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Proceedings of the National Peer Review on Environmental and Indirect Human Health Risk Assessment for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI

December 11-13, 2018
Ottawa, Ontario

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Editor: Lily Weber

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Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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TABLE OF CONTENTS

SUMMARY

The *Canadian Environmental Protection Act* (CEPA), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health prior to their manufacture or import into Canada. In accordance with a Memorandum of Understanding (MOU) between Fisheries and Oceans Canada (DFO), ECCC, and HC, DFO assists in implementing the *New Substances Notification Regulations (Organisms)* [NSNR(O)] by providing science advice that is based on an environmental risk assessment for fish products of biotechnology, and, along with HC, on the indirect human health risk assessment for fish products of biotechnology. DFO may also make recommendations regarding any necessary measures to manage risks, if required.

On July 27, 2018, AquaBounty Canada Inc. submitted a regulatory package to ECCC under the NSNR(O) of the *Canadian Environmental Protection Act* (CEPA) for the manufacture and production of EO-1α Salmon, a fast growing, genetically engineered Atlantic Salmon (*Salmo salar)*, at a new land-based aquaculture facility, near Rollo Bay, PEI. AquaBounty Canada submitted a similar notification in 2013, detailing its intent to commercially manufacture the EO-1α Salmon, in a land-based contained facility near Fortune Bay, PEI. For the current assessment, DFO and HC collaborated to conduct the environmental and indirect human health risk assessments, respectively, and to develop recommendations, taking into account the previous Science Advice for the previous notification to support a regulatory decision by the Ministers of ECCC and HC.

The CSAS national science advisory process was used to peer review the two risk assessments and to develop scientific consensus on the risk assessment outcomes and recommendations provided to ECCC and HC. A peer review meeting was held on December 11-13, 2018 in Ottawa, Ontario. The terms of reference and agenda for this process are found in Appendix 1 and 2, respectively. Meeting participants included experts and scientists from DFO, ECCC and HC, the government of PEI, academia, the Atlantic Salmon Federation and the US National Oceanographic and Atmospheric Administration (Appendix 3). The conclusions and advice resulting from this meeting are provided in the form of a Science Advisory Report, as well as two research documents containing the details of the risk assessments and will be publically available on the CSAS website. The purpose of this document is to summarize the key discussions points and conclusions from the peer-review meeting.

INTRODUCTION

The *Canadian Environmental Protection Act* (CEPA), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. In accordance with a Memorandum of Understanding (MOU) between Fisheries and Oceans Canada (DFO), ECCC, and HC, DFO assists in implementing the *New Substances Notification Regulations (Organisms)* [NSNR(O)] by providing science advice that is based on an environmental risk assessment for fish products of biotechnology, and, along with HC, on the indirect human health risk assessment for fish products of biotechnology. DFO may also make recommendations regarding any necessary measures to manage risks, if required.

On July 27, 2018, AquaBounty Canada submitted to Environment and Climate Change Canada (ECCC), a notification of its intent to manufacture the AquAdvantage® Salmon, a salmon genetically engineered for fast growth, at a facility near Rollo Bay, PEI, under contained conditions. Commercial grow-out of sterilized AquAdvantage® Salmon at the same facility, and under the same conditions of containment, is also proposed. The current notification follows a previous notification submitted in 2013 to manufacture AquAdvantage® Salmon at a contained facility near Bay Fortune, PEI.

The CSAS peer-review process included participants with relevant expertise, who gathered to review and discuss the draft “Environmental Risk Assessment for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI ” prepared by DFO, and the draft “CEPA Human Health Assessment Report on the AquAdvantage® Salmon”, prepared by HC (herein after referred to as the environmental risk assessment and indirect human health (IHH) risk assessment, respectively). The meeting was held December 11-13, 2018 in Ottawa, Ontario, where discussion focused on the main components of the two draft risk assessments, including exposure assessment, hazard assessment and associated levels of uncertainty, to reach final conclusions on risk. Consensus was reached on the document output of the meeting; a draft Science Advisory Report entitled “Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-Out of EO-1α Salmon, including the AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI”. The final Science Advisory Report was submitted to ECCC as science advice in support of the regulatory decision taken by ECCC and HC.

CSAS SCIENCE NATIONAL REVIEW PROCESS

Presenter: Gilles Olivier, Chair; Fisheries and Oceans Canada

The meeting chair, Gilles Olivier provided an overview of the “CSAS Science National Peer-Review Process”, the principles of CSAS, and explained the role of all meeting participants as reviewers. He emphasized the strictly scientific basis of consensus for CSAS processes, as well as the ground rules for the meeting and expected publications.

CSAS provides science advice in support of DFO policy, and management plans and decisions. The approach is based on the SAGE (Scientific Advice for Government Effectiveness) principles and guidelines for the effective use of science and technology advice in government decision making. The main objectives are to provide sound, objective, and impartial science advice. Participation in the CSAS process is by invitation to those with the expertise and knowledge on the subject matter. Scientific working paper(s) and other inputs (analysis, findings, conclusions) are subject to rigorous review and quality control in a peer-based forum. The resultant peer-

reviewed documents are released to the public as Scientific Advisory or Response Reports, Research Documents (Risk Assessments), and Proceedings through publication on the DFO CSAS website.

All participants were asked to review the material provided, participate fully in discussion, contribute relevant expertise, experience, data, and knowledge on the subject of review and consider the application of the data and methods. It was noted that participants should work toward developing consensus on the conclusions and advice.

REGULATORY CONTEXT, RISK ASSESSMENT PROCESS, AND PROPOSED USE SCENARIO SUMMARY

Presenter: Sherry Walker, Fisheries and Oceans Canada

The “Regulatory Context, Risk Assessment Process, and Proposed Use Scenario Summary” presentation addressed the legislative and regulatory context under which the risk assessments were conducted, the risk assessment process itself, and the translation of the risk assessment findings into a recommendation (i.e., the Science Advisory Report), in support of a regulatory decision under CEPA, given the proposed scenario of the manufacture and production of the AquAdvantage® Salmon.

PUBLIC TRANSPARENCY NOTICE

Presenter: Marie Breton, Environment Canada and Climate Change

Environment and Climate Change Canada and HC are working together to promote more public engagement in the risk assessment of higher organisms (e.g., genetically modified plants and animals). Under a new voluntary initiative, the New Substances Program publishes summaries of higher organism notifications, and invites stakeholders to share scientific information and test data related to potential risks to the environment or human health, to help inform the risk assessment process. Notice of the notifications for the manufacture and production of the AquAdvantage® Salmon at a land-based and contained facility near Rollo Bay, PEI was posted on the ECCC website on September 14, 2018 and stakeholders were invited to provide relevant scientific data and information during a 30-day comment period. ECCC compiled all scientific information and provided a summary to the evaluators prior to completion of the risk assessment.

The public engagement notice received a total of nine submissions, which included the identification of a potential new source of environmental exposure. The shipment of non-transgenic eyed-eggs raised concerns about the potential of human error in inadvertently mixing up the non-transgenic eggs with the transgenic organisms. The new potential source of exposure was taken in to consideration and addressed accordingly in the environmental and indirect human health (IHH) risk assessments and the Science Advisory Report (SAR). Under Scenario A, non-transgenic fish for external parties are produced alongside transgenic fish. Under Scenario B, no non-transgenic fish are produced at the facility for external parties.

CHARACTERIZATION OF THE EO-1α SALMON

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Characterization of the EO-1α Salmon, including AquAdvantage® Salmon” presentation addressed the molecular structure and function of the genetic modification in EO-1α Salmon. Strain propagation, targeted and off-target phenotypes, and history of use of the EO-1α Salmon were also addressed. The comparator species, *Salmo salar,* and the associated phenotypic

differences between domesticated and wild Atlantic Salmon were addressed, as well as the status of wild Atlantic Salmon populations in Eastern Canada.

Discussion

Discussion after the presentation focused on whether there have been any changes to the opAFP-GHc2 transgene and EO-1α strain since the 2013 assessment. It was clarified that there has been continued selection under commercial rearing in the homozygous lines.

A question was raised as to whether the epigenome had been characterized, including potential for silencing effects on the transgene. It was clarified that the notifier tests the broodstock for gene presence and the location of the gene to verify that it has not shifted. The transgene has a stable Mendelian inheritance. It was indicated that environmental conditions can influence gene expression, which ultimately relates to the phenotype and stability over time.

Key summary points of the discussion

- The nature of the transgene construct or integrant is unlikely to cause any harm to the environment or indirect human health;
- Mendelian inheritance and molecular stability of the opAFP-GHc2 at the EO-1α locus have been adequately demonstrated;
- There is still uncertainty regarding environmental effects on phenotype and genotype stability over time.

CHARACTERIZATION OF THE RECEIVING ENVIRONMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The “Characterization of the Receiving Environment” presentation examined the location of the Rollo Bay facility and the potential bodies of water that could receive the EO-1α salmon. A small stream located on the facility, Rollo Bay Brook is part of a larger drainage system that empties into the Rollo Bay and the Northumberland Strait.

Discussion

Participants asked if any baseline data had been collected from the Rollo Bay brook in terms of substrate, habitat and species identification, to assess the potential effects from production and the effluent from the facility. It was clarified the water outflow occurs in small volumes and is well oxygenated. However, there were very little data provided on the brook and there have been no known electro-fishing surveys performed. Two unpublished surveys were brought to the attention of the evaluators for inclusion in the risk assessment document.

Participants discussed whether there should be provisions for containment in the brook, such as regular electro-fishing surveys. There are currently no measures of containment once outside of the buildings; however, there are provisions for collecting solid waste.

Key summary points of the discussion

- The stream outside of the facility is suitable habitat for salmonids;
- The volume of the discharged water from the facility should be clarified;
- The literature on the identified baseline data for the Rollo Bay brook will be cited;

- It was suggested that provisions for containment in the brook should be in place.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “IHH Exposure Assessment” presentation addressed the exposure of EO-1α Salmon to humans through the environment. The IHH exposure assessment process involved identifying the sources of exposure, the individuals likely to be exposed (healthy, immunocompromised, children, those with underlying medical conditions), and potential routes of exposure. Oral ingestion is considered a food safety issue and is not captured under CEPA. While there are similarities in how exposure and hazards are ranked in the IHH Risk Assessment and the Environmental Risk Assessment, the IHH assessment is a standalone assessment with independent processes to obtaining the rankings for exposure, hazard, risk, and the associated uncertainties. Detailed descriptions of how rankings were obtained can be found in the IHH Risk Assessment and the Science Advisory Report.

Discussion

There was confusion among participants as to whether the uncertainty associated with the exposure rating is based on the fitness of the EO-1α Salmon or the containment of the facility itself. It was clarified that the exposure rating and uncertainty are based on the proposed containment measures.

Concerns were raised regarding a potential source of environmental exposure identified through the public engagement notice. The sale of non-transgenic eggs to external parties opened up the potential for human error and the accidental mixing of non-transgenic eggs with transgenic eggs, which are then sold as non-transgenic. Participants concluded the exposure rating will likely not change, though uncertainty may increase if the production of non-transgenic and transgenic fish occur simultaneously.

Consensus

Participants reached consensus on the following:

- The human exposure potential of the EO-1α Salmon is low, as physical, biological and operational measures are in place or planned at the Rollo Bay facility to prevent unintentional environmental release, thus greatly limiting human exposure to the notified organism. This ranking is not expected to change when taking scenarios A and B into consideration.
- Uncertainty associated with the IHH exposure assessment is low since adequate information is available regarding exposure scenarios in the Canadian environment given the containment measures. However, this uncertainty could likely be higher in the event that production of non-transgenic and transgenic fish occur alongside each other (scenario A).

INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

Presenter: Stephen Dugan, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the capacity of the EO-1α Salmon to act as a vector for human pathogens, as well as its toxicity, allergenicity, and general health status. The IHH Risk assessment does not include potential hazards associated

with consumption of the AquAdvantage® Salmon as food (considered under the *Food and Drugs Act*) or occupational health hazards (considered under the *Occupational Health and Safety Act*). Detailed descriptions of how rankings were obtained can be found in the IHH Risk Assessment and the Science Advisory Report.

Discussion

After the presentation, questions were raised regarding surrogate information that suggests a higher susceptibility of pathogens in transgenic fish. It was clarified that evidence will be added to support the statement in the IHH assessment document.

There were concerns that the statement, “there has been no adverse indirect human health reported by the staff of the notifier after more than 20 years”, implies a false sense security and is anecdotal information. It was suggested there should be clarification of how the standard operating procedures (SOPs) and staff training support this statement.

The potential for a mix up of transgenic eggs and non-transgenic eggs due to human error is not expected to affect the hazard rating or the associated uncertainty.

Consensus

Participants reached consensus on the following:

- It was agreed that the indirect human hazard potential of the EO-1α Salmon with respect to zoonotic potential and potential of indirect human health allergenicity and toxicity assessed to be low.
- The consensus of the uncertainty associated with the potential indirect human health hazards is low.

INDIRECT HUMAN HEALTH RISK ASSESSMENT

Presenter: Kassim Ali, Health Canada

The “Indirect Human Health Risk Assessment” presentation addressed the exposure to indirect human health hazard outcomes and concluded on the indirect human health risk. The presentation concluded that there is no evidence to suggest a risk of adverse human health effects from EO-1α Salmon at the exposure levels predicted for the general Canadian population from the commercial aquaculture production in land-based contained facilities in Rollo Bay, PEI.

Discussion

It was clarified that there are different definitions for the uncertainties associated with the Indirect Human Health exposure and hazard and they are standardized across all assessments performed by Health Canada. The uncertainty criteria used in the IHH risk assessment and the environmental risk assessment also vary because they are from different processes.

It was mentioned there should be greater detail on the background and legal context in the IHH risk assessment .

It was discussed that it should be clarified the Bay Fortune facility is used as a proxy to assess the Rollo Bay facility.

There were discussions on whether the IHH risk would change based on the two scenarios to address the concern of the potential mix up of non-transgenic and transgenic eggs due to human error.

Consensus

The overall indirect human health risk associated with the manufacture and grow-out of EO-1α at a land-based facility was concluded to be low.

ENVIRONMENTAL RISK ASSESSMENT

ENVIRONMENTAL EXPOSURE AND WAIVER ASSESSMENT

Presenter: Colin McGowan, Fisheries and Oceans Canada

The “Environmental Exposure and Waiver Assessment” presentation provided an overview of the basis of a waiver request and potential stages of events for environmental exposure from release to spread of the AquAdvantage® Salmon in the natural environment: release, survival, persistence, reproduction, proliferation, and geographic spread. An overview was provided of the basis of the exposure assessment and the uncertainties associated with the physical and biological containment measures. A summary of all the possible pathways of entry into the environment and a failure mode analysis (FMA) for the hatchery, grow-out and broodstock buildings were presented.

It was noted that the company had submitted information and data in response to information element 5(a) of Schedule 5 of the NSNR(O), *data from a test conducted to determine pathogenicity, toxicity or invasiveness,* in accordance with Section 106 (8) of CEPA, with the expectation that if the information provided was not sufficient, a waiver for the information element was requested based on containment. A CSAS Science Response process was conducted to establish whether information provided by the company in the regulatory package was sufficient to determine invasiveness. It was concluded that the information provided by the notifier was not sufficient to systematically assess EO-1α Salmon. However, based on redundant containment measures and operational oversite indicating the organism will be sufficiently contained (see below), it was recommended that the waiver should be accepted.

Discussion

It was clarified that the information provided by the company in the notification on planned and existing containment measures at the Rollo Bay facility was used in the exposure assessment, and that the Bay Fortune facility was not being used as a proxy for Rollo Bay to assess containment measures. The redundant containment measures used in the completed hatchery building will be transferred over to the other buildings as well as the standard operating procedures (SOP) and compliance documentation.

There were discussions as to whether the uncertainty associated with the fate of the organism should be higher or lower. The definitions of uncertainty rankings were reviewed, and it was agreed that the level was moderate, provided the definition includes limited to low knowledge of GXE interactions. This addition acknowledges that there are high quality data demonstrating GXE forces influencing the phenotypic differences exist, but there is poor understanding of how the interactions would perform under natural conditions.

Clarification regarding containment at the drum filter and the potential for fry to survive in and escape from this point was requested along with greater detail in the SOPs and additional protocols for extreme weather, and catastrophic events.

Concern was raised over the shipment of non-transgenic eggs and fry to external parties, and the potential for an accidental mix up of non-transgenic eggs with transgenic eggs. This

potential source of exposure was taken into consideration and two scenarios were formulated collaboratively by participants.

- Under Scenario A, company activities would include the production of non-transgenic fish, for external parties, occurring along-side transgenic fish production using existing and planned procedures for keeping eggs organized and separated and for keeping transgenic organisms contained.
- Under Scenario B, there is no production of non-transgenic fish for external parties, with all non-transgenic salmon housed at the facility used only for the production of AAS, as described in the regulatory package submitted by the company.

Under Scenario A, consensus was reached on a ranking of low for environmental exposure. Participants raised concerns that there was missing information regarding the frequency and volume of non-transgenic egg shipments and what their final fate may be. It was suggested that further detail and compliance on this issue should be added to the company SOPs.

Under Scenario B, there was consensus on a ranking of negligible exposure, though it was agreed that a footnote to the definition of negligible exposure should be added to clarify it does not mean absolutely zero chance of exposure to the environment, but rather no foreseeable exposure.

It was indicated the company would like flexibility in what tasks will be performed in which buildings. This raised concerns about the potential transfer of EO-1α Salmon between buildings in the Rollo Bay facility. It was suggested that SOPs need to be stronger and clearer with regards to this issue. It was noted that this concern would be covered through the DFO Introductions and Transfers Committee.

Consensus

Participants reached consensus on the following:

- Under Scenario A, the likelihood of exposure of AquAdvantage® Salmon to the Canadian environment is ranked low with low uncertainty due to the potential exposure from human error.
- Under Scenario B, the the likelihood of exposure of AquAdvantage® Salmon to the Canadian environment is ranked negligible with low uncertainty if no non-transgenic eggs are sold at the facility.

ENVIRONMENTAL HAZARD ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Environmental Hazard Assessment” presentation examined the potential for the EO-1α Salmon to cause harmful effects to the environment due to the targeted phenotype or off-target effects. It considered potential hazards to environmental components (e.g., potential to act as a vector of disease agents) and ranked hazards depending on the presence and reversibility of harmful effects. There are no available data for EO-1α Salmon from natural environments and thus a reliance on laboratory data and/or comparator species for hazard assessment. There are knowledge gaps, lack of empirical data, and effects of genotype, environment, and GxE interactions when relying on laboratory data. This contributes to uncertainty in hazard assessment.

Eight different hazard endpoints were assessed: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through trophic interactions; 4) through hybridization; 5) as a vector of disease; 6) to biogeochemical cycling; 7) to habitat; and 8) to biodiversity.

1. Potential environmental toxicity

The potential differences in hormone levels, potential of the EO-1α Salmon to bioaccumulate toxicants, and toxicological concerns with triploidy and sex reversal were reviewed. In order for an escaped EO-1α Salmon to be hazardous through environmental toxicity, they would need altered body chemistry, be ingested by predators, and cause harm to the predators from the altered body chemistry. Given the lack of studies on the potential for EO-1α Salmon to bioaccumulate toxicants, and no toxicological concerns associated with triploidy or sex reversal under proposed procedures, it was concluded that there is no evidence indicating EO-1α salmon would pose higher hazard through environmental toxicity than the wild-type. Consequently, there is a negligible hazard potential through environmental toxicity. Due to the limited data on full life-cycle levels of hormones and reliance on indirect data, there is a moderate uncertainty associated with this rating.

Discussion

It was clarified that the 2013 risk assessment did not have the benefit of the HC food safety assessment and the CFIA toxicity assessment of AquAdvantage® Salmon. It was recommended that these assessments be cited.

A hormonal treatment for sex reversal of broodstock is a common industry procedure and not specifically associated with the genetic modification, any hazards through this pathway are not covered under CEPA NSNR(O).

Consensus

- It was agreed to conclude negligible toxicological hazard to populations or the structure and function of the ecosystem, with moderate uncertainty.

2. Potential for hazards through horizontal gene transfer

Hazards through horizontal gene transfer (HGT) requires 1) exposure/uptake of the free transgene to a novel organism, 2) stability and expression of the gene within the novel organism, 3) neutral or positive selection of the novel organism expressing the transferred gene, and 4) harm to the organism or the environment from the expression of the transferred gene. While much is unknown about potential for uptake, expression and then potential harm from HGT of salmon genes to prokaryotes, the well defined transgene does not contain any elements that indicated HGT and resulting hazards would be any different for the EO-1α transgene than a native Atlantic Salmon gene. Consequently, the potential for hazards through HGT is negligible. The lack of mobile elements in well-defined transgene and insert site results in low uncertainty in this rating.

Discussion

The stability of the EO-1α transgene was discussed. Only the immediately adjacent native sequences are known, and nearby sequences could potentially contain transposable elements.

Consensus

- There was consensus on a negligible rating of hazards through HGT, with moderate uncertainty due to the possibility of the surrounding repeat sequences containing transposable elements.

3. Potential for hazards through trophic interactions

Hazard considerations regarding the potential impacts of the EO-1α Salmon through trophic interactions with other organisms were reviewed. There is the possibility that the EO-1α Salmon may impact native organisms through trophic interactions as a competitor, predator, or prey. For specific trophic interactions it was put forward that there was high hazard through competition, moderate hazard through predation, and low hazard as prey. The overall hazard rating for potential impacts through trophic interactions was determined to be high, however the rating is context-specific and may be negligible to high depending on specific sets of conditions. The limited data specific to the EO-1α Salmon, limited knowledge of which factors influence marine survival and growth and the limited ability to define the GXE interactions in surrogate organisms resulted in a moderate uncertainty.

Discussion

It was suggested to reference a study in Norway concerning escaped reared salmon that have a higher mortality and do not compete well with wild salmon. This study could be related to the EO-1α Salmon and incorporated in the potential for hazards through trophic interactions.

It was suggested to provide information about previous surveys of the Rollo Bay Brook and baseline data to provide more background. It was clarified that the brook is compatible habitat for salmon.

There were discussions whether the ranking should be high for potential hazard through predation on other organisms. The original proposed moderate ranking definition states the harmful effects are reversible. It was noted that the potential hazard of the EO-1α Salmon should be assumed irreversible unless there is supporting evidence to show that the system could rebound.

It was clarified there is moderate uncertainty associated with the hazard because there is significant reliance on data from surrogate organisms (other transgenic fish, domesticated Atlantic salmon), and presence but poor understanding of genotype by environment interactions in surrogate organisms.

Consensus

Participants reached consensus on the following conclusions:

- The hazard for potential impacts of the EO-1α Salmon through interactions with other organisms was concluded to be high hazard with moderate uncertainty.

4. Potential impacts through hybridization

4.1 Hybridization with Atlantic Salmon

Impacts from the hybridization of domestic genotype with wild Atlantic Salmon populations are poorly understood, but include decreased productivity of wild populations due to lowered fitness, and increased stray rate of hybrid offspring. Studies in other models suggest the EO-1α Salmon could reproduce with wild populations, with potentially long-term evolutionary-scale impacts from introduction of the growth hormone transgene. The presentation concluded there is high hazard to wild Atlantic Salmon populations through hybridization with moderate uncertainty due to no relevant data on the EO-1α Salmon, and lack of data on effects over multiple generations in nature in comparator models.

Discussion

There were discussions on whether the uncertainty should be lowered since it is known that hybridization with Atlantic Salmon can occur. It was clarified there is still a lack of understanding and knowledge of the harmful effects of the EO-1α Salmon transgene on wild Atlantic Salmon through hybridization.

Consensus

Participants reached consensus on the following conclusion:

- The assessment concluded with moderate uncertainty that the EO-1α Salmon represented a high potential for hazards via gene transfer through hybridization with Atlantic Salmon.

4.2 Hybridization with other species

Atlantic Salmon is known to hybridize naturally with Brown Trout and it has been demonstrated that the EO-1α Salmon x Brown Trout hybrids can express the opAFP-GHc2 transgene. In artificial streams, presence of hybrids (transgenic and non-transgenic combined) greatly decreased growth of both transgenic and non-transgenic Atlantic Salmon. Consequently, both types of hybrid offspring may negatively impact wild Atlantic Salmon in the same niches. There is moderate hazard through hybridization with other species and a moderate uncertainty associated with the ranking due to the inability to separate potential impacts of EO-1α transgenic versus non-transgenic hybrids, and limited data regarding hazards from interspecific hybridization.

Discussion

There were no comments to the presentation content or conclusions.

Consensus

Participants reached consensus on the following conclusion:

- Participants concurred with the moderate hazard of the potential impacts of hybridization of the EO-1α Salmon with other species with a moderate uncertainty.

5. Potential to act as a vector of disease agents

It was proposed that a land-based facility, with 97% recirculation and UV/ozone treatment, has a lower potential of being a source of pathogens to natural populations, relative to typical net-pen aquaculture. The EO-1α Salmon could impact wild fish if the organism acts as a reservoir in the environment for naturally occurring diseases. Though relative disease susceptibility of EO-1α Salmon has not been formally examined, preliminary work indicates no consistent differences from wild-type. Given the expected health profile of escaped fish and lack of alterations in disease susceptibility of EO-1α Salmon, a negligible hazard to cause harm as a vector of disease was suggested. There is a high uncertainty associated with the hazard ranking due to lack of studies examining vector capabilities in EO-1α Salmon, and a limited understanding of how data from other models can be applied.

Discussion

Discussion focused primarily on effluent, and the potential for the release of pathogens into Rollo Bay Brook. It was pointed out that the UV and ozone treatment do not completely sterilize the water. Though other models demonstrate GH transgenesis decreases immune function, which would likely impact vector capabilities, the potential harmful effects are unknown. Consequently, it was suggested the hazard ranking for vector capabilities should be low.

Consensus

- The hazard rating on potential of the EO-1α Salmon to act as a vector of disease was concluded to be low with high uncertainty.

6. Potential to impact biogeochemical cycling

The potential effects of EO-1α Salmon on nutrient cycling has not been examined. The sterilized AquAdvantage® Salmon is unlikely to return to spawn and could be a net exporter of nutrients from freshwater, but this would only have an impact on Rollo Bay Brook. If EO-1α Salmon were to impact wild Atlantic Salmon through other hazard pathways, it could indirectly effect the role of wild salmon in nutrient cycling. However, in Eastern Canada, Atlantic Salmon likely have a limited role in biogeochemical cycling due to poor returns. Therefore, a negligible hazard of the EO-1α Salmon through impacts to biogeochemical cycling was postulated, with a moderate uncertainty due to a limited understanding of the role Atlantic Salmon play in nutrient cycles in Canada, and the potential effects of EO-1α Salmon on wild population densities.

Discussion

It was noted the current returns of the Atlantic Salmon should not be used as a baseline due to the possibility they contributed significant nutrients to the system in the past.

Consensus

- There was consensus of a negligible hazard of EO-1α salmon through impacts to biogeochemical cycling. There is moderate uncertainty with the caveat that current returns of the Atlantic Salmon should not be used a baseline comparison.

7. Potential to impact habitat

The EO-1α Salmon could potentially influence habitat structure on a local level if they establish a reproducing population that spawn at significant densities relative to spawn area, or decrease wild populations that significantly contribute to habitat structure. The presentation concluded there is a low hazard to habitat structure from EO-1α Salmon and high uncertainty due to lack of information on spawning behaviour of EO-1α females and the role of Atlantic Salmon on habitat structure in Eastern Canada.

Discussion

The uncertainty associated with the hazard of the EO-1α Salmon's potential to impact habitat should be changed to moderate since there is information available on the decreased level of redd digging in transgenic fish. As redd digging is a form of ecosystem engineering, this could potentially impact the ecosystem at a local level though the effects may not be harmful.

Consensus

- The hazard ranking on potential of the EO-1α Salmon to impact habitat is low with moderate uncertainty.

8. Potential to affect biodiversity

The potential pathways through which EO-1α Salmon could influence biodiversity were presented. Genetic alteration through introgression and hybridization was ranked moderate to high, while the competitive exclusion or displacement of other fish species and changes in species composition due to EO-1α Salmon feeding behaviour ranked high. The transfer of disease was ranked low, and changes to nutrient cycling that may alter food-web dynamics and

community biodiversity was ranked negligible to low. These ratings indicate the mostly likely pathways to affect biodiversity is through genetic and competitive interactions with wild fish populations. Overall the presentation concluded on moderate hazard to biodiversity with high uncertainty as only one study is known to examine the impacts of GH transgenic fish on community dynamics. Even in well-studied models such as the domesticated Atlantic salmon, effects on overall biodiversity are still poorly understood.

Discussion

There were discussions as to whether the overall rating should be left inconclusive. However, given there are ecological models looking at the potential of transgenic to affect biodiversity, the potential pathways and information presented here should be adequate to form a decision.

Consensus

- The hazard potential of the EO-1α Salmon to affect biodiversity was concluded to be moderate with high uncertainty.

Summary Discussion and Consensus on Environmental Hazards

The hazard assessment for the previous notification for AquAdvantage® Salmon made an overall conclusion on risk. It was put forward that individual hazard assessment components should be kept separate in the current assessment when concluding on risk. It is important to articulate to regulators ratings and uncertainty associated with hazard and exposure assessments. As well, exposure routes may be different in certain hazards (i.e., hazards through HGT or as a vector of disease may not require escape of EO-1α Salmon to pose risk). Hazard ratings align with the previous notification assessment for AquAdvantage® Salmon, except for where conclusions were made in the current assessment but not the previous assessment (as a vector or disease and to biodiversity), and uncertainty decreased in a few pathways where additional information has been made available since the last assessment. There is uncertainty in some hazard ratings, where rating level may be context specific. In these cases the highest conceivable rating was used.

Discussion and Consensus

There were discussions on the differences between the previous and current assessment, and it was clarified that anywhere the two assessments differed, the justification for this should be well outlined. The consensus was to leave hazard ratings separate and to not make a single final conclusion on risk.

ENVIRONMENTAL RISK ASSESSMENT

Presenter: Rosalind Leggatt, Fisheries and Oceans Canada

The “Environmental Risk Assessment” presentation addressed the exposure outcome, the environmental hazard outcomes, and concluded on the environmental risk for both proposed Scenarios. Under Scenario A, non-transgenic fish for external parties are produced alongside transgenic fish. Under Scenario B, no non-transgenic fish are produced at the facility for external parties.

The exposure assessment concluded that, for the notified and other potential activities, exposure of the EO-1α Salmon to the environment is expected to be negligible to low, depending on the use scenario. Potential environmental hazards were assessed for eight points (toxicity, horizontal gene transfer, gene transfer through hybridization, interactions with other organisms, vectors of disease agents, biogeochemical cycling, habitat and biodiversity). The

potential hazards of the EO-1α Salmon to the assessment endpoints were concluded to range from negligible to high. Risk was assessed for each scenario.

Discussion

Discussions focused on clearly formulating the two proposed scenarios, to address concern over the possible mix up of non-transgenic and transgenic eggs if they were sold to external parties.

It was agreed that any changes to containment, or expansion of the production facility could change the outcome of the assessment and would require additional information be provided to ECCC by the company.

Proposed mitigation measures to reduce the potential of mixing up the transgenic and non-transgenic eggs under Scenario A were discussed and are included in the Science Advisory Report.

Consensus

- Based on the exposure and hazard assessments, and previous discussions, the overall environmental risk associated with the manufacture and production of EO-1α Salmon at a facility near Rollo Bay, PEI was concluded to be negligible to moderate depending on the proposed use scenario and hazard pathway.
- Under Scenario A, where non-transgenic eggs are sold to external parties from the Rollo Bay facility, the EO-1α Salmon pose Low to Moderate Risk to Canadian environments.
- Under Scenario B, where non-transgenic eggs are for internal use only, the EO-1α Salmon pose Negligible to Low Risk to Canadian environments.

FINAL CONCLUSIONS ON RISK ASSESSMENT

Reviewers reached consensus and concluded on risk to the environment and indirect human health through two proposed use scenarios. Under Scenario A, where non-transgenic fertilized eggs will be produced for external parties, the potential for human error in shipping eggs increases potential exposure, therefore results in low to moderate risk of EO-1α Salmon to Canadian environments. An alternate use scenario (Scenario B) where no non-transgenic eggs are sold to external parties would result in negligible to low risk of EO-1α Salmon to Canadian environments. A final conclusion of low risk to indirect human health of Canadians was reached under both Scenario A and B.

Mitigation measures were proposed that could further reduce exposure and risk of Scenario A. There was consensus that the exposure rating could be reduced with these mitigation measures, but there was not consensus on whether the exposure could be reduced to negligible. Any changes to containment or expansion of the manufacture and grow-out facilities could change the outcome of the environmental risk and indirect human health assessments and could require additional information to be provided to ECCC.

APPENDIX 1: TERMS OF REFERENCE

Environmental and Indirect Human Health Risk Assessment for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI

National Peer Review – National Capital Region

December 11-13, 2018

Ottawa, Ontario

Chairperson: Gilles Olivier

Context

The *Canadian Environmental Protection Act*, 1999 (CEPA 1999), administered by Environment and Climate Change Canada (ECCC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including living organisms, are assessed for their potential harm to the environment and human health. The New Substances Notification Regulations (*Organisms*) [NSNR (Organisms)] under CEPA 1999 prescribe the information that must be provided to ECCC prior to the import to or manufacture in Canada of new living organisms that are animate products of biotechnology, including fish products of biotechnology.

ECCC and HC are responsible for conducting the **CEPA risk assessment** to evaluate whether the notified fish product of biotechnology is "CEPA toxic" in accordance with Section 64 of CEPA 1999: where a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Fisheries and Oceans Canada (DFO), ECCC and HC signed a Memorandum of Understanding respecting the implementation of the NSNR (Organisms) for new living fish products of biotechnology. DFO assists in implementing the NSNR (Organisms) by providing science advice based on an environmental risk assessment for fish products of biotechnology, and, supports HC, on the indirect human health risk assessment for fish products of biotechnology. In addition, DFO will recommend any necessary measures to manage risks, if required.

Based on the environmental and indirect human health risk assessments, DFO provides science advice to ECCC and HC in support of their CEPA risk assessment and decision making process for products of biotechnology that have been notified under the NSNR (Organisms).

In 2013, AquaBounty Canada submitted a notification to ECCC detailing its intent to commercially produce genetically-modified (GM) Atlantic salmon in Canada in a contained facility. The proposed production scenario consisted of egg production and broodstock maintenance at a land-based and contained facility near Fortune, PEI, and commercial grow-out

at a land-based and contained facility in Panama. Under the well-defined containment conditions proposed by AquaBounty, Fisheries and Oceans Canada determined that the AquAdvantage® Salmon poses low risk to the Canadian environment and indirect human health (DFO 2013).

On July 27 of 2018, AquaBounty Canada submitted to ECCC, a notification of its intent to manufacture the AquAdvantage® Salmon at a second facility near Rollo Bay, PEI, under contained conditions. Commercial grow-out of sterilized AquAdvantage® Salmon at the same facility, and under the same conditions of containment, is also proposed.

Objective

The objective of this Science Advisory Process is to peer-review the draft environmental and indirect human health risk assessments for the manufacture and production of sterile AquAdvantage® Salmon at a land-based and contained facility near Rollo Bay, PEI, and provide science advice and recommendations to manage risk taking into account the previous Science advice for the first proposed facility near Fortune, Bay PEI (DFO, 2013).

Working papers to be reviewed will include:

- Environmental Risk Assessment for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based Facility near Rollo Bay, PEI; and
- Indirect Human Health Risk Assessment Report on AquAdvantage® Salmon.

The DFO environmental risk assessment will include consideration of potential risks to fish, fish habitat and the environment in general. The HC indirect human health risk assessment will not consider potential risks related to human consumption, but will consider potential risks from environmental exposures to the living organism such as risks from exposure to toxins and allergens and the transmission of zoonotic diseases.

The Science Advisory Process will evaluate the conclusions, rankings, and recommendations of the draft risk assessments, taking account of the weight of scientific evidence, quality of data, identified knowledge gaps and uncertainties associated with the:

- Characterization of the AquAdvantage® Salmon;
- Environmental exposure: characterization and assessment including an assessment of any request for a possible waiver;
- Indirect human health exposure: characterization and assessment;
- Environmental hazard: characterization and assessment;
- Indirect human health hazard: characterization and assessment;
- Environmental risk assessment; and
- Indirect human health risk assessment.

Expected Publications

- Science Advisory Report
- Research Document(s)
- Proceedings

The publications will be subject to third party confidential business information claims by the regulatory proponent and nondisclosure requirements in accordance with the *Access to Information Act* and the *Canadian Environmental Protection Act,* 1999.

Expected Participation

- Fisheries and Oceans Canada (Ecosystems and Oceans Science Sector; Pacific Region; Central and Arctic Region, Gulf Region, Newfoundland and Labrador Region; Aquaculture Management-Gulf Region)
- Environment and Climate Change Canada and Health Canada
- Province of Prince Edward Island
- Academia
- Other invited experts

References

DFO. 2013. Summary of the Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon. DFO Can. Sci. Advis. Sec. Sci. Resp. 2013/023.

APPENDIX 2: AGENDA

Agenda of the CSAS Science National Peer-Review Process
Environmental and Indirect Human Health Risk Assessment for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI

December 11th -13th, 2018
Delta Hotel By Marriot Ottawa City Centre
101 Lyon Street North
Ottawa, ON

DAY 1 – TUESDAY, DECEMBER 11

8:30 - 8:40	Welcome and introductions (*Gilles Olivier)*
8:45 – 8:55	Introduction to CSAS Science National Peer-Review Process (*Gilles Olivier)*
9:00 – 9:15	Context: Regulatory, risk assessment, proposed use *(Sherry Walker)*
9:15 – 10:00	Public Transparency Notice: summary of public comments *(Marie Breton)*
10:00-10:15	BREAK
10:15-10:30	Characterization of the receiving environment *(Colin McGowan)*
10:30 - 11:00	Characterization of AquAdvantage® *(Rosalind Leggatt)*
11:00- 12:00	Indirect human health exposure assessment *(Kassim Ali)*
12:00 – 1:00	LUNCH
1:00– 1:30	Consensus: Indirect human health exposure assessment *(All)*
1:30 – 2:15	Indirect human health hazard assessment *(Stephen Dugan)*
2:15 – 2:45	Consensus: Indirect human health hazard assessment *(All)*
2:45 – 3:00	BREAK
3:00 – 3:45	Indirect human health risk assessment *(Kassim Ali)*
3:45- 4:15	Consensus: Indirect human health risk assessment *(All)*
4:15- 4:30	Summary of Day 1 and adjournment *(Gilles Olivier)*

DAY 2 – WEDNESDAY, DECEMBER 12

8:30 – 8:45	Review and summary of conclusions so far *(Gilles Olivier)*
8:45 – 9:15	Waiver Assessment *(Colin McGowan)*
9:15 – 10:30	Environmental exposure assessment *(Colin McGowan)*
10:30 – 10:45	BREAK
10:45 – 11:15	Consensus: Environmental exposure assessment *(All)*
11:15 - 12:00	Environmental hazard assessment *(Rosalind Leggatt)*
12:00 - 1:00	LUNCH
1:00 – 1:30	Consensus: Environmental hazard assessment *(All)*
1:30 – 2:15	Environmental risk assessment *(Colin McGowan, Rosalind Leggatt)*
2:15 – 2:30	BREAK
2:30 – 3:00	Consensus: Environmental risk assessment *(All)*
3:00-3:45	Consideration of public comments
3:45-4:00	Summary of Day 2 and adjournment *(Gilles Olivier)*

DAY 3 – THURSDAY, DECEMBER 13

8:30 – 8:45	Review and summary of conclusions so far *(Gilles Olivier)*
8:45 – 9:30	Proposed risk management measures, if needed *(Colin McGowan)*
9:30 – 10:30	Science Advisory Report development *(All)*
10:30 – 10:45	BREAK
10:45 – 12:00	Science Advisory Report development *continued (All)*
12:00 - 1:00	LUNCH
1:00 - 2:30	Science Advisory Report development *continued (All)*
2:30 -3:00	Final Consensus *(All)*
3:00– 3:15	Conclusions and adjournment *(Gilles Olivier)*
3:15	**END OF MEETING**

APPENDIX 3: MEETING PARTICIPANTS

Table 1. Participants of the CSAS Science National Peer-Review Process *Environmental and Indirect Human Health Risk Assessments for the Manufacture and Production of Sterile AquAdvantage® Salmon at a Land-Based and Contained Facility near Rollo Bay, PEI.*

Name	**Affiliation**
Ali, Kassim	Health Canada
Arvanitakis, George	Health Canada
Baillie, Shauna	Fisheries and Oceans Canada
Bradbury , Ian	Fisheries and Oceans Canada
Breton, Marie	Environment and Climate Change Canada
Breau, Cindy	Fisheries and Oceans Canada
Byrne, Philip	Fisheries and Oceans Canada
Carr, Jonathon	Atlantic Salmon Federation
Devlin, Bob	Fisheries and Oceans Canada
Dugan, Stephen	Health Canada
Fleming, Ian	Memorial University, St. John’s, NL
Hard, Jeff	National Oceanic and Atmospheric Administration (NOAA), USA
Leggatt, Rosalind	Fisheries and Oceans Canada
Lortie, Michel	Environment and Climate Change Canada
Louter , Jim	Environment and Climate Change Canada
MacNair, Neil	Province of Prince Edward Island
McGowan, Colin	Fisheries and Oceans Canada
McKay, Stephanie	University of Ottawa, Ottawa, ON
Mills, Chris	Fisheries and Oceans Canada
Olivier, Gilles	Fisheries and Oceans Canada
Parsons, Jay	Fisheries and Oceans Canada
Saikali, Zeina	Environment and Climate Change Canada
Siboo, Ian	Environment and Climate Change Canada
Walker, Sherry	Fisheries and Oceans Canada
Weber, Lily	Fisheries and Oceans Canada
Winterborn, Andrew	Queen’s University, Kingston, ON