DRAFT STUDY ON RISK ASSESSMENT: APPLICATION OF ANNEX I OF DECISION CP 9/13 TO LIVING MODIFIED FISH

Report for the Secretariat of the Convention on Biological Diversity, UN Environment Programme

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1. Introduction and Terms of Reference: Study on Risk Assessment: application of Annex I of decision CP 9/13 to living modified fish (LMF)

In decision CP-9/13, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety (COP-MOP) decided to establish a process for the identification and prioritization of specific issues regarding risk assessment (RA) of living modified organisms with a view to developing further guidance on risk assessment on the specific issues identified, taking into account Annex I. The Annex sets out the process for recommending specific issues of risk assessment for consideration by the COP-MOP. This process includes a structured analysis to evaluate whether the specific issues fulfil a set of criteria and a stock-taking exercise of resources to determine if resources on similar issues have been developed by other bodies and if so, whether these resources may be revised or adapted to the objective of the Protocol, as appropriate. It also decided to consider at its next meeting, whether additional guidance materials on risk assessment are needed for living modified fish.

In this context, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety requested the Executive Secretary to commission a study informing the application of Annex I of the decision to living modified fish, to facilitate the process referred to above.

The living modified fish (LMF) referred to in this report are from the classes Agnatha (jawless fish), Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish). Other marine and aquatic species of crustaceae and molluscs, such as bivalves and snails, are not included in this study. Fish cell culture transgenesis (Rubio et al., 2019) is not considered in this report as it does not result in whole living fish.

2. List of abbreviations

AAS: AquAdvantage Salmon

AFP: Anti-freeze protein

AHTEG: Ad Hoc Technical Expert Group

AP: Asia Pacific (group of countries)

BCH: Biosafety Clearing-House

CBD: Convention on Biological Diversity

CEE: Central and Eastern European (group of countries)

CEPA: Canadian Environmental Protection Act

COP-MOP: Conference of the Parties serving as a meeting of the Parties to the Cartagena Protocol on Biosafety

DEA: Department for Environmental Affairs (South Africa)

DFO: Fisheries and Oceans Canada

EC: European Commission

EFSA: European Food Safety Authority

EIS: Environmental impact statement

ERA: Environmental Risk Assessment

EPA: Environmental Protection Agency (US)

ERM: Environmental Risk Management

EU: European Union

FDA: The Food and Drug Administration (US)

FISK: Fish Invasiveness Screening Kit

FL: Female-specific lethality

FS: Female-specific sterility

GFP: Green flouresence protein

GH: Growth hormone: used in context of LM fish expressing enhanced levels of GH

GRULAC: Latin America and Caribbean (group of countries)

ILSI: International Life Science Institute

INAD: Investigational new animal drug

IPPC: Integrated Pollution Prevention and Control (EU)

LM: Living modified

LMA: Living Modified Animal

LMF: Living Modified fish

LMO: Living modified organism[1]

MIAMBIENTE: Ministry of Environment (Panama)

NADA: New animal drug application

NSNR (Organisms): New Substances Notification Regulations (Organisms)

OECD: The Organisation for Economic Co-operation and Development

OGTR: Office of gene technology regulator (Australia)

PMM: Post Market Monitoring

PRM: Post Release Monitoring

RA: Risk Assessment

[1] Please note that the terms ”genetically modified” and “genetically modified organism” are used in some bibliographic references in this study. However, for the purposes of this study which is in line with the Cartagena Protocol on Biosafety, the terms “living modified” and “living modified organism” are used instead.

RARM: Risk Assessment and Risk Management

RM: Risk Management

UK: United Kingdom of Great Britain and Northern Ireland

UN: United Nations

USDA: USA Department of Agriculture

USA: United States of America

WEOG: Western European and others group (of countries)

WHO: World health organisation

WTO: World trade organisation

3. Executive Summary

The main objective of this study was to gather information to undertake the exercise of informing the application of the criteria from Annex I of decision CP-9/13. Information was gathered through a literature review, a survey and from other sources such as information published on the Biosafety Clearing-House (BCH) website related to submissions of information and online forum discussions on risk assessment.

The literature search revealed that a wide range of living modified fish (LMF) are being researched and developed in many locations around the world and this report shows that a wide range of novel traits have been developed for a range of both research and commercial use. Two groups of LMF are being commercialised: various species of ornamental fish and growth enhanced salmon for food. LMF expressing novel colours and fluorescence were originally developed to support biological research but subsequently developed as ornamental fish for aquaria. These ornamental LMF have received regulatory approval in Canada and the United States of America (USA), and the regulatory decisions on these fish by these two countries considered that there are no indications that they will have environmental impacts different from non-LM conspecifics. In other cases, national authorities have assessed ornamental LMF and set conditions for use which include requirements to contain the fish in aquaria and do not allow environmental release. Living modified (LM) Atlantic salmon modified with a growth hormone gene and expressing increased growth rates and earlier finishing, has received regulatory approval in 3 countries but under restrictive containment conditions with no environmental release permitted and post release monitoring required in 2 countries. It has also been commercialised for food production in 2 countries.

In relation to the anlysis against the criteria set in annex 1 of decision CP-9/13, some responses to the Secretariat´s notification[2] calling for the submission of information as well as the questionnaire from this study, showed that LMF were identified as a priority for the development of further guidance by some Parties. The responses from the survey and the literature showed that some countries have developed regulations and risk assessment (RA) procedures which can be applied to LMF. However, several countries reported that they do not have risk assessment procedures appropriate for LM animals, including fish. These tend to be countries with less well-developed regulatory systems for living modified organisms (LMOs) and little experience of assessing LMOs. In addition, several reported that they have very limited expertise and no experience in assessing LMF. Several considered that they had little or no

[2] SCBD/SPS/DC/MPM/MW/86376

access to risk assessment guidance for the risk assessment of LMF and that guidance was required. Other responders with more experience in risk assessment, considered that adequate guidance was available in many regions and that this should be made available to authorities with limited experience and that expertise and capacity should be provided to these authorities.

Also, in the survey, the majority of responders considered that LMF fall within the scope of the Cartagena Protocol on Biosafety.

In relation to challenges to existing risk assessment frameworks, guidance and methodologies, this report has shown that risk assessments of LMF for environmental release require data on intended and pleiotrophic changes to their phenotype and information on any changes in behaviour, survival, adaptation, competitiveness, reproduction, interactions with wild type and other species in the range of potential receiving environments. The challenges presented by the complexities of assessing the interactions of LMF with their wild or domesticated comparators and with different components of the environments in which they could move and survive were pointed out by various sources. The scientific literature, information from other sources (e.g. submissions of information to the CBD Secretariat and an online forum) and comments from responders to the survey, has indicated that providing this data on these environmentally sensitive characteristics of fish presents new challenges and can introduce high levels of uncertainty into the risk assessment of some fish species.

The majority of responses from the survey considered that some LMF may have the potential to cause environmental harm depending on the fish species, the traits, the receiving environments and the conditions of release. Some responders referred to experiences with fish farming and introductions of novel types indicating that they had produced mixed outcomes and environmental impacts, including levels of fish diseases. It is therefore expected that the production and releases of some types LM fish could have a similar range of outcomes. In contrast, inland contained aquaculture facilities could have less environmental impact than aquaculture systems located in waterways or marine environments.

In relation to introductions into the environment either deliberately or accidentally, the literature study and the information supplied by survey responders indicated that there have been unapproved introductions and releases of ornamental LM fish in some countries and unapproved ornamental fish have crossed national boundaries due to human activities. In addition, the approved salmon and some ornamental LMF are being legally traded across borders.

With regard to the potential to disseminate across national borders, there was a general consensus from questionnaire responders that some LMF released into the environment can cross national borders, depending on the species, trait and areas of release, which is also supported by scientific literature on the behaviour of fish species.

Concerning commercialization of LM fish, the LM Atlantic salmon have been approved and commercialised under conditions of biological and physical containment because of the difficulties in providing data that informs predictions of environmental impacts of releases. This approach has been questioned by some experts, especially as it is anticipated that production of the LM salmon will be increased. Currently, there are no indications that the specific conditions applied to commercialised LM salmon will be changed or that other commercial fish species are being developed for environmental release.

The final part of Annex I of decision CP-9/13 provides that the process for recommending specific issues of risk assessment for consideration by the COP-MOP should consider a stock-taking exercise. Section 6 of this report describes the risk assessments performed on LMF by Canada and USA and the guidance documents relating to risk assessment of LM animals available in those two countries. In addition, it discusses the guidance documents that are available such as those produced by EFSA, ILSI, OECD and

CBD. There are also guidance documents available in many countries for risk assessment of LM animals for contained use and the UK risk assessment system is presented as an example (Section 6.4.4).

4. Methods

4.1 Review of Literature

A literature search using Google Scholar, CAB Abstracts (CABI, Wallingford, UK), and JSTOR (Digital library of academic journals, reports, books, and other primary sources) was conducted. The Biosafety Clearing-House database was examined for reports of transboundary movements and regulation and risk assessment of LMF. The comprehensive literature search conducted by Cowx et al. (2011) for the European Food Safety Authority (EFSA) as part of the EFSA exercise in developing guidance for the environmental risk assessment (ERA) of LM fish, was used for setting search terms and for testing the comprehensiveness of this literature search.

This literature was studied for information relevant to the development and risk assessment of LMF and this report reviews the current situation, including published comments on the risk assessments conducted to date. The review considers the data requirements which have been identified by different regulatory authorities for the RA of fish and how data gaps and uncertainty are considered.

This provides information on whether the risk assessment (RA) of LMF pose challenges to existing RA frameworks, guidance and methodologies. It will also indicate if LMF have been assessed with existing RA frameworks but pose specific technical or methodological challenges that require further attention.

4.2 Gathering information from national biosafety authorities, institutions and stakeholders in relation to the criteria in Annex I

Annex I in CP 9/13 describes the process for recommending that specific issues of risk assessment are identified and prioritized. This process for recommending specific issues of risk assessment should include a structured analysis to evaluate the specific issues against a set of criteria as well as a stock-taking exercise of resources on similar issues. In order to collect information that could be useful for this analysis, a questionnaire was prepared (see Annex 1 to this study) around the criteria specified in Annex I in CP 9/13. The questionnaire asked for information about the development, testing, assessment and approval of LMF. The questionnaire was produced in English, French, Spanish and Russian and was sent out in mid-September to a regionally-balanced group of biosafety authorities and institutions in 74 national biosafety authorities and institutions, 6 inter-governmental organisations, 12 civil society organisations and 2 industry organizations. Two reminders were sent out to non-responders on October 18 and 25. In total, 29 responses were received: including 23 from countries, 3 from international organisations, and one from a civil society organization. The analysis of the responses is presented in section 7, and additional information is also presented in Annex 1.

5. Literature Review

5.1 Background

Research on genetic modification of fish through transgenesis started in mid 1980s and some of the first LM fish were reported in China in 1985 (Dunham & Winn, 2014) and by Maclean and Talwar (1984), Zhu et al. (1985) and Ozato et al. (1986) by modifying rainbow trout (*Oncorhyncus mykiss*), goldfish (*Carassius auratus*) and Japanese medaka (*Oryzias latipes*) respectively, by micro-injection of cloned gene sequences into fish eggs. These researchers as well as, Guyomard et al. (1998) and Maclean et al.

(1987) working with common carp (*Cyprinus carpio)* and tilapia (*Oreochromis sp.*), demonstrated that the transgenes were integrated into the genome with stable expression and heritability. Gibbs et al. (1988) described the transfer of reporter genes into zebrafish and rainbow trout. Initially, mammal and viral genes were transferred as the functions of fish genes were not well studied. However, by the 1990s, gene functions and sequences of fish genes were more extensively studied so that it became possible to use molecular techniques and bioinformatics to identify genes and promoters that are homologous to specific genetic and promoter regions in fish. In the early 1990s, Funkenstein et al. (1991) transferred a growth hormone (GH) gene from trout to sea bream and Du et al. (1992a & 1992b) developed an all-fish gene cassette for gene transfer and induced growth enhancement in transgenic Atlantic salmon using an all-fish chimeric growth hormone gene construct.

5.2 Transformed Fish species

In their review of LM fish Cowx et al. (2010) provided a list of 50 fish species that had been transformed and over 400 fish trait combinations prior to 2010. These are summarised in their Table 1* below. In addition, prior to 2010, Chan and Devlin (1993) had produced sockeye salmon (*Oncorhynchus nerka*) with metallothionein promoter and histone 3 (H3) promoter fused to the type 1 growth hormone (GH) gene from the same species to produce enhanced growth (see also: Leggatt et al., 2012). In recent years there have been reports of genetic transformation of other fish species such as fluorescent flying barb (*Esomus danricus*; Jha, 2011), red fluorescent white cloud mountain minnow (*Tanichthys albonubes*, Jiang et al., 2011), green fluorescence in killifish (*Nothobranchius furzeri*; Hartmann & Englert, 2012), albino rainbow shark (*Epalzeorhynchos frenatum*; Leggatt, 2019), and growth hormone modification in *Betta imbellis* (Peaceful Betta: Kusrini et al., 2018) .

Table 1. Summary of main fish species, species traits and foreign genes involved in GM research and development

GM trait	Foreign gene	Species
Disease resistance	Insect genes	Striped bass
	hLF + common carp ß-actin promoter	Grass carp
	Silk moth (*Hyalophora cecropia*) cecropin genes	Channel catfish
	Insect cecropin or pig cecropin-like peptide genes + CMV	Arctic charr
	Rainbow trout lysozyme gene +ocean pout AFP promoter	Japanese medaka
	Mx genes	Atlantic salmon
	Shark (*Squalus acanthias* L.) IgM genes	Tilapia
	Antisensesalmon GnRH + common carp ß-actin promoter	Zebrafish
Growth enhancement	Salmon and human GH;	Atlantic salmon
	rainbow trout GH	Artic char
	Grass carp GH and common	Common carp
	carp ß-actin promoter	Indian major carps
	GH + Arctic flatfish AFP	Channel catfish
	Mudloach GH + mud loach and mouse promoter genes	Goldfish
	Bovine GH or chinook salmon GH	Mudloach
	Arctic flatfish AFP + salmon GH	Northern pike
	Chinook salmon GH with ocean-pout type III	Sea bream
	AFPpromoter	Chinook salmon
	Arctic flatfish AFP + salmon GH	Coho salmon
	Sockeye MT-B or H3 promoters driving sockeye GH1	Cutthroat trout
		Rainbow trout
		Tilapia
Sterility	Antisense-GnRH mRNA	Common carp
Pharmaceutical	Japanese flounder promoters (complement component C3, gelatinaseB, keratin and tumor necrosis factor) linked to GF	Zebrafish
Cold tolerance	Ocean pout type III AFP Human GH	Goldfish
	Arctic flatfish AFP	Atlantic salmon
Dietary performance – including increased food conversion efficiency	Aspergillus niger phytase gene + human CMV or sockeye	Japanese medaka
	salmon histone type III promoter	Atlantic salmon
	Chinook salmon GH	Rainbow trout
	Tilapia GH+ human CMV	Coho salmon
	Arctic flatfish AFP +salmon GH	Tilapia
	Human glucose transporter + rat hexinose type II with viral or sockeye salmon promoters	
Ornamental	rerecombinase driven by T7 promoter + fluorescent protein flanked by two loxP sites crossed with T7 RNA Polymerase (gonad specific)	Zebrafish

*Table from Cowx I.G. et al., 2010. Defining environmental risk criteria for genetically modified fishes to be placed on the EU market. Scientific and Technical Report for EFSA CT/EFSA/GMO/2009/01 May 2010

Fish of commercial interest which have been transformed fall into two main groups:

1. Ornamental fish species used in aquaria.
2. Fish species used for food production.

Ornamental fish such as zebrafish, tetra and barb fish have been transformed with colour changes and fluorescence characteristics. Some of these ornamental species have been commercialised and traded across borders, initially with little control over their movements or use. Transgenic ornamental zebrafish (*Danio rerio*) and barb fish are now being marketed in the United States of America (USA) and Canada. Many of the modifications achieved in ornamental fishes have been for fundamental studies of biology (see Section 5.4), but a spin-off has been to market these unusual ornamentals to collectors and hobbyists. At present, this is confined to modifications in colouration and fluorescence, but research has been carried out to enhance particular traits or to accentuate certain characteristics, such as resistance to cold (Maclean & Laight, 2000). Several countries have reported the presence of LM ornamental fish, have

conducted assessments and have banned or restricted their distribution. Ornamental fish submitted for regulatory approval have undergone risk assessment in some countries (USA and Canada). In USA and Canada, they have been approved for use in aquaria, but the regulators also considered that their release would have environmental impacts similar to those of non-LM conspecifics. They are mostly warm water species and considered unlikely to survive if released; but there have been concerns raised in countries and regions with warmer climates. Ornamental LMF are discussed in detail in Sections 5.3.7 and 6.1.

The main food fish species transformed include salmon, trout, carp, tilapia, bass, bream and catfish and are mostly transformed with genes that enhance growth hormone production in order to improve productivity. Genes for improved food conversion efficiency, disease resistance and cold tolerance have also been introduced into several species. Researchers in East Asia have published a large proportion of the carp, catfish and tilapia studies while North American and European researchers lead on publications on salmon and trout studies. However, no reports of commercialisation of edible LMF in Asia have been found.

LM Atlantic salmon (*Salmo salar*) expressing growth hormones, which confer faster growth rates, have been approved for production in Canada, USA and Panama with restrictions on their release and management. They have been placed on the market in Canada and USA. There have been considerable studies made of these fish as changes in hormone expression and growth rates in salmonids and carp species can also affect other characteristics, which may result in changes in their behaviour, survival, environmental adaptation, competitiveness, invasiveness, fertility and reproductive success. Because of these changes to the LM fish and the complexities of natural environments, there is considerable discussion in the scientific literature about the data requirements for assessing the potential environmental impacts of LM fish. In addition, researchers have demonstrated the environmental sensitivity of many of these changes and the difficulties to test for them under contained experimental conditions. These issues are discussed in detail in Sections 6.2 - 6.5.

5.3 Transgenic Traits

5.3.1 Enhanced Growth

Growth enhancement through the introduction of growth hormone genes has been developed in several stable lines of growth-enhanced transgenic fish including tilapia (Martinez et al., 1996, 1999; Rahman et al., 1998 & 2001), mud loach (*Misgurnus mizolepis*) (Nam et al., 2001, 2002), Atlantic salmon (Du et al., 1992a; Fletcher et al., 2004) sockeye and Coho salmon (*Oncorhynchus kisutch*) (Devlin et al., 2004) and common carp (Zhong et al., 2012).

Growth in many of these modified species is significantly increased resulting in improvements in productivity. In addition, there are indications that, in some species, the growth hormones change fish physiology, metabolism (Hill et al 2000; Leggatt et al., 2009; Kim et al., 2018) and gut microbiome (Li et al., 2013) such that the fish feed conversion efficiency is improved
. This means that faster growth rates are achieved with similar inputs of feed used in non-transformed fish.
In 1989, a salmon line was created by injecting an Atlantic salmon egg with a gene construct opAFP-GHc2 that contained a promoter and termination region from the ocean pout (*Zoarces americanus*) anti-freeze gene and a growth hormone gene from Chinook salmon (*Oncorhynchus tshawytscha*). The ocean pout anti-freeze promoter was previously shown to be constitutive, or continually expressing, in salmon (Devlin et al., 1995), in contrast to the native growth hormone promoter in salmon, which only expresses in response to certain environmental cues such as day length and temperature (Bjornsson, 1995).

The chinook and Atlantic salmon growth hormone genes are very similar and a single copy of the construct was integrated into the Atlantic salmon genome. During transgene integration, a rearrangement

of the construct took place, which resulted in the integration of a small fragment of the plasmid into the salmon genome (Butler & Fletcher, 2009). Part of the promoter was integrated downstream of the termination region. This truncated promoter has reduced expression compared to the full promoter in salmon but remains functional (Butler & Fletcher, 2009). The initial transformed individual (founder) was backcrossed to wild-type Atlantic salmon, and the EO-1α gene sequence was identical in the second and fourth generations, indicating that the insertion is stable and revealing no additional mutational effects during insertion other than the two desired genes. These two genes enable the LM salmon to grow more rapidly over longer seasonal periods, growing to market size in 16 to 18 months rather than three years.

5.3.2 Fatty acid composition: Production of Omega 3 fish

The potential for the production of different long chain polyunsaturated fatty acids (PUFAs) has been considered by several researchers (e.g. Monroig et al., 2018). Zhang et al. (2019) reported substantial production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in common carp and they considered that "*the all-fish CA:fat1-transgenic*[3] *common carp can serve as a novel healthy dietary source of omega-3 PUFA, especially EPA and DHA*".

5.3.3 Enhanced and Double Muscle

Lee et al. (2009) produced homozygous transgenic zebrafish which were 45% heavier than non-transgenic controls. The area of the muscle fiber of the transgenic fish was twice that of non-transgenic controls and this was the first model zebrafish with a hereditarily stable myostatin-suppressed genotype and a double-muscle phenotype. Medieros et al. (2009) produced transgenic rainbow trout overexpressing follistatin (Fst1) in muscle tissue which exhibited increased epaxial and hypaxial muscling similar to that observed in double muscled cattle and myostatin null mice. Sawatari et al. (2010) created a transgenic medaka strain that exhibited increased production of skeletal muscle fibers at the adult stage (hyperplasia), although gross muscle mass was not altered. Li et al. (2011) generated Fst1 transgenic zebrafish, which exhibited elevated expression levels of myogenic regulatory genes myosin-D (MyoD) and paired box-7 (Pax7) in muscle cells. High levels of Fst1 expression increased myofiber numbers in skeletal muscle, without significantly changing the fiber size and they concluded that Fst1 overexpression can promote zebrafish muscle growth by enhancing myofiber hyperplasia. Jiang et al. (2017) introduced follistatin 2 gene to cause myofiber hypertrophy which develops double muscle in blunt snout bream (*Megalobrama amblycephala*).

5.3.4 Disease Resistance

5.3.4.1 Virus Diseases

Anderson et al. (1996) provided the first evidence of the potential for transgenic enhancement of a fish resistance when they used the expression of viral coat protein genes or antisense of viral genes to improve viral resistance in rainbow trout to infectious hematopoietic necrosis virus (IHNV). Chiou et al. (2002) examined in vitro effectiveness of native cecropin B and a synthetic analogue, CF17, for killing several fish viral pathogens: infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), snakehead rhabdovirus (SHRV), and infectious pancreatic necrosis virus (IPNV). Zhong et al. (2002) introduced a human lactoferrin gene into grass carp (*Ctenopharyngodon idellus*) to increase resistance against grass carp heamorragic virus. Chiou et al. (2014) produced disease-resistant, homozygous rainbow trout strains, which were resistant to IHNV, as well as *Aeromonas salmonicida* bacterial infection, the causal agent of furunculosis.

[3] Fish codon-optimized omega-3 desaturase gene (fat1) driven by the common carp β-actin promoter (CA).

5.3.4.2 Bacterial Diseases

Transfer of genes overexpressing antibacterial compounds from distant taxa has been used to transfer anti-bacterial resistance into fish. An example is the transfer of antibacterial peptide genes, which have the advantage of providing protection during early development, before the immune system has matured to a stage where specific immunity can be elicited by other forms of immunization, such as vaccination (Dunham, 2009). Examples of enhanced transgenic resistance to bacterial diseases are:

- Columnaris and enteric septicemia resistance in channel catfish (*Ictalurus punctatus)*;
- *Pseudomonas fluorescens*, *Vibrio anguillarum* and *Flavobacterium columnare* resistance in medaka (Sarmasik et al., 2002);
- *Vibrio vulnificus* resistance in tilapia (Hsieh et al., 2010);
- *Aeromonas* resistance in rainbow trout (Chou et al., 2014; Han et al., 2018);
- *Vibrio anguillarum* resistance in grass carp (Mao et al., 2004); and
- *Flavobacterium columnare, Streptococcus agalactiae, Vibrio vulnificus* and *Edwardsiella tarda* resistance in zebrafish (Pan et al., 2011; Suet al., 2018).

5.3.5 Biocontainment

In order to address concerns about release or escape of LMF, several transgenic strategies to reduce fertility or viability of LMF have been considered.

5.3.5.1 Fertility reduction and sterility

Transgenic sterilization has the potential to sterilize transgenic fish without the drawbacks of polyploidy. However, only limited research has been done on this topic. Maclean et al. (2002) described a transgenic method of reducing fertility in Nile tilapia (*Oreochromis niloticus*). Uzbekova et al. (2000 & 2003) described production of a transgenic rainbow trout containing salmon-type antisense gonadotropin-releasing hormone (GnRH) from Atlantic salmon, which appears to be sterile. Zhang et al. (2015) developed an on-off reproductive containment strategy for fish that renders the offspring sterile but leaves their parents fertile. This on-off strategy is a potentially effective means of generating sterile fish for commercialization while retaining fertility in brood stocks and offers a novel method to mitigate the ecological risks of fish introductions. Li et al. (2017 & 2018) reported investigation of repressible knockdown approaches to manipulate transgenic sterilization in channel catfish. They reported reduced spawning and gonad size. Li et al. (2017 & 2018) considered that repressible transgenic sterilization is feasible for reproductive control of fish, but that negative pleiotropic effects can result.

Wong and Zohar (2015) discussed several mechanisms, many of them transgenic, that have been developed for inducing sterility. These include both maternal and male sterility systems produced by transgenic and non-transgenic techniques. They reported: "*The most advantageous benefit of Maternal Sterility Technique is that sterility is a default outcome. This default sterility approach thus generates sterile fish without the need of any induction treatment, which is a more cost-effective method for large scale operations*." These studies indicate potential mechanisms that can be used but that reliably achieving full infertility with these systems is unlikely and that these strategies need to be combined with other non-transgenic techniques as well as careful screening of each generation.

5.3.5.2 Reduced Viability

A number of systems relying on creating dependencies in fish by removing or adding enzymes involved in essential metabolism functions have been tested. For example, Noble et al. (2017) developed LM zebrafish expressing thiaminase so that they are dependent on thiamine in their diet and so die if they escape.

5.3.6 Cold Tolerance

Hew et al. (1992) introduced winter flounder anti-freeze protein (AFP) genes into Atlantic salmon resulting in stable genomic integration and low levels of expression of winter flounder (*Pseudopleuronectes americanus*) AFP genes in a small number of salmon, demonstrating that stable germ-line transformed Atlantic salmon can be produced. Fletcher et al. (1992 & 1999) and Hobbs & Fletcher (2008) showed that levels of AFP expression were inadequate for improving the freeze resistance of AFP transgenic salmon. However, Wang et al. (1995) reported that they microinjected ocean pout AFP genes into the oocytes of goldfish to produce more cold tolerant fish and that the AFP gene can also provide freeze resistance in some fish species. Abass et al. (2016) transformed channel catfish, hybrid catfish (channel catfish female × blue catfish (*Ictalurus furcatus*), male) and transgenic channel catfish with catfish growth hormone gene driven by the antifreeze protein promoter and noted that some catfish showed enhanced cold tolerance at -0.5^{0}C.

5.3.7 Fluorescence and colour changes in LMF

Green fluorescence protein (GFP) produced by a gene in jellyfish (*Aequoria spp.*) absorbs blue light at 395nm and emits green light at 509nm. Amsterdam et al. (1995) introduced the GFP gene into zebrafish embryos to produce fluorescence which was expressed in fish muscles and other tissues as they developed and in subsequent generations (Gong et al., 2003). The GFP reporter system facilitates optical microscopy of embryogenesis in model fish species such as zebrafish and medaka (Zeng et al., 2005a). This GFP transgenic fish technology has been employed in many areas such as analyses of gene expression and development at cellular and organ levels and activity of promoters/enhancers etc. GFP transgenic fish have also been used in analysis of regulatory factors, mutagenesis screening and characterization, and as a promoter/enhancer indicator. GFP transgenic fish can be used as biosensors and bio-indicators for surveillance of environmental contaminants (Section 5.4.8). Wan et al. (2002) produced two-color transgenic zebrafish using the green and red fluorescent protein reporter genes.

Kinoshita (2004) developed transgenic medaka with brilliant fluorescence in skeletal muscle under normal light expressing a fluorescent green colour which were the first fluorescent fish marketed as ornamentals (Lian and Chung, 2005). Gong et al. (2013) developed new varieties of ornamental fish with different fluorescence patterns, e.g., skin fluorescence, muscle fluorescence, skeletal muscle-specific and/or ubiquitous fluorescence. Blake et al. (2019) described the transfer of natural red fluorescence from coral (*Discosoma sp.*) and hybridisation to combine orange, blue and yellow colour variants of zebrafish with GFP fish, in order to produce enhanced colour expression in the hybrid fish. Nguyen et al. (2014) reported the transformation of a marine medaka (*Oryzias dancena*) with a mutant version of the cyan fluorescent protein isolated from the non-bioluminescent anthozoa species (*Anemonia majano*) to produce fluorescent blue fish (Matz et al. 1999).
Other species that have been transformed include black tetra (*Gymnocorymbus ternetzi)*, tiger barb (*Puntius tetrazon*) and albino rainbow shark. They were originally produced for medical and developmental biology research purposes, but later the commercial potential of the various fluorescent zebrafish was recognised and developed.

5.3.8 Transgenic biosensors, bio-indicators and bio-medical uses of LMF

Transgenic fish have been developed and used in ecotoxicology, where they have the potential to provide more advanced and integrated systems for assessing health impacts of chemicals. The zebrafish is the most popular fish for transgenic models, for reasons including their high fecundity, transparency of their embryos, rapid organogenesis and availability of extensive genetic resources (Carvan et al., 2000, 2001 & 2006). The GAL4-UAS system, where *Saccharomyces cerevisiae* GAL4 transcription activator is placed under the control of a desired promoter and an upstream activation sequence (UAS) is fused with a fluorescent marker, has greatly enhanced model development for studies in ecotoxicology (Lee et al.,

2015). Transgenic fish have been developed to study the effects of heavy metal toxicity (via heat-shock protein genes), oxidative stress (via an electrophile-responsive element), for various organic chemicals acting through the aryl hydrocarbon receptor, thyroid and glucocorticoid response pathways, and estrogenicity. Transgenic fish (principally zebrafish) have been used for studies in environmental toxicology and in biological and biomedical studies for research purposes (Chen et al., 2015) and to a lesser extent for the production of some biomedical and pharmaceutical products.

5.4 Biological control of fish

Transgenic techniques for controlling fish populations have been studied, which include the introduction of sterility genes or genes which change the fitness or reproductive ability of target fish populations. Muir & Howard (2004) and Howard et al. (2004) discussed methods (e.g. Trojan Gene – Muir, 1999), which involved male LM fish introducing infertility or reduced fitness genes that could drive these traits into fish populations by outcompeting with wild types. Bax & Thresher (2009), Kapuscinski & Sharpe (2014) and Thresher et al. (2014) discussed a range of techniques, including the Trojan gene approach. They examined two approaches to reduce effective female population sizes: female-specific sterility (FS) and female-specific lethality (FL), focusing on the FL strategy because of the successful application of this approach in insects. They tested the direct-drive FL construct in transient assays in common carp and obtained results consistent with enhanced female mortality. Wang et al. (2018) showed that a system of inducing ectopic gene expression could be used to introduce a "Trojan gene", which is detrimental to that species and used in biological control.

Thresher et al. (2014) concluded that *"Integrated management involving FL, FS and other sex-ratio-distorting genetic options coupled with classic biological control could prove useful against various lower vertebrate pests*". Finding suitable biological control agents for vertebrates has proven difficult since the agents themselves may cause economic or environmental damage.

6. Commercialisation, Regulations, Risk Assessment and Risk Management of LMF

Two types of transgenic fish have been commercialised; these are the coloured fluorescent fish described in section 5.3.7 and the AquaAdvantage salmon expressing a growth hormone gene to enhance its productivity.

6.1 Commercialisation, Regulation and Risk Assessment of Fluorescent and Coloured Ornamental LMF

The first fluorescent zebrafish were marketed in Taiwan Province of China in 2003 and subsequently followed by fluorescent medaka, tetra, tiger barb, albino rainbow shark, convict cichlids (*Amatitlania nigrofasciata*) and angelfish (*Pterophyllum scalare*) expressing different colours. They were marketed by Taikong Corporation and labelled as TK-1, TK-2 and TK-3.

Fluorescent zebrafish marketed as "GloFish" were introduced to the United States of America market later in 2003 by Yorktown Technologies. Yorktown Technologies sold the rights of "GloFish" to Spectrum Brands, Inc. in May 2017.

Illegal imports of fluorescent fish were reported into Singapore and Canada in 2002/3 and marketing of GloFish occurred in Canada prior to approval through the regulatory process. The import, sale and possession of these fish is not permitted within the European Union, as no marketing application has been made. On November 9, 2006, the Netherlands' Ministry of Housing, Spatial Planning and the Environment (VROM) reported 1,400 fluorescent fish were sold in various aquarium shops in the Netherlands. The report additionally mentioned that other illegal introductions occurred in Austria,

Germany and Czech Republic in 2006. Furthermore, Finland, Norway and Belgium communicated illegal importations of fluorescent zebrafish in the BCH occurring in 2007, 2012 and 2017, respectively. The Netherlands has risk assessed introductions and considers they pose no environmental risk and has allowed their use in its territory under conditions of part B of the EU regulations, which allow contained use of these LM fluorescent fish, but do not permit their marketing, sale or release.

The illegal imports of fluorescent fish detected in 2006 by Czech Republic (2007) were in two sources of transgenic zebrafish. Tests detected the red fluorescence gene that originated from Singapore in these fish. There were some indications of import of LM zebrafsh from the Russian Federation. Fluorescent fish were also reported in New Zealand, notified to BCH/CB and destroyed (New Zealand, 2011). In 2017, zebrafish, tiger barb, black tetra, and Japanese medaka with unnatural colors for the species (green, yellow, red, orange), were reported in Denmark and the zebrafish were confirmed as transgenic (Anon, 2017).

In the responses to the survey, 11 responders (including two non-Parties Canada and USA), reported that they had assessed the risks of ornamental and some other LMF. In the 2019 submissions of information on risk assessment to the CBD, the following countries reported that they had assessed LMF: Côte D'Ivoire, Czech Republic, Finland, Malaysia, Mexico and the Netherlands and the species included Arctic char (*Salvelinus alpinus*), carp, rainbow trout, tilapia and zebrafish. The risk assessments were for contained use in research facilities or aquaria only, or because of the potential for illegal imports. Some had published the results on their websites, while others had not.

LM Glofish are permitted in USA and Canada and are being marketed in China and Sri Lanka, as they are available through some websites in these countries. Fluorescent LMF have been reported in India, Malaysia, the Russian Federation, Thailand and some EU countries, but without regulatory approval (van den Akker & Wassenaar, 2012). In Singapore, it is an offence to import Fluorescent LM fish even though the Glofish was originally developed there. They are not permitted in New Zealand nor in Australia, where an application for release by Yorktown was withdrawn.

Illegal marketing has been detected in the EU and stopped by the National Authorities as described above and reported in the survey responses. However, transboundary movements of LMF have occurred into several countries originating from the countries where these fish were first bred and multiplied, and subsequently distributed as legal or illegal trade and by enthusiasts and collectors. These issues are discussed in a report by RIVM in the Netherlands (van den Akker & Wassenaar, 2012) and examples of introductions and illegal trading are reported by the Animal and Plant Health Inspectorate, UK (2016), in Belgium (Johansson, 2015), in Denmark (Anon, 2017), in Mexico and in Peru (Scotto, 2013, 2016 & 2018).

In the case of ornamental zebrafish, an environmental risk assessment and decision not to regulate was made by the U.S. Food and Drug Administration (FDA), which has jurisdiction over all living modified animals due to the pharmaceutical nature of the transformations (See Annex 3). The FDA determined in December 2003 no reason to regulate these particular fish in the absence of evidence that the fish pose any more threat to the environment than unmodified counterparts (FDA, 2003).

In 2018 and 2019, Fisheries and Oceans Canada (DFO, 2018 & 2019) presented scientific opinions of low risk on a range of species and types of ornamental fish. For example, in 2019 the overall assessment of the use of GloFish® Tetras in the ornamental aquarium trade or other potential uses in Canada was declared as low risk. This meant that these LM ornamental fish could now be traded in both Canada and USA.

6.2 Commercialisation, Regulation and Risk Assessment of AquAdvantage Salmon

A selection from the Atlantic salmon line created by injecting a salmon egg with a gene construct opAFP-GHc2 that contained a promoter and termination region from the ocean pout anti-freeze gene (opAFP) and a growth hormone gene from Chinook salmon (CHc2), was developed as the commercial "AquAdvantage Salmon" (AAS). For the commercial production of AAS, eggs are treated with pressure, to create triploid batches of fish eggs, which are sterile (Devlin et al., 2010). Any batch that contains 5 percent or more diploid fish, is destroyed because diploid fish are capable of reproducing. In addition, AquaBounty developed mostly female triploid transgenic salmon for commercialisation with reduced fertility, as well as enhanced growth rate due to their polyploidy (Benfey, 2016).

Applications for the production for this salmon in contained facilities were made in 3 countries (Canada, USA and Panama). To date, there are no notifications or applications to market or produce AAS in countries outside the 3 mentioned above. According to the AquaBounty web site, experimental production of AAS was tested in Brazil in 2019 and in Argentina in 2018 under the regulations for contained experimental use in those countries.

This LM salmon was deregulated in USA in 2015 and fully approved (for ecological and human health assessments) in Canada in 2016, however, the approvals required that there be physical confinement to supplement the fish's biological containment characteristics. AAS were initially only allowed to be raised in land-based tanks at sites in Canada and Panama (Tizard et al., 2016). The fish eggs were produced in a land-based, fresh-water research facility on Prince Edward Island, Canada, so that if eggs escaped this facility, they would be unable to survive in surrounding sea water. However broodstock of mature, fertile fish modified for growth hormone expression are also maintained at this facility, which are capable of survival in sea water. The eggs were then transported in coolers to a confined production unit in a land-based aquaculture facility at Boquete, Province of Chiriqui, at high altitude in Panama near a river that drains into the Pacific Ocean. The salmon were grown to market size, slaughtered and first transported to various marketing points, including Canada, in 2017.
According to the assessments conducted by FDA (USA), Environment Canada and Fisheries and Oceans Canada, the breeding, hatching and rearing facilities all have biosecurity and confinement arrangements so that escape is unlikely. In addition, the fish are mostly sterile females so that little reproduction is anticipated in any surviving escaped fish. Canada permitted only confined use of AAS, and the FDA and the National Commission of Biosafety for Genetically Modified Organisms of Panama also required monitoring of the breeding and production of AAS. In April 2018, FDA gave approval for the raising of AAS on mainland USA under confinement and monitoring conditions. The National Commission of Biosafety for Genetically Modified Organisms of Panama agreed with the FDA and is overseeing the monitoring in their territory. AAS were sold and consumed in Canada, where AAS do not need to be labelled as genetically modified and so cannot be distinguished from non-modified fish by the consumer, though the company is considering a labelling strategy.

On March 8, 2019, the FDA released a statement on AAS deactivating the import alert and allowing import of AAS into USA (see section 6.3.5 and Annex 3). This means that AAS eggs can now be imported to the company's contained grow-out facility in Albany, Indiana to be raised into salmon for food. In June 2019, 3 months after the FDA lifted the import alert, AquaBounty Technologies Inc. began commercial production of AAS at its Albany facility in Indiana for food markets in USA and Canada. In addition, the company announced in 2018 that it intends further development at Rollo Bay in Prince Edward Island and the construction of a research and development hatchery, broodstock facilities and a 250-metric ton production unit at this site. In addition, AAS research facilities are operating in Brazil and Argentina with plans for expanding research to China and Israel.

AAS is being moved across borders of Canada, Panama and USA as living modified fish as well as its products. It is likely that fish products will be marketed outside these 3 countries in future and production may expand to some other countries. No reports of unlicensed movements of live AAS or movements to or from countries that have not approved AAS have been reported.

6.3 Risk Assessment Resources for LMF

6.3.1 Guidance Documents

In relation to risk assessment of LMF, many variable parameters affecting the potential environmental interactions between fish and different receiving environments. The complexity involved in predicting outcomes and impact have been identified. Methods for studying the impacts of salmon farms on wild stocks have also been developed (Keyser et al., 2018). Guidance documents for LMF produced by different countries and entities attempt to incorporate these considerations, but can only provide general guidance, as the actual RA of an LMF is always case-specific and a guidance document cannot provide protocols for individual events or situations.
Some of the main national or regional guidance documents are described:

6.3.2 Canada: Risk Assessment Guidance of LM Animals

The Canadian Environmental Protection Act, 1999 (CEPA, 1999) provides the federal government with the authority to address pollution issues which includes animate products of biotechnology (i.e. LMOs). The Act requires that substances be identified and assessed, to determine whether they are "toxic" or capable of becoming toxic. Toxic, as defined in CEPA 1999, refers to potential hazards to human health, the environment or its biological diversity.
Environment Canada administers LMF applications under the Canadian Environmental Protection Act, 1999 (CEPA, 1999), and the New Substances Notification Regulations (Organisms). Environment Canada evaluates the environmental aspects of the notification. The Fisheries and Oceans Canada (DFO) is also involved in the process because of its role in protecting fish health and habitat, and the environment. Any request to develop fish using modern biotechnology for commercial purposes is subject to the New Substances Notification Regulations under CEPA, 1999. DFO and Environment Canada work together to have any such products assessed for notification and compliance with those regulations. The Canadian Food Inspection Agency (CFIA) additionally has jurisdiction for animal health matters during the process of assessing the LM animals. In addition, the CFIA is responsible for assuring that diseases do not spread between animals. ERA Guidance is provided by Environment Canada (2010) and details of the Canadian Guidance are provided in Annex 4.

6.3.3 European Union: Guidance on the Environmental Risk Assessment of LM animals (including fish)

EFSA (European Food Safety Authority) conducts risk assessments of LMO applications for commercialisation in the European Union on behalf of the European Commission, who are the regulators. Applications for experimental and contained use of LMOs are considered by member states individually. EFSA has produced guidance documents on the risk assessment of LM animals for both food and feed uses (EFSA, 2012a) and for release into the environment (EFSA, 2013). These give guidance in relation to applications for food/feed import of animal products and for the commercialisation of live modified animals (including fish) in the EU in compliance with Regulation (EC) No 1829/2003 or Directive 2001/18/EC.

The guidance documents on the risk assessment of LM animals for release into the environment (EFSA, 2013) provides guidance to applicants and risk assessors for assessing potential adverse effects of LM animals on the environment, human and animal health and the rationales for data requirements for a comprehensive risk assessment. It also provides general guidance for producing post-market environmental monitoring plans. This guidance considers issues specific to LMF, as well as issues common to the risk assessment of a wide range of LM animals. The EFSA (2013) Guidance on the risk assessment of LM animals is described in more detail in Annex 5.

6.3.4 United Kingdom of Great Britain and Northern Ireland: Guidance on the Risk Assessment of LMOs for contained use

In the United Kingdom of Great Britain and Northern Ireland (UK), the Health and Safety Executive, Scientific Advisory Committee on Genetic Modification, has published a Compendium of Guidance, Part 5, Genetic modification of animals (HSE, 2014). This document contains regulations concerning the risk assessment (environment and human health issues) and containment and control measures required to work with LM animals. It contains sections which discuss:

- Risk assessment for the environment;
- Mechanisms by which the LMO might pose a hazard to the environment;
- Capacity to survive, establish and disseminate;
- Hazards associated with the inserted gene/element;
- Transfer of harmful sequences between organisms;
- Phenotypic and genetic stability;
- Likelihood that the LMO will be a risk to the environment;
- Assessment of likelihood;
- Assessment of consequence;
- Determination of risk;
- Containment measures needed to sufficiently protect against harm to the environment;
- Mechanisms by which the LMO could be a risk to human health;
- Likelihood that the LMO will be a risk to human health;
- Control measures needed to sufficiently protect human health;
- Review of procedures and control measures;
- Containment and control measures for activities involving genetically modified animals; and
- Animal containment measures.

The document indicates that higher risks might be presented by LM animals that are able to persist or become established in the environment. Particular attention should be given to:

- LM animal species likely to disturb natural ecosystems, especially derivatives of naturally-occurring species that may have a selective advantage;
- LM derivatives of non-indigenous species that are able to become established and might prey upon native species or compete for the niche they currently occupy;
- LM derivatives of non-indigenous species that might consume indigenous plant life and disrupt the ecology; and
- LM animals that express potentially harmful biologically active products, especially if they are likely to be preyed upon.

It indicates that hazard identification should give particular attention to:

- The capacity of the LM animal to survive, become established and disseminate. This includes its ability to compete with other animals and any other adverse effects on animal and plant populations;
- Hazards associated with the inserted gene/element. This will be particularly relevant if the insert encodes a toxic product and could have adverse effects due to its biological activity;
- Potential for transfer of genetic material between the LM animal and other organisms; and
- Phenotypic and genetic stability.

This guidance is relevant to LMF, which are to be maintained in confined conditions and identifies the levels of containment and isolation required. It advises on identification of hazards associated with

release in order to establish containment levels but does not give guidance on risk assessing a full environmental release.

6.3.5 USA: Risk Assessment Guidance for LM Animals

FDA regulates animals with intentionally altered genomic DNA as containing new animal drugs, since the inserted DNA is intended to affect the structure or function of the animal. This meets the legal definition of a new animal drug in USA. Other agencies become involved depending on the application and use of the animal. For example, if the LMO was being used to control pests, then EPA and the Animal and Plant Health Inspection Service of the US Department of Agriculture would become involved. The approach taken, and the methods recommended by FDA for risk assessment and post release monitoring, are described in Annex 3.

6.3.6 Convention on Biological Diversity (CBD): Guidance on the Risk Assessment of LMOs

A document on "Guidance on Risk Assessment of Living Modified Organisms and Monitoring in the Context of Risk Assessment" was developed through a process under the Cartagena Protocol on Biosafety (Secretariat, 2016). This develops a general risk assessment approach and describes the main steps of risk assessment as:

1. "An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health."
2. "An evaluation of the likelihood of adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism."
3. "An evaluation of the consequences should these adverse effects be realized."
4. "An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized."
5. "A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks."

This Guidance is more generic than some others and lays down general principles rather than specific procedures or data requirements.

6.3.7 Other Guidance on the Risk Assessment of LMF

In their report to EFSA, Cowx et al. (2010) described and discussed the risk assessment methods that have been used for assessing impacts of both intentional and incidental introductions of fish species into new environments for those species (e.g. National Science and Technology Council, 1999; Copp et al., 2005 & 2008; Herborg et al., 2007a, b). A range of methods have been used for fresh and marine species and there have been different outcomes from these risk assessments (Van Eenennaam & Olin, 2006). The methods developed for LMF by some national authorities have evolved from methods such as those described by Devlin et al. (2006), in the book edited by Kapuscinski et al. (2007) and reported by Cowx et al. (2010).

In addition, Copp et al. (2005 & 2009) describe the Fish Invasiveness Screening Kit (FISK) risk assessment method for releases of fish which has been applied to LMF by Castillo et al. (2009) and Hill et al. (2014). EcoPath and Ecosim modelling have been used to try to predict effects of releases of LM salmon with variable levels of release and over different time periods (Li et al., 2014).

The South African Department of Environmental Affairs (2012) produced "Risk analysis for contained use research and development activities with genetically modified aquatic organisms", which is a follow up to the ABRAC (1995) document on "Performance standards for safely conducting research with genetically modified fish and shellfish".

Several documents from national and international organisations have addressed scientific aspects relevant to the development of regulations for LMF (GIC, 2019). For example, the Organisation for Economic Co-operation and Development (OECD) (2017) has published a consensus document on the Atlantic salmon which describes its biology, phenology, life cycle and behavioural characteristics, and forms a useful basis for understanding the characteristics that can also be modified by transformation of fish.

6.4 Risk Assessment: Issues and Challenges

The risk assessment of LM fishes for commercialisation considers the consequences of release or escape since it is considered likely in most cases of commercial production of fish. Containment of fish is feasible when distribution is limited and they are closely managed under experimental or specialist containment conditions such as in certain types of aquaria, research laboratories or in special confinement facilities.

6.4.1 Fluorescent and colour change LMF

Aquaria fish occasionally escape due to inadequate management and human error, and fish and eggs may be disposed of into water courses (Hill et al., 2014). Thus, the risk assessment of the ornamental LMF considers consequences of release into the environment. These LMF are mostly tropical fresh water species and there is no indication that their adaptation to other environments has been changed.

Khee (2006), Cortemeglia & Beitinger (2006b) and Hill et al. (2011) assessed that the fluorescent zebrafish had reduced reproduction success and viability and was more susceptible to predation. Hill et al. (2014) reported that they applied FISK to zebrafish, black tetra, and tiger barb, which are transgenic fluorescent ornamental fish commercially available in USA. They found that the three transgenic fluorescent ornamental fishes represent a low risk of invasiveness in USA and that any risk is limited to the warmer regions of the country. No potential for hybridization with native species, little history of invasiveness elsewhere, a lack of traits associated with persistence, and small body size coupled with predation-enhancing fluorescence all indicated that the ability of these species to become established and have impacts is limited even in warm regions. Cortemeglia & Beitinger (2006a) and Cortemeglia et al. (2008) examined temperature tolerance of transgenic and wild-type zebrafish and Leggatt et al. (2018) examined the minimum temperature tolerance of 3 species of transgenic fluorescent ornamental fish as wild types, as well as four lines of green fluorescent protein transgenic and wild-type zebrafish used in research. Their results indicated that tropical transgenic fish models used in research and in the aquarium trade are not expected to persist over winter in temperate climate water systems.

Hill et al. (2017) conducted a risk screen of 34 important freshwater ornamental fish species using FISK version 2 for the United States of America. Screens resulted in categorization of 91–100% of the species as non-invasive. The low climate match of these mostly tropical species largely confines establishment to subtropical regions, primarily peninsular Florida, and to isolated thermal refuges (e.g. geothermal springs). They reported that there had been a few reports of the existence of tropical ornamental fish in the United States of America and some limited local impacts but little evidence for the occurrence of large, long-term effects. They concluded that the freshwater tropical ornamental fish trade is less risky for the United States of America than has been concluded in most previous studies. They advised that further risk assessment for management decisions might be required for regional or localized, high-risk situations such as in Florida.

Fluorescent zebrafish introduced into Mexico (Castillo et al., 2009) and Peru (Scotto, 2016) were reported to be likely to be released into environments similar to those found in the geographic origin of wild types. Scotto (2018) later reported similar concerns for the fluorescent black tetra in Peru and described problems of identifying the transformation events.

Thus, it appears that the commercialised fluorescent LMF could survive in warmer climatic zones in niches where they are not subjected to limiting levels of predation. Surviving fish could cross with any compatible wild relatives in these environments and so genes could flow into populations. However, there are no indications that fluorescence genes would confer fitness or breeding advantages and so any gene flow is likely be limited, particularly by enhanced predation levels. There have been no reports that non-transgenic forms of these species released from aquaria have established in warmer regions or have created environmental problems.
Nguyen et al. (2014) reported the transformation of a marine medaka with a mutant version of the cyan fluorescent protein amFP486, isolated from the non-bioluminescent Anthozoa species (Matz et al., 1999). This is a euryhaline species adapted to both marine and fresh water environments. Release or escape of this fish could provide the opportunity for it to move over a wider range of marine environments and different river systems, in favourable climatic environments.

The results of the risk assessments conducted in the cooler climates of North America and Europe are governed by the inability of these fluorescent LMF to survive outside aquaria. In warmer climates the FISK analysis has shown that other factors such as size, fecundity, fitness, presence of wild-type fish and predation could limit survival.

Thus, the risk assessment of fluorescent LMF is challenged by requirements for information on the survival characteristics of the LMF in different environments, which includes data on adaptation to river and/or marine environments, levels of predation and competition, presence of wild type or compatible species and comparative fitness of LM and non-LM fish in compatible environments.

6.4.2 Growth Hormone expressing LM Salmon

Domestication of Atlantic salmon was initiated in 1969 in Norway, and subsequently in other Northern European and Northwestern Atlantic regions. This domestication has resulted in genetic differences between cultured and domesticated Atlantic salmon, which exhibit lower relative fitness and spawning success compared to wild Atlantic salmon in the wild (Krueger and May, 1991; Zhang et al. 2016). In domesticated Atlantic salmon, while migration to spawning grounds is diminished, large numbers of escaped domesticated aquaculture fish are reported at wild Atlantic salmon spawning grounds (Gausen & Moen, 1991; Jensen, 2013; Glover et al., 2017), which suggests a fast-growing phenotype only reduces but does not prevent spawning migration. Interbreeding results in heritable, population-level reductions in fitness to wild populations (Lehnert et al., 2013). Escapes from Atlantic salmon net-pen aquaculture occur frequently, and the number of escapees can equal or exceed wild fish in certain areas. Genetic changes in wild populations due to these high levels of introgression from domesticated salmon have been detected in nearly all regions where salmon aquaculture and wild populations occur (Naylor et al., 2005; Cowx et al., 2010; Wringe et al. 2018). In many regions, wild salmon stocks have declined because of reduced fitness and fertility, as well as due to diseases and parasites originating from aquaculture fish. In addition, Youngson et al. (1993) showed that escaped female farmed salmon can hybridise with trout species.

The OECD has produced consensus document Number 7 describing the Atlantic salmon. In this it is stated: *Despite the extensive current and growing body of knowledge on Atlantic salmon, there is still insufficient information to adequately describe the critical or limiting environmental conditions controlling the survival and distribution of this species. In addition, the underlying genetics that allow for phenotypic*

adaptations to those limiting environmental conditions has not been adequately characterised. Thus, establishing baselines and meaningful comparisons may be difficult in salmon so that assessing the impacts of genetic modifications, which can alter behaviour as well as phenotype, may be problematic, resulting in uncertainty (see also: Verspoor et al., 2005; Houston & Macqueen, 2019).

It is against this background that the development of AquAdvantage salmon has to be considered, since the release of LM salmon could allow transgenes to be taken up and dispersed into wild populations of both trout and salmon, if adequate measures are not taken to prevent this. The methods for inducing polyploidy (Devlin et al., 2010; Zhou & Gui, 2017) in embryos can result in a small percentage remaining as diploid fertile fish expressing growth hormone (GH salmon), so that escaping salmon could contain a low percentage of fertile individuals. Oke et al. (2013) demonstrated that this fertility will also include hybridisation with brown trout and introgression of transgenes into trout populations. The GH salmon have enhanced growth rates and improved feed conversion efficiency (Devlin et al., 2015). In addition, GH transgenesis in fish is reported to produce a range of unintended (pleiotrophic) effects, including altered foraging behaviour, life-history timing, gene expression levels and disease resistance (Devlin et al., 2015), which may alter susceptibility to predation (Abrahams & Sutterlin,1999) and general ability to survive (e.g. due to effects on disease resistance) (Moreau, 2011; Moreau et al., 2011a & 2011b). When considering potential environmental risks associated with use of GH salmon, the effects from both the targeted growth enhancement and the other changes in the phenotype due to the hormonal and incidental (pleiotrophic) effects need to be considered.

The environmental effects of the GH salmon if released/escaped include direct competition and predation effects on wild salmon populations and also effects on the various receiving environments they can inhabit. Salmon are migratory fish completing the early stages of their life cycles in rivers, entering oceans, growing while at sea to sexual maturity and returning to rivers to spawn. Salmon are predators feeding on a wide range of organisms in their different environments.

For AAS to cause harm to wild populations of Atlantic salmon through hybridisation, a “pathway to harm” must occur (Devos et al., 2019): release of AAS from land-based facilities; survival in nature; migration to wild spawning grounds; and successful reproduction with wild fish and negative impacts on wild populations as a result of hybridisation and introgression of genes. If the biology of AAS or other factors prevent or partially influence any one of these steps from occurring, this would affect potential harm occurring to wild populations through hybridisation. For example, studies in semi–natural arenas with Coho salmon suggest that cultured GH salmon are reproductively out-competed by wild-reared salmon (Bessey et al., 2004; Fitzpatrick et al., 2010). Moreau and Fleming (2011) and Moreau et al. (2014) showed that GH Atlantic salmon had decreased relative competitiveness, survival and fitness characteristics compared to wild-type fish. However**,** Devos et al. (2019) reported that the challenge to risk assessment is to determine whether survival, migration, spawning, hybridisation and introgression will occur under natural conditions and in different environments. Unfortunately, this is not feasible to test under experimental conditions as it is not practicable to simulate the range of natural and environmental conditions that influence these factors such as disease incidence, food type and availability, predation, development size at escape, receiving environments, habitat complexity, etc. (Sundstrom et al., 2007; Leggatt et al., 2017; Vandersteen et al., 2019). Environmental impacts of the presence of the GH transgene have been found to be context-specific, where LM fish may have lesser, equal or greater survival than non-LM siblings dependent on numerous factors (Vandersteen et al., 2019). Consequently, the impacts of hybridisation on wild populations may vary depending on the environmental conditions present. Regarding the impacts of introgression of the transgene into wild salmon populations, computer modelling simulations found presence of the transgene could potentially shift genetic backgrounds and phenotypes of both LM and non-LM individuals away from the naturally selected optima (Ahrens & Devlin, 2011). Quantitative trait loci mapping demonstrated that the presence of the GH transgene altered the genetic basis of growth-related traits, which may indicate the potential for the transgene to influence evolutionary changes in salmon, with potential ecological consequences (Kodama et al., 2018). Overall,

assuming diploid fertile AAS escape aquaculture facilities, there is no step in the examined pathway that would completely prevent harm to wild salmon populations through hybridisation. However it is important to note that the likelihood of harm from releases of LMF is very context specific. Studies in laboratory and varying semi-natural conditions demonstrate that the pathway to harm may be influenced by numerous factors including time or life stage of escape and biological conditions present in the natural environment. Consequently, there is significant uncertainty in final predictions of harm to wild populations from AAS. These studies have also demonstrated major difficulties associated with using data solely from culture conditions to predict environmental risk. Genotype-by-environment interactions have been observed for most phenotypes examined, where wild and LMF respond to different environments in different ways (Devlin et al., 2015). GH and non-LM salmon have been shown to respond to cultured and simulated-natural conditions very differently (Sundstrom et al., 2007). These strong genotype-by-environment interactions mean that using data only from experimental studies are not adequate for supporting predictions of environmental impacts. In their recent study, Vandersteen et al. (2019) conclude that there is "*complexity of integrating these factors to allow accurate prediction of fitness and consequences of novel genotypes within the many complex conditions found in nature. These data continue to highlight the difficulty in using laboratory-based experiments with limited ability to fully replicate nature to accurately predict risk in the wild."*

Thus, it can be seen that the risk assessment of AAS presents many challenges. Risks were reduced by creating AAS as triploid sterile females for biocontainment of the transgene and by restricting its production to facilities with physical containment to prevent escape into the environment (ZKBS, 2011). However, the methods for inducing polyploidy in embryos can result in a small percentage remaining as diploid, fertile GH fish. In addition, the likelihood of escapes may increase in future as production levels increase. Thus, some have argued that a full environmental impact statement (EIS) is required as the environmental risk assessments by AquaBounty and the regulatory authorities did not adequately consider that escapes of fertile AAS might occur, and that they might survive, reproduce and move to a range of receiving environments (Smith et al., 2010; FDA, 2011, Kapuscinski and Sundstrom[4]).

6.4.3 Capacity for Risk Assessment

In the online forum of 2018 and the 2019 submission of information (https://bch.cbd.int/onlineconferences/submissions.shtml ; https://www.cbd.int/doc/c/43ce/abd6/32052119cec99bb1891af127/sbstta-22-inf-12-en.pdf) and in the responses to the information gathering exercise (see Sections 7 and 8) most responders stated that they had not performed risk assessments of LMF and the authorities that had conducted risk assessments stated that these risk assessments were for LMF for contained use conditions and not for release. In addition to this lack of experience, many responders considered that they lacked the relevant expertise to perform a risk assessment of LMF, particularly concerning the need to understand the biology and phenology of fish, as well as their interactions with marine and aquatic organisms and environments. Some responders questioned whether there was guidance available for assessing LMF or considered that the available guidance was not adequate for assessing LMF. They requested that additional guidance should be provided. However, responders that had assessed LMF or had access to suitable guidance, considered that authorities who lacked experience or expertise should be provided with access to the relevant guidances. In addition, they should be provided with training and other assistance from experienced authorities or organisations involved in capacity-building.

6.5 Risk Management

[4] https://envs.dartmouth.edu/sites/en vs.dartmouth.edu/files/comments.pdf

National authorities will often place conditions on the release of LMOs in order to minimise exposure or to reduce the likelihood of the potential adverse impacts identified in the risk assessment. In addition, post-market or post-release monitoring for environmental impacts may be required. In the case of AAS, FDA and Panama required a number of containment conditions and environmental monitoring by the applicant. For instance, in order for Aqua Bounty Panama to conduct studies and produce AAS in Panama, certain conditions were made by the authorities. These conditions included that there should be an environmental management plan based on the environmental impact plan containing, *inter alia*, the following requirements:

- Permits for carrying out the activities relating to the project including the import of AAS eggs and fry from Canada, water use and waste disposal (solid and liquid);
- Payment for ecological damage;
- Environmental assessment of the grow-on facility prior to the project;
- Environmental management plan in relation to prevention, mitigation, control, compensation and compliance of environmental requirements for the facility;
- Notification of any anticipated changes to the project;
- Compliance with the Health Ministry Code and animal health requirements; and
- Cleanliness and disinfection plan.

6.5.1 Post-Release Monitoring

Some regulatory and risk assessment authorities, such as FDA and EU/EFSA, routinely require post-release monitoring (see Annexes 3 & 5) and in addition, some request monitoring on a case-by-case basis depending on the outcomes of the risk assessment and the conditions set for release.

In the case of AAS, both FDA and the MIAMBIENTE Panama (Panamanian Ministry of Environment) required monitoring for escaped fish and for reporting any events that might lead to unintended release as well as for any adverse environmental or health effects associated with the fish production facility. The applicant had to prepare and submit a monitoring plan detailing the data recorded and reporting intervals.

In the responses to the survey, some responders indicated their lack of experience and expertise to conduct monitoring and that additional guidance and training were required.

6.5.2 Biocontainment of AquAdvantage Salmon: Female and Sterile AAS

AquAdvantage Salmon are produced as triploid sterile females to provide biocontainment of the transgene. All-female AAS are produced from female salmon and irradiated sperm of Arctic char by a process known as gynogenesis. Irradiated sperm are introduced to eggs, followed by a pressure treatment to result in diploid offspring containing two maternal sets of chromsomes. Arctic char milt is used which has been irradiated so that no Arctic char DNA is present in the gynogen population. If the milt irradiation is not successful, the offspring are Arctic char x Atlantic salmon hybrids which are readily identifiable by their markings and phenotypic appearance and removed.

The remaining all-female population are subjected to "masculinization" using 17-methyltestosterone. The females become "neomales" (genetically female fish that produce milt instead of eggs). Upon sexual maturity, the neomales are bred with non-LM Atlantic salmon females. Then, fertilized eggs are subjected to pressure shock treatment, which involves treating the egg in meiosis Ⅱ with hydrostatic pressure, to prevent extrusion of the second polar body so that triploid embryos (Zhu & Cui, 2017) with two sets of chromosomes from the non-LM female salmon and one set of chromosomes from the neomale LM salmon are produced. The ploidy of these embryos is determined by flow cytometric analysis of the DNA content of erythrocytes. Samples of embryos are tested from each batch and any batch showing more than 5% diploids is destroyed. These female triploids are the commercialized AquAdvantage salmon.

6.5.3 Physical Containment of AAS

The AquaBounty facilities for AAS production are designed to prevent escape of fish with multiple redundant systems of tanks, cages, filters and waste water filtration and treatment. The systems are continually monitored either with closed-circuit television or by human presence for any spillage, leakage or other malfunction of the system. Casimiro et al. (2018) reported that extreme weather events and flooding are hazards to inland fish farms and can lead to escapes. They comment that biosafety requirements of these facilities should allow for these events in their design and management.

Monitoring is linked to these risk management measures and includes monitoring levels of triploid female fish production and any leakage, spillage or escape from the grow-out facility. Recapture and/or monitoring of escaped fish may be required if there is a failure in the production system or to determine levels of undetected or unreported escapes. This is problematic as it requires both efficient systems for sampling environments receiving escaped fish and methods for their identification. Senenan et al. (2007) discuss risk management of LMF including methods of monitoring and remediation in case of environmental release and harm. Chittenden et al. (2011) describe methods for the recapture of escaped fish and various methods of identification including visual, image analysis and molecular testing have been examined (Rhebein and Heller, 2003; Hamels et al., 2009; Sundstrom et al., 2015).

7. Gathering information from national biosafety authorities and institutions and stakeholders

The questionnaire was sent out as described in section 4.2 to 74 national biosafety authorities and institutions, 6 inter-governmental organisations (IO), 12 civil society organisations (CSO) and 2 industry organizations.

7.1 Responses to the Survey

Written responses were received from a total of 23 national authorities and biosafety institutions from Africa (3), Asia and the Pacific (AP; 3), Central and Eastern Europe (CEE; 2), Latin American and the Caribbean (GRULAC; 3), and Western European and Others Group (WEOG; 12). In addition, there were responses from intergovernmental organisations (IO: 2), one industry organisation and one civil society organisation (CSO). The responses to the questions and an analysis of the responses are described in Annex 6.

7.2 Discussion of the Survey Results

Several respondents from national biosafety authorities and institutions had reported or observed transboundary movements. In two cases, these relate to countries that had approved LMF movements and there were reports of licensed introductions of LMF for confined uses in research facilities. However, there were also reports of unapproved or illegal introductions of ornamental LMF and some national biosafety authorities and institutions had intervened to prevent incursions. The majority of responders had not reported or were not aware of any reports of transboundary movements.

The majority of responders consider that some LMF, depending on species, phenotype and conditions of production could have the potential to cause adverse environmental effects. They commented that LMF have been commercialised in a few countries and their use is anticipated in others. In addition, LMF are being used in biological and bio-medical research in contained laboratory conditions in several countries.

Approximately half the responders considered that LMF would be produced only in contained facilities and thus not deliberately released. However, many thought that fish could escape so that there would be unintentional releases. They commented that LM ornamental fish are available in some countries without regulatory approval, that ornamental fish are often released into ponds or waterways and that farmed and

hatchery fish also often escape. The other half of the responders thought that LMF were likely to be deliberately released in the future in some areas and that this could result in releases into areas where approvals had not been given.

The majority of respondents from national biosafety authorities and institutions and other responders considered that LMF fall within the scope and objectives of the Cartagena Protocol and no respondents from national biosafety authorities and institutions considered that they did not.

Capacity and resources to risk assess and manage LMF varied with region and country. Some national biosafety authorities and institutions that had given approval for commercialisation of LMF or use of LMF in contained facilities had appropriate capacity and resources. However, some of these national biosafety authorities and institutions indicated that they were only assessing LMF for contained/confined use and for determining the levels of isolation/confinement required. Responders from other regions said they had little or no capacity for risk assessing LM animals. Thus, several authorities considered said that their experience with LMF was nil or limited and that panels containing experts on fish related topics would need to be established.

There was general recognition that guidance documents on risk assessment relevant to LM animals were available but that some of these documents were not specific enough for LMF risk assessment or only applicable to contained use of LMF. The IOs and the industry organization indicated that existing guidances from countries with relevant experience (e.g. Canada, EU/EFSA and USA/FDA) should be made more widely available to competent national authorities, especially those in countries with less developed regulatory systems for LMOs. There was also general agreement from IOs, the the industry organization and national biosafety authorities and institutions with access to appropriate LMF guidance (e.g. EFSA, FDA, Environment and Health Canada) that it was in line with Cartagena Protocol on Biosafety. Generally, respondents from national biosafety authorities and institutions from countries with less experience of risk assessment of LMOs considered the need for the development of guidance to be a priority. International organisations and respondents from national biosafety authorities and institutions from AP and WEOG countries with more mature risk assessment and regulatory systems and with experience in assessing LMOs considered that sufficient guidance is available and that it should be harmonised and made more widely available to regions with limited experience with risk assessments. There was a general view that there needed to be international agreement on the guidance and data requirements for the risk assessment of LMF.

Some respondents from national biosafety authorities and institutions in AP, CEE and WEOG reported that they had performed risk assessments on LMF. National biosafety authorities and institutions in 2 WEOG countries had conducted and published risk assessments of AAS and ornamental LMF. The other AP, CEE and WEOG responders had conducted risk assessments of LMF for contained use only or because of the potential for illegal imports.

Most responders considered that the risk assessment of LMF was either constrained or presented challenges. The constraints related to lack of capacity and competence, while the challenges were confronting the complexities of aquatic and marine organisms and their environments in order to assess impacts of LMF. Several responders from different regions considered that LMF pose challenges to existing risk assessment frameworks because of the complexities of assessing the release of LMF. However, 2 respondents from national biosafety authorities and institutions in WEOG considered that these challenges could be overcome by using quarantine systems to prevent or restrict entry of LMF into countries instead of LMO regulations. The the industry organization, an IO and two respondents from national biosafety authorities and institutions in WEOG that had assessed the risks of both AAS and LM ornamental fish considered that current risk assessment guidance, frameworks and methods are adequate for LMF.

8. Application of Annex I of decision CP-9/13 to living modified fish

This analysis is aimed at informing the application of Annex I of decision CP-9/13 by considering information from the sources refered to in the methodology of this study. The analysis draws on the information presented in the preceding sections to inform the application of Annex I of decision CP-9/13 to the topic of living modified fish.

8.1 Structured analysis against the criteria in Annex I of decision CP-9/13

a. They are identified by Parties as priorities, taking into account the challenges to risk assessment, particularly for developing country Parties and countries with economies in transition

The submissions of information in response to SCBD notification SCBD/SPS/DC/MPM/MW/86376, as well as responses to the survey, have shown that LMF has been identified as a priority for the development of further guidance by some Parties. Some of the phenotypic changes made to LMF and the associated pleiotrophic and behavioural effects described in this report indicate that LMF could have different environmental impacts than non-LM domesticated and wild-type fish. Most responders to the questionnaire and the online forum considered that risk assessments are a priority for determining these environmental impacts. The challenges presented by the complexities of assessing the interactions of LMF with their wild or domesticated comparators and with different components of the environments in which they could move and survive were pointed out by various sources. In addition, the lack of relevant experience and expertise to assess LMF was indicated by some countries as a challenge.

b. They fall within the scope and objective of the Cartagena Protocol on Biosafety

Most respondents to the survey considered that LMF are within the scope and objective of the Cartagena Protocol and there were no contrary statements. As described in numerous scientific papers cited in this report, LMF generally contain DNA transferred from other species of fish or organisms, which is incorporated into the genomes of the fish and stably inherited creating new genetic lines that are genetically distinct from wild-type fish and other domesticated forms. They thus fall within the definition of an LMO and are within the scope of the Cartagena Protocol.

c. They pose challenges to existing risk assessment frameworks, guidance and methodologies, for example, if the issue at hand has been assessed with existing risk assessment frameworks but poses specific technical or methodological challenges that require further attention

In responses to the survey and in comments to the 2018 online forum, some countries with relatively little experience of risk assessments of LMOs felt that either the available guidance was too general or not adequate for LMF. However, others indicated the range of materials (i.e risk assessment books, publications and guidances) that have been produced on LM animals and LMF were sufficient and should be made more accessible to authorities with little risk assessment experience or expertise. Examples of risk assessment guidance produced by some countries and organisations are described in this report and annexes. Other sources of potential importance for risk assessment have been mentioned in the report of the 2018 risk assessment online forum (i.e. IPPC, OECD, FAO, WHO, OGTR, among others)[5]. Three national biosafety authorities and institutions have assessed the risks of and approved AAS for use under

[5] https://www.cbd.int/doc/c/43ce/abd6/32052119cec99bb1891af127/sbstta-22-inf-12-en.pdf

contained conditions, two have permitted release of ornamental LMF and several have permitted contained use of LMF such a zebrafish, carp and trout in their countries for research purposes.

This report has shown that LMF present challenges to the risk assessment process because of their environmental sensitivity, phenotypic variability and genetic diversity. All stages of fish life cycles, fish development, their sex, their behaviour in terms of competiveness, reproduction and survival are environmentally sensitive. The OECD description of Atlantic salmon comments that knowledge of the complex life cycle, the interactions with different species and environments and environmental factors which influence fish reproduction, development and behaviour is very limited. Thus, understanding the complexities of fish in order to establish comparator base lines for risk assessment purposes is problematic. Some genetic modifications can result in pleiotrophic effects, which are also environmentally sensitive, and fish wild types are genetically very diverse with adaptations to certain environments. These interacting factors are difficult to test under controlled experimental conditions. Several responders also commented on these aspects of risk assessment and agreed with the scientific literature indicating that predicting the environmental impacts of releases of LMF into different potential receiving environments with different interacting biota is problematic.

(d) The challenges in addressing the specific issue are clearly described

Addressing the environmental impacts and consequences of releases of LMF present the challenges mentioned in (c). In addition, some of the challanges that are clearly described relate to the complexities of fish biology and behaviour and it has also been shown in this report that some transformations of fish can result in pleiotrophic and secondary effects, which can have pronounced effects on the the phenology and behaviour of fish. Often these unintended or incidental effects are also environmentally sensitive so that testing them and determining their environmental consequences presents additional challenges. In the example case of Atlantic salmon, OECD has shown that establishing base lines for wild-type and cultivated salmon is challenging. Atlantic salmon is a predatory, migratory fish transitioning between fresh and salt water environments with a complex life cycle and extraordinary migratory and homing characterisitics, which we do not fully understand. Therefore, predicting impacts of changing the hormone expression levels in GH Atlantic salmon is indeed challenging.

(e) The specific issues concerning living modified organisms that:

(i) Have the potential to cause adverse effects on biodiversity, in particular those that are serious or irreversible, taking into account the urgent need to protect specific aspects of biodiversity, such as an endemic/rare species or a unique habitat or ecosystem, taking into account risks to human health and the value of biological diversity to indigenous peoples and local communities;

(ii) May be introduced into the environment either deliberately or accidentally;

(iii) Have the potential to disseminate across national borders;

(iv) Are already, or are likely to be, commercialized or in use somewhere in the world;

(i) Most responses considered that, case-by-case, and dependant on the species, traits and conditions of release, there could be adverse effects from released LMF. The literature in the report and some of the respondents indicated that experiences with fish farming and introductions of novel types had produced mixed outcomes and environmental impacts, including levels of fish diseases. These have had impacts on fisheries, fish stocks and on the economics of fishing in some areas. It is therefore expected that the production and releases of some types LM fish could have a similar range of outcomes. The industry organisation respondent to the questionnaire commented that inland contained aquaculture facilities would have less environmental impact than aquaculture systems located in waterways or marine environments. However, the LM salmon development and production is at an early stage and so there is

no information on the biodiversity or socio-economic impacts of the current and intended levels of commercialisation.

Concerns were raised in countries with favourable climates and environments that there was no data on whether released fluorescent LMF could establish.

Therefore it was not possible to identify relevant information regarding indigenous peoples and local communities in relation to LMF. Because of this lack of information, it is not possible to comment on impacts of LMF on biodiversity and consequences for indigenous peoples and local communities.

(ii) The ornamental LMF permitted for aquaria use in USA and Canada are considered to have the same characteristics as non-LM conspecifics and thus, no additional environmental impacts when released. In addition, there are several reports indicating that LM ornamental fish are being released into the environment through human activities in other regions than North America. There are no reports of AAS releases into the environment and the current approvals are for confined use only. Currently, there are no indications that LM salmon or other commercial fish species are being developed for environmental release. Several responders considered that, with scaling up of commercial production, certain confined LMF would be more likely to escape their containment conditions.

(iii) Both AAS and LM ornamental fish are crossing borders under licensed conditions, the latter sometimes for research purposes. In addition, there are several reports in the literature and in the responses to the questionnaire showing that there is unlicensed and unapproved transboundary movement of LM ornamental fish. There was a general consensus from questionnaire responders that some LMF released into the environment can cross national borders, depending on the species, trait and areas of release. This is supported by the literature on the behaviour of domesticated fish.

(iv) This report and information from several countries have shown that several species of LM ornamental fish and an LM Atlantic salmon (AAS) have been commercialised. AAS is only permitted in 3 countries under contained conditions, while LM ornamental fish are permitted for release in Canada and USA. Several countries have permitted LMF in confined aquaria conditions for ornamental uses or for research purposes.

8.2 Stocktaking of resources on similar issues

The final part of Annex I of decision CP-9/13 provides that the process for recommending specific issues of risk assessment for consideration by the COP-MOP should consider a stock-taking exercise to determine if resources on similar issues have been developed by national, regional and international bodies and, if so, whether such resources may be revised or adapted to the objective of the Cartagena Protocol, as appropriate.

Section 6 of this report describes the risk assessments performed on LMF by Canada and USA and the guidance documents relating to risk assessment of LM animals available in those two countries. In addition, it discusses the guidance documents that are available such as those produced by EFSA, ILSI, OECD and CBD. There are also guidance documents available in many countries for risk assessment of LM animals for contained use and the UK risk assessment system is presented as an example (Section 6.4.4). Responses to the survey and information from the 2018 online forum show that several countries have experience with risk assessment of LMOs and have developed risk assessment systems, which allow introduction or prohibition of LMOs in their territories. In most cases, they considered that these risk assessment systems are applicable to LMF.

As indicated in Section 7, the levels of experience in different countries with risk assessment of LMOs varies. Many respondents indicated that their countries had no experience and lacked capacity for the risk assessment of LMF, including access to guidance and scientific expertise. Other respondents from national biosafety authorities and institutions and an international organisation indicated that guidance is available and that they have sufficient expertise. They considered that this guidance and expertise should be shared with other countries in order to improve the overall capacity of all Parties. There was no indication from these responders that revision or adaptation of the guidances was required to meet the objectives of the Cartagena protocol, though the guidances should be prepared in a range of languages to make them more accessible.

Annex 1. Survey Questionnaire

This document, containing a questionnaire, was sent to 74 national biosafety authorities and institutions, 6 inter-governmental organisations (IO), 12 civil society organisations (CSO) and 2 industry organizations.

J. T. Environmental Consultants, Cambridge, UK.

STUDY ON RISK ASSESSMENT: APPLICATION OF ANNEX I OF DECISION CP 9/13 TO LIVING MODIFIED FISH

In decision CP 9/13, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol decided to establish a process for the identification and prioritization of specific issues regarding risk assessment of living modified organisms with a view to developing further guidance on risk assessment on the specific issues identified, taking into account Annex I which contained a list of criteria. It also decided to consider at its next meeting, whether additional guidance materials on risk assessment are needed for living modified fish.

In this context, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol requested the Executive Secretary to commission a study informing the application of Annex I of the decision to living modified fish, to facilitate the process referred to in paragraph 6 of that decision.

Gathering information from biosafety national competent authorities and stakeholders, in relation to the Criteria in Annex 1

Annex 1 describes the process for identifying specific issues of risk assessment that are priorities. This process for recommending specific issues of risk assessment should include a structured analysis to evaluate whether the specific issues fulfil a series of criteria.

The Questionnaire

A range of countries and other stakeholders are being contacted by JT Environmental Consultants (JTEC) and asked a series of questions relating to each of these criteria in relation to living modified fish (LMF). The questions also ask for information about the development of LMF in their country or regions.

The Questionnaire is provided in English, French, Spanish and Russian. Recipients of questionnaires can also submit responses orally in English. If you wish to make an oral response, please email jtenvironmentalconsultants@gmail.com. Responses will be presented in tabular form by each question to facilitate examination by the Expert Group and by the Parties to the Protocol. Individual responses and comments will not be attributed to individuals or organisations in the subsequent report submitted by JT Environmental Consultants to CBD.

If you have questions or concerns about this survey please address them to Jeremy Sweet at JTEC (jtenvironmentalconsultants@gmail.com).

Questionnaire

Please answer the following questions as completely as you can

1. Name:
2. Address:
3. Organisation:
4. Email:
5. Telephone (including country code):
6. Position and role in organisation:
7. Are you/your organisation involved in risk assessment or regulation of Living Modified organisms (LMOs)?
8. Are you/your organisation involved in LMO research and development?
9. Are you/your organisation involved in production or testing of LMOs?
10. Do you consider that Living Modified Fish (LMF) have the potential to cause adverse effects biodiversity, in particular those that are serious or irreversible, taking into account the urge need to protect specific aspects of biodiversity, such as an endemic/rare species or uniq habitats or ecosystems, taking into account risks to human health and the value of biologic diversity to indigenous peoples and local communities?
11. Do you think that LMF are, or are likely to be, released into the environment deliberately accidentally?
12. Do you think LMF have the potential to disseminate across national borders?
13. Do you consider that LMF fall within the scope and objectives of the Cartagena Protocol?
14. Are LMF being developed and are they likely to be commercialized or in use in your country region?
15. What capacity and resources do you have in your country to risk assess and manage LMF?

16. What guidance documents on risk assessment, particularly for LM fish, do you have access or consult? Are these guidance documents in line with the objectives of the Cartage Protocol? If not, can these resources be revised or adapted to be in line with the objectives the Protocol?

17. Do you consider the development of guidance for the risk assessment of LMF to be a priority?

18. What do you consider to be the main challenges and constraints in risk assessing LMF?

19. From your experience, do LMF pose challenges to existing risk assessment framework guidance and methodologies? Have you experienced specific technical or methodologic challenges that require further attention?

20. Has your national authority reported trans-border movement of LMF? Please describe a reports.

21. Has your national authority risk assessed LMF for experimental use and/or release? If s please reference published reports or describe the event and risk assessment outcomes.

22. Are there research and development programmes on LM fish in your country or organisation? so, please describe them.

23. Do you wish to make any other comments or observations on LM Fish?

Please email your responses to jtenvironmentalconsultants@gmail.com by 19 November 2019

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Annex 3. USA: Risk Assessment, Monitoring and Regulation of LM Animals (including LMF)

The Federal Drug Agency (FDA) regulates animals with intentionally altered genomic DNA as containing new animal drugs, since the inserted DNA is intended to affect the structure or function of the animal. This meets the legal definition of a new animal drug in USA. Other agencies become involved depending on the application and use of the animal. For example, if it was being used to control pests then the Environmental Protection Agency (EPA) and the Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA APHIS) would become involved.

In 2015 FDA produced a Guidance to Industry (FDA 2015b) which describes the non-binding recommendations for applications to market genetically engineered animals in USA. In relation to environmental impacts, it recommends that an Environmental Assessment (EA) (Office of new animal drug evaluation reviewer's chapter, 2010) or an Environmental Impact Statement (EIS) are prepared, unless it can be shown that the animal will be "categorically excluded" from the environment.

Environmental Assessments (EA) are conducted to identify any environmental effects that might occur and an EIS is produced to describe the impacts of the identified effects. FDA will examine the EA and/or EIS and "*FDA will examine the potential for environmental impacts, including the potential for inadvertent release or escape of the GE animal and/or its products into the environment, and whether certain measures may mitigate any potential significant impacts that would adversely affect the human environment. Additionally, sponsors may be subject to applicable environmental requirements with respect to runoff from animal production facilities and land receiving animal waste under the Clean Water Act. 33 U.S.C. 1251 et. seq. and other statutes."* (FDA, 2015).

FDA (2015) recommend that applicants ("sponsors") contact them to discuss any environmental issues and whether management or mitigation measures are required both at the experimental stage of development and for subsequent commercial production. The 2015 Guidance to Industry is being revised and a new Draft Guidance to Industry (not for implementation) (FDA 2017) has been published for consultation. The recommendations for EA and EIS remain the same.

In relation to LMF FDA is mainly concerned about the following environmental issues:

- Is there anything about the LMF itself that poses a human, animal, or environmental risk. For example, does the altered genomic DNA contain sequences that can cause human or animal disease either intrinsically or by recombination?

- For environmental releases, does the LMF with intentionally altered genomic DNA pose any more of an environmental risk than its counterpart?
- Are there any concerns about the disposition of animals with intentionally altered genomic DNA that could pose human, animal, or environmental risks?
- Are there any other safety questions that have not been adequately addressed by the sponsor?

There are certain exceptions to the requirements. For example, the FDA has not and does not intend to enforce investigational new animal drug (INAD) and new animal drug application (NADA) requirements for animals of non-food-producing species whose genomes have been intentionally altered that are raised and used in contained and controlled conditions such as laboratory animals with intentionally altered genomes used in research institutions. However, the FDA retain the discretion to take enforcement action if they learn of safety concerns and evaluate certain risk factors, and they may exercise enforcement discretion over INAD and NADA requirements for additional kinds or uses of non-food-producing species of such animals, as they did after reviewing information about zebrafish genetically engineered to fluoresce in the dark (GloFish) (FDA 2003 and Int'l Ctr. for Tech. Assessment v. Thompson, 421 F. Supp. 2d 1 (D.D.C. 2006)). The FDA stated:

"Because tropical aquarium fish are not used for food purposes, they pose no threat to the food supply. There is no evidence that these genetically engineered zebra danio fish pose any more threat to the environment than their unmodified counterparts which have long been widely sold in the United States. In the absence of a clear risk to the public health, the FDA finds no reason to regulate these particular fish"(FDA, 2003).

Post-Release Monitoring of LM Animals: Post-approval monitoring requirements and arrangements are similar to those required for conventional animal drugs in USA. Developers are required to register and provide a list of all LMF that they have produced and keep records of any additional information they develop related to the safety of the LMF and the claim on which the approval was based. The FDA recommends that applicants work closely with them to be clear on the post-approval requirements and recommendations.

FDA guidance also recommends the development of a "durability plan" by applicants and approval of that plan by FDA. Applicants should develop a plan for assessing the genetic and phenotypic generational stability by monitoring the genotype and phenotype of the LM animal over time to assess whether the LM animal remains equivalent to the LM animals that were initially approved. Applicants are solely responsible and accountable for this monitoring.

Annex 4. Canada: Risk Assessment Guidance

Environmental risk assessment guidance is provided by Environment Canada (2010) and informs that: Applications to manufacture, import, or sell to Canada any animal derived through biotechnology, are required to provide technical documentation relating to the animal's health to support their application. The Canadian Food Inspection Agency (CFIA) analyzes the documentation and helps to evaluate the submissions. When the application is for a LM fish, Fish and Oceans Canada (DFO) will participate in the assessment and provide advice.

The New Substances Notification Regulations (Organisms) [NSNR (Organisms)] implement Part 6 Animate Products of Biotechnology of CEPA 1999 (sections 104-115) and prescribes the information as well as the time lines for the notification to Environment Canada of the manufacture or import of living organisms that are animate products of biotechnology.

Living organisms are first categorized by generic class, and then by factors such as conditions or circumstances of introduction. This system of notification groups allows the government to match

information requirements with anticipated concerns about the characteristics of specific notification group of living organisms and to ensure appropriate assessment of potential environmental and human health risks.

Living modified organisms can be exempt from notification under the NSNR (Organisms) and the following exemption criteria can apply:

1. The LMO is a research and development substance; and
2. There is no release from the facility to the environment of the LMO, the genetic material of the organism or material from the organism involved in toxicity.
3. The Information requested for an ERA is listed in the Notification Form attached to the Guidance document (Environment Canada (2010). The main ERA requirements are as follows:
4. Estimated quantities of the organism in the environment and the estimated population trends.
5. Description of habitats where the organism may persist or proliferate.
6. Identification of other species that are likely to be exposed to the organism and other species that are likely to be affected.
7. Information in respect of the ecological effects of the organism.
8. Data from a test conducted to determine the pathogenicity, toxicity or invasiveness of the organism. Notifiers are encouraged to contact Environment Canada to determine whether microcosm or mesocosm tests are appropriate for obtaining notification data. Data from an appropriately designed microcosm or mesocosm test may be considered on a case-by-case basis, if the test could provide meaningful effects data. However, considerable understanding of the characteristics of the organism and its intended use may be required before a microcosm or mesocosm test system can be properly designed. The duration of the test should be based upon whether there is a suspicion of adverse effects. For cases of suspected invasiveness, the duration of the test should permit time for colonization and manifestation of effects in the test system.
9. Controls: Negative controls should be identical in every respect to the treated test organisms except for exposure to the notified organism or treatment. If possible, notifiers should establish positive controls with relevant closely related organisms to ensure that the test system is capable of detecting an adverse effect.
10. Reporting: Notifiers should detail all information for a complete and accurate description of the test procedures, and all data, information, and analysis necessary for Environment Canada to reach an independent conclusion. This should include a justification for choosing a particular test species and test method and a statistical analysis of differences between the test group and control groups.
11. Adverse effects: Where adverse effects are found, additional testing over a range of concentrations or doses should be considered in order to establish an effect threshold.
12. Ecological effects of organism residues: Known information on whether the residues of the organism can have an ecological effect, such as allelopathy, on other organisms should be provided.
13. Potential of the organism to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity. A summary of predicted ecological effects should be provided, including any effects on biodiversity. This should include a description of the expected beneficial or adverse ecological effects that result from the growth of the organism, as well as any other potential ecological effects likely to occur from its introduction.
14. Potential for the organism to have adverse human health effects, including most likely routes of human exposure to the LMO and an estimate of human exposure to the introduced organism including disclosure of the number of persons potentially exposed in growing, handling, using or disposing of the organism or parts of it and the number of persons potentially exposed in the general population.
15. Environment Canada and Health Canada evaluators will assess the notification package to determine levels of toxicity and potential environmental harm. In addition, the agencies involved

in the risk assessment conduct an uncertainty analysis of the data and outcomes of the RA and publish this.

Assessment for “toxicity “: determines whether an organism is, or is suspected of being toxic or capable of becoming toxic involves assessing the potential for exposure to humans and components of the environment, and the potential for adverse effects of the organism on humans, the environment or biological diversity (including other living organisms, interacting natural systems, and the abiotic components of the environment). An organism may be suspected of being toxic or capable of becoming toxic if there is concern about either the adverse effects of the organism or the potential exposure to the organism. For example, organisms with considerable potential for exposure because of continuous release in high quantities, or persistence in the environment, may be suspected of being toxic although there may be uncertainty regarding a biological or environmental hazard from the information available for the assessment.

When an assessment has led to a "suspicion of CEPA toxic”, or suspicion that a significant new activity (SNAc) in relation to the living organisms may result in the living organism becoming toxic, the government has authority to impose terms of use or control measures to which a manufacturer, importer or user of the organism must adhere. These terms of use and control measures may be applied to minimize any risk to human health, the environment or biological diversity. The government must take action and make measures under section 109 of CEPA 1999 before the assessment period expires. The notifier must comply with these measures.
Unlike in USA there is no requirement for post approval monitoring but there is a requirement that any new information concerning the safety or environmental impact should be provided to Environment Canada or Health Canada.

Annex 5. European Union: Guidance on Environmental RA of LM animals or fish

EFSA (European Food Safety Authority) conducts risk assessments of LMO applications for commercialisation in the European Union on behalf of the European Commission who are the regulators. Applications for experimental and contained use of LMOs are considered by member states individually. EFSA has produced guidance documents on the risk assessment of LM animals for both food and feed uses (EFSA, 2012a) and for release into the environment (EFSA, 2013). These give guidance in relation to applications for food/feed import of animal products and for the commercialisation of live modified animals (including fish) in the EU in compliance with Regulation (EC) No 1829/2003 or Directive 2001/18/EC.
The environmental risk assessment (ERA) guidance document provides guidance to applicants and risk assessors for assessing potential adverse effects of LM animals on the environment, human and animal health and the rationales for data requirements for a comprehensive risk assessent. It also provides general guidance for drawing conclusions on the post-market environmental monitoring (PMEM). This guidance considers issues specific to fish as well as issues common to the risk assessent of all potential LM animals.

The EFSA ERA guidance advises that the risk assessent of LMF involves collecting relevant information on the LMF, comparing it with non-modified fish or appropriate comparators, examining differences in impacts and determining whether these impacts will have adverse and harmful effects on human and animal health and the environment. The risk assessent should be carried out on a case-by-case basis, meaning that the required information will vary depending on the type of LMF and the modified trait(s), the potential receiving environments and the intended uses.

In the case of LMF for human or animal consumption, some data will be already compiled for the comparative safety assessment of food and feed derived from the LMF, including data on the molecular characterisation, on the compositional analysis and on the phenotypic characterisation of the LMF. This

will inform the initial steps of the risk assessment of LMF and, in particular, the identification of possible unintended effects.

The risk assessent of LMF should follow a six-step approach: (1) problem formulation including hazard and exposure identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) overall risk evaluation. As a general principle, the use of a step-by-step approach beginning with problem formulation is required whereby scientifically reliable evidence, based on qualitative and, whenever possible, quantitative analyses, is combined with an explicit uncertainty analysis in order to support the final conclusions of the risk assessent. The following areas of risk should be considered : (1) persistence and invasiveness of the LMF, including vertical gene transfer; (2) horizontal gene transfer; (3) interactions of the LMF with any target organisms; (4) interactions of the LMF with non-target organisms (NTOs); (5) environmental impacts of the specific techniques used for the management of the LMF; (6) impacts of the LMF on biogeochemical processes; and (7) impacts of the LMF on human and animal health through non-food/feed exposure.

In addition, the EFSA (2013) Guidance Document describes several generic cross-cutting considerations that need to be accounted for throughout the whole risk assessment. These include consideration of the identification and characterisation of relevant receiving environments which may be exposed to the LMF, the choice of adequate comparators and, where appropriate, the use of non-GM surrogates with similar characteristics that can inform the risk assessmentof the LMF. Applicants are advised to follow the requirements for proper experimental design, modelling as well as the general statistical principles outlined in the document, such as the specification of the effect size and the power analysis. Experimental studies should allow testing for difference and equivalence. The treatment of uncertainty and the results and conclusions of the uncertainty analysis should be described.

Potential long-term effects and their methods of study should be considered. In addition, health aspects should be considered in relation to LMF and their wild types and the welfare of LMF should be taken into account (EFSA 2012b).

EFSA advises that the risk assessment should be carried out in a scientifically sound manner based on available scientific and technical data and following the common methodology for the identification, gathering and interpretation of the relevant data. Sufficient scientific data enabling qualitative/quantitative risk estimates is required in order to draw conclusions on the possible environmental risks posed by an LMF.

The EU gives consents for all LM products and organisms for 10 years before there is a review and a consideration for renewal of the consent. Applicants are required to conduct General Surveillance to determine whether any adverse effects have occurred during this period. In addition, if specific hazards or risks are identified during the risk assessment, then the conditions of the consent may include requirements to manage these risks and to conduct Case Specific Monitoring of these LM organisms to determine whether they are having any adverse impacts.

Annex 6. Information gathered from biosafety national authorities and stakeholders

The Questions and Responses

Written responses were received from a total of 23 national authorities and biosafety institutions from Africa (3), Asia and the Pacific (AP; 3), Central and Eastern Europe (CEE; 2), Latin American and the Caribbean (GRULAC; 3), and Western European and Others Group (WEOG; 12). In addition, there were responses from Intergovernmental Organisations (IO: 2), one Industry Organisation (IB) and one civil society organisation (CSO). The questions and responses from the 23 national biosafety authorities and

institutions are separated from those of the 2 Intergovernmental Organisations (IO), the Industry Organisation (IB) and the CSO. The responses from national biosafety authorites and institutions are analysed by CBD region.

1) *Are you/your organisation involved in risk assessment or regulation of Living Modified organisms (LMOs)?*

All responders from national biosafety authorities and institutions said they were involved in risk assessment and/or regulation of LMOs, except one from GRULAC. One IO and the IB said they were involved in risk assessment and/or regulation of LMOs while the other IO said it was not involved in risk assessment and/or regulation of LMOs. The CSO indicated that it was involved in risk assessment and/or regulation of LMOs.

2) *Are you/your organisation involved in LMO research and development?*

Six responses from national biosafety authorities and institutions (1 AP, 1 CEE & 4 WEOG) said they were responsible for contracting and/or funding research, but not actually conducting research. 17 responses from national authorities and institutions said they were not involved in LMO research and development. One IO and the IB were involved in biosafety research.

3) *Are you/your organisation involved in production or testing of LMOs?*

No national biosafety authorities and institutions were involved in producing LMOs, but 6 responses indicated they were directly or indirectly involved in testing or validating testing methods for LMOs and products (1 African, 1 CEE, 1 GRULAC and 3 WEOG). One IO and the IB were involved in developing or applying testing methods to LMOs

4) *Do you consider that Living Modified Fish (LMF) have the potential to cause adverse effects on biodiversity, in particular those that are serious or irreversible, taking into account the urgent need to protect specific aspects of biodiversity, such as an endemic/rare species or unique habitats or ecosystems, taking into account risks to human health and the value of biological diversity to indigenous peoples and local communities?*

National biosafety authorities and institutions (19) replied yes to this question and several of these respondents said that this was case by case, dependant on the species, traits, conditions of release etc. Some respondents referred to the current experiences with harm from fish farming, levels of escape and hybridisation. Two responses from national biosafety authorities and institutions in WEOG commented only on the two currently approved LMF and said they considered that they would not cause harm. Two other responses from national biosafety authorities and institutions in WEOG said they would or could not comment on potential harm but one of these commented that no LMF would be permitted if it could cause harm.

5) *Do you think that LMF are, or are likely to be, released into the environment deliberately or accidentally?*

Seven respondents from national biosafety authorities and institutions (1 AP, 1 GRULAC, 5 WEOG) considered it was unlikely that LMF would be deliberately released into the open environment in the near future but kept in contained facilities. However, they considered that this was very context related and that these fish could or would be unintentionally or accidentally released into the environment. Examples were

that LM ornamental fish are available in some countries without regulatory approval, ornamental fish are often released into ponds or waterways and that farmed and hatchery fish often escape.

10 respondents from national biosafety authorities and institutions (2 Africa, 2 AP, 1 CEE, 2 GRULAC, 3 WEOG), and 2 IOs considered that LMF would be deliberately released in the future and that this could also result in accidental or unintentional releases into areas where approvals had not been given. 2 respondents from national biosafety authorities and institutions (1 WEOG and 1 Africa) and one international organisation commented that currently approved LMF would not be deliberately or unintentionally released. The remaining respondents from national biosafety authorities and institutions did not comment.

6) *Do you think LMF have the potential to disseminate across national borders?*

21 respondents from national biosafety authorities and institutions (3 African, 2 AP, 2 CEE, 3 GRULAC, 11 WEOG) and all other responders answered yes to this question, depending on the type of fish and trait, and this included 3 respondents from national biosafety authorities and institutions (1 AP, 2 WEOG) who pointed out that AAS are being moved between Canada, Panama and USA under licencing arrangements and that there was no unlicensed movement of LMF across borders. Others commented that, in addition to the licensed movement, ornamental LMF had been transported into countries and across national borders without the required permits. Two respondents from national biosafety authorities and institutions (1 AP, 1 WEOG) considered that there is no potential for unlicensed movement across borders.

7) *Do you consider that LMF fall within the scope and objectives of the Cartagena Protocol?*

21 respondents from national biosafety authorities and institutions and all other responders consider that LMF fall within the scope and objectives of the Cartagena Protocol and no authorities considered that they did not.

Two respondents from national biosafety authorities and institutions did not respond to this question and an WEOG national biosafety authority and institution said that it could not comment because this is a legal question and that ”The context of potential use or transit of LM fish would be important in answering this question, noting the provisions of Article 6 regarding transit and contained use of LMOs (i.e. advanced informed agreement procedure does not apply), and of Article 7 regarding intentional transboundary movement and intentional introduction to the environment”.

8) *Are LMF being developed and are they likely to be commercialized or in use in your country or region?*

The 2 IOs the IB and 3 respondents from national biosafety authorities and institutions in WEOG responded that LMF are currently commercialised in their country or globally. Seven respondents from national biosafety authorities and institutions (2 AP 5 and WEOG) said they are also available under contained, often laboratory, conditions for particular uses such biomedical research in their country/region. 15 respondents from national biosafety authorities and institutions (2 Africa, 1 AP, 2 CEE, 3 GRULAC, 7 WEOG) responded that LMF are not being developed or commercialised in their country.

9) *What capacity and resources do you have in your country to risk assess and manage LMF?*

The IOs and IB indicated that risk assessment capacity and resources were available in some countries and regions. 19 respondents from national biosafety authorities and institutions, (2 African, 2 CEE, 1 AP, 2 GRULAC,12 WEOG), and the CSO said that they had risk assessment procedures and systems in place for the risk assessment and management of LMF in their country or region. However, of the 19

authorities, eight indicated that their rules and procedures were only for assessing LMF for contained/confined use and for determining the levels of isolation/confinement required (2 Africa, 3 AP, 2 CEE, 1 GRULAC). These responses also said that their experience with LM animals was limited and that panels containing experts on fish related topics would need to be established. 2 respondents from national biosafety authorities and institutions in GRULAC said that they did not have the capacity to risk assess or manage LMF and 2 others (1 Africa, 1 GRULAC) said they had very limited capacity and resources.

10) *What guidance documents on risk assessment, particularly for LM fish, do you have access to or consult?*

A total of 19 respondents from national biosafety authorities and institutions (2 Africa, 1 AP, 2 CEE, 2 GRULAC, 12 WEOG) and the CSO said they had access to guidance on risk assessment of LMF. Of these, 6 respondents from national biosafety authorities and institutions in WEOG said they use or would use EU/EFSA guidance, but some had also developed their own approach for contained use. However, 1 African, 2 AP, 2 CEE, 2 GRULAC said they had limited access or access only to the voluntary risk assessment guidance developed under the Cartagena Protocol or similar guidance which is rather generic to LMAs. They also commented that these guidances were mainly applicable to contained use of LMF. A response from GRULAC said they had no access to guidance and 2 other respondents from national biosafety authorities and institutions (1 African and 1 GRULAC) said that the availability of guidance was very limited. The IOs and IB indicated that there were several guidance documents available internationally.

11) *Are these guidance documents in line with the objectives of the Cartagena Protocol? If not, can these resources be revised or adapted to be in line with the objectives of the Protocol?*

There was general agreement from those with access to guidance that it was in line with the Cartagena Protocol.

12) *Do you consider the development of guidance for the risk assessment of LMF to be a priority?*

Nine respondents from national biosafety authorities and institutions (2 Africa, 2 AP, 2 CEE, 3 GRULAC) and the CSO considered the need for the development of guidance to be a priority. In addition, some countries from other regions considered that there needed to be international agreement on the guidance and data requirements for the risk assessment of LMF.

13 other respondents from national biosafety authorities and institutions (1 Africa,1 AP, 11 WEOG) the IOs and the IB considered that sufficient guidance is available and that it should be harmonised and made more widely available to regions with limited experience with risk assessment and LMF. A response from WEOG indicated that it would use quarantine measures to prohibit LMF and so as it would never permit release of LMF, guidance was not required.

13) *What do you consider to be the main challenges and constraints in risk assessing LMF*?

A number of respondents from national biosafety authorities and institutions from Africa, AP, CEE and GRULAC commented on capacity requirements for LMF and indicated that the main challenges were associated with inadequate capacity on risk assessment, lack of specific and appropriate guidelines on LMF, no established expertise, lack of experience in risk assessment and in use of guidance. They also commented that the regulatory systems and guidance need to be harmonised internationally. Training is also needed in some regions and there are funding constraints.

Ten respondents from national biosafety authorities and institutions (2 AP, 2 CEE, 6 WEOG) commented on the challenges associated with conducting risk assessments. The comments included concerns about unfamiliarity with marine and aquatic environments and background and baseline information on fish biology and ecology in different environments. They felt challenged by the need for data on the effects of intended and unintended or consequential changes on environmental interactions, behaviour, fitness, and competitiveness changes in LMF, impacts on food chains, biotic components and processes in different ecosystems, including competition with other species. In addition, they were concerned about information required on reproduction, gene flow, spread, persistence and invasiveness in different environments and impacts of different scales of releases, over large time scales. Some considered that it would be difficult to determine whether effects were manageable, temporary or reversible and they were concerned that these factors created greater uncertainties in predicting outcomes. One IO also commented that risk management of LMF also presented a range of challenges.

Two respondents from national biosafety authorities and institutions from WEOG countries with strict quarantine regulations to restrict novel or invasive species, felt that implementing these regulations can be used to prevent LMF from being introduced into their countries and so saw no real challenges to the risk assessment process. In addition, they considered that only LMF that could be restricted to contained facilities were likely to be permitted in their country/region.

14) *From your experience, do LMF pose challenges to existing risk assessment frameworks, guidance and methodologies? Have you experienced specific technical or methodological challenges that require further attention?*

8 respondents from national biosafety authorities and institutions (1 Africa 2 AP, 1 CEE, 1 GRULAC, 3 WEOG) and the CSO considered that LMF pose challenges to existing risk assessment frameworks because of the issues listed in responses to the previous question, particularly considering releases of LMF.

11 responders said they had not experienced technical or methodological challenges because they had either only conducted RAs on LMF to be kept in containment (5 WEOG) or they had not conducted any RAs of LMF (3 African, 2 GRULAC, 1 CEE). An IO and the IB considered that current risk assessment guidance, frameworks and methods are adequate for LMF. The remainder could not comment as they had no experience with risk assessment of LMF.

15) *Has your national authority reported trans-border movement of LMF? Please describe any reports.*

9 respondents from national biosafety authorities and institutions (1 CEE, 8 WEOG,) replied that they had reported or observed trans-boundary movements. In two cases, these were WEOG countries that had approved LMF and movements were approved or licensed. Five respondents from national biosafety authorities and institutions (4 WEOG and 1 AP) had reported introductions of LMF for confined uses in research facilities. In other cases, there were reports of unapproved or illegal introductions of ornamental LMF and the authority had intervened to prevent incursions. 14 respondents from national biosafety authorities and institutions (3 African, 4 WEOG, 3 AP, 3 GRULAC, 1 CEE) had not reported or were not aware of any reports of transboundary movements. One IO commented on the approved transboundry movements of LMF and one answer was not applicable to this question.

16) *Has your national authority risk assessed LMF for experimental use and/or release? If so, please reference published reports or describe the event and risk assessment outcomes.*

Eleven respondents from national biosafety authorities and institutions (1 AP, 1 CEE, 1 GRULAC, 8 WEOG) reported that they had performed risk assessments on LMF. Two respondents from national

biosafety authorities and institutions in WEOG had conducted and published risk assessments of AquAdvantage Salmon and ornamental LMF. The remaining 9 respondents had assessed the risks of LMF for contained use only or because of the potential for illegal imports. Some had published the results on their web sites, while others had not. 11 respondents from national biosafety authorities and institutions (3 African, 2 AP, 1 CEE, 1 GRULAC, 4 WEOG) and the CSO had not conducted risk assessments of LMF.

17) *Are there research and development programmes on LM fish in your country or organisation? If so, please describe them.*

Research and development studies conducted with LMF were reported by 11 respondents from national biosafety authorities and institutions (1 AP, 1 CEE, 9 WEOG). In all cases, LMF were being used in biological or biomedical studies, while in 2 WEOG countries work on developing LMF for markets were also being studied. Some respondents from national biosafety authorities and institutions and the CSO reported that fish breeders were also using gene editing and other biotechnologies to develop novel fish types. 12 respondents from national biosafety authorities and institutions (3 Africa, 2 AP, 1 CEE, 3 GRULAC, 3 WEOG,) reported that they were not aware of research and development programmes using LMF in their region or organisation.

18) *Do you wish to make any other comments or observations on LM Fish*?

The comments added here mostly referred to issues raised in the other questions and have been added where appropriate. However, an IO and the IB also made the following comments: there are other major issues associated with fish breeding, aquaculture, biodiversity and the environment which are more important and that LMF are likely to have minor impacts compared with these. Because of resistance to transgenic technologies it is likely that more breeding effort will go into gene editing and already some gene edited fish are being tested for commercialisation (e.g tilapia). In addition, the IB commented that inland and confined production of fish (including LMF) can minimise environmental impacts and impacts on wild populations.

An IO also commented that production of transgenic plant-based fish feeds with modified protein and fatty acid content (e.g. omega-3) could reduce harvesting of small fish to feed fish farms and improve the nutritional value of the fish.