SUMMARY OF THE ENVIRONMENTAL AND INDIRECT HUMAN HEALTH RISK ASSESSMENT OF AQUADVANTAGE® SALMON

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:

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.

A notification under the NSNR (Organisms) was submitted to EC for AquAdvantage® Salmon by AquaBounty Canada Inc. on April 30, 2013. DFO conducted environmental and indirect human health risk assessments of AquAdvantage® Salmon in order to make recommendations on any necessary risk management measures to EC to support a regulatory decision by the Minister of the Environment on AquAdvantage® Salmon.

This Science Response is the result of the July 17-19, 2013 National Science Response Process on the Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon. The purpose of this meeting was to peer review the conclusions presented in DFO's preliminary comprehensive Environmental and Indirect Human Health Risk Assessment of AquAdvantage® Salmon.

Background

AquaBounty and AquAdvantage® Salmon

AquaBounty Technologies Inc. is an American biotechnology company with a land-based, contained research and development facility in Prince Edward Island (PEI). AquaBounty has genetically engineered an Atlantic Salmon (*Salmo salar*) referred to as *AquAdvantage* salmon (AAS hereinafter) intended for human food consumption that is claimed to grow faster than its non-genetically engineered counterpart.

AAS was developed by micro-injecting a gene construct (opAFP-GHc2) comprised of an ocean pout (*Macrozoarces americanus*) anti-freeze protein (AFP) promoter and a Chinook salmon (*Oncorhynchus tshawytscha*) growth hormone (GH) gene into the egg of a wild Atlantic salmon.

AquaBounty has indicated its intent to commercially produce all-female triploid, transgenic AAS eyed-eggs at their PEI facility and to export no more than 100,000 eggs annually to a contained, land-based, grow-out facility in the highlands of Panama (ABT 2013). AAS will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility, where they will be processed for retail sale in approved markets for food consumption.

Although the proposed AAS product for export to Panama is all-female triploid eyed-eggs from the EO-1α line bearing a single copy of the opAFC-GHc2 transgene, other life-stages (gametes through to sexually mature adults), genotypes (i.e. diploids, triploids, hemizygotes, homozygotes) and genders (females and masculinized females) are required for the production of the eyed-eggs and are therefore included in the risk assessment.

The risk assessment is conducted on AquaBounty's proposed use scenario to grow AAS under the containment conditions specified in the regulatory submission for the PEI and Panamanian facilities. The exposure assessment focuses on the potential for exposure of AAS to the Canadian environment; activities in Panama are only relevant where they may result in exposure to the Canadian environment (i.e. the potential for fish to be released in Panama and swim back to Canadian waters). The indirect human health hazard assessment and the environmental hazard assessment are conducted separately. The risk assessment integrates the exposure and hazard assessments to determine the likelihood that a harmful effect will be realized. Uncertainty associated with each element of the risk assessment is reported and taken into consideration for regulatory decision-making.

Molecular Characterization of AAS

AAS is a genetically engineered Atlantic salmon (*Salmo salar*) containing a single copy of the opAFP-GHc2 transgene at the EO-1α locus (ABT 2013).

Characterization of the transgene construct

AquaBounty appropriately described the opAFP-GHc2 transgene construct. Briefly, the transgene construct was assembled through the use of standard molecular biology tools and techniques including plasmids, bacteriophage, restriction enzymes, linearization and ligation. No mobile genetic elements were used. The opAFP-GHc2 construct includes a 5'- antifreeze

protein (AFP) promoter from the ocean pout, the complementary DNA sequence of growth hormone (GH) from the Chinook salmon and a 3'-terminator from the ocean pout (Figure 1). AquaBounty provided *in-vitro* and *in-vivo* evidence of the functionality of the opAFP promoter to drive gene expression in salmonid species. Through complete sequencing of the integrant, AquaBounty provided evidence for (1) the presence of expected regulatory elements in the promoter and terminator regions, (2) the presence of a full-length sequence encoding a mature hormone homologous to the endogenous GH-1 Chinook salmon gene which is 95% homologous to the Atlantic salmon GH and (3) the absence of sequences for known toxic proteins. It is concluded that the nature of the transgene construct is not of concern.

Strain history and genealogy

AAS comprises the genetic background of several strains of Atlantic salmon. Early generations of AAS were derived from and crossed with individuals from the Exploits, Colinet and Northeast Rivers in the Province of Newfoundland and Labrador. However, since 2000, subsequent generations used in the development of the AAS line intended for commercial application have been crossed predominantly with domesticated fish from the St. John River strain. AAS is therefore a domesticated transgenic Atlantic salmon strain.

Characterization of the transgene integrant

Two integration sites, referred to as the α- and β-integrants, were identified in the founder animal (EO-1). As only the α-integrant confers the enhanced growth phenotype the non-functional β-integrant was removed from the AAS EO-1α line through selective breeding. Sufficient evidence was provided to demonstrate the absence of the β-integrant in all AAS broodstock maintained at the PEI facility.

AquaBounty thoroughly characterized the transgene integrant. Evidence demonstrates that the opAFP-GHc2 construct was rearranged upon insertion into the host genome (Figure 1). The 4205 base pair (bp) EO-1α transgene integrant includes the last 613 bp of the ocean pout 5'-AFP promoter sequence, followed by the intact Chinook salmon GH cDNA, the complete ocean pout antifreeze 3' regulatory sequence, 25 bp from pUC9, 20 bp from pUC18 and the first 1678 bp of the ocean pout antifreeze 5' region. Excluding the above rearrangements, sequencing of the transgene integrant confirms complete identity with the transgene construct. The non-coding pUC sequences are not of concern. Although microinjection is not a transgene delivery method of concern, the reported co-injection of the transgene and plasmid DNA raised questions about the potential integration of the plasmid, or fragments of it, into the host genome. Southern Blot Analyses using AAS blood genomic DNA probed with pUC19 failed to detect plasmid DNA. In addition, multiplex polymerase chain reaction (PCR) analyses conducted in multiple families over 5 generations with primers designed to anneal to the ampicillin resistance gene (161 bp) also failed to detect plasmid DNA in AAS. From the above, it is concluded that no large fragments from the plasmid and no fragments from the ampicillin resistance gene larger than 161 bp were integrated into the host genome. However, based on recent evidence using modern biotechnology techniques (Zhang et al. 2012), uncertainty remains about the potential integration of incomplete fragments of the transgene or small fragments of the plasmid in the host genome. Although the exact location of the integrant is not known, sequencing of the flanking regions of the integrant provides sufficient evidence to conclude that the integrant was not inserted in the coding region of an endogenous gene. Remaining uncertainty about the potential integration of plasmid fragments smaller than 161 bp and the potential for the transgene integrant to disrupt surrounding genes is alleviated by the

nature of the construct and consideration of unintended pleiotropic effects reviewed under the biological and ecological characterization of AAS (*see below*). Therefore, it is concluded with reasonable certainty that the nature of the transgene integrant at the EO-1α locus is not of concern.

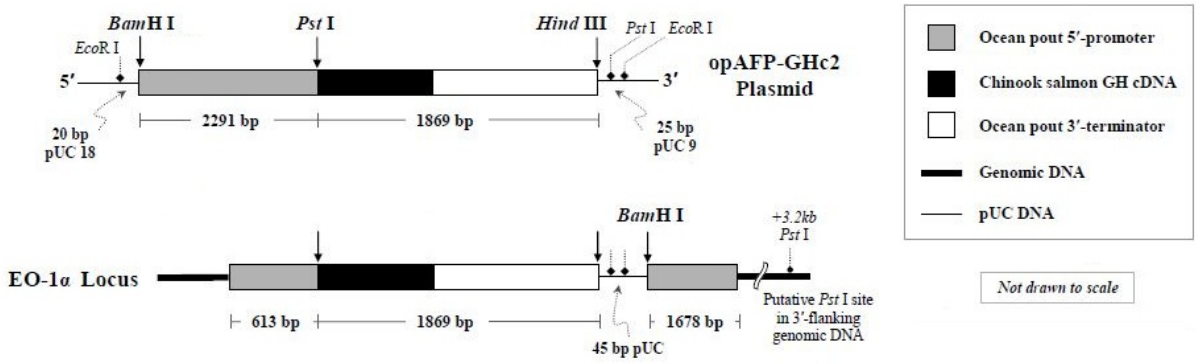

Figure 1. Comparison of the physical characterizations of the microinjected transgene construct (opAFP-GHc2 Plasmid) and the transgene integrant (EO-1α locus) in the Atlantic salmon (Salmo salar) genome (ABT 2013).

Inheritance and stability of the transgene integrant

Mendelian inheritance of the opAFP-GHc2 transgene inserted at the EO-1α locus was demonstrated through ratios of transgenic to non-transgenic progeny that were identified using PCR. AquaBounty provided detailed data for the percentage of inheritance in several crosses over five generations including all families from the broodstock. Reported ratios of transgenic to non-transgenic offspring were as predicted by mendelian inheritance (Shears and Yaskowiak 2004). Crosses between transgenic hemizygous and non-transgenic individuals led to 50% or 75% inheritance when the hemizygous individuals had one or two integrants respectively. Predicted transgene inheritance ratios for crosses between non-transgenic individuals (0%) or crosses involving transgenic homozygous (100%) individuals were also demonstrated. It is concluded with high certainty that the opAFP-GHc2 transgene inserted at the EO-1α locus is transmitted in accordance with mendelian inheritance ratios.

Genotypic stability of the opAFP-GHc2 transgene inserted at the EO-1α locus was demonstrated over three generations, through consensus nucleotide sequencing at the integrant and genomic flanking regions of individuals from the F_2 and F_4 generations (Yaskowiak et al. 2004, 2006). Additional evidence was provided through PCR amplification of the 5' and 3' junctions of the EO-1α integrant in fish selected from the F_2, F_4 and F_6 generations (Buchanan and Hobbs 2007). Therefore, it is concluded with high certainty that the opAFP-GHc2 transgene is stable at the EO-1α locus in AAS. However, it is noted that the insertion of the transgene in a simple sequence repeat region of the genome has the potential to alter locus structure, but only over evolutionary timeframes (Greckho 2011).

Additional Modifications to AAS

In addition to transgenesis, the production of all-female triploid eyed-eggs from the EO-1α line bearing a single copy of the opAFC-GHc2 transgene necessitated the application of gynogenesis, sex-reversal and triploidy induction techniques.

Gynogenesis was used in the early development of an all-female broodstock, but is no longer used for maintenance of the commercial all-female broodstock. Although current gynogenetic techniques can be expected to reliably produce all-female populations (Johnstone and Stet 1995; Quillet and Gaignon 1990; Pepper et al. 2004), its efficacy should be confirmed under all conditions of use. While confirmation of sex is not routinely conducted, AquaBounty reports never to have found a “true” male among the gynogens produced at the PEI facility and have provided evidence in which all sampled fish were determined to be females. Therefore, it is concluded that the generation of an all-female population through gynogenesis has been successful, though sampling to confirm the female status of the monosex broodstock population under rearing conditions at the PEI facility has been limited to phenotypic examination of the neomale gonads. Since sex determination in Atlantic salmon may not be solely genetic (Eisbrenner et al. 2013) and no information confirming the sex of fish reared at the Panamanian facility was provided, some uncertainty about the maintenance of all-female populations remains. However, despite the limited evidence of gender maintenance of fish at the Panamanian facility, it is concluded that the process of gynogenesis used in the development of broodstock is not a concern. It is recommended that AquaBounty Canada adopt a standard operating procedure to verify the genetic sex of neomale broodstock through DNA-based tests on an on-going basis, if such a test becomes available.

Sex-reversal of genetic females is achieved through17α-methyltestosterone treatment to produce phenotypic males. Since levels of exogenous 17α-methyltestosterone are reported to be transient and decline to trace levels by 14 days post-treatment (Curtis et al. 1991), potential toxicological effects through the consumption of AAS by predators would be over an extremely limited time frame. It is concluded that sex-reversal treatment is not of concern.

Induced triploidy through pressure shock shortly after fertilization of the eggs is another modification made to AAS. Due to uneven chromosome segregation during meiosis, triploid fish are functionally sterile (Benfey 1999), providing a useful method of biological containment, however the procedure is not always 100% effective. There are no toxicological concerns associated with induction of triploidy.

Biological and Ecological Properties of AAS

Biological and ecological properties of AAS are summarized below. When relevant, the phenotypes of AAS-relatives (Atlantic salmon microinjected with the opAFC-GHc2 construct) are also reported.

Body size, growth rates and hormone levels

In Atlantic salmon, body size is the phenotype most relevant to overall fitness, and is positively correlated with freshwater and marine survival, fecundity, egg size, reproductive success and offspring survival (Garcia de Leaniz et al. 2007). Since increased growth rate is the intended phenotype of the genetic modification to AAS, size, growth rates and growth hormone expression profiles are important considerations.

AquaBounty provided *in-vitro* and *in-vivo* evidence of the ability of the truncated promoter of the transgene integrant to activate gene expression. With over 95% homology between the Chinook and the Atlantic salmon growth hormone amino acid sequences, it is not possible to distinguish between the transgenic and endogenous GH. Consequently, investigations of the expression of the GH transgene rely on the comparison of total GH levels in transgenic and non-transgenic individuals. Very little information is available about GH levels in AAS. Early studies by Du et al. (1992) in AAS-relatives found no differences in plasma GH levels with non-transgenic counterparts at the parr stage. However, plasma GH levels varied greatly both within and between the experimental and control groups, GH levels were not related to growth rates and sample sizes were small. Erisman (2004) reported that GH levels in the muscle-skin of commercial size AAS were below the detection limit of 6.24 ng/ml. The same study also detected no difference between the experimental and control groups for the insulin-like growth factor (IGF-1).

The primary phenotypic change of AAS is increased growth and size at equivalent age relative to non-transgenic siblings. This phenotype is consistently observed in standard hatchery practices by AquaBounty and in numerous published papers (Deitch et al. 2006; Levesque et al. 2008; Moreau and Fleming 2012, Oke et al. 2013). Variation in growth of AAS between and within generations has not been extensively examined, but appears to be slightly greater than the variation observed in non-transgenics. The accelerated growth phenotype of AAS appears to be very plastic, and is strongly influenced by environmental conditions. Although accelerated growth may be limited in many circumstances, it cannot be concluded that AAS will never express growth rates that provide AAS a fitness advantage in the natural environment. Uncertainty remains around the maximum size of AAS.

Morphology, metabolism and physiology

Morphological irregularities reported in AAS are of low frequency and of a non-debilitating nature. At commercial size, and under commercial feed conditions, AAS have a body composition within the range of commercially grown Atlantic salmon. However, there is no information available regarding the body composition of AAS at other life stages, or for AAS fed natural prey (ABT 2013).

Oxygen consumption in AAS is similar to non-transgenic fish during early life stages up to the beginning of exogenous feeding (Moreau 2011), but is higher in adults (Deitch et al. 2006). Increased oxygen uptake and consumption rates have also been reported in juvenile AAS-relatives (Stevens and Sutterlin 1999; Cook et al. 2000a, 2000b). Other metabolic and physiological differences between AAS and non-transgenic counterparts include higher feed consumption rates, lower feed conversion ratios, reduced metabolic scope and reduced swimming performance in juveniles raised under hatchery conditions (Deitch et al. 2006; ABT 2013). Increased feed consumption rates have also been reported in AAS-relatives compared to non-transgenic counterparts (Abrahams and Sutterlin 1999; Cook et al. 2000a).

Health status

There is insufficient data to conclude whether AAS has an altered susceptibility to pathogens as compared to wild Atlantic salmon. AAS is known to be susceptible to *Infectious Salmon Anemia Virus* (ISAv) and *Aeromonas salmonicida* (furunculosis). Based on Fish Health Certificate data, it is concluded that fish disease risk at the AquaBounty facility in PEI is well managed.

Life history, behaviour and reproduction

Available information suggests that, although the GH transgene has a minimal effect on fitness-related traits during early stages of development (embryo to beginning of exogenous feeding juveniles) (Moreau 2011), it does appear to influence important life history traits as juveniles grow and mature. Specifically, AAS males have a reduced tendency to mature sexually as parr and appear to reach smolt status faster than non-transgenics under artificial conditions (Moreau 2011; Moreau et al. 2011a). There is no information available on the maturation of female AAS relative to non-transgenic conspecifics.

Limited information about the behaviour of AAS is available. AquaBounty reported normal avoidance, feeding and postural behaviour of juvenile AAS in a hatchery environment (ABT 2013). In a study with first-feeding AAS juveniles, territorial behaviour of transgenic and non-transgenic individuals was similar, suggesting no significant differences in competitive foraging at this critical life history stage (Moreau et al. 2011b). Abrahams and Sutterlin (1999) demonstrated that AAS-relatives are willing to incur greater risk of predation while foraging than non-transgenic comparators, a behaviour that has not been assessed for AAS. There is no information available about the predatory behaviour of AAS or AAS-relatives in the natural environment.

Triploid AAS females are expected to be functionally sterile; however the process of generating triploids at a commercial scale is not always 100% effective. AquaBounty's proposed sampling procedure to select eggs for export ensures a minimum of 95% triploid induction efficacy (ABT 2013). There is no information on the reproductive behaviour of female AAS (both diploid and triploid); a significant knowledge gap. Despite their reduced reproductive performance relative to wild conspecific males, hatchery reared, diploid AAS males that are sexually mature will compete for access to wild females, can participate in natural spawning events, and are capable of producing offspring that will survive past the first feeding stage under food limited conditions (Moreau et al. 2011a).

Exposure

Exposure Characterization

The assessment of AAS exposure to the Canadian environment includes both its potential to enter the environment and its fate once in the environment. Specifically, it considers: (1) the potential for unintentional release(s) of AAS at both the PEI and Panamanian facilities and during transport between the two locations, (2) the potential for AAS to survive, disperse and persist in the Canadian and Panamanian receiving environments, (3) the potential of AAS to reproduce, establish and spread in the Canadian and Panamanian environments and (4) the potential for the disposal of AAS carcasses in Canada to act as an exposure pathway. Though the characterization of exposure considers the potential for AAS to enter, survive, reproduce and establish in both the Canadian and Panamanian environments, the final assessment only considers exposure to the Canadian environment.

Potential for unintentional release(s) of AAS

Both acute failures in physical containment that may result from natural events or security violations, and chronic failures in physical containment were considered in the assessment of

exposure. With respect to chronic failure, the presence of three redundant mechanical barriers was accepted as adequate physical containment (ABRAC 1995) and a Failure Mode Analysis (FMA) was used to provide insight into the efficacy of all barriers and all operational procedures involving containment (Stamatis 2003; McDermott et al., 2009). All life history stages and all pathways of entry into the environment for both sterile triploid (3n) and fertile diploid (2n) AAS were considered.

The potential for an acute failure of physical containment at the PEI facility is concluded to be negligible with reasonable certainty. Natural events such as hurricanes and tidal surges are common to the region; their frequency and magnitude documented for over 100 years. The facility's location, siting, construction and design, as well as its emergency and standard operating procedures, have been selected and designed to effectively mitigate events of this nature. Also, the facility has never experienced an unauthorized entry of the building. Extensive security measures, such as perimeter fences, alarms, video surveillance and key control, are in place to prevent illegal access.

The potential for chronic release of AAS from the PEI facility is also concluded to be negligible with high certainty. All pathways of entry into the environment have a minimum of three redundant mechanical barriers with effective and documented oversight in place to ensure that all barriers are well maintained and that any potential failure is rapidly detected and corrected.

At the Panamanian facility, the potential for an acute release of AAS resulting from a natural event is concluded to be low, with reasonable certainty. Though seismic activity in this region of the world is not uncommon, earthquakes in Chiriquí Province are rare and are typically of insignificant magnitude. Flash floods are common to the region and of particular significance during the rainy season. The facility's siting, at a high altitude and near the headwaters of the watershed should effectively mitigate any potential damage from such an event; however, the lack of long-term historical data on the frequency and magnitude of events at this location make it difficult to predict the consequences with high certainty. The facility's remote location, restricted access, standard operational procedures to prevent unauthorized access and security measures such as guard dogs and steel perimeter fences topped with barbed wire, make the likelihood of security violations negligible with reasonable certainty.

The potential for chronic release of AAS from the Panamanian facility is concluded to be low with reasonable certainty. All pathways of entry into the environment have a minimum of four redundant mechanical barriers and there are standard operating procedures to maintain barriers and detect failures. However, there is a lack of procedural, documented oversight in place (e.g., use of checklists and sign-off sheets for routine duties).

The likelihood of release while in transit between the PEI and Panamanian facilities is concluded to be negligible with reasonable certainty. AAS eggs will be contained in a sturdy plastic cooler with a secured lid and placed inside an additional shipping crate. Air transport will be facilitated by a commercial freight-forward company to maintain chain-of-custody and the shipment will be received in Panama and transported to the facility under the supervision of an official from the Ministry of Agriculture's Quarantine Department (ABT 2013). Shipments of eggs will be unpacked and inspected at the AquaBounty Panama facility under the supervision of an official from the National Animal Health Authority (ABT 2013).

Potential for AAS to survive, disperse and persist in the receiving environments

In the unlikely event of an unintentional release, the principle factors limiting the survival, dispersal and persistence of AAS will be the environmental conditions (e.g. water temperature, salinity) of the receiving environment. Though the conditions of triploidy, gynogenesis, sex-reversal, domestication and growth hormone transgenesis may have an effect on the overall fitness of AAS in the wild, they are not expected to prevent AAS from reaching the adult life-stage given a favorable environment.

The saline condition of the marine environment is the principal factor limiting the survival and dispersal of AAS at the potential point of entry in Canada. Though salinities of 20 to 30 parts per thousand will likely prevent the survival of AAS during obligate freshwater life-stages (embryos to fry), conditions in the Bay Fortune estuary are not expected to prevent the survival and dispersal of AAS at later life-stages (parr to adult). Therefore, potential exposure resulting from the survival, dispersal and persistence of AAS parr, smolts, post-smolts or adults that may be unintentionally released from the PEI facility is concluded to be high. The availability of peer reviewed data describing the environmental requirements and tolerances of Atlantic salmon parr, smolts, post-smolts and adults and detailed information about the environmental parameters of the receiving environment, make this conclusion highly certain.

High water temperature is the principal environmental factor limiting the dispersal of AAS from the potential point of entry in Panama, to the Canadian environment. Though environmental conditions at the point of entry in Panama will likely permit the survival of any AAS that are unintentionally released, regional freshwater temperatures at lower altitudes and regional temperatures in the ocean would prevent AAS from dispersing to lower sections of the watershed and from surviving long enough to reach a suitable marine environment. Given its reduced metabolic scope, AAS is not expected to have greater upper temperature tolerance than wild Atlantic salmon. Therefore, though the likelihood of survival, dispersal and persistence of AAS locally, at the potential point of entry in Panama is concluded to be high with high certainty, the capacity of AAS to disperse beyond the local watershed and enter the Canadian environment from Panama is concluded to be negligible with high certainty.

These inferences are based on the availability of peer reviewed data describing the environmental requirements and tolerances of Atlantic salmon, as well as detailed information about the environmental parameters of the receiving environment.

AAS eggs will be shipped from the facility in PEI to the facility in Panama by a combination of ground and air transport. In the unlikely event that AAS eggs are unintentionally released from containment during transport, they would most likely enter a terrestrial or marine environment and die. Consequently, under the current production schedule, the likelihood of exposure resulting from the survival and persistence of AAS embryos during transport is negligible. The availability of peer reviewed data describing the environmental requirements and tolerances of Atlantic salmon embryos and information about the shipping route and environmental parameters of potential receiving environments result in a conclusion that is reasonably certain.

Potential of AAS to reproduce, establish and spread in the receiving environments

In the unlikely event of an unintentional release, the principle factors limiting the reproduction, establishment and spread of AAS will be its reproductive fitness, the availability of suitable spawning habitat and the availability of suitable mates. Though the condition of triploidy will

effectively render AAS sterile and unable to reproduce, the process of generating triploid populations at a commercial scale is not 100% effective and may leave some individuals fertile (0 to 5% based on statistical sampling procedures to select batches of eggs for export, ABT 2013). Triploid populations will also be 100% genetically female and consequently unable to reproduce or establish populations in the absence of male conspecifics. Regardless, commercial broodstock held at the PEI facility will be diploid and fertile (homozygous females and homozygous neomales) and though the effects of domestication and growth hormone transgenesis are expected to diminish reproductive fitness through behavioural, rather than physiological means, they will not preclude AAS from successfully reproducing in the wild with an appropriate conspecific.

Exposure resulting from the reproduction, establishment and spread of fertile AAS parr that are unintentionally released from the PEI facility, but are restricted to the Fortune River, is concluded to be high with reasonable uncertainty. Successful reproduction is likely to be limited given the behavioural anomalies associated with domestication and the low incidence of conspecifics. However, despite a general decline in the regional abundance of wild salmon, the local habitat may still be capable of supporting viable salmon populations. This conclusion is based on the availability of peer reviewed data describing the reproductive requirements of Atlantic salmon and the reproductive behavior of AAS, information about the environmental parameters of the receiving environment, history of Atlantic salmon introductions, and detailed information on potential propagule pressure.

Exposure that could result from the reproduction, establishment and spread of fertile AAS smolts, post-smolts or adults that disperse from the Fortune River watershed into the marine environment is also concluded to be high with reasonable uncertainty. There is abundant evidence supporting the opinion that escaped domesticated Atlantic salmon can migrate to suitable habitat and successfully reproduce with wild Atlantic salmon. There is also experimental evidence suggesting that, despite diminished reproductive fitness, AAS has the capacity to successfully reproduce with wild Atlantic salmon. However, limited knowledge regarding the fate of AAS, AAS relatives and Atlantic salmon in the marine environment and the high likelihood of low propagule pressure add substantial uncertainty to this conclusion.

The likelihood of exposure to Canada resulting from the reproduction, establishment and spread of AAS in Panama, is concluded to be negligible. Most (95 to 100%) of the all-female AAS in Panama will be sterile triploids. In the rare event of a fertile AAS being released, the absence of male conspecifics in the region will preclude its ability to successfully reproduce. The availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy, the effectiveness of generating all-female stocks, and detailed information about the biogeographical parameters of the local and regional environments, result in a conclusion that is highly certain.

During transport between the two facilities, the possibility of AAS embryos developing, reproducing and establishing viable populations is limited by their narrow range of tolerance to environmental parameters in the freshwater environment and no capacity to survive in the terrestrial or marine environment. Their reproductive capacity is further diminished by the process of induced triploidy. Consequently, the likelihood of exposure resulting from the reproduction, establishment and spread of AAS embryos that may enter the environment during transport from the facility in PEI to the facility in Panama is concluded to be negligible. This conclusion is made with high certainty, given the availability of peer reviewed data describing the effectiveness of sterilization using induced triploidy, the environmental requirements and

tolerances of AAS and Atlantic salmon embryos and information about the environmental parameters of potential receiving environments.

Potential for the disposal of AAS carcasses in Canada to act as an exposure pathway

Potential exposure resulting from the disposal of AAS eggs or carcasses in Canada is concluded to be negligible with high certainty. The proposed methods for disposal (incineration or private landfill) are in compliance with municipal waste disposal standards and practices and will not result in the release of live AAS, its genetic material or material from AAS involved in toxicity into the environment. These statements are based on detailed information provided in the regulatory submission regarding the proposed methods for disposal of transgenic AAS eggs and carcasses.

Exposure Assessment

The final ranking for exposure requires consideration of multiple elements related to the biological, biogeographical, and physical containment of AAS, including a variety of pathways that determine the entry and fate of AAS in the Canadian environment. In some cases, the likelihood of one element is limited by the likelihood of other elements. For example, the likelihood of reproduction, establishment and spread, will be limited by the likelihood of survival, dispersal and persistence, which are limited by the likelihood of entry. In such cases, the overall exposure will be determined by the lowest ranked element. In other cases, the likelihood of one element is not limited by the likelihood of another element. For example, the likelihood of entry into the Canadian environment for one pathway, such as entry from the facility in PEI, will not influence the likelihood of entry for a different pathway, such as dispersal from the facility in Panama. In this case, the final exposure is determined by the highest ranked element.

The various elements of exposure are consolidated in Table 1. The final ranking for exposure is the exposure element that limits the overall exposure and its associated uncertainty. The likelihood of AAS exposure to the Canadian environment is concluded to be negligible with reasonable certainty.

Table 1. Summary of the exposure assessment of AAS to the Canadian environment

Pathways of entry into the Canadian environment	Entry	Survival, dispersal, persistence	Reproduction, establishment, spread	Exposure
Acute failure in PEI	Negligible (reasonable certainty)	High (high certainty)	High (reasonable uncertainty)	Negligible (reasonable certainty)
Chronic failure in PEI	Negligible (high certainty)			Negligible (high certainty)
Return from Panama	Negligible (high certainty)			Negligible (high certainty)
Failure in transit	Negligible (reasonable certainty)	Negligible (reasonable certainty)	Negligible (high certainty)	Negligible (reasonable certainty)
Disposal in Canada	Negligible (high certainty)	n/a	n/a	Negligible (high certainty)
Final				**Negligible (reasonable certainty)**

Uncertainties in the Exposure Assessment

The high degree of certainty associated with the physical containment (i.e. entry) of AAS results from available information that adequately demonstrates the efficacy and redundancy of mechanical barriers, and the efficacy of standard operating procedures and operational oversight. It includes detailed diagrams of facility design, mechanical barriers and containment systems, incident reports, and training and compliance documentation. It also includes information on the potential occurrence of chance events such as fires, floods, hurricanes, earthquakes and security violations that could lead to a failure of containment.

In contrast, uncertainty associated with the biological and geographical containment of AAS that may enter the environment is derived largely from the limited availability of empirical data regarding the survival, fitness and ability of AAS to reproduce in the natural environment. However, robust scientific information related to the biological tolerances of valid surrogates and the environmental parameters of the receiving environment moderate this aspect of uncertainty.

Indirect Human Health Hazard

The indirect human health hazard assessment characterizes the potential for AAS to cause adverse effects to humans in Canada relative to wild Atlantic salmon as a consequence of dermal contact through environmental exposure (e.g. recreational swimming and fishing) to AAS. The potential toxicity, allergenicity and the capacity of AAS to act as a vector for human pathogens are assessed for their potential to affect human health. Although food safety and occupational human health hazards are not subjects of the current risk assessment, information

from the scientific literature on these aspects can be a valuable indicator of potential effects to humans from environmental exposure.

Indirect Human Health Hazard Characterization

Potential human toxicity of AAS

There have been no reports of adverse human health effects associated with toxins during human occupational exposure to AAS. Basic Local Alignment Search Tool (BLAST) searches for nucleotide and amino acid sequence homology with the inserted sequence did not detect any known toxins or proteins. There are no known endogenous toxins associated with Atlantic salmon and triploidy, gynogenesis and sex-reversal are not expected to introduce or alter any indirect human health hazards. It is therefore concluded with reasonable certainty that the potential indirect human health hazard associated with exogenous or endogenous toxins from AAS is negligible.

Potential human allergenicity of AAS

Experimental evidence related to endogenous allergenic potency of AAS is weak and does not include consideration of potential allergenicity relative to wild Atlantic salmon. There is limited data comparing allergenic potencies of diploid and triploid AAS with domesticated Atlantic salmon. However, even if endogenous allergen production were altered in AAS, allergic responses in humans to dermal contact with various fish species is generally limited in nature and severity. Therefore, it is concluded with reasonable certainty that the potential increased hazard to indirect human health related to endogenous allergens in AAS triploids is negligible and that related to AAS diploids is low. This conclusion is supported by the fact that there have been no reports of adverse human health effects associated with AAS during human occupational exposure to AAS at the AquaBounty facility. Based on BLAST searches for nucleotide and amino acid sequence homology with known allergens, it is concluded with high certainty that the potential indirect human health hazard associated with exogenous allergens is negligible. Individuals already allergic to fish protein may however also be highly likely to have allergic responses if exposed to AAS. Taken together, the potential indirect human health hazard related to allergenicity relative to wild Atlantic salmon is concluded to be low with reasonable certainty.

Potential to act as a vector for human pathogens

The potential for AAS to act as a vector for human pathogens is related to both the susceptibility of AAS to human pathogens (and subsequent introduction of such pathogens into the environment from the PEI facility) and the capacity of AAS to act as a reservoir for human pathogens (once in the environment). No pathogens of human health significance have ever been detected in the PEI facility, and no adverse human health impacts attributable to exposure to AAS have ever been reported in AquaBounty staff over almost two decades. Due to a lack of data, no conclusion can be drawn as to whether AAS would have an increased capacity to act as a reservoir for the transmission of disease agents to humans. However, even if AAS were to have increased capacity to act as vector for human pathogens, adverse effects in humans related to topically acquired fish zoonoses are generally limited in nature and severity. Consequently, it is concluded with high certainty that the hazard to indirect human health related to AAS acting as a vector for human pathogens is low.

Indirect Human Health Hazard Assessment

The various elements of indirect human health hazard are consolidated in Table 2. The final ranking for indirect human health hazard is the highest ranked element and its associated uncertainty. The final indirect human health hazard is concluded to be low with reasonable certainty.

Table 2. Summary of the indirect human health hazard assessment of AAS

Assessment endpoints	Hazard	Uncertainty
Toxin	Negligible	Reasonable certainty
Allergen	Low	Reasonable certainty
Vector for human pathogens	Low	High certainty
Final	**Low**	**Reasonable certainty**

Uncertainties in the Indirect Human Health Hazard Assessment

Uncertainty in the indirect human health hazard assessment results from limited data on allergenicity and the capacity of AAS to act as a vector for human pathogens. However, there is a high degree of certainty associated with the data supporting the absence of coding sequences for known exogenous toxins and allergens on the transgene integrant. There is also reasonable certainty associated with the nature and severity of allergenic effects related to dermal contact due to the consistency with which human health effects are reported for other fish species in the scientific literature. Consequently, there is reasonable certainty associated with the final indirect human health hazard conclusion.

Environmental Hazard

The assessment of the potential environmental hazard of AAS includes consideration of available information on AAS as well as appropriate consideration of information from surrogates such as AAS-relatives (e.g. Abrahams and Sutterlin 1999; Cook et al. 2000a, 2000b), other growth hormone enhanced transgenic salmonids (e.g. Devlin et al. 2000; Devlin et al. 2004; Sundström et al. 2004; Tymchuk et al. 2005; Lõhmus et al. 2008; Devlin et al. 2009; Devlin 2011; Sundström and Devlin 2011), and other relevant studies and reports (e.g. Rodgers and Beamish 1981; Moriyama 1995; Habibi et al. 2004; DFO 2006; DFO 2013b; EFSA 2013).

Environmental Hazard Characterization

The environmental hazard assessment characterizes the nature, and severity of potential harmful effects that AAS may cause to the Canadian environment. Potential toxicity, capacity to act as a vector for diseases/parasites, and horizontal gene transfer, were all considered in the environmental hazard assessment. The potential hazards to the following assessment endpoints were assessed: (1) wild populations of Atlantic salmon, (2) prey of Atlantic salmon, (3) predators of Atlantic salmon, (4) competitors of Atlantic salmon, (5) habitat, and (6) biodiversity.

Hazard considerations

Environmental toxicological considerations include potential consumption of AAS by natural predators. Although GH has been shown to be bioactive across fish species (Moriyama et al 1993; Xu et al. 2001), proteolytic digestion and the high doses required to elicit a biological response make it reasonably certain that the toxicological hazard to potential predators from the consumption of AAS containing potentially elevated levels of GH or IGF-1 is negligible. Data on the bioaccumulation of contaminants in AAS is not available. Though higher food consumption rates of AAS may lead to higher bioconcentration of waterborne contaminants, the magnitude of this potential hazard cannot be predicted. Consequently, the environmental hazard resulting from potential toxicity of AAS to predators is concluded to be low with reasonable uncertainty.

There is 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.

Horizontal gene transfer (HGT) between higher eukaryotes is rare and can involve mobile genetic elements. Given that no characteristics of the transgene suggest potential changes in mobility, the potential for HGT of DNA containing the EO-1α transgene is expected to be similar to that of naturally occurring HGT in Atlantic salmon. Were HGT to occur, it would most likely involve prokaryotes and is of little concern since the GH gene is naturally occurring and the transgene integrant does not contain any sequences that confer toxicity or pathogenicity, nor functionality in prokaryotes. AquaBounty has also demonstrated the absence of a complete ampicillin resistance gene in the AAS genome (ABT 2013). Therefore, it is concluded that the environmental hazard related to the potential HGT is negligible with reasonable uncertainty.

Potential impacts on environmental hazard assessment endpoints

The potential hazard of AAS to wild populations of Atlantic salmon is concluded to be high with reasonable uncertainty. The greatest hazard to wild populations of Atlantic salmon is expected to result from competition and from potential genetic introgression of fertile broodstock with wild populations. The magnitude of the hazard is expected to be greatest in small populations of wild Atlantic salmon. The reasonable degree of uncertainty is attributed to the lack of information on phenotypic characteristics of AAS in the natural environment, ecological interactions of AAS at different life stages, the reproductive capacity of AAS and the reliance on information from GH transgenic surrogate species.

The greatest potential hazard to prey of wild Atlantic salmon is expected to be related to the feeding motivation of AAS. In the natural environment, AAS are expected to have similar or increased feeding motivation relative to wild Atlantic salmon. However, it is not possible to foresee the fitness of AAS in the natural environment or the number of AAS that may be present, making it difficult to predict the magnitude of potential pressure of AAS on prey.

Consequently, the potential hazard of AAS to prey of wild Atlantic salmon is concluded to be moderate with high uncertainty. The high degree of uncertainty is attributed to the lack of information on AAS feeding behavior and its ability to avoid predators in the natural environment.

The predicted low potential toxicity of AAS (see above) to predators of wild Atlantic salmon is concluded to be low with high uncertainty. The high degree of uncertainty is attributed to

inconclusive evidence about the ability of AAS to avoid predators in the natural environment, the lack of information about the nutritional value of AAS to predators and the limited information on hormone and allergen levels in AAS.

The effects of AAS on competitors of Atlantic salmon are expected to result from direct, competitive interactions with AAS at various life stages, rather than from genetic introgression through interspecies hybridization with non-native brown trout. The potential hazard of AAS on competitors of wild Atlantic salmon is concluded to be moderate with reasonable uncertainty. The reasonable uncertainty is attributed to the lack of information on phenotypic characteristics of AAS in the wild and on the relative competitive ability of AAS with coexisting species in the natural environment.

The potential hazard of AAS to habitat is concluded to be low with high uncertainty on the basis of expert opinion. The high degree of uncertainty is attributed to the lack of information on fitness, population size, migration and spawning behaviors, body size of adult AAS spawners, propensity for spawning, and overall longevity of repeat AAS spawners.

The potential hazard of AAS to biodiversity in Canada is unknown. AAS is expected to have low effects on nutrient cycles in rivers unless AAS adults have a greater propensity to semelparity (death after spawning) than wild Atlantic salmon. Effects of AAS to biodiversity through the displacement or exclusion of non-salmonid species from their habitat is unknown. The effects of AAS feeding on biota not typically consumed by wild Atlantic salmon are also unknown. Potential hazards of escaped farmed fish to biodiversity are poorly understood (Leggatt et al. 2010) making reliable prediction of the effects of AAS on overall community dynamics, ecosystem function and biodiversity very difficult.

Environmental hazard assessment

The various elements of environmental hazard are consolidated in Table 3. The final ranking for environmental hazard is the highest ranked element and its associated uncertainty. The final environmental hazard of AAS to the Canadian environment is concluded to be high with reasonable uncertainty.

Table 3. Summary of the environmental hazard assessment of AAS

Assessment endpoints	**Hazard**	**Uncertainty**
Wild populations of Atlantic salmon	High	Reasonable uncertainty
Prey of Atlantic salmon	Moderate	High uncertainty
Predators of Atlantic salmon	Low	High uncertainty
Competitors of Atlantic salmon	Moderate	Reasonable uncertainty
Habitat	Low	High uncertainty
Biodiversity	Unknown	
Final	**High**	**Reasonable uncertainty**

Uncertainties in the environmental hazard assessment

Uncertainty in the final environmental hazard assessment results from the lack of information on phenotypic characteristics of AAS in the natural environment, genotype x environment interactions, and the reliance on information from surrogate GH transgenic species. Predictions regarding the potential ecological and genetic effects of GH transgenic fish in variable natural environments are complex. Studies over the last two decades provide solid evidence that rearing conditions, resource levels, backgrounds genetics, life history stages, and predation levels can all affect the potential ecological consequences of GH transgenic salmonids. Consequently, the magnitude of the potential environmental hazard of AAS is difficult to predict and remains reasonably uncertain.

Risk Assessments

Both the indirect human health and the environmental risk assessments are conducted in accordance with the classical risk assessment paradigm where risk is directly related to the exposure and hazard of the organism, or **R = E x H**. Final indirect human health and environmental risk assessments are reported separately.

Indirect Human Health Risk Assessment

The exposure assessment has examined the potential for AAS to enter the Canadian environment through four different pathways. The findings of the exposure assessment are summarized in Table 1, and conclude that, for the use scenario specified in the regulatory submission, exposure of AAS to the Canadian environment is expected to be negligible with reasonable certainty.

Human contact with naturally occurring Atlantic salmon during swimming is rare and catches in the recreational and aboriginal Atlantic salmon fisheries provide limited opportunity for dermal exposure through handling of fish. For the purposes of the indirect human health risk assessment, the likelihood of any incidental human contact to AAS through activities such as recreational swimming or fishing is considered to be extremely remote given that entry of AAS into the Canadian environment is negligible. Therefore, the exposure of humans in Canada to AAS is concluded to be negligible with reasonable certainty.

The indirect human health hazard assessment characterized and ranked the incremental human health hazards that could result from environmental exposure to AAS, relative to wild Atlantic salmon. It was based on the potential toxicity and allergenicity of AAS, and its capacity to act as a vector for human pathogens. The final indirect human health hazard of AAS was concluded to be low with reasonable certainty (Table 2).

The outcome of the indirect human health risk assessment is summarized in Table 4 and concludes that, under the proposed use scenario specified by AquaBounty in the regulatory submission, the risk to human health resulting from environmental exposure to AAS is low with reasonable certainty.

Table 4. Indirect human health risk assessment of AAS under the proposed use scenario

Assessment	Rank	Uncertainty
Exposure	Negligible	Reasonable Certainty
Hazard	Low	Reasonable Certainty
Risk	**Low**	**Reasonable Certainty**

Environmental risk assessment

The exposure assessment has examined the potential for AAS to enter the Canadian environment through four different pathways. The findings of the exposure assessment are summarized in Table 1, and conclude that, for the use scenario specified in the regulatory submission, exposure of AAS to the Canadian environment is expected to be negligible with reasonable certainty.

The environmental hazard assessment characterizes the nature, and severity of potential harmful effects that AAS may cause to wild populations of Atlantic salmon, prey of Atlantic salmon, predators and competitors of Atlantic salmon, habitat, and biodiversity. The final environmental hazard of AAS to the Canadian environment is concluded to be high with reasonable uncertainty (Table 3).

The outcome of the environmental risk assessment is summarized in Table 5 and concludes that, under the proposed use scenario specified by AquaBounty in the regulatory submission, the risk to the Canadian environment associated with the manufacture and production of AAS is low with reasonable certainty.

Table 5. Environmental risk assessment of AAS under the proposed use scenario

Assessment	Rank	Uncertainty
Exposure	Negligible	Reasonable Certainty
Hazard	High	Reasonable Uncertainty
Risk	**Low**	**Reasonable Certainty**

Changes to the proposed use scenario or to the proposed containment measures specified in the regulatory submission may result in the entry or release of AAS into the environment in a quantity, manner or circumstance significantly different to the potential exposure of AAS assessed in the current risk assessment. Given the potential hazard of AAS to the Canadian environment and associated uncertainty, including potential invasiveness, any significant new activity may result in an altered exposure, and consequently, a different risk assessment conclusion than provided in this report.

Waiver Request

AquaBounty has requested a waiver for information required under information item 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)* in accordance with Section 106(8) of CEPA 1999. This information item requires that data be submitted from tests conducted to determine the invasiveness of AAS. The waiver request is based on AquaBounty's

assertion that the organism is manufactured at a location where the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health (ABT 2013).

Therefore, the waiver should only be granted if it can be demonstrated that AAS is contained such that it will not enter the Canadian environment.

Waiver Assessment

AAS is intended for use under strictly controlled conditions that include physical containment at two clearly defined facilities. At the PEI facility, there are 16 pathways of entry into the environment for all life-stages of AAS. To prevent an unintentional release from the PEI facility, there are a minimum of 3 (and as many as 6) mechanical barriers along each pathway. In all cases, suitable operational measures and oversight are in place to avert or mitigate potential failures and prevent living AAS at all life-stages from entering the Canadian environment. In addition, the PEI facility is sited at a location and constructed to standards that effectively prevent the unintentional release of AAS that may result from naturally occurring catastrophic events. Finally, extensive security measures are in place to prevent unlawful entry that may result in theft or damage to property.

During transport from the PEI facility to the facility in Panama, AAS eggs will be securely packed and labeled for shipment by air. Chain-of-custody will be maintained through to its arrival in Panama using a commercial freight–forward company. AAS eggs will be received and transported to the facility in Panama under the supervision of an official from the Ministry of Agriculture's Quarantine Department and will be unpacked and inspected at the facility under the supervision of an official from the National Animal Health Authority.

At the Panamanian facility, there are 4 pathways of entry into the environment for all life-stages of AAS. To prevent an unintentional release from the facility, there are a minimum of 4 (and as many as 12) mechanical barriers along each pathway. In most cases, suitable operational measures are in place to avert or mitigate potential failures and prevent living AAS at all life-stages from entering the Panamanian environment. Further, in the unlikely event of AAS escaping from the facility in Panama, geographical isolation and regional water temperatures that are beyond the range of tolerance for Atlantic salmon will prohibit AAS from entering the Canadian environment.

AquaBounty has provided well-defined parameters for the scope of their proposed activity, as outlined above. Proposed containment measures (physical, biological and geographical containment) were assessed to result in a negligible risk of entry into the Canadian environment.

AquaBounty has committed to ensuring that live eggs exported from the PEI facility to the facility in Panama will be reared only at the production site described in the notification and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out.

Based on the above, it is concluded that AAS is manufactured at a location where AquaBounty is able to contain the living organism so as to satisfactorily protect the environment and human health.

Conclusions

1 - Indirect Human Health Risk

The finding of negligible with reasonable certainty for the exposure assessment and low with reasonable certainty for the indirect human health hazard assessment resulted in a risk assessment outcome of low with reasonable certainty and a conclusion of not “CEPA toxic”.

2 - Environmental Risk

The finding of negligible with reasonable certainty for the exposure assessment and high with reasonable uncertainty for the environmental hazard assessment resulted in a risk assessment outcome of low with reasonable certainty and a conclusion of not “CEPA toxic”.

3 - Waiver

Given the proposed use scenario specified in the regulatory submission and that 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, data on invasiveness as specified in paragraph 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)* is not needed to determine whether the organism is toxic as defined under section 64 of CEPA 1999.

Any activities outside of the well-defined parameters described in the complete waiver assessment in Appendix A of the risk assessment (DFO 2013b) may be considered a significant new activity and could require a Significant New Activity Notice.

4 - Significant New Activities

AquaBounty Canada has indicated its intent to commercially produce pressure-shocked female AAS eggs at its land-based facility in PEI for export to a land-based, grow-out facility in the highlands of western Panama. No more than 100,000 eggs will be exported to Panama in any given year. In Panama, AAS will be grown to a commercial weight of 1 to 3 kg, then harvested, euthanized and transported to a processing plant in close proximity to the Panamanian grow-out facility where they will be processed for retail sale in approved markets for food consumption.

AquaBounty has also committed to ensuring that live eggs exported from the facility in PEI to the facility in Panama, will be reared only at the production site described in the regulatory submission and that no live fish of any life stage will be sold or given by AquaBounty Panama to a third party for grow-out.

AAS is intended for use under strictly controlled conditions that include physical confinement in two clearly defined facilities. AquaBounty has provided well-defined parameters for the scope of their proposed activity, as outlined above. The proposed parameters, which include physical, biological and geographical containment provisions, have been deemed sufficient to result in a negligible likelihood of entry into the Canadian environment with reasonable certainty.

Changes to the proposed use scenario or to the proposed containment measures may result in the entry or release of AAS into the environment in a quantity, manner or circumstances significantly different to the potential exposure of AAS assessed in the current risk assessment. Given the potential hazard of AAS to the environment and associated uncertainty, including potential invasiveness, any significant new activity may result in an altered exposure and consequently in a different risk assessment conclusion than provided in this report.

The emphasis that has been placed on containment to prevent exposure to the Canadian environment and in particular on physical containment of AAS, makes it imperative that the use scenario proposed by AquaBounty be maintained including all physical, biological, geographical and operational containment measures. Therefore, any activities outside of the well-defined parameters that have been described in the regulatory submission may be considered a significant new activity and could require a Significant New Activity notification.

Based on the scope of the use scenario specified by AquaBounty in their regulatory submission and the outcome of DFO's risk assessment, in accordance with the DFO/EC/HC Memorandum of Understanding, DFO offers the following recommendations to EC in respect of a Significant New Activity notice:

A Significant New Activity in relation to AAS could include any activity other than the following:

1. Commercial production at the AquaBounty Canada facility, near Souris, 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 St. 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 Panama facility in Chiriquí Province, 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;

3. Biological containment as described in AquaBounty's regulatory submission.

If a significant new activity in relation to AAS is proposed, information to be provided by AquaBounty to the Minister of the Environment, at least 120 days prior to the commencement of the proposed significant new activity, should include the following:

1. a detailed description of the proposed significant new activity in relation to AAS;

2. a detailed description of all physical, biological and geographical containment measures proposed to be used and data to support their efficacy;

3. the information specified in paragraph 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)*; and

4. any other information or data in respect of AAS that is in AquaBounty's possession or to which they have reasonable access, that is relevant to determine whether AAS is "CEPA toxic" or capable of becoming "CEPA toxic".

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