes with proportions depending on selection on the phenotypes and underlying genotypes. Applicants should provide information and justification for omitting one or more of the aforementioned comparators.

2.2.2 Comments to applicant regarding chapter 2.2.1

In the application, the applicant needs to describe in detail the breeding history of the VIRGIN® salmon used in the experiments, in order to assess the genetic difference between the VIRGIN® salmon and the farmed-salmon comparator. It should be clear from the application where the VIRGIN® rescue broodstock (F0 used in 2021) are located, and how many VIRGIN® rescue broodstock (F0) that was used to produce the 303 experimental VIRGIN® salmon. Also, the parents (P) of the F0 generation needs

to be described. The applicant also needs to detail other potential comparators, e.g., if experimental VIRGIN® salmon should escape from the research facilities, the guidelines suggest wild salmon as an appropriate comparator.

2.3 Animal trials for comparative analysis - experimental design and statistical analysis

From the EFSAs Guidance on the environmental risk assessment of genetically modified Animals (2013):

Production of animal material for comparative analysis is performed in order to assess similarities and differences between the GM animal and its appropriate comparator(s). For any particular endpoint, there should be a difference test between the GM animal and the conventional counterpart. Where there is sufficient and appropriate animal material (see below), an equivalence test with a null hypothesis of non-equivalence may be applied, using the methodology already described by EFSA (EFSA, 2010a). Equivalence should be considered as the absence of differences other than those expected naturally through variation between traditionally-bred animals with a history of consumption as food and feed. Identified differences should be placed into a biological context. Such differences may point to biological changes caused by the genetic modification which should subsequently be further assessed for their toxicological and/or nutritional relevance.

2.3.1 Experimental design and statistics

2.3.1.1 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 with its comparator(s) and to measure their magnitude.

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, 2010b) to verify whether or not the GM animal is equivalent to its comparator (EFSA, 2010a) within certain bounds (see definition of so-called limits of concern' below).

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 characterisation 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 (2011c). 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. 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 (2010b). 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.

2.3.1.2 Principles of experimental design

… It may be important to subject animals that are being compared to the same management practices. 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 minimised. Applicants should explain and justify 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…

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 placing on the market or accidental 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 animal and its comparator and which will form the basis of the risk assessment…

"..The ideal situation is where the optimal conditions reflected by typical commercial practice for the GM animal and its comparator overlap.."

2.3.2 Statistical analysis

…Recommended procedures for statistical analysis involving difference and equivalence tests are discussed in EFSA (2010b) and EFSA (2010a). 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…

2.3.3 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, feed composition and sources of feed ingredients, 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…

2.3.4 Comments to applicant regarding chapter 2.3

In the application, the applicant needs to describe in detail how the experimental fish (303 VIRGIN® salmon and 485 control fish) fish will be distributed among the four net pens? The numbers do not fit with the description of 250 individuals in each net pen.

Moreover, the experimental design needs to be described for analysing standard stress factors and measuring acute physiological responses. A description is needed for how mortality will be handled if it occurs during the stress response trials or for other reasons.

3 Specific areas of risk to be addressed in the ERA

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 and birds) and the potential traits covered by this Guidance Document. For example, in the ERA of GM fish, section 4.1.3 on biotic interactions includes the assessment of the interactions of the GM fish with target and NTOs as in Directive 2001/18/EC. 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 NTOs. 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 should be read in conjunction with the cross-cutting sections . 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.

Forskrift om konsekvensutredning etter genteknologiloven:

IV Opplysninger om vekselvirkninger mellom de genmodifiserte organismene og miljøet

A. Egenskaper som påvirker overlevelse, formering og spredning

1. Biologiske egenskaper som har innvirkning på overlevelse, formering og spredning.

2. Kjente eller antatte miljøforhold som kan ha innflytelse på overlevelse, formering og spredning (vind, vann, jord, temperatur, pH osv.).

3. Følsomhet overfor særskilte agenser.

B. Vekselvirkninger med miljøet

1. De genmodifiserte organismenes antatte habitat.

2. Undersøkelser av de genmodifiserte organismenes atferd og egenskaper samt deres økologiske betydning utført i simulert naturlige miljøer som mikrokosmos, vekstkamre eller drivhus.

3. Genetisk overføringsevne:

a. Overføring, etter utsetting, av genmateriale fra de genmodifiserte organismene til organismer i de berørte økosystemer,

b. overføring, etter utsetting, av genmateriale fra naturlig forekommende organismer til de genmodifiserte organismene.

4. Sannsynlighet for at seleksjon etter utsetting kan føre til uttrykk av uventede eller uønskede egenskaper i den modifiserte organismen.

5. Tiltak som benyttes til å sikre og verifisere genetisk stabilitet. Beskrivelse av genetiske egenskaper som kan forhindre eller redusere til et minimum spredning av genmateriale. Metoder for verifisering av genetisk stabilitet.

6. Biologiske spredningsveier, kjente eller mulige former for vekselvirkning med den spredende agensen, herunder innånding, inntak, overflatekontakt, nedgraving osv.

7. Beskrivelse av økosystemer der de genmodifiserte organismene vil kunne spre seg.

8. Potensial for uforholdsmessig stor bestandsøkning i miljøet.

9. De genmodifiserte organismenes konkurransefortrinn i forhold til ikke-modifiserte mottaker- eller foreldreorganismer.

10. Identifisering og beskrivelse av målorganismene (om relevant).

11. Antatt mekanisme for og resultat av vekselvirkning mellom de utsatte genmodifiserte organismene og målorganismene (om relevant).

12. Identifisering og beskrivelse av ikke-målorganismer som kan bli påført skadevirkninger ved utsetting av den genmodifiserte organismen, og de antatte mekanismene ved alle kjente skadelige vekselvirkninger.

13. Sannsynlighet for endring i biologiske vekselvirkninger eller i vertsområdet etter utsettingen.

14. Kjente eller antatte virkninger på ikke-målorganismer i miljøet, herunder på konkurrerende organismer, byttedyr, verter, symbionter, predatorer, parasitter og patogener.

15. Kjent eller antatt medvirkning i bio-geokjemiske prosesser.

16. Andre potensielt betydningsfulle vekselvirkninger med miljøet.

V Opplysninger om planer for overvåking, kontroll og avfallsbehandling, samt om beredskapsplaner

A. Overvåkingsteknikker

1. Metoder for sporing av de genmodifiserte organismene og overvåking av virkningene.

2. Overvåkingsteknikkenes spesifisitet (for å identifisere de genmodifiserte organismene og for å skjelne dem fra donor-, mottaker- og eventuelt foreldreorganismene), følsomhet og pålitelighet.

3. Teknikker for å påvise overføring av det innførte genmaterialet til andre organismer.

4. Overvåkingens varighet og hyppighet.

B. Kontroll av utsettingen

1. Metoder og rutiner for å unngå og/eller redusere til et minimum spredningen av de genmodifiserte organismene utover utsettingsstedet eller det planlagte bruksområdet.

2. Metoder og rutiner for å verne stedet mot uvedkommende personers inntrenging.

3. Metoder og rutiner for å forhindre andre organismer i å trenge inn på stedet.

C. Avfallsbehandling

1. Type produsert avfall.

2. Antatt mengde avfall.

3. Beskrivelse av den planlagte behandlingen.

D. Beredskapsplaner

1. Metoder og rutiner for kontroll av de genmodifiserte organismene ved uventet spredning.

2. Metoder for dekontaminering av de berørte områder, f.eks. utryddelse av de genmodifiserte organismene.

3. Metoder for disponering eller rengjøring av planter, dyr, jord osv. som er blitt eksponert under eller etter spredningen.

4. Metoder for isolering av stedet som berøres av spredningen.

5. Planer for vern av menneskers helse og miljøet ved uønskede virkninger.

3.1 Persistence and invasiveness of GM fish, including VGT

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

In this section, applicants shall address the consequences of the placing on the market or accidental escape, establishment, gene transfer and changes in the fitness of the GM fish (see step 2) and any recipient of the recombinant DNA. This might result in changes in persistence, competitiveness and invasiveness of the GM fish line itself, of hybrids between GM and wild individuals, and of backcrossed descendants inheriting the recombinant DNA, within and outside confined aquaculture facilities, and might lead to environmental harm. Note that, if the GM fish line is hemizygous, then only one-half of first-generation hybrids between a GM fish and a wild fish will inherit the recombinant DNA construct. The possible introgression of the recombinant DNA from the GM fish into wild species raises the need to assess how introgression affects conservation of genetic diversity in any affected wild population, including changes to allelic frequencies, population genetic structure and variation. It is important to assess effects of introgression—due to phenotypic changes in individuals bearing the recombinant DNA—on individual survival and reproductive capability and hence on local adaptation of the wild population (reviewed in Kapuscinski et al., 2007c) and on the resources used from and provided to the ecosystem by fish bearing and expressing the recombinant DNA. **See page 49 for more information**.

3.1.1 Comments to applicant regarding chapter 3.1

The evaluation of environmental risk if the VIRGIN® salmon escape is very different if there is any uncertainty whether the fish are sterile or not. The application should contain sufficient information to evaluate if it is 100% certain that these individuals are sterile. This includes the number of examined individuals underlying the conclusion and a description of potential, unwanted outcomes.

If, for reasons that are rare and unwanted, a functionally fertile but genetically sterile fish escape, it is necessary to understand the potential inheritance patterns. For example, a cross between a homozygous VIRGIN® rescue salmon and a wildtype salmon should produce a diploid dnd-KO/wildtype heterozygote and it is interesting to know if the inheritance is co-dominant or not. Also, it could be possible that such a cross resulted in triploid or gynogenetic diploid offspring. Knowledge from such crosses and crosses with mosaic genomes would aid the risk assessment.

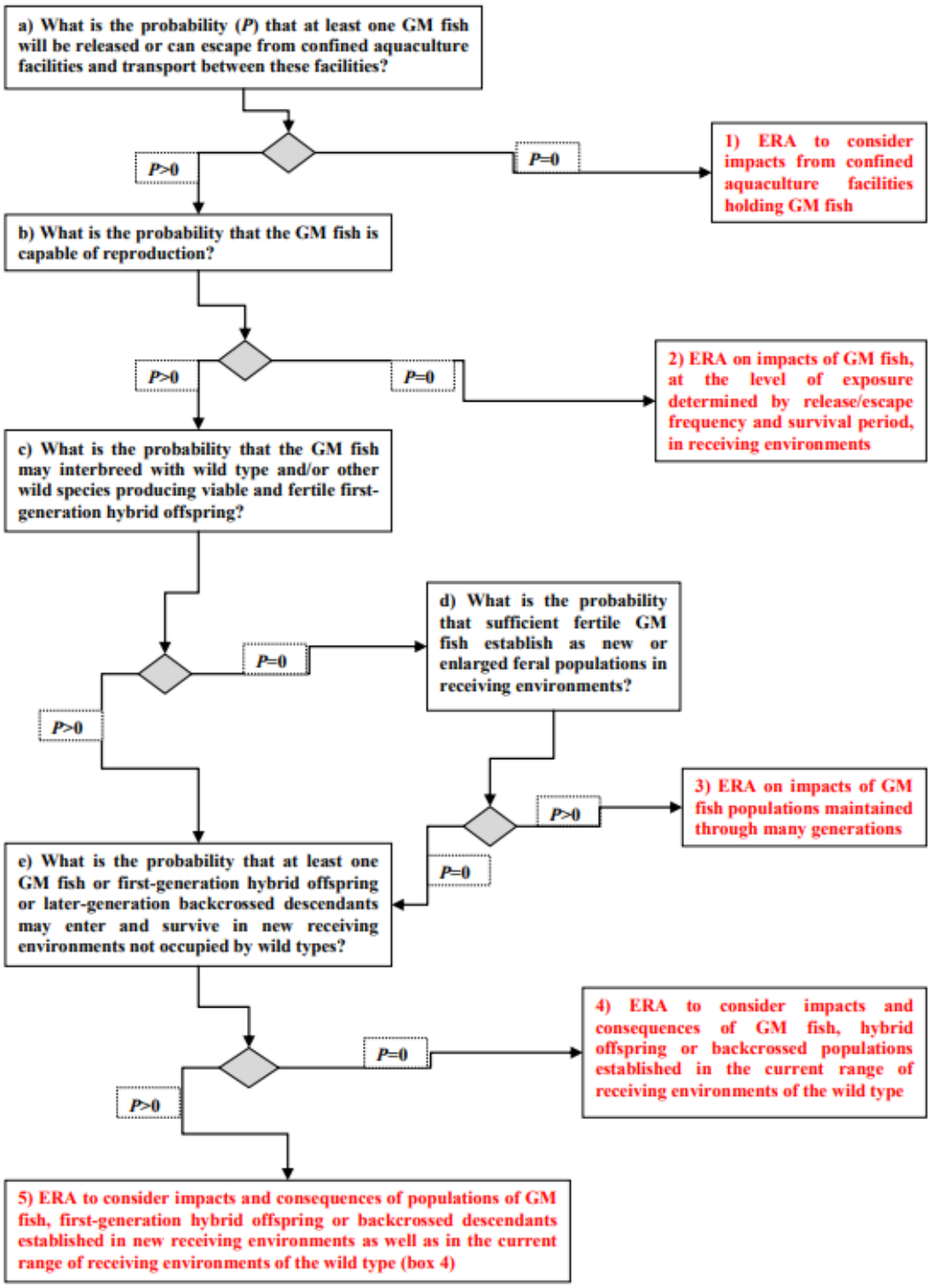

Figure 6: Example of a staged approach to problem formulation for the identification of hazards associated with the dispersal of GM fish and gene introgression and environmental exposure. These characteristics provide information on the extent of the ERA required for GM fish in each receiving environment.

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 and any offspring from outcrosses. 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.

It is advisable to pursue a step-wise approach to assess measurable endpoints in a chain of events that have to occur, to end up with incorporation of recombinant DNA from GM fish into a population of wild relatives in a receiving environment. The assessment should address two major endpoints: entry of sexually mature, fertile GM individuals into a receiving environment; and introgression of recombinant DNA genotypes into the gene pool of wild relatives. Thus, applicants can organise a step-wise approach around this basic relationship: probability of gene flow = probability of entry into receiving environment probability of introgression. Similarly, a step-wise approach for assessingpossible establishment of a self-sustaining feral GM population can be organised around the relationship: probability of feral population establishment = probability of entry into receiving environments probability of successful reproduction among GM individuals. Following from these basic relationships, applicants should address six steps in the following assessment pathway:

1. Assess the probability and magnitude of release or escape of sexually mature and immature GM fish from the confined facility.

2. Assess the probability and magnitude of immature escaped fish surviving to sexual reproduction in the receiving environments.

3. Assess the probability and magnitude of encounter between sexually mature GM and wild fish (or, for feral population establishment, between sexually mature GM fish).

4. Assess the probability and magnitude of successful mating between GM fish and wild conspecifics or adults of a closely related species (or, for feral population establishment, between sexually mature GM fish).

5. Assess the probability and magnitude of first-generation hybrid offspring surviving and successfully reproducing.

6. Assess the probability and magnitude of survival and reproduction in subsequent backcrossed generations of introgressed fish (or, for feral population establishment, in subsequent descendant generations).

See page 53 for more information.

Step 3: Exposure characterisation (Likelihood)

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 relatives in these different environments. 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).

Gene flow: The likelihood of spread (introgression) of genes from any invading genotype, including GM fish, into a fish population is a function of the reproductive and life-history traits of the invasive genotype and recipient population. Thus, 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 relatives in the receiving environments; applicants can find relevant detailed guidance in Kapuscinski et al. (2007c). This will in turn indicate the exposure 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.

Receiving environments: Changes in the phenotypic and biological characters identified in the hazard characterisation will indicate the potential geographical range of populations of GM fish, first-generation hybrids and backcrossed generations that carry the recombinant DNA. 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).

Step 4: Risk characterisation

In this step applicants should quantitatively or semi-quantitatively estimate the probability of occurrence and magnitude of harmful effect(s) based on problem formulation, hazard and exposure characterisation. Applicants should characterise the risk by combining the magnitude of the consequences of each hazard and the likelihood of the consequences related to these hazards occurring in the receiving environments. Furthermore, applicants should assess the overall uncertainty for each identified risk (see section 3.8).

Step 5: Risk management strategies

If the ERA identifies risks related to reproduction or behaviour (e.g. 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 physical, geographical or biological confinement (Mair et al., 2007). For example, biological confinement could involve lowering mating frequency and/or sexual fertility, or be directed at controlling the progeny of GM fish resulting from gene flow. 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 with estimates that are as quantitative as possible regarding (1) the extent to which the recombinant DNA can move from the GM fish into other fish populations or species within and outside confined aquaculture facilities; (2) the extent to which the fitness of GM fish and any offspring from outcrosses are more or less successful in the relevant receiving environments; (3) whether any changes in fitness of the GM fish or any offspring from outcrosses result in changes in population size of non-GM fish in the receiving environments; and (4) risk management measures required to mitigate any identified environmental harm. This information should be taken forward so that the full biotic and abiotic interactions

and consequences of the changes in populations and biology of the GM fish can be considered (see sections 4.1.3 and 4.1.5).

Uncertainties associated with the ERA conclusions of this section should be identified and assessed (see section 3.8), 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.

3.2 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 exposure and transfer of recombinant DNA and further dissemination to other organisms. Furthermore, since HGT cannot be excluded, the consequences of such transfer events for human and animal health and the environment must be evaluated.

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 that are representative of the aspects/parts of the environment(s) that need to be protected from adverse effects.

See page 55 for more information

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 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 temporo-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 sections 3.7 and 3.8).

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 fish production systems (e.g. confined aquaculture facilities): 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: 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:16 GM fish products (e.g. DNA in raw (sushi) fish will be exposed to the microbiota of the gastrointestinal tract of the consumer; and exposure will depend on storage and type and level of processing. GM fish by-products may also be utilised as a feed source.

When relevant, other sources leading to exposure to similar genes as the examined transgene(s) should be identified and considered.

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 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.

Step 6: Overall risk evaluation and conclusions

Identified knowledge gaps should be briefly summarised 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 remaining uncertainty and the efficacy of any proposed risk management strategies.

The risks and uncertainties described in the overall conclusions of the ERA provide the basis for the PMEM plan to be proposed by applicants.

3.3 Impacts of GM fish 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 placed on the EU market or escaped into the environment, compared with appropriate comparators (see section 3.3). Biotic interactions include those defined as target and non-target impacts in Directive 2001/18/EC. A target organism (TO) is one with which the GM fish is specifically designed to interact in order to manage the population of the TO or its environmental effects, as indicated by applicants. TOs could include, for example, 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 NTOs. 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, through various means, such as predation, competition, habitat alteration, inter- and intraspecific hybridisation and introduction of new parasites and diseases that influence behaviour and/or survival of the wild biota. Depending on the characteristics of the GM fish, there may be changes in the secretion of substances, actively or passively, or release of substances upon death of the GM fish or as metabolites, should the GM fish be consumed by a predator. 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. Indirect effects are those effects even though the GM fish is not in contact with the individual being affected. It is particularly important to examine whether this can give rise to trophic cascades whereby an initially small direct effect, caused by the GM fish, can lead to larger effects on the ecosystem by shifting the balance in the system. These effects typically occur through a limited number of species, so called keystone species (see Glossary), 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 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. Assessment of indirect effects therefore requires careful planning of experiments and sufficient time, and the experimental conditions should be complex enough for effects to mimic those that may exist in nature.

The assessment of the biotic effects of the GM fish is necessarily carried out from the perspective of the environment in which they are marketed or 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 with 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.

3.3.1 Comments to applicant regarding chapters 3.2 and 3.3

In the application, the applicant must detail the potential effects on biotic components and processes that may occur independently of whether or not the experimental fish are 100% sterile. This includes effects in the immediate marine environment but also a discussion of the potential for VIRGIN® salmon to enter rivers, as it is known that farmed fish escaping from net pens may enter fresh water even if not sexually mature. The description of effects on other biota needs to include several biological processes like predation, competition, and disease transmission, and needs to assess variation in the outcome based on when the experimental fish escape, their body size, their behaviour and most likely habitat, and their longevity and maximum body size.

Step 2: Hazard characterisation

Applicants should assess whether the GM fish has changed foraging behaviour (for example, the amount and nature of food) and quantify 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. This includes possible competition with other ecosystem members, either by competing for similar foods, diet space or 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 occur only 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 when assessing the impact of the GM fish.

To obtain a preliminary indication of whether competitive interactions might occur, applicants should assess similarity in resource use between potentially invasive GM fish and wild species, in order to determine the degree to which the GM fish and wild species utilise the same range of resources (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 (see also section 4.1.1). These should include potential 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, whose change may lead to increased stress to potentially affected species and ultimately their decline. Applicants should also consider the effects of the additional numbers of individuals added 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.

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

Symbiotic associations also occur within and between species. Examples are shoaling for both feeding and predator avoidance, 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; therefore, applicants should consider using models and scenario testing to determine possible environmental consequences (see sections 3.5 and 3.7).

An assessment is also required of whether the GM fish and its effluents present a new hazard for the health of other animals (see section 4.1.4) and this should be also taken into consideration while assessing consequences of biotic interactions.

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, to settle in a range of potential receiving environments (see section

3.1) and to 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, so the link between physiology and behaviour requires examination. Dispersal can also be through involuntary transport by birds or animals capture, fishing or other human activities that facilitate dispersal (e.g. through water ballast, by purposeful introduction of pet species or as escaped food species). Thus, the management, transport and handling of the GM fish need to be considered fully (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 (see section 4.1.1). 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; thus, the frequency of invasions and the numbers of fish invading should be taken into consideration. Further, applicants should consider the possibility that the GM fish may change the ecology of the receiving environments and thereby expose the GM fish to novel biotic conditions. These can include products of the metabolism (e.g. carbon dioxide, ammonia), effluents (e.g. faeces) and products from decaying plants and animals. This includes microorganisms (primarily algae) that can influence fish survival by secreting bioactive toxins into surrounding water or by causing physical

irritation to gill membranes. Some GM fish will have been developed to better endure certain biotic/abiotic factors (see section 4.1.1), but it also becomes important to assess if this comes at a cost of enduring other factors in receiving environments.

Applicants should consider methods for assessing dispersal behaviour under confined conditions and 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 ecological niches they are likely to influence.

Step 4: Risk characterisation

Applicants should consider the biota present in the receiving environments of the GM fish and determine the likely direct interactions that will occur in terms of food, prey, predation, competition, displacement, disease, local population change, etc. The indirect effects from these direct effects should then be considered in terms of food chain effects and the possible consequences for different biota in these ecosystems in the medium term and hence the long-term prospects for these environments. Applicants should consider using the methods and approaches described in section 3.2 and by Devlin et al. (2006) and Kapuscinski et al. (2007a).

Applicants should assess whether keystone 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 receiving environments 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 (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, after 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 (see section 3.8). Applicants should describe identified risks or critical uncertainties that require further information from post-market monitoring studies.

3.4 Fish pathogens, infections and diseases

Fish live in an environment together with viruses, bacteria, fungi, protozoa, helminths, nematodes, copepods and other lower organisms. Most of these organisms are harmless or even beneficial to their hosts (mutualism or commensalism). However, some may cause diseases (parasitism or amensalism) by their presence either inside or outside the fish body, or more indirectly exhibit negative effects such as depriving the water of oxygen (algal blooms). The term "pathogen" in this section refers to an agent that can cause disease.

Infectious diseases are among the major obstacles in aquaculture, causing losses in productivity or mortality and poor animal welfare. The high stocking densities at which fish are normally 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. 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 of making them disease resistant or tolerant (direct effects), either to a specific disease or to many diseases (group 1). Fish may also be genetically modified to express other traits which may change their susceptibility to infectious diseases more indirectly (group 2). All GM fish not in group 1 belong to group 2 according to this Guidance

- Group 1 GM fish are created with the intention of increasing resistance to 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 receptor for a toxin producedby the pathogen (disease resistance). This group can be divided into two subgroups: (a) GM fish with increased resistance to a specific pathogen (or a specific group of pathogens) and (b) GM fish with a more generalised resistance to several pathogens.

- Group 2 GM fish are not created with the primary intention of increasing resistance topathogens, but a consequence of the genetic modification is an effect on the susceptibility of the GM fish to infection. This may be due to an interaction between the immune system and the genetic modification in question. **For more information see page 62.**

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.

3.4.1 Comments to applicant regarding chapter 3.4

In general, the applicant has not described or assessed whether VIRGIN® salmon have inherent characteristics that make them more susceptible to infections than the Comparator. How VIRGIN® salmon interact with pathogens may constitute a higher risk for transmission of pathogens to wild or farmed fish, or even contribute to establishment of endemic disease or higher impact of the disease (higher virulence). T

Reference is made to how gamete-free salmon avoid puberty and thus the immunosuppression associated with sexual maturation. More comprehensive assessments are required on other conditions, pertaining to the VIRGIN® fish itself, or the way it is farmed that could lead to reduced health and increased susceptibility to pathogens. It is the applicant's responsibility to present a complete set of arguments for their claims in the application.

VKM calls for a complete medical history (including all signs of impaired health) for all the individuals that were the basis for the 303 individuals applied for. Reports from health controls, investigations and analyses made and results from these should be provided by the applicants. The application should provide transparent information on how many individuals the experiment started with (on day 0), and specific information on how and why the omitted animals were excluded (Cause specific mortality etc). This information is relevant for the assessment of the remaining individuals' immune defences and possible effects on the environment linked to infection and further spread from these.

The application contains a description on manipulation of light and on vaccination prior to sea transfer. Specific information about the vaccine is however lacking for the experimental group and for the comparator. In addition, there is a discrepancy in the number of degree days obtained in the described experiment (13C og 4 weeks) and the number of degree days recommended or required to obtain protection from most vaccines used in Atlantic salmon farming in Norway today.

VKM is also calling for a more thorough discussion related to the applicant’s statement that VIRGIN® salmon die at 5 years of age. The causal relationship and any early effects are important to uncover.

Furthermore, there is a need for the same type of analyses in the experimental group in this trial as described in the full-scale trial by Kleppe et al 2022.

The cohort study carried out and published by Kleppe et al. (2022) refers to mortality due to technical causes. The reason for and outcome of these technical challenges are interesting if they resulted in selection of more a robust test group. The question also arises whether the same technical challenges could be present in the up-coming experiment.

Finally, there is a need for clarification regarding how the project will monitor and assess the health in the test group and the comparator after sea transfer.

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 a GM fish with its appropriate comparator(s) under the range of receiving environments. Applicants need to justify the environmental conditions used in their

studies to capture a range of receiving environments into which the GM fish and their effluents may be released both intentionally and unintentionally.

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

If the answer is "likely", applicants should further consider for example:

a) Would the phenotype of the GM fish alter the virulence of fish pathogens?

b) Would the GM fish alter transmission range and frequency of pathogens?

c) Would the GM fish become a silent carrier for pathogens?

d) Would the GM fish release metabolites and effluents that alter the pathogen population?

e) If the GM fish can enter other environments or living conditions, would the GM fish introduce pathogens to these other environments, and would the GM fish become pathogen reservoir?

f) Would the aquatic management practices (see step 5) alter the interaction between the GM fish and pathogens?

g) Would 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 include genetics (e.g. species or strains), physiological state of a fish (e.g. age, size, sexual maturity), environment (e.g. temperature, season, photoperiod), stress (e.g. water quality, pollution, density, handling and transport, breeding cycles), nutrition (feed quality and quantity, nutrient availability, use of immune-stimulants, antinutritional 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) characterisation of the pathogen, including description of the 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 or change the spread of the pathogen in comparison with its non-GM counterpart; and (c) description of other organisms in the environment that are susceptible to the introduced pathogens (see also section 4.1.3). Information is required on the infectivity of pathogens to the disease-tolerant GM fish and the subsequent transmission from the infected GM fish to other fish (e.g. any species eating the GM fish or other fish occurring in the same environment as the GM fish; see also Nerland et al., 2011). Transmission studies are required to demonstrate whether GM fish can transmit the pathogen to other non-GM fish and so the GM fish can act as a symptomless carrier of infection. **For more information see page 63.**

Step 3: Exposure characterisation

This step is to evaluate the likelihood and/or frequency of occurrence for each identified hazard and it is important that applicants consider the specific trait of the GM fish itself (e.g. group 1 or group 2), the receiving environments of the GM fish and the presence of non-GM fish in the receiving environments. For confined GM fish, factors affecting the introduction and exposure to diseases within aquaculture units should be considered, such as stocking density, mobility, etc. In addition, the likelihood and frequency of escape needs to be estimated. For semi-confined GM fish, the time fraction and developmental stage for confinement and non-confinement periods should be estimated.

Applicants should describe in detail the different steps of handling 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 of 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 in 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 cultivate only GM fish. Moreover, to reduce the frequency of transmission of pathogens from a farm housing GM fish to other farms and wild populations, stringent bio-security measures can be implemented on the farm to prevent release of pathogens. These can include sufficient levels of confinement to prevent animal escape, adequate waste treatment to prevent release of GM materials through farm runoff, adequate disposal of carcasses from diseased fish, etc. For disease-resistant or -tolerant GM fish, applicants should consider that dead fish may be carriers of pathogens with the ability to infect the GM fish and therefore implement strategies of handling carcasses to prevent the further spread of pathogen and disease (e.g. incineration).

Applicants should also describe 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 confined aquaculture facilities 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 (see section 3.8). 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 PMEM, as well as an explanation of why identified environmental impacts are considered acceptable and do not present risks.

3.5 Interactions of GM fish with the abiotic environment

There are two aspects of abiotic interactions that are relevant for the ERA of GM fish:

- The GM fish may have an altered (increased or decreased) tolerance to abiotic factors. This can be either the desired consequence of the genetic modification or a pleiotropic consequence of it.
- The GM fish may affect the abiotic environment in a different way from non-GM fish, for example by making different nests or by altering the digging behaviour of females. This second aspect can be divided into direct effects of the GM fish itself and indirect effects cascading from the direct effects (as described for biotic effects), which may act either on abiotic factors or onbiotic components.

The genetic modification can alter the sensitivity and behavioural response of GM fish 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 abilityof 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, at least at the egg stage (Sundt-Hansen et al., 2007).

Direct abiotic effects are those effects that the fish itself generates through various means, for example physical parameters such as 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 genetic modification), during its lifetime or after

death, this could have effects on abiotic components, either directly, for example by lowering pH, or indirectly, influencing biota that in turn affect abiotic components.

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

By affecting the abiotic environment, GM fish can alter the ecosystem's trophic structure (i.e. energy flow and food web relationships). Biotic effects of the GM fish are also likely to give rise to 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 factors 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 its tolerance and response to abiotic factors. Firstly, applicants should consider whether the GM fish has a different abiotic tolerance in relation to the relevant comparator, such as the ability to toleratehigher and/or lower temperatures or oxygen levels. Next, the GM fish response to these changes in the

abiotic factors should be assessed to determine not only if the GM fish can survive specific conditions, but also if it will change its behaviour in these conditions. For instance, applicants should assess whether the GM fish, during its different development stages, 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 3.9).

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 examination needs 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. the 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 to construct nests. Such interactions may also lead to GM fish spreading into areas not normally inhabited by the species and/or other abiotic factors being exposed to the GM fish.

Indirect 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. The assessment should include possible 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 to examine whether a physiological capacity to endure a specific abiotic factor also leads to a behavioural decision to actually exploit it. Further, it should be assessed whether dispersal

into environments with a new range of abiotic conditions can occur through involuntary transport 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) and whether the genetic modification has influenced the likelihood of involuntary dispersal. **For more information see page 66.**

Step 4: Risk characterisation

Applicants should consider the abiotic factors present in the receiving environments of the GM fish and determine the likely direct interactions that will occur. The impacts of indirect effects arising from these direct interactions on other abiotic and biotic characteristics of these ecosystems in the medium term and, hence, the long-term prospects for these environments should be considered. 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, 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 outlined in section 3.8.

In addition, applicants should explain why identified environmental impacts are considered acceptable and do not present risks.

Step 5: Risk management strategies

Applicants should propose methods to reverse or reduce adverse impacts on abiotic factors and key ecological functions identified in the risk assessment. The practicality and efficacy of these methods should be evaluated and 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 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.

3.6 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 management, rearing and production. There is a requirement in Directive 2001/18/EC (EC, 2001) to assess the environmental impacts of the specific management practices associated with the GM fish compared with non-GM fish. Considering that the characteristics of the GM fish may differ from those of the non-GM comparator, the management of the confinement measures, welfare, health and feeding regimes of the GM fish may be altered and/or adapted to particular locations. In addition, if GM fish are adapted to different environmental conditions (e.g. lower temperature), production units (e.g. confined aquaculture facilities) could be located in novel locations and have different impacts. An important aspect of the management of the confined aquaculture facilities is to prevent the accidental escape of the GM fish and so the impacts of changes to confinement measures of the facilities should be considered including the breeding, rearing, production and any transport between them (see also section 4.1.1).

Production units also produce effluents and can harbour pathogens. Any differences in waste products and pathogen release from aquaculture facilities should also be considered (see 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 management of the production units (e.g. confined aquaculture facilities). Any changes identified should then be studied to determine their immediate consequences and also any downstream, knock-on, cumulative or long-term effects (see section 3.6). For example, if the consequence of a change in management of production units is a change in diet and/or feed consumption, then the impacts of this on use of natural resources and emissions of 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 units (e.g. emissions of GM fish and waste, water usage, reduction of water quality) and also indirect effects associated with the introduction of new pathogens and parasites into the new areas (see also section 4.1.4).

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

In summary, the ERA should:

- describe the management (e.g. changes in diet composition or amount of food consumed, waste products, water quality) of GM-based aquaculture facilities likely to occur across receiving environments, including new receiving environments, and how the management differs from that of current aquaculture facilities;

- describe the potential adverse environmental impacts associated with the differences in management of the aquaculture facilities of the GM fish (e.g. waste products and pathogens) compared with the management of the non-GM comparator;

- determine the overall risks associated with the changes in management of the aquaculture facilities and their environmental consequences.

3.6.1 Comments to applicant regarding chapters 3.5 and 3.6

In the application, the applicant needs to detail what will be done to limit the spread of VIRGIN® salmon if some or all of them escape from the research facility. Also, the applicant needs to assess whether common routines for reporting and recapturing escaped farmed salmon are sufficient when doing experiments with VIRGIN® salmon in net pens. A pertinent question may be if the experimental fish have any external mark or tag that can identify them, in addition to an internal PIT tag.

Step 2: Hazard characterisation

The hazards associated with the changes to the management of aquaculture facilities 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 management measures to reduce environmental exposure (e.g. for treating effluents or controlling diseases) should be considered. The ERA should consider how changes to aquaculture facilities could impact surrounding accessible ecosystems (see section 3.1.2) at both the local and regional scale. In addition, temporal effects over longer timescales should be taken into account. For

scaling up of ERA, modelling, simulation and analysis of production units and accessible ecosystems may be required, in addition to the analysis of small-scale studies (EFSA, 2008).

Step 4: Risk characterisation

The risks posed by any changes in management of the aquaculture facilities 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 to be considered for other areas of risk described in section 4.1.

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 effluents from the aquaculture facilities.

Measures could be taken to restrict the size or the location of the aquaculture facilities. 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 of the aquaculture facilities for the GM fish considering both direct and indirect 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. The risks and uncertainties described in the overall conclusions of the ERA provide the basis for PMEM.

3.7 Impacts of GM fish on human health

Applicants should evaluate whether the GM fish presents a new hazard for human health compared with appropriate comparators. Applicants should consider both immediate and delayed effects on human health resulting from potential direct and indirect interactions with GM fish. This should include any increased risk of disease to people in contact with GM fish and fish products. Applicants shall follow the step-by-step approach described in section 2.1.

For GM fish applications for food and feed purposes, applicants should refer initially 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 scientific opinions of the EFSA GMO Panel dealing with, for example, allergenicity (EFSA, 2010c).

This Guidance Document considers primarily effects of GM fish on human health through routes of exposure other than ingestion or intake; these include ocular and nasal as well as exposure through dermal contact and inhalation. However, applicants should assess the likelihood of oral exposure of humans to GM animals or their products which are not intended for food or feed uses. If such exposure is likely and ingestion or intake will occur at levels which could potentially place humans at risk, then applicants should apply the assessment procedures described in the EFSA Guidance Document on the risk assessment of food and feed from GM animals and on animal health and welfare aspects (EFSA, 2012a).

Furthermore, fish are capable of carrying pathogens and parasites that can infect humans, and these may be present in the water as well as in the fish. Increased risks to human health from all sources and routes of infection, including the oral route, are considered in this section.

About half of the recognised species of human pathogens are zoonotic, and zoonotic pathogens are twice as likely as non-zoonotic ones to be in the category of emerging and re-emerging pathogens (Woolhouse and Gowtage-Sequeria, 2005). Despite the fact that information is still relatively scarce, fish have been identified as hosts for certain zoonotic pathogens that can cause disease or infections via natural transmission: examples are bacteria such as Mycobacterium marinum, Aeromonas hydrophila and Streptococcus inieae and the nematodes Anisakis simplex and Diphyllobothrium latum(tapeworm). Viruses of fish are usually much more species specific and have not been reported to be zoonotic.

Some fish can produce proteins and other compounds that can cause irritations or allergenic responses to exposed humans working with fish. In addition, some fish have spines, teeth and scales that can harm or irritate human handlers. It is important to determine whether GM fish differ in any of these characteristics and so could place human operators at greater risk when GM fish are handled.

Consideration may need to be given to morphological (e.g. increased size) or behavioural changes that might result in increased hazards to humans handling GM fish. Therefore, applicants should assess whether phenotypic characteristics are changed in GM fish to the extent that they may cause additional harm to people during handling of 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 or with water or other components of the fish environment (e.g. effluents). Human infections caused by pathogens transmitted from fish or the aquatic environment vary depending on the season, human contact with fish and the related environment, dietary habits and the immune system status of the exposed individual (reviewed in Novotny et al., 2004). **For more information see page 70.**

Step 2: Hazard characterisation

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

Altered disease transmission capacity to humansApplicants should determine whether the pathogen load for a specific pathogenic agent will reach levels that can cause human diseases (see section 4.1.4). Where a potential hazard is identified, laboratory animal experiments may be required in order to determine infectivity and transmission capacity. The environmental conditions under which the GM fish will be produced should be considered when determining the pathogen load, for example stocking density, temperature, feed composition, growth rates and medication.

- Emergence/selection of new pathogens and/or strains with the potential to cause human diseases
- Potentially altered allergenicity or toxicity
- Phenotypic changes in the GM fish
- Changes in specific management practices for GM fish

For more information see page 72.

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. **For more information see page 72.**

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 of 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 3.8).

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.

The risks and uncertainties described in the overall conclusions of the ERA provide the basis for the PMEM plan to be proposed by applicants.

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 effluents from the aquaculture facilities.

Measures could be taken to restrict the size or the location of the aquaculture facilities. 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 of the

aquaculture facilities for the GM fish considering both direct and indirect 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. The risks and uncertainties described in the overall conclusions of the ERA

provide the basis for PMEM.