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# Proceedings of the National Science Response Process for the Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon

**July 17-19, 2013**
**Ottawa, Ontario**

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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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## SUMMARY

In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), Environment Canada, and Health Canada, DFO is responsible for conducting the environmental and indirect human health risk assessments of fish products of biotechnology in support of the *New Substances Notification Regulations (Organisms)* [NSNR(O)] under the *Canadian Environmental Protection Act, 1999* (CEPA 1999). On April 30, 2013, a regulatory notification was submitted to Environment Canada by AquaBounty Canada Inc. for the production of eggs for AquAdvantage® Salmon (AAS), a genetically-modified salmon, at a facility in Prince Edward Island, Canada. The company planned to ship the eggs to a facility in Panama, where the fish would be raised. DFO conducted environmental and indirect human health risk assessments of AAS, and developed recommendations to support a regulatory decision by the Minister of the Environment. The purpose of the Science Response Process was to undertake a national peer review of the risk assessment and develop scientific consensus on the risk assessment outcomes and recommendations to be provided to Environment Canada. The National Science Response Meeting was held July 17-19, 2013 in Ottawa, Ontario. The terms of reference and agenda for this process are found in Appendix 1 and 2, respectively. Participants included scientists from Fisheries and Oceans Canada, Environment Canada and Health Canada, Provincial Government, academia and other invited experts (Appendix 3). Documents resulting from this peer review process include the Science Response Report (DFO 2013) and these proceedings.

## SOMMAIRE

Conformément à un protocole d'entente conclu entre Pêches et Océans Canada (MPO), Environnement Canada et Santé Canada, Pêches et Océans Canada est chargé de réaliser des évaluations des risques pour l'environnement et des risques indirects pour la santé humaine posés par les produits du poisson issus de la biotechnologie, à l'appui du *Règlement sur les renseignements concernant les substances nouvelles (organismes)* [RRSN(O)] en vertu de la *Loi canadienne sur la protection de l'environnement, 1999* (LCPE 1999). Le 30 avril 2013, AquaBounty Canada Inc. a soumis un avis réglementaire à Environnement Canada concernant la production d'œufs de saumon AquAdvantage® (SAA), un saumon génétiquement modifié, dans une installation de l'Île-du-Prince-Édouard, au Canada. L'entreprise prévoyait expédier les œufs à une installation au Panama, où les poissons seraient élevés. Le MPO a effectué des évaluations des risques pour l'environnement et des risques indirects pour la santé humaine posés par le SAA, et a formulé des recommandations pour appuyer la décision réglementaire prise par le ministre de l'Environnement. Le processus de réponse des Sciences visait à entreprendre un examen national par les pairs de ces évaluations des risques et à parvenir à un consensus scientifique sur les résultats de l'évaluation des risques et les recommandations à présenter à Environnement Canada. La réunion nationale consacrée à la réponse des Sciences s'est tenue du 17 au 19 juillet 2013 à Ottawa (Ontario). Le cadre de référence et l'ordre du jour de ce processus sont décrits aux annexes 1 et 2, respectivement. Parmi les participants, on comptait des scientifiques de Pêches et Océans Canada, d'Environnement Canada et de Santé Canada, du gouvernement provincial, du milieu universitaire et d'autres experts invités (annexe 3). Les documents découlant de ce processus d'examen par les pairs sont notamment le Rapport de réponse des Sciences (MPO 2013) et le présent compte rendu.

## INTRODUCTION

On April 30, 2013, AquaBounty Canada Inc. submitted a regulatory package to Environment Canada (EC) under the *New Substances Notification Regulations (Organisms)* [NSNR (Organisms)] of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) for the AquAdvantage® Salmon (AAS). In accordance with a Memorandum of Understanding between Fisheries and Oceans Canada (DFO), EC, and Health Canada (HC), DFO conducted the environmental and indirect human health risk assessment for AAS and developed recommendations to support a regulatory decision by the Minister of the Environment.

The national Science Response process included participants with relevant expertise to review and discuss the draft "Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon" (herein after referred to as risk assessments) prepared by DFO. Participants were provided with the draft risk assessment in advance, and contributed comments for consideration prior to the meeting. Subsequently, a face-to-face “National Science Response Process for the Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon” meeting was held July 17-19, 2013 in Ottawa, Ontario. Discussion at this meeting included the main components of the draft risk assessment including exposure assessment, waiver assessment, indirect human health hazard assessment, environmental hazard assessment, environmental and indirect human health risk assessments, uncertainty, and proposed measures. For the duration of the meeting, participants were invited to focus on comments that could change outcomes of the assessment. Consensus was reached on a draft Science Response entitled “Summary of the Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon”.

## NATIONAL SCIENCE RESPONSE PROCESS

The Canadian Science Advisory Secretary (CSAS) participant and rapporteur, Erika Thorliefson (DFO – National Capital Region) provided an overview of the CSAS, outlining the Secretariat, the CSAS Science Response process, role of participants, definition of consensus for CSAS processes, and ground rules for the meeting.

CSAS provides oversight and direction for the Science Response and provision of science advice in support of Fisheries and Oceans Canada management 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 to the CSAS process is by invitation to those with the expertise, knowledge and experience on the subject matter. Scientific working paper(s) and other inputs are subject to rigorous review and quality control of all inputs, analyses, findings and recommendations in a peer forum. Final Scientific Advisory and Response Reports, Research Documents and Proceedings are published on the 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 appropriateness and application of the data and methods. It was noted that participants should work toward developing consensus on the conclusions, recommendations, and advice. Economic and social considerations are not to bear on the conclusions and advice.

In response to questions from participants, the following clarifications were provided:

- The objective of the meeting was to review the draft Science Response entitled “Summary of the Environmental and Indirect Human Health Risk Assessment of AquAdvantage®

Salmon" based on the draft "Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon". Key text in the draft summary document was bolded and was to be used as the basis of the meeting presentations and discussion that followed each presentation. Written editorial comments on the draft risk assessment document were to be incorporated once the Science Response was completed.

- The National Science Response Process and meeting were confidential, because of the need to protect third-party scientific, technical and business information included in the proponent's notification under the *Canadian Environmental Protection Act* and *Access to Information Act*.
- Confidential information of the Crown was to be protected until a regulatory decision was made by the Minister of the Environment, which was anticipated for August 27, 2013, whereas confidential business information of the proponent would need to be protected in perpetuity.
- Recognizing the need to maximize openness and transparency in the science advisory process, it was reported that DFO intended to work with EC to ensure that the Science Response was published on the Canadian Science Advisory Secretariat website (or alternatively on EC's website). The proponent would be provided with an opportunity to identify any confidential information in the Science Response that would need to be protected in advance of its posting, consistent with the Government of Canada's obligation to protect confidential third party information under the *Access to Information Act*.
- Socio-economic considerations are not incorporated into EC's science-based decision making process to determine whether an organism is "CEPA toxic".
- The completed Science Response was to be submitted to EC on August 13, 2013. The Science Response was to be revised during the course of the meeting, with additional comments from the Science Response that were to be incorporated shortly afterward. The Science Response was then to be sent to participants for review, with a short turn-around time, and then finalized and submitted to EC.

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

The "Regulatory Context, Risk Assessment Process, and Proposed Use Scenario", presented by Jan Beardall (DFO - National Capital Region), addressed the legislative and regulatory context under which the risk assessment was conducted, the risk assessment process itself, and the translation of the risk assessment findings into a recommendation for a regulatory decision given the proposed use scenario for the AquAdvantage® Salmon.

The risk assessment was conducted under the *Canadian Environmental Protection Act, 1999* (CEPA), an Act to prevent pollution, protect the environment and human health, and contribute to sustainable development. More precisely, the risk assessment was conducted under the biotechnology provisions of CEPA (Part 6), which require that all new living organism products of biotechnology, including genetically-engineered (GE) fish, be notified and assessed prior to manufacture or import in Canada, to determine whether they are "CEPA toxic" or capable of becoming "CEPA toxic". Under CEPA, a living organism may be considered "CEPA toxic" if it is entering or may be entering the environment in a quantity or concentration or under conditions that:

a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;

b) constitute or may constitute a danger to the environment on which life depends; or

c) constitute or may constitute a danger in Canada to human life or health.

The *New Substances Notification Regulations (Organisms)* [NSNR(O)] specify the information requirements and timelines for the notification of manufacture or import of living organisms that are animate products of biotechnology. In some cases, a waiver may be requested for specific information requirements. For instance, if the organism is to be manufactured at a location where the proponent requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health, a waiver for some information requirements may be granted.

DFO is responsible for conducting the environmental risk assessment and the indirect human health risk assessment for fish products of biotechnology and to provide recommendations to EC, who retains ultimate authority for regulatory decision-making. The outcome of the risk assessment process is used for deciding whether the organism is “CEPA toxic” (prohibitions or condition(s) may be imposed; or additional information may be requested), not “CEPA toxic” (manufacture may proceed as proposed), or not “CEPA toxic” as proposed, but could become “CEPA toxic” as a result of a significant new activity. The latter case may lead EC to impose a significant new activity notice (SNAc). A final decision must be rendered within a 120-day legislative timeframe.

The risk assessments were conducted under the classic paradigm where:

Risk = Hazard X Exposure

For each endpoint, hazard and exposure were ranked as: negligible (1), low (2), moderate (3), or high (4). Uncertainty associated with either the exposure and hazard assessments was ranked as highly certain, reasonably certain, reasonably uncertain, or highly uncertain. Risk was estimated by plotting hazard on the X axis and exposure on the Y axis of a two-dimensional risk matrix, for which the increasing numerical value within each cell indicates an increasing level of risk (see Figure 1). Uncertainty associated with both the exposure and hazard assessments was assigned to the final risk assessments. If uncertainty varied between hazard and exposure, the greatest level of uncertainty between the two was allocated to the final assessment of risk.

Conclusions of the environmental and the indirect human health risk assessment would provide the basis for recommendations to EC for consideration in regulatory decision-making by the Minister of the Environment. A recommendation of “CEPA toxic” would be provided if the risk assessments concluded that the risks attributed to the AquAdvantage® Salmon were moderate or high. A recommendation of not “CEPA toxic” would be made if the assessments concluded that the risks attributed to the AquAdvantage® Salmon were negligible or, with reasonable certainty, low. However, greater uncertainty could be tolerated if the outcome of either the hazard or the exposure assessment was negligible (*see Assessment Outcomes*).

The risk assessment was case-specific, in that they were limited to the company’s proposed use scenario of commercial production at the AquaBounty Canada facility in Prince Edward Island **(**PEI), as described in AquaBounty’s notification. The company’s intent was to commercially produce all-female triploid (sterile), transgenic AquAdvantage® Atlantic Salmon (AAS) eyed-eggs at their PEI facility, and export 100,000 eggs annually to a contained, land-based, grow-out facility in the highlands of Panama. There, the fish would be grown to market weight, harvested, euthanized and processed. Pending its approval by the United States Food and Drug Administration (US FDA), the processed fish would be shipped into the United States and sold

as Atlantic Salmon. Only sterile (triploid) female Atlantic Salmon eyed-eggs bearing one copy of the opAFP-GHc2 construct at the EO-1α locus would be exported to Panama. Other life-stages (gametes through to sexually mature adults), genotypes (i.e. diploids, triploids, hemizygotes, homozygotes) and genders (females and masculinized females), that are required for the production of the AAS eyed-eggs, would remain under the described physical containment at the AquaBounty Canada facility in PEI. Finally, sterile AAS eggs would be produced using milt from homozygous masculinized AAS females (neomales), and eggs from non-transgenic Atlantic Salmon females that are derived from the domesticated Saint John River strain.

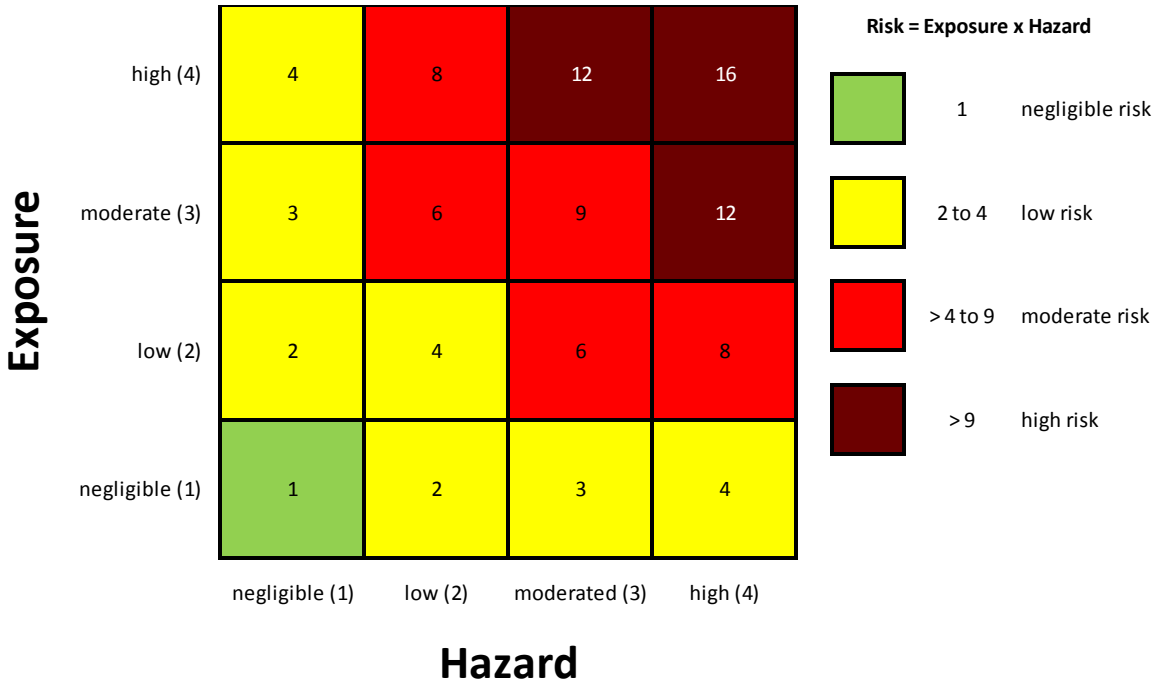


*Figure 1. Risk matrix used in the integration of exposure and hazard to establish an overall estimate of risk.*

Physical containment would be as efficacious as described in AquaBounty's notification. All life-stages of AAS at the AquaBounty Canada facility in PEI, the AquaBounty Panama facility, and while in transport between the two locations, would remain under the singular and direct control of AquaBounty Technologies. The company also specified that the current efficacy of sterilization procedures would be maintained.

In response to questions from participants, the following clarifications were provided:

- The scope of the proposal is relatively small in comparison to the estimated number of eggs that are produced at the AquaBounty PEI facility annually. Surplus eggs are used for ongoing research and development activities or disposed of as appropriate.
- The ongoing regulatory process for AquAdvantage® Salmon in the United States, which also included a peer review, is completely separate from the regulatory process under CEPA and the NSNR(O).

## CHARACTERIZATION OF THE AQUADVANTAGE® SALMON

The “Characterization of the AquAdvantage® Salmon”, presented by Caroline Mimeault (DFO – National Capital Region), addressed the molecular structure and function of the transgene, additional modifications, and the biological and ecological properties of the AquAdvantage® Salmon.

The molecular characterization reviewed the 6721 bp opAFP-GHc2 construct, which includes the 2660 bp plasmid vector and the 4061 bp transgene insert. The transgene was reported to contain the appropriate regulatory sequences, including a functional promoter, and a coding region for expression of a mature growth hormone (GH) protein, as confirmed by DNA sequencing. The construct was assembled through standard molecular biology techniques and did not include toxic sequence or mobile genetic elements. It was concluded that the transgene construct was unlikely to cause any harm to the environment or indirect human health.

Integration of the transgene into the genome of several wild Atlantic salmon was achieved by micro-injection of digested construct through the micropyle of recently fertilized eggs. Two transgene integrants (α and β) were detected in the EO-1 founder female and its F1 progeny. Careful examination of pedigreed descendant genotypes confirmed the successful elimination of the β-integrant from the EO-1 line through selective breeding.

Sequence analysis confirmed that the integrated transgene is Chinook GH-1, while a Basic Local Alignment Sequence Tool (BLAST) analysis showed no sequence homology between the transgene and other known biologically active proteins. Further, Chinook GH-1 is not known to cause toxicity in humans or animals.

The DNA sequence of regions flanking the integration site suggested that no endogenous genes were disrupted. The additional short sequence derived from the vector multiple-cloning site is unlikely to cause a toxic or allergic response in humans as it is non-coding and would not produce a protein product.

Concerns regarding the simultaneous micro-injection of transgene and vector into the eggs were mostly alleviated by Southern blot analysis and multiplex polymerase chain reaction analyses, which failed to detect any additional vector sequence or ampicillin resistance genes in the genome of the EO-1α line. It was concluded that inheritance and stability of the opAFP-GHc2 transgene at the EO-1α locus was adequately demonstrated by the company.

Gynogenesis is not a part of the production process and was used only once, when first establishing a female only broodstock. Consequently, it is unlikely to cause any harm to the environment or to indirect human health. The company’s ability to maintain female gender under the rearing conditions at the PEI facility was satisfactorily demonstrated. The processes and outcomes of sex reversal and induced triploidy were also accepted as non-hazardous.

The presentation focused mainly on growth hormone (GH) levels and growth rates, though other traits were reviewed in the risk assessment document. The GH profile for AAS was considered incomplete since plasma GH levels were reported only for transgenic Atlantic salmon juveniles that were the result of independent injection events. Growth hormone concentrations in tissue-skin samples of adult AAS were detected using current standard techniques and reported to be below the limit of detection. Enhanced growth rates relative to non-transgenic controls were reported to be consistently observed under hatchery conditions, however, growth in salmonids tends to be plastic, and uncertainty remains regarding growth rates of AAS in the natural environment.

## DISCUSSION

Discussion after the presentation identified the possibility that insertion of the transgene in certain regions of the genome may have the potential to alter locus structure, but only over evolutionary timeframes. This did not alter the conclusion that the opAFP-GHc2 transgene is stable at the EO-1α locus in AAS.

Some participants noted that although the female genotype may be biologically stable, some uncertainty exists with respect to the long term stability of female only broodstock, if husbandry conditions were modified.

It was clarified that, in addition to the information provided by the company regarding containment, the research and development facility in PEI was previously inspected by EC under the authority of CEPA. EC is responsible for enforcing the regulations. DFO consulted with EC inspectors and had access to past inspection reports. DFO has also previously conducted inspections of the facility under the *Fish Health Protection Regulations*. It was also clarified that, under section 106(11) of CEPA, the company must notify the government of any new information, or corrections to information, as soon as possible after learning of them.

Participants expressed concerns about the selection process for fish included in the morphological irregularities study conducted by the proponent. Since both captive and transgenic fish tend to have morphological irregularities, participants questioned whether the selected fish were an appropriate representation of production fish.

Participants recommended several minor changes to the text of the Science Response Report and the Risk Assessment, primarily to clarify the life-stages of reported phenotypes. In the absence of data on AAS, the phenotypic characterisation relied on data gathered on suitable surrogates, of which Atlantic Salmon microinjected with the opAFC-GHc2 construct (AAS relatives), were the most appropriate.

## CONCLUSIONS

Participants reached consensus on the following conclusions:

- The nature of the transgene construct is unlikely to cause any harm to the environment or to indirect human health;
- The nature of the transgene integrant at the EO-1α locus is unlikely to cause any harm to the environment or to indirect human health;
- Mendelian inheritance and molecular stability of the opAFP-GHc2 at the EO-1α locus have been adequately demonstrated;
- The gynogenesis process used in the early development of the broodstock is unlikely to cause any harm to the environment or indirect human health;
- Successful generation and maintenance of an all-female broodstock population through gynogenesis and hormonal sex-reversal at the PEI facility have been adequately demonstrated;
- Sex-reversal of AAS is unlikely to cause any harm to the environment or to indirect human health;
- Induced triploidy is unlikely to cause any harm to the environment or to indirect human health;

- Functional sterility in most of the treated individuals (with 0.5 to 2% failure) is accepted as a biological containment strategy; and
- Though growth rates of AAS are expected to be limited in many circumstances, it cannot be concluded that AAS would never express growth rates in the wild that provide a fitness advantage relative to wild Atlantic Salmon in the natural environment.

## EXPOSURE: CHARACTERIZATION AND ASSESSMENT

The “Exposure Characterization and Assessment of the AquAdvantage® Salmon”, presented by Colin McGowan (DFO – National Capital Region), addressed the rankings used for exposure and associated uncertainties, physical containment at the Canadian and Panamanian facilities, and potential fate of AAS in the environment.

Exposure ranking used for both the environmental and human health risk assessment was described as the following:

- **negligible,** if AAS was not expected to be present in the Canadian environment (i.e., there would be no entry or no survival at point of entry);
- **low,** if AAS was expected to enter in very low numbers and survive in the Canadian environment, but are not expected to reproduce (i.e., low-level, single generation presence);
- **moderate,** if AAS was expected to enter in significant numbers and survive in the Canadian environment, but was not expected to reproduce (i.e., significant single generation presence); or
- **high,** if AAS was expected to reproduce, establish, or spread within the Canadian environment (i.e., established presence).

Uncertainty related to the physical containment was ranked as follows:

- **highly certain,** when detailed information on facility design, containment structures, water treatment equipment, standard operating procedures (SOPs), internal compliance documentation, facility incident reports, and inspection reports are available, and long-term, reliable historical data on relevant chance events at or near the location of each facility are available;
- **reasonably certain,** when detailed information on facility design, containment structures, water treatment equipment, and SOPs are available, and historical data on relevant chance events in the region of each facility are available;
- **reasonably uncertain,** when information on facility design, containment structures, water treatment equipment is available; and
- **highly uncertain,** when limited information of facility design, containment structures, and water treatment equipment is available.

Uncertainty related to the fate of the organisms was ranked as follows:

- **highly certain,** when high quality data on AAS, data on environmental parameters of the receiving environment and at the point of entry, absence of gene x environment (GxE) effects or a complete understanding of GxE effects across relevant environmental conditions or evidence of low variability are available;

- **reasonably certain,** when high quality data on AAS-relatives or valid surrogate, data on environmental parameters of the receiving environment, understanding of potential GxE effects across relevant environmental conditions or evidence of some variability are available;
- **reasonably uncertain,** when limited data on AAS, AAS-relatives or valid surrogates, limited data on environmental parameters in the receiving environment are available and when knowledge gaps had been identified and reliance on expert opinion was required; and
- **highly uncertain,** when there are significant knowledge gaps and reliance on expert opinion.

Characterisation and assessment of the physical containment at the PEI facility was based on standard methodologies and included a failure mode analysis. Assessment of physical containment included possible accidental release scenarios and considered potential natural events, security violation, and chronic (continuous) containment failure. Hurricanes and tidal surges were identified as natural events of relevance at the PEI facility. However, mitigation measures in place to prevent the acute release of AAS were determined to be appropriate and the likelihood of exposure through an acute break in the physical containment was concluded to be negligible with high certainty. Although never documented, vandalism and theft were identified as potential security violations that could lead to a containment breach. Mitigation measures in place were determined appropriate and the likelihood of exposure through a security breach was concluded to be negligible with high certainty. Sixteen (16) potential pathways of entry into the environment were identified in the characterization of physical containment at the PEI facility. The failure mode analysis identified 3 to 6 redundant mechanical barriers per pathway, all with appropriate safeguards to detect or prevent potential failures. Several operational precautions, such as training, written SOPs, daily checklists, and records of containment failure, also contributed to a conclusion that there is, with high certainty, a negligible likelihood of exposure from chronic failure at the PEI facility.

The characterization and assessment of physical containment at the Panamanian facility examined the potential for AAS to enter the environment in Panama, though it was acknowledged that in order to enter the Canadian environment, AAS in Panama would also have to survive and disperse to Canada's territorial waters. Mitigation to prevent the acute release of AAS due to natural events of relevance (e.g., seismic activity and flash flooding) was considered adequate. Consequently, the likelihood of acute exposure was concluded to be low with reasonable certainty. Although never documented, vandalism and theft were also identified as potential security violations at the Panamanian facility. However, given the security measures in place at the facility in Panama, it was concluded with reasonable certainty that the likelihood of a release through a breach of security was negligible. Four potential pathways of entry were identified at the Panamanian facility. The failure mode analysis identified 4 to 12 redundant mechanical barriers per pathway and several operational procedures, such as training and written SOPs, to detect or prevent potential failures in physical containment. However, a lack of oversight documentation at the Panamanian facility resulted in a conclusion that, with reasonable certainty, there is a low likelihood of escape from chronic containment failure.

While triploid eggs are in transit between the PEI and Panamanian facilities, a combination of redundant physical containment, SOPs, oversight documentation, and brokered chain-of-custody maintenance are in place to ensure that neither accidental nor intentional release occurs prior to a shipment reaching its destination. This led to a conclusion, with reasonable certainty, that the likelihood of exposure to AAS while in transit was negligible.

The characterization and assessment of AAS fate in the environment considered:

1. the capacity of live AAS to survive, disperse, and persist in the receiving environment,
2. the capacity of live AAS to reproduce, establish, and spread in the receiving environment, and
3. the disposal of dead AAS in Canada.

Though transgenesis and domestication may reduce fitness and affect migration, they would not prevent survival, dispersal and persistence of AAS, given favourable circumstances. At both facilities, the likelihood of the survival, dispersal and persistence of AAS in the surrounding environments were reported, with high certainty, to be high. However, during transport, the likelihood of survival, dispersal and persistence of AAS eggs that escape containment were reported, with high certainty, to be negligible.

AAS that mature sexually were reported, with reasonable uncertainty, to have a high likelihood of reproducing, establishing, and spreading, if they were to escape from the PEI facility. If escaping in Panama, the likelihood of AAS reproducing, spreading, and establishing was concluded, with high certainty, to be negligible; an assessment based on a minimum triploid induction efficacy of 95%, the use of all-female stocks, and the absence of established Atlantic Salmon populations or other species in the region that may be suitable for reproduction. Given the geographical isolation of the Panamanian facility, and regional water temperatures that are lethal to Atlantic Salmon, the likelihood of AAS swimming from Panama to Canadian territorial waters was determined to be negligible with high certainty.

The disposal of non-living AAS was reported to be accomplished through incineration or private landfill according to municipal waste disposal standards, and would not result in the release of live AAS. Since non-living AAS do not meet the definition of “organism” under the NSNR(O), the likelihood of exposure in Canada from the disposal of AAS carcases was determined, with high certainty, to be negligible.

Overall, it was concluded with reasonable certainty that the likelihood of AAS exposure to the Canadian environment is negligible.

## DISCUSSION

Discussion following the presentation clarified that consideration of exposure in Panama is only relevant in assessing the potential of exposure to the Canadian environment. It was also clarified that a permit to ship AAS eggs to the Panamanian facility had been granted previously under a research and development exemption. It was noted that Canada is not legally bound to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, however, acting as a model non-Party, the Panamanian embassy was notified of the transboundary movement of AAS eggs from PEI to Panama.

Clarifications were also requested regarding the procedure by which a final ranking for exposure to the Canadian environment was determined in accordance with the problem formulation; a DFO internal document that was circulated to the peer-review committee for comments prior to the company’s regulatory submission. Final exposure ranking was contingent on the relationship between the various pathways of exposure. When pathways were independent from one another, the final ranking of exposure (and associated uncertainty) was the same as the highest ranked pathway. However, when pathways were dependent on one another (i.e., sequential), the final ranking of exposure (and associated uncertainty) was equal to the lowest ranked component.

The relevance of including a negligible category in the ranking of exposure was discussed. Similar debates over ranking categories were reported for previous risk assessment exercises

without significant impact on the outcome. Participants concluded that the proposed ranking categories were adequate.

Participants recommended the following changes:

- Due to the unpredictable nature of some natural disasters, the certainty associated with the negligible likelihood of an acute failure of physical containment at the PEI facility should be ranked as reasonable certainty, instead of high certainty;
- Rank the certainty associated with the negligible likelihood of release of AAS while in transit between the PEI and the Panamanian facilities as reasonable certainty, instead of high certainty, to account for the potential risks of land transportation;
- Rank the certainty associated with the negligible likelihood of exposure resulting from the survival and persistence of AAS embryos during transport as reasonable certainty, instead of high certainty, to account for seasonal variation in potential survival, especially during the summer; and
- Rank the likelihood of exposure resulting from the reproduction, establishment and spread of fertile AAS parr in the event of an unintentional release from the PEI facility as high, instead of negligible, to account for the suitable nature of the potential receiving environment. As a result of this change, the certainty associated with this element was reviewed and subsequently changed from high certainty to reasonable uncertainty, in order to remain consistent with similar elements of the exposure assessment such as the likelihood of exposure resulting from the reproduction, establishment, and spread of AAS fertile smolts, post-smolts, and adults. The latter change was made after the meeting, in consultation with the peer-review committee.

## CONCLUSIONS

Participants reached consensus on the following conclusions:

- The assessment concludes with reasonable certainty that the potential for AAS to enter the Canadian environment through an acute failure of containment at the PEI facility is negligible.
- The assessment concludes with high certainty that the potential for AAS to enter the Canadian environment through chronic failure of containment at the PEI facility is negligible.
- The assessment concludes with high certainty that the potential for AAS to enter the Canadian environment through a return from Panama is negligible.
- The assessment concludes with reasonable certainty that the potential for AAS to enter the Canadian environment through a failure of containment in transit between Canada and Panama is negligible.
- The assessment concludes with high certainty that the potential for AAS to enter the Canadian environment through disposal of AAS eggs and carcases in Canada is negligible.
- Overall, the assessment concludes with reasonable certainty that the likelihood of an exposure of AAS to the Canadian environment is negligible.

## WAIVER AND SIGNIFICANT NEW ACTIVITY

The "Waiver" presentation, by Colin McGowan (DFO – National Capital Region), addressed AquaBounty Canada's request for a waiver of information required under information element 5(b) of Schedule 5 of the *New Substances Notification Regulations (Organisms),* in accordance with Section 106(8) of CEPA 1999. The company requested a waiver for data from tests conducted to determine invasiveness of the notified organism based on the rationale that the living organism is physically contained within land-based facilities so as to satisfactorily protect the environment and human health. This assertion was based on claims by the company that:

- regulatory oversight is in place;
- reasonable security is in place;
- the PEI and Panamanian facilities are sited, constructed, and managed to mitigate naturally occurring catastrophic events;
- AAS has no capacity to establish in the wild; and
- AAS is physically contained in specified land-based facilities with acceptable confinement procedures and management practices.

Further, the company stated that AAS would only be used under the strictly controlled conditions at the two facilities that are clearly defined and described in the regulatory submission.

The assessments of exposure to the Canadian environment concluded that suitable physical containment measures were in place at both facilities to prevent living AAS from entering the Canadian environment, geographical barriers would prevent AAS from entering the Canadian environment from Panama, and suitable measures were in place during transport to prevent the entry of AAS into the Canadian environment.

Based on the above considerations, DFO recommended that the waiver request be granted under paragraph 106(8) (b) of CEPA.

DFO also recommended that any activities involving AAS outside of the well-defined specifications provided in the regulatory submission should be considered a significant new activity requiring a Significant New Activity Notice, as defined under CEPA.

## DISCUSSION

During discussions, it was reiterated and clarified that AAS was only assessed as per the strictly controlled conditions described by the company, in the two clearly defined facilities in PEI and Panama. In PEI, it was concluded that suitable measures were in place to prevent living AAS from leaving the facility and entering the Canadian environment. In Panama, it was similarly concluded that suitable measures were in place to prevent living AAS from entering the Panamanian environment and geographical barriers would also prevent AAS from entering the Canadian environment. Finally, it was concluded that during transport, suitable measures were in place to prevent entry of AAS into the Canadian environment.

It was also clarified that the waiver assessment was conducted for the proposed use scenario outlined in the regulatory submission, and evaluated whether the proponent had demonstrated satisfactory containment of AAS.

It was explained that a Significant New Activities Notice specifies the conditions under which a new Notification would be required by specifying the new information to be submitted by a proponent to determine whether a proposed new activity or use can proceed.

## CONCLUSIONS

Participants reached consensus on the following conclusions:

- The waiver requested by AquaBounty should be granted under paragraph 106(8)(b) of CEPA because the information provided in support of the waiver request was considered sufficient to demonstrate that the organism will be contained so as to satisfactorily protect the environment and human health.
- Any activities outside of the well-defined parameters described in the waiver assessment may be considered a significant new activity and should require a Significant New Activity Notice.

# INDIRECT HUMAN HEALTH RISK ASSESSMENT

## INDIRECT HUMAN HEALTH HAZARDS: CHARACTERIZATION AND ASSESSMENT

The “Indirect Human Health Hazard Assessment of the AquAdvantage® Salmon”, presented by Jan Beardall (DFO – National Capital Region), addressed the capacity of AAS to act as a vector for human pathogens, its toxicity, its allergenicity, and its general health status.

The indirect human health risk assessment only considered the hazards that could result from environmental exposure to AAS through activities such as recreational fishing or swimming. It did not include potential hazards associated with the consumption of AAS as food (which are considered under the *Food and Drugs Act*), or occupational health hazards (which are considered under the *Occupational Health and Safety Act*) as these hazards were beyond the scope of the risk assessment. A comparative hazard assessment approach was taken to determine the potential of AAS to act as a vector for pathogens, and its potential toxicity and allergenicity, relative to wild Atlantic Salmon.

Hazard rankings used for the indirect human health risk assessment were described as follows:

- **negligible,** if no effects on human health are expected;
- **low,** if effects on human health are expected to be mild, asymptomatic, or benign in healthy individuals, if effective prophylactic treatments are available, and if case reports of human diseases are rare and without potential for community-level effects;
- **moderate,** if the effects on human health are expected to be moderate, but rapidly self-resolving in healthy individuals or, if effective prophylactic treatments are available, but there is some potential for community-level effects; and
- **high,** if the effects on human health are expected to be severe, of long duration and/or could result in sequelae in healthy individuals, or may be lethal if prophylactic treatments are not available or are of limited benefit, or if there is a high potential for community-level effects.

Uncertainty associated with each hazard assessment was ranked as follows:

- **highly certain,** when there are many reports of human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e. low variability), or when the potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects;
- **reasonably certain,** when there are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent, or there are no

reports of human health effects and there are no effects related to the hazard reported for other mammals;

- **reasonably uncertain,** if there are some reports of human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent, or there are reports of effects related to the hazard in other mammals, but not in humans; and
- **highly uncertain,** if there are significant knowledge gaps (e.g. few reports of effects in individuals exposed to the organism, but the effects have not been attributed to the organism).

In general, the fish health status at the PEI facility appeared to be well managed; a claim supported by fish health certificates that date from 1996 to 2013. Participants at the meeting concluded that there are insufficient data to determine whether AAS has an elevated susceptibility to pathogens in the environment or the potential to act as a reservoir for pathogens relative to wild Atlantic Salmon in the natural environment.

Morphological irregularities were assessed through a study conducted to identify phenotypic changes associated with triploidy and transgenesis. Diploid AAS, triploid AAS, and domesticated size-matched controls that were pre-qualified for the study based on their general health assessment and whole body weight, were used in the analysis. Internal reports disclosed an increased incidence of focal inflammations and a low occurrence of jaw erosion in diploid AAS. Other morphological irregularities such as gill and fin abnormalities, soft tissue mineralization, hepatic vacuolization, and cardiac shape abnormalities were determined to be the result of triploidy. It was concluded that morphological irregularities reported in the AAS were of low magnitude, limited distribution, and of a non-debilitating nature.

The potential to act as a vector for human pathogens was reviewed. Bacteria are the only fish zoonotics transmitted to humans, through swimming, topical contact, or puncture wounds. Events are of infrequent occurrence and are usually mild and localized. However, in rare instances symptoms can be severe (e.g., meningitis). Reports in the scientific or medical literature are usually consistent in nature and severity. There are no reports of Atlantic Salmon zoonoses. No adverse human health effects and no pathogens of human health significance have been detected at the PEI facility. It was concluded with high certainty that the potential indirect human health hazard related to AAS acting as a vector for human pathogens was low.

The potential toxicity of AAS that could indirectly affect human health was also reviewed. No reports of adverse human health effects had ever been reported at the PEI facility. The potential for expression of a known exogenous toxin resulting from the introduction of the opAFP-GHc2 construct was addressed through Basic Local Alignment Search Tool (BLAST) searches that were performed on the entire inserted sequence and revealed no unexpected known genes or toxins. There are no endogenous toxins associated with Atlantic Salmon and no indirect human health hazards related to triploidy or gynogenesis and sex reversal have been reported. It was, therefore, concluded with high certainty that the potential indirect human health hazard associated with toxins was negligible.

The assessment of the potential allergenicity of AAS that could indirectly affect human health was reported. The two principal allergens found in fish are parvalbumin and fish type-1 collagen. Although serious health consequences have been reported in a small proportion of individuals occupationally exposed to fish allergens, the nature and severity of adverse effects in humans are generally mild, are consistently reported in the literature, and do not pose a community-level risk. There have been no reports of adverse human health effects at the PEI facility, and BLAST analyses failed to reveal allergens, or unexpected genes, in the inserted sequence. A preliminary radioallergosorbent test (RAST) revealed a 1.5-fold increase in endogenous

allergens in diploid AAS compared to domesticated fish. Though the results from the study were found to be highly uncertain, this relatively modest increase in endogenous allergens would not be expected to be biologically or clinically relevant to individuals that accidentally come into contact with AAS due to fishing or swimming. Overall, the potential indirect human health hazard related to allergenicity of AAS was concluded, with reasonable certainty, to be low. Though the level of uncertainty in the indirect human health hazard assessment was affected by the quality and quantity of available data, the lack of an appropriate comparator, and natural variability, it was agreed that there was still a reasonable level of certainty associated with the conclusion.

### Discussion

It was noted after the presentation that the hazard assessments are conducted independently from the exposure assessment.

Concerns were raised about the pre-selection of animals in the morphological irregularity study. It was noted that the main reason for considering morphological irregularities in the context of the current risk assessment was to determine their potential effects on the fitness of the animals for their subsequent environmental effects, rather than for animal welfare considerations.

### Conclusions

Participants reached consensus on the following conclusions:

- The assessment concludes with reasonable certainty that the potential indirect human health hazard associated with novel or endogenous toxins from AAS is negligible.
- The assessment concludes with reasonable certainty that the potential indirect human health hazard related to allergenicity from AAS is low.
- The assessment concludes with high certainty that the potential indirect human health hazard associated with AAS acting as a vector for human pathogens is low.
- Overall, the assessment concludes with reasonable certainty that the potential indirect human health hazard associated with AAS is low.

## INDIRECT HUMAN HEALTH RISK

The “Indirect Human Health Risk Assessment of the AquAdvantage® salmon” presentation, by Jan Beardall (DFO – National Capital Region), addressed the exposure and indirect human health hazard outcomes, and concluded on the indirect human health risk and associated uncertainties.

The risk assessment was based on the risk paradigm of:

Risk = Exposure x Hazard

The exposure assessment was conducted for all possible routes of entry (PEI facility, Panamanian facility, transport, and disposal) and concluded that, for the notified activity, exposure of AAS to the Canadian environment is expected to be negligible. It was also noted that human exposure to AAS in the environment would be further limited to incidental encounters during recreational fishing and swimming. The potential hazards of AAS were reported to range between negligible and low, with reasonable to high certainty resulting from the consistency with which the prevalence, nature, and severity of indirect human health hazards were reported in the literature.

Consequently, based on the risk assessment and previous discussion, the overall indirect human health risk associated with the manufacture and production of AAS, as per the proposed use scenario, was concluded to be low with reasonable certainty.

## ENVIRONMENTAL RISK ASSESSMENT

## ENVIRONMENTAL HAZARDS: CHARACTERIZATION AND ASSESSMENT

The “Environmental Hazard Assessment of the AquAdvantage® salmon”, presented by Caroline Mimeault (DFO – National Capital Region), addressed the rankings of hazard and uncertainty, hazard considerations, environmental assessment endpoints, and the outcome of the environmental hazard assessment.

Hazard ranking used for the environmental hazard assessment were described as:

- **negligible,** if no effects were expected;
- **low,** if no harmful effects were expected;
- **moderate,** if reversible harmful effects were expected; and
- **high,** if irreversible harmful effects were expected.

Uncertainty associated with potential harmful effects on the environment was ranked as:

- **highly certain,** when high quality data on AAS was available and when the absence of GxE effects, or evidence of low variability, was demonstrated across relevant environmental conditions;
- **reasonably certain,** when high quality data on AAS-relatives or a valid surrogate was available, and if an understanding of GxE effects, or limited variability, was reported across relevant environmental conditions;
- **reasonably uncertain,** when limited data on AAS, AAS-relatives, or valid surrogate was available, and if there was a limited understanding of GxE effects across relevant environmental conditions, or if knowledge gaps existed and reliance on expert opinion was necessary; and
- **highly uncertain,** when significant knowledge gaps existed and significant reliance on expert opinion was required.

Hazard considerations regarding the potential toxicity of AAS and the potential for horizontal gene transfer (HGT) were reviewed. Based on the molecular characterisation of the transgene integrant, it was concluded with high certainty that no known toxic genes had been introduced into the genome of the AAS. The potential for HGT of the EO-1α transgene was concluded to be equal to the natural occurrence of HGT in Atlantic Salmon. Therefore, the potential for environmental hazards related to HGT in Atlantic Salmon was concluded with reasonable uncertainty to be negligible. However, high rates of oxygen consumption reported in adult AAS could lead, with reasonable certainty, to increased bioconcentration of waterborne contaminants, relative to wild conspecifics.

Potential effects to natural predators of Atlantic Salmon, especially other species of fish, which may result from the consumption of AAS with increased hormone levels, were also considered. Although *in silica* analysis indicates the digestibility of Chinook Salmon GH in potential predators, and *in vivo* proteolytic digestion of GH has been demonstrated, high doses of orally administered GH have been reported to be bioactive in fish. Still, the maximum potential concentrations of GH reported in transgenic salmonids remain much lower than doses needed

to elicit a biological response through oral consumption. It was, therefore, concluded, with reasonable certainty, that consumption of AAS with potentially high GH levels represent a negligible hazard to natural predators. It was also concluded, with reasonable certainty, that consumption of AAS with potentially high IGF-1 and thyroid hormone levels would also present a negligible hazard to natural predators. It was not possible to conclude on the potential environmental hazard that may result from consumption of AAS with increased estradiol and testosterone levels. Overall, it was concluded with reasonable uncertainty that potential toxicity of AAS poses negligible to low environmental hazard.

Potential hazards that could result from interactions with AAS were assessed for wild populations of Atlantic Salmon, prey of Atlantic Salmon, predators of Atlantic Salmon, competitors of Atlantic Salmon, habitat, and biodiversity.

The greatest potential hazards to wild populations of Atlantic Salmon were expected to result from either competition with AAS within food limited environments, or from genetic introgression (of both the transgene and the domesticated genetic background), following natural reproduction with fertile AAS broodstock, within environments that are not food limited. Uncertainties regarding these interactions resulted from a general lack of knowledge about potential GxE interactions with AAS, including its growth rate in natural environments, female reproductive fitness, and the ecology of adult Atlantic Salmon during the marine phase. Consequently, the potential hazards of AAS to wild populations of Atlantic Salmon were expected to range from low to high, with reasonable uncertainty.

Potential hazards to prey of Atlantic Salmon would depend on the predation pressure that AAS might exert in the wild. Though it was reported that AAS are expected to have similar or greater feeding motivation compared to that of wild conspecifics, and is likely to exhibit greater dispersal (hence a potential to feed on additional prey), it was difficult to conclude if AAS would exert increased, similar or decreased predation pressure relative to wild conspecifics. Uncertainty resulted from the absence of information regarding the growth rate and maximum size of AAS in the natural environment. Consequently, the potential hazards to prey of Atlantic Salmon were expected, with reasonable uncertainty, to range from low to moderate.

Based on the previous consideration of AAS potential toxicity to predators, uncertainty regarding its nutritional value at all life stages, and uncertainty regarding the ability of AAS to avoid predators in the natural environment, the potential hazards of AAS to predators of Atlantic Salmon were expected, with high uncertainty, to range between negligible and low.

The assessment of hazard to competitors of Atlantic Salmon considered the interspecific competitiveness of AAS at all life stages in the natural environment, as well as interspecific hybridization. Interspecies competition of juvenile AAS was expected to be lower or similar to wild conspecifics, but would also depend on previous rearing conditions (wild or hatchery). Uncertainties resulted from the absence of information regarding the growth rate and body size of AAS in the wild. Also, little is known about the interspecies competitiveness of Atlantic Salmon during the adult marine stage of its life cycle. Transmission or introgression of the transgene through hybridization with Brown Trout was considered possible, but remote, given the natural pre- and post-reproductive isolating mechanisms between these two species in Canada. Consequently, the hazardous impacts were expected to result from ecological interactions, rather than genetic interactions. It was concluded with reasonable uncertainty that hazards to competitors of Atlantic Salmon would range from negligible to moderate.

The assessment of potential hazards considered the limited role of AAS in habitat alteration. As is the case with domesticated Atlantic Salmon, AAS were expected to have diminished nest building behaviour, though uncertainty around this conclusion is high, given that no information is available regarding the reproductive behaviour of AAS females in a natural habitat.

Consequently, the potential hazards of AAS to habitat were predicted to be negligible with high uncertainty.

Although the effects of AAS on biodiversity were considered, and it was reported that AAS is expected to have negligible effects on the nutrient cycles of rivers, the paucity of knowledge regarding the impacts of escaped domesticated salmonids on biodiversity, or the impacts of any single ecosystem member on overall biodiversity, made it impossible to draw any substantive conclusions. Consequently, the potential hazard of AAS to biodiversity was reported to be unknown.

Overall, the potential environmental hazards of AAS were reported to range from negligible to high. Uncertainty in the assessment resulted from the necessity to derive conclusions using surrogate information, and limited information regarding GxE interactions and the effects of background genetics on phenotypic variation under various rearing conditions. These factors were reported to be of paramount importance to the risk assessment.

## Discussion

Discussion after the presentation covered a variety of issues. Regarding the potential toxicological effects of AAS, participants thought the assessment should focus on levels of growth hormone and insulin-like growth factor, since most of the data on other hormones was inconclusive. It was also recommended that the potential for bioaccumulation of contaminants in AAS be addressed, rather than bioconcentration of waterborne contaminants, to account for different scenarios.

Discussions around the potential hazards of AAS to wild populations of Atlantic Salmon concluded that potential hazards are not restricted to food limited environments, since competition may take place for other resources such as refuge and spawning sites.

Though the potential for AAS to affect wild populations of Atlantic Salmon via the Trojan Gene Effect was discussed, it was agreed that effects of AAS on populations will be broad, and should include all aspects of fitness. Consequently, it was resolved that overly simplistic models, such as the Trojan Gene model where all predictive power is based on a single relationship (predator-prey), should not be included as part of the Science Response. However, participants agreed on the difficulties of predicting fitness in the natural environment and highlighted the uncertainty associated with this exercise.

Considerable discussion and clarifications regarding the potential for horizontal gene transfer did not change the conclusion that the related environmental hazard is negligible with reasonable uncertainty. Participants agreed that the potential effects of AAS on competitors of Atlantic Salmon would occur through competition rather than through genetic introgression with non-native Brown Trout.

An extensive discussion regarding the ranking of potential hazards to habitat considered the positive and negative effects of bioturbation, and the reversible nature of salmonid activities in concluding that the effects of AAS on habitat are more likely to be low with high uncertainty.

Participants also agreed that there was not enough evidence to conclude on the nature and magnitude of AAS effects on biodiversity. The ranking of the biodiversity assessment endpoint as unknown, which had not been previously defined in the problem formulation, was debated and accepted.

The following changes to the environmental hazard assessment were recommended:

- Apply a conservative ranking to the hazard characterization of assessment endpoints. Where the hazard level had been reported as a range (e.g., low to high), the participants

recommended to use the highest hazard characterization. Consequently, the potential hazard of AAS to wild populations of Atlantic Salmon is high instead of low to high; to prey of Atlantic Salmon is moderate instead of low to moderate; to predators of Atlantic Salmon is moderate instead of negligible to moderate; and to competitors of Atlantic Salmon is moderate instead of negligible to moderate.

- Rank the potential hazard of AAS to habitat as low instead of negligible.

## Conclusions

Participants reached consensus on the following conclusions:

- The assessment concludes with reasonable uncertainty that AAS represent a negligible toxicological hazard to potential predators.
- There are insufficient data to determine whether AAS has an elevated susceptibility to pathogens in the environment, or the potential to act as a reservoir for pathogens, relative to wild Atlantic Salmon in the natural environment.
- The assessment concludes with reasonable uncertainty that the environmental hazard that may result from horizontal gene transfer is negligible. The assessment concludes with reasonable uncertainty that the potential hazard of AAS to wild populations of Atlantic Salmon is high.
- The assessment concludes with high uncertainty that the potential hazard of AAS to the prey of wild Atlantic Salmon is moderate.
- The assessment concludes with high uncertainty that the potential hazard of AAS to predators of wild Atlantic Salmon is low.
- The assessment concludes with reasonable uncertainty that the potential hazard of AAS on competitors of wild Atlantic Salmon is moderate.
- The assessment concludes with high uncertainty that the potential hazard of AAS to habitat is low.
- The potential hazard of AAS to biodiversity in Canada is unknown.
- Overall, the assessment concludes with reasonable uncertainty that the potential environmental hazard associated with AAS is high.

## ENVIRONMENTAL RISK

The “Environmental Risk Assessment of the AquAdvantage® salmon” presentation, by Caroline Mimeault (DFO – National Capital Region), addressed the exposure outcome, the environmental hazard outcomes and concluded on the environmental risk and associated uncertainties.

The risk assessment was based on the risk paradigm where:

Risk = Exposure x Hazard

The exposure assessment was conducted for all possible routes of entry (PEI facility, Panamanian facility, transport, and disposal) and concluded that, for the notified activity, exposure of AAS to the Canadian environment is expected to be negligible. Reasonable certainty associated with the exposure assessment resulted from redundant containment strategies supported by high quality information. Potential environmental hazards were assessed for six endpoints (wild populations of Atlantic Salmon; prey, predators, competitors of

Atlantic Salmon; habitat and biodiversity). The potential hazards of the AAS to the assessment endpoints were reported to range from low to high, with one unknown. The overall high degree of uncertainty associated with the environmental hazards is a consequence of multiple knowledge gaps, limited understanding of AAS phenotype plasticity, and the potential for genotype x environment interactions.

Consequently, based on the risk assessment and previous discussion, the overall environmental risk associated with the manufacture and production of AAS, as per the proposed use scenario, was concluded to be low, with reasonable uncertainty.

## ASSESSMENT OUTCOMES

The risk assessment outcomes, presented by Jan Beardall (DFO – National Capital Region), summarized the results of the exposure assessment, the indirect human health hazard and risk assessments, the environmental hazard and risk assessments, and the proposed regulatory interpretation of the risk assessment outcomes ("CEPA toxic" or not "CEPA toxic").

According to the original guidance provided in the problem formulation, the outcome of low with reasonable uncertainty for the environmental risk assessment should result in a conclusion of "CEPA toxic". However, participants observed that the conclusion of "CEPA toxic" was inconsistent with both the outcome of the exposure assessment (negligible with reasonable certainty) and the consensus conclusion earlier in the meeting that the proponent's waiver request be granted on the basis that they had demonstrated sufficient containment of AAS to satisfactorily protect the environment and indirect human health.

Participants reviewed the results of the exposure assessment (particularly those regarding the "acute failure in PEI" and "failure in transit" pathways of entry into the Canadian environment) and agreed that the proposed risks and uncertainties were appropriate.

Participants questioned whether the methodology of the problem formulation was correct and comparable to other risk assessment frameworks. Extensive discussion followed and participants identified two errors.

First, it was determined that the problem formulation presented a model for calculating risk that did not fully describe how uncertainty for both the exposure assessment and the hazard assessments are considered in developing a conclusion on risk. Logical completion of the model suggested that where the hazard assessment is high with high certainty, exposure must be negligible with high certainty to result in a conclusion of not "CEPA toxic". However, if the hazard is high, with high *un*certainty, the actual hazard could range from negligible to high.

Second, the problem formulation proposed that the level of uncertainty for an overall risk assessment can be determined by that associated with either the exposure or hazard assessment, whichever is greatest. Instead, the Science Response participants recommended that logically, it would be more appropriate if the uncertainty assigned to risk should be that associated with the element, either hazard or exposure, that limits risk most in the risk assessment paradigm (Risk = Hazard X Exposure).

Consensus was reached amongst Science Response participants to address these errors in the problem formulation, the Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon and the Science Response to ensure that the risk assessment outcomes are appropriately founded upon the conclusions of the exposure assessment, the hazard assessment, and their associated uncertainties.

Therefore, participants agreed to the following changes (in italicized text) to the problem formulation which clarified that:

1. "In the case where the hazard assessment is high *with high certainty*, then the exposure must be negligible with high certainty to result in a recommendation of not "CEPA toxic" to be made."
2. "If the rankings for uncertainty in the hazard and exposure assessments differ, the *uncertainty for risk should be that associated with the element that limits risk most in the risk assessment paradigm: Risk = Hazard X Exposure*."

With these issues resolved, all participants agreed that a consensus could be reached on the conclusions of both the human health and the environmental risk assessments.

## CONSIDERATIONS FOR SIGNIFICANT NEW ACTIVITIES

The components and considerations of potential new activities were presented by Jan Beardall, who explained that, following a decision of not CEPA toxic but where there was a potential for new activities to result in new exposures, the Minister of Environment had 90 days to publish in the *Canada Gazette*, a Significant New Activity Notice (SNAc). Consequently, this peer-review meeting was an opportunity to make recommendations, for consideration by EC decision makers, regarding such measures that should be taken into consideration. It was acknowledged, that the Minister of the Environment would be the final authority on deciding the precise wording and elements if a decision was taken that a SNAc would be needed.

Potential new activity requirements discussed at the meeting included the possibilities of limiting commercial production of AAS to the AquaBounty Canada Facility in PEI, limiting the export of hemizygous triploid female AAS eyed-eggs to no more than 100,000 as described in the notification, and standards for physical containment of AAS at all life-stages at the facility in PEI, the facility in Panama, and while in transport between the two facilities.

AquaBounty Canada's regulatory submission proposed to limit export activities to 100,000 AAS eyed-eggs, an element of the proposed activity that shaped the scope of the risk assessment. While participants recognized the importance of the export limit in determining the outcomes of the risk assessment, they also acknowledged the difficulty of proposing, within the limited time available, a relevant numeric threshold for the manufacture and export of AAS eyed-eggs that should trigger a review. Consequently, participants agreed not to use the 100,000 AAS eyed-egg limit, instead making broader recommendations regarding the maintenance of physical and biological containment measures at the existing facilities.

Participants reached consensus (through review of a revised Science Response following the meeting) that the use scenario should be limited to:

1. Commercial production at the AquaBounty Canada facility, in PEI, that has been described in AquaBounty's notification, of hemizygous triploid female Atlantic Salmon eyed-eggs bearing the opAFP-GHc2 construct at the EO-1α locus using milt from homozygous masculinized AAS females (neomales) and eggs from non-transgenic Atlantic Salmon females that are derived from the domesticated Saint John River strain;
2. Physical containment, as efficacious as described in AquaBounty's notification, of all life-stages of AAS at the PEI facility and at the AquaBounty facility in Panama, that are under the singular and direct control of AquaBounty Technologies, and while in transport between the two facilities as described in the notification; and
3. Biological containment as described in AquaBounty's regulatory submission.

Participants reached consensus on recommendations that, in the event of a proposed new activity in relation to the AquAdvantage® Salmon, information provided by AquaBounty to the Minister of Environment should include:

1. A description of the proposed significant new activity in relation to the living organism;
2. A detailed description of all physical, biological and geographic containment measures proposed to be used;
3. The information specified in paragraph 5(b) of Schedule 5 of the New Substances Notification Regulations (Organisms); and
4. Any other information or data in respect of this living organism in AquaBounty's possession or to which they have access, that is relevant in order to determine whether the living organism is invasive or capable of becoming invasive.

## CONCLUSIONS

Science Response participants reached consensuses on the final conclusions of low risk with reasonable certainty for both the Indirect Human Health Risk Assessment and the Environmental Risk Assessment, and on the conclusions of not "CEPA toxic" to the environment or indirect human health.

Participants also reached consensus on the conclusion that potential changes in the activities or containment provisions described by AquaBounty Canada in its regulatory submission under the NSNR(O), have not been assessed, and may require the submission of new information to satisfy any requirements in the recommended Significant New Activities Notice.

## APPENDIX 1 - TERMS OF REFERENCE

**Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon**

**Science Response Process – National Capital Region**

**July 17-19, 2013**
**Ottawa, Ontario**

Co-chairs: Stephen Stephen and Mark Hovorka

### Context

The *Canadian Environmental Protection Act*, 1999 (CEPA 1999), administered by Environment Canada (EC) and Health Canada (HC), is the key authority for the Government of Canada to ensure that all new substances, including 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 EC prior to the import to or manufacture in Canada of new organisms that are animate products of biotechnology, including fish products of biotechnology.

Fisheries and Oceans Canada (DFO), EC and HC signed a Memorandum of Understanding respecting the implementation of the NSNR (Organisms) for fish. DFO assists in implementing the NSNR (Organisms) by conducting an environmental and indirect human health risk assessment for fish products of biotechnology and recommending any necessary measures to manage risks. The risk assessments evaluate whether the notified fish product of biotechnology is "CEPA toxic" in accordance with Section 64 of CEPA 1999: 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.

Based on the scientific risk assessments, DFO would provide science advice to EC to support a regulatory decision by the Minister of the Environment on the fish product of biotechnology that has been notified under the NSNR (Organisms).

### Objectives

The objective of this Science Response Process is to peer review the draft Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon and any recommended measures to manage risks and provide relevant science advice on the assessments and recommendations.

Working papers to be reviewed will include:

- a draft Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon; and
- a Summary of the Environmental and Indirect Human Health Risk Assessment of the AquAdvantage® Salmon.

The environmental component of the risk assessment will include consideration of potential risks to fish, fish habitat and the environment in general. The indirect human health component

of the risk assessment will not consider potential risks related to consumption, but will consider potential risks such as toxins, allergens and the transmission of zoonotic diseases.

The Science Response Process will evaluate the conclusions, rankings and recommendations of the draft risk assessment and any recommended measures to manage risks, including the weight of scientific evidence, quality of data, identified gaps and associated uncertainties of the:

- Characterization of AquAdvantage® Salmon;
- 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 Response

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.

**Participation**

- Fisheries and Oceans Canada (Ecosystems and Oceans Science Sector; Newfoundland and Labrador Region, Gulf Region, Maritimes Region, Central & Arctic Region, Pacific Region)
- Environment Canada and Health Canada
- Provincial Governments
- Academia
- Other invited experts

## APPENDIX 2 - AGENDA

Agenda of the National Science Response Process

Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon

July 17-19, 2013
Fisheries and Oceans Canada
200 Kent Street, Ottawa, Ontario
Room: BCC 1-2 (Ground Floor)

**Wednesday, July 17th**

8:30 Welcome and introductions (Stephen Stephen, Mark Hovorka)
8:45 Introduction to National Science Response Process (Erika Thorleifson)
9:00 Context: Regulatory, risk assessment, proposed use (Jan Beardall)
9:30 Characterization of the AquAdvantage® Salmon (Caroline Mimeault)
10:30 Break
10:50 Characterization of the AquAdvantage® Salmon *continued* (Caroline Mimeault)
11:30 Lunch
12:30 Exposure: characterization and assessment (Colin McGowan)
14:30 Break
14:50 Exposure: characterization and assessment *continued* (Colin McGowan)
15:20 Waiver (Colin McGowan)
16:30 Review, summary, and recapitulation of the day / end of day 1

**Thursday, July 18th**

8:30 Review and summary of conclusions (Stephen Stephen, Mark Hovorka)
8:45 Indirect human health hazards: characterization and assessment (Jan Beardall)
9:45 Indirect human health risk assessment (Jan Beardall)
10:30 Break
10:50 Environmental hazards: characterization and assessment (Caroline Mimeault)
12:15 Lunch
13:15 Environmental hazards: characterization and assessment *continued* (Caroline Mimeault)
14:15 Environmental risk assessment (Caroline Mimeault)
14:30 Break
14:45 Environmental risk assessment *continued* (Caroline Mimeault)
16:30 Review, summary, and recapitulation of the day / end of day 2

**Friday, July 19th**

8:30 Proposed risk management measures (Colin McGowan)
10:30 Break
10:50 Review and summary of conclusions (Stephen Stephen, Mark Hovorka)
12:00 End of meeting

## APPENDIX 3 - MEETING PARTICIPANTS

Participants of the National Science Response Process

Environmental and Indirect Human Health Risk Assessment
of the AquAdvantage® Salmon

| Name | Affiliation |
|---|---|
| Beardall, Janet | Fisheries and Oceans Canada |
| Bradbury, Ian | Fisheries and Oceans Canada |
| Byrne, Philip | Fisheries and Oceans Canada |
| Chaput, Gerald | Fisheries and Oceans Canada |
| Devlin, Robert | Fisheries and Oceans Canada |
| Dugan, Stephen | Health Canada |
| Fraser, Dylan | Concordia University |
| Hovorka, Mark | Fisheries and Oceans Canada |
| Leggatt, Rosalind* | Fisheries and Oceans Canada |
| MacKinnon, Anne-Margaret | Fisheries and Oceans Canada |
| MacNair, Neil | Province of Prince Edward Island |
| Mandrak, Nicholas | Fisheries and Oceans Canada |
| Marshall, Larry | Fisheries and Oceans Canada |
| McGowan, Colin | Fisheries and Oceans Canada |
| Meerburg, David | Atlantic Salmon Federation |
| Mills, Chris | Fisheries and Oceans Canada |
| Mimeault, Caroline | Fisheries and Oceans Canada |
| Moreau, Darek | Independent consultant |
| Shahsavarani, Arash | Environment Canada |
| Stefanov, Ivan | Fisheries and Oceans Canada |
| Stephen, Stephen J. | Fisheries and Oceans Canada |
| Thorleifson, Erika | Fisheries and Oceans Canada |

*provided peer review by correspondence only and did not attend the meeting.