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Canadian Environmental Protection Act Indirect Human Health Assessment Report on the AquAdvantage® Salmon

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

An indirect human health risk assessment was conducted on a genetically modified Atlantic Salmon (*Salmo salar*) known as the AquAdvantage® EO-1α Salmon (AAS) that was notified under the *Canadian Environmental Protection Act* (CEPA). This risk assessment examined the potential for the AAS to cause harmful effects to humans in Canada relative to wild-type Atlantic Salmon as a consequence of environmental exposure, including exposure in natural environments and environments under its intended use of manufacture and grow-out in a contained land-based facility in Prince Edward Island. Multiple redundant physical and biological containment measures are in place to prevent release and establishment in the Canadian environment. There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use of AAS for commercial grow-out in a contained land-based facility as well as other identified potential uses. As such, there is no expectation that AAS poses any more risks to human health than wild-type Atlantic Salmon.

BACKGROUND INFORMATION

In 2013, AquaBounty Canada submitted a notification (NSN 16528) to Environment and Climate Change Canada (ECCC), under the New Substances Notification Regulations (Organisms) [NSNR(O)] of the *Canadian Environmental Protection Act* (CEPA), detailing its intent to commercially manufacture EO-1α Salmon broodstock in a land-based contained facility near Bay Fortune, PEI and produce eyed eggs of the AquAdvantage® Salmon (AAS) for export to Panama. AAS is the commercial form of EO-1α Salmon that is used for grow-out operations to produce market-size fish for sale as food. Under the containment conditions proposed by notifier, it was determined that production of EO-1α Salmon broodstock and AAS eyed eggs poses low risk to the Canadian environment and indirect human health (DFO 2013).

In 2016, Health Canada (HC) and the Canadian Food Inspection Agency (CFIA) approved the AquAdvantage® Salmon for human food and animal feed use, respectively, on the basis that it is nutritionally the same as non-genetically modified Atlantic Salmon.

On July 27, 2018, AquaBounty Canada Limited submitted a regulatory package to ECCC explaining its intent to manufacture EO-1α Salmon broodstock to produce AAS eyed eggs for export to Panama and United States and to grow-out AAS at a land-based aquaculture facility near Rollo Bay, PEI.

Pursuant to section 64 of CEPA, the objective of this indirect human health assessment is to determine if the notified living organism (AquAdvantage® EO-1α Salmon) is toxic. That is, to determine if the living organism is entering or may enter the environment in a quantity or concentrations or under conditions that constitute or may constitute a danger in Canada to human life or health.

This assessment is divided into two parts. The hazard assessment part of this report focuses on the potential of the AquAdvantage® EO-1α Salmon to constitute a danger in Canada to human life or health. On the other hand, the exposure assessment part of this report explores the different scenarios under which AquAdvantage® EO-1α Salmon could enter the Canadian environment and result in human exposure and the quantity, concentrations or conditions associated with that exposure. To conclude the assessment, risk to human life or health that may arise following exposure through the environment is characterized using the paradigm that Risk = Hazard x Exposure.

HAZARD ASSESSMENT

IDENTIFICATION AND CHARACTERIZATION OF THE AQUADVANTAGE® SALMON

Binomial name

Salmo salar L.

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Gnathostomata
Class	Actinopterygii

Order	Salmoniformes
Family	Salmonidae
Genus	*Salmo*
Species	*Salar*
Strain	EO-1α Salmon

Synonyms, common and superseded names

Common name: Atlantic Salmon

Trade name: AquAdvantage® Salmon [Atlantic Salmon (*Salmo salar* L.) bearing a single copy of the stably integrated α-form of the opAFP-GHc2 gene construct at the α-locus in the EO-1α line].

Characterization and substantiation of the taxonomic identification

The notified organism is a genetically modified Atlantic Salmon (*Salmo salar* L.) identified as the AquAdvantage® Salmon (AAS) previously assessed in 2013 under NSN 16528. AAS are a triploid, all-female, hemizygous line of salmon engineered to reach market size more rapidly than non-modified counterparts. AAS contains a single copy of the opAFP-GHc2 transgene at the EO-1α locus. The transgene consists of a growth hormone (GH) gene from the Chinook Salmon (*Oncorhynchus tshawytscha*) and an anti-freeze promoter from the Ocean Pout (*Zoarces americanus*) (DFO 2013). The assessment also includes diploid females and neomales that will be used as broodstock.

Atlantic Salmon is one of approximately 20 species in the sub-family *Salmoninae*, of the *Salmonidae* family. The most closely related species is the Brown Trout (*Salmo trutta*) and while not native to North America, it is known to hybridize with Atlantic Salmon in areas where it has been introduced. The two most reliable external features for distinguishing the two species are the scale count between the base of the adipose fin and the lateral line and the length of the upper jaw bone in relation to the eye. Wild type Atlantic Salmon identification may also be confirmed through differences in chromosome numbers, enzyme electrophoresis of protein variants (allozymes) and DNA analyses (e.g., mitochondrial DNA) (OECD 2017).

STRAIN HISTORY

A detailed description of the strain history for the notified line was provided in NSN 16528. Briefly, development began in 1989 with Atlantic Salmon from the Exploits, Colinet and Northeast Rivers in Newfoundland and Labrador. Since 2000, fish used in the development of the notified line have been predominantly domesticated fish from the Saint John River strain and therefore, AAS may now be considered as a domesticated transgenic Atlantic Salmon strain. Since 2013, the line has continued undergoing family-based breeding.

GENETIC MODIFICATIONS

Phenotypic and genotypic changes resulting from the modifications and the stability of the genetic modifications

The methods used to produce the notified organism, the resulting phenotypic and genotypic changes as well as the sources and functions of the inserted genetic materials were provided in NSN 16528 and described in DFO (2013). The notifier reported there have been no additional genetic modifications to AAS since NSN 16528. Genetic stability of the transgene was

demonstrated over multiple generations in NSN 16528. To demonstrate genetic stability for the notified line, PCR results were provided for 2015, 2016, and 2017 spawning seasons and Southern blot results for the 2015 spawning season. Assessment of genetic stability done for NSN 16528 and the updated information for the 2015, 2016, and 2017 spawning seasons concluded that there is molecular stability of the transgene at the EO-1α locus and a low potential for mobilization of or recombination of the EO-1α.

AAS may be distinguished from non-modified salmon by PCR. Study reports were submitted by the notifier validating ability of the PCR protocol to confirm the presence of a single integrated form of the inserted genetic construct.

BIOLOGICAL AND ECOLOGICAL PROPERTIES

Atlantic Salmon is native to the temperate and subarctic regions of the North Atlantic Ocean and its marginal seas. In Canada, the range is approximately one-third of the global range extending from the St. Croix River to the outer Ungava Bay and eastern Hudson Bay. Estimates suggest that Canada has at least 700 rivers which either currently or once did support Atlantic Salmon populations (COSEWIC 2011).

AAS display an enhanced growth phenotype with more individuals of the strain growing to at least 100 g within 2,700 degree-days of first-feeding than non-transgenic comparators. Description of the growth rates at the various life stages is provided in DFO (2013).

Atlantic Salmon is anadromous with the young migrating from the river to the sea for feeding and returning to their natal river to spawn as adults. However, there are populations that complete their entire life cycle in freshwater and are known to be landlocked (OECD 2017). Smoltification is size dependent and may occur at 10 cm fork length with fast growing parr smolting at younger ages. Adults can reach a size of up to 120 cm and 150 cm for females and males, respectively, with a maximum weight of 40 kg (OECD 2017). There are no size data for AAS beyond four years of age.

The life cycle ranges from three to twelve years and while there is a genetic component to generation time, it may be strongly modified by environmental factors such as temperature, food abundance and density. Sexual maturity varies between the sexes with males frequently reaching maturity at the parr stage during their second or third autumn while landlocked females typically mature at smaller sizes compared to anadramous populations (OECD 2017). The majority of AAS females are reported by the notifier to sexually mature at approximately three years of age. In males, no difference in maturation rate was observed between AAS and non-trangenics in the first year of life and an approximate 50% decrease in the rate for the transgenic fish seen in the second year (Moreau and Fleming 2012). Salmonids are annual autumn/winter spawners relying mainly on seasonal cues to entrain gamete maturation and spawning cycle (OECD 2017).

Atlantic Salmon inhabit cool temperature streams and tolerate freshwater temperatures ranging from 0 to 28°C. Summer acclimatized juveniles show positive growth from approximately 5 to 26°C with fastest growth occurring between 16 to 20°C, while winter acclimatized fish under laboratory conditions can show positive growth temperatures as low as 1°C (OECD 2017). There are no studies comparing the temperature tolerances of AAS to those of non-transgenic Atlantic Salmon.

HUMAN HEALTH EFFECTS

Zoonotic potential

Fish-borne zoonoses are rare and tend to be restricted to a small number of opportunistic bacterial pathogens (Boylan 2011). Bacterial species that have been isolated from wounds and systemic infections following aquatic injuries and exposures include *Aeromonas hydrophyla, Chromobacterium violaceum, Edwardsiella tarda, Erysipelothrix rhusiopathiae, Mycobacterium marinum, Shewanella* species, *Streptococcus iniae* and *Vibrio vulnificus* (Diaz and Lopez 2015; Savini et al. 2017). Zoonotic bacteria may be acquired through stings, bites, spine/pincer injuries or open wounds on the handler with persons of immunocompromised health status being at greater risk (Weir et al. 2012). However, there are no reported cases in the scientific literature of infections from these bacterial species resulting from Atlantic Salmon exposure.

Bacterial pathogens that have caused serious epidemics in farmed salmonids include *Renibacterium salmoninarum*, the causative agent in bacterial kidney disease, and *Aeromonas salmonicida* which causes furunculosis (OECD 2017). Other bacterial pathogens that have been recorded causing disease in Atlantic Salmon include *Flavobacterium psychrophilum*, *Flavobacterium columnare*, *Yersinia ruckeri*, *Piscirickettsia salmonis*, *Pseudomonas fluorescens*, *Vibrio salmonicida, Vibrio anguillarum*, *Vibrio ordali*, *Moritella viscosa*, and *Tenacibaculum maritimum* (OECD 2017).

While there are no reported cases in the scientific literature of human infections by *R. salmoninarum*, there are recently reported cases from *A. salmonicida*. Acosta-García and Aguilar-García (2014) reported on a soft tissue infection in a 64 year old woman in Mexico with no history of chronic disease following a foot and leg injury resulting in septic shock and loss of the limb. *A. salmonicida* was isolated from the blood of a 34 year old immunocompetent woman in India (Tewari et al. 2014). A case of *A. salmonicida* bacteremia was reported in a diabetic patient following chronic well water consumption (Moore et al. 2017). *A. salmonicida* was found to be the causative agent in a case of postoperative endophthalmitis in a 55 year old woman two weeks after cataract surgery (Varshney et al. 2017). As well, *Y. ruckeri* has been isolated from a leg wound infection in a 16 year old male in Belgium (De Keukeleire et al. 2014). However, the authors reported that the cause of the infection was unclear as other bacterial species also isolated included *Aeromonas* spp., *Lactobacillus* spp., and *Clostridium perfringens*. *P. fluorescens* is considered to be an opportunistic pathogen of low virulence in immunocompromised patients and people with underlying health issues. In these patients, this micro-organism has been found to colonize the airways, urinary tract and blood (Rossignol et al. 2008). It has been implicated in a bacteremia outbreak in oncology patients (Hsueh et al. 1998) and in coronary care units (Benito et al. 2012; Oba et al. 2017). A fatal *V. anguillarum* infection was reported in a 65 year old immunocompromised woman (Sinatra and Colby 2018). The source of infection could not be determined but the authors provided two possibilities based on the available epidemiologic data: 1) exposure of a skin wound or fly bite to contaminated sea water, or 2) consumption of contaminated seafood. While *Aeromonas* spp. are consistently considered to be opportunistic pathogens (Dias et al. 2018), there are no reported zoonotic cases in the literature of infections from *A. salmonicida* or the other bacterial pathogen species listed above.

The relative susceptibility of AAS to fish zoonotics compared to non-genetically modified Atlantic Salmon is not known. Higher susceptibility of GH transgenic Coho Salmon to *A. salmonida* compared to non-transgenics has been reported (Kim et al. 2013). However, the Bay Fortune facility has never detected any pathogens of human health significance and no adverse human health effects attributable to AAS exposure have been reported by the notifier's staff after more than two decades. The notifier provided standard operating procedures (SOPs) for the Rollo

Bay facility outlining the pathogen barrier procedure for staff and visitors as well as for handling mortalities and moribund salmonids. While altered resistance to pathogens have been reported in other GH transgenic salmonids (Jhingan et al. 2003), even if AAS were to have an increased capacity to act as a reservoir for human pathogens, the nature and severity of adverse effects related to topically acquired fish zoonoses reported in the scientific literature is relatively mild.

In addition to bacterial infections, humans suffer from numerous parasitic fishborne zoonoses (e.g., opisthorchiasis, intestinal trematodiasis, anisakiasis or diphyllobothriasis) many of which are caused by helminths (Chai et al. 2005). Some fish parasites particularly at their infective stages (third-stage larvae of nematodes, metacercariae of trematodes, plerocercoids of tapeworms) may be of human health significance (Scholz 1999). Anisakid larvae and *Diphyllobothrium* sp. infections have been reported in Atlantic Salmon (Rodger 1991; Marty 2008; Crotta et al. 2016). However, in most of these cases involving waterborne parasites, infections are acquired through the consumption of raw or improperly cooked or processed fish (Boylan 2011).

Allergenicity/toxigenicity

Atlantic Salmon are not known to produce endogenous toxins and there is no evidence to suggest the genetic modification will result in the production of any exogenous toxins by AAS. Basic Local Alignment Search Tool (blastn and blastx) analyses of the opAFP-GHc2 construct for NSN 16528 found the sequence to not code for any known toxins or proteins other than the intended GH (DFO 2013). Similar results were seen following BLAST analyses of the inserted transgene sequence for this NSN.

In-house amino acid sequence analyses of the expressed Chinook Salmon GH using the AllergenOnline Database (v18B; 23 March, 2018) found no matches to known allergens with greater than 35% identity nor exact matches for 80 and 8 sliding window amino acid segments, respectively. Analyses were conducted for all six open reading frames. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA plants (WHO/FAO 2009). The U.S. Food and Drug Administration (USFDA 2010) concluded that there are no potential allergen concerns identified with salmon GH based on a search of the AllergenOnline Structural Database of Allergenic Proteins.

Prevalence of fish allergy in the general population ranges from 0.2 to 2.29% and up to 8% in fish processing workers and salmon is among the major species of fish reported to cause allergic reactions (Sharp and Lopata 2014). While the major route of fish sensitization is through ingestion, people with a fish allergy can react to aerosolized proteins generated by cooking or processing resulting in dyspnea, wheezing, throat tightness, urticaria, edema and light headedness (Sharp and Lopata 2014). Sensitization through aerosol and dermal exposure to fish protein allergens has been reported in occupational settings (Onesimo et al. 2012; Lopata and Jeebhay 2013) including in a salmon processing plant (Dahlman-Höglund et al. 2012). In addition, dermatitis and bronchial hyperreactivity have been reported in fish processing workers following exposure to fish infected with *Anisakis* sp. (Nieuwenhuizen et al. 2006). However, no allergic reactions have been reported by staff of the notifier after more than 20 years of occupational exposure.

Compositional studies were conducted on AAS for NSN 16528 with the presence of the inserted construct being the only significant difference found between AAS and non-transgenic, farm-raised Atlantic Salmon. While no additional studies have since been conducted, in 2014, the notifier requested an independent evaluation of the extant allergenicity data provided with NSN 16528 from the co-directors of the Food Allergy Research and Resource Program at the

University of Nebraska - Lincoln. Their review concluded that both the diploid and triploid genetically modified salmon did not present any greater risk than non-modified salmon to those with fish allergies.

Rehbein and Devlin (2009) found no indication of an increase in parvalbumin expression at either the mRNA or protein level in transgenic rapid-growing Coho Salmon (*Oncorhynchus kisutch*) compared to non-transgenics. Similarly, Nakamura et al. (2009) reported no difference in endogenous allergen expression in genetically modified Amago Salmon (*Oncorhynchus masou ishikawae*). However, susceptible individuals that are already allergic to fish proteins may also be highly likely to have an allergic response if exposed to AAS.

HISTORY OF USE

Since the completion of NSN 16528, the notifier has produced eggs of AAS through three spawning cycles at its Bay Fortune facility and started six production cycles at its facility in Panama with three harvests. In May 2016, the notifier received approval from Health Canada for the sale of AAS filets in Canada as well as Canadian Food Inspection Agency approval for use of AAS in animal feeds with the first sale of the product in Canada occurring in 2017.

HAZARD CHARACTERIZATION

The human hazard potential of the AquAdvantage® Salmon is assessed to be low because:

1. The notified organism is a transgenic Atlantic Salmon containing a single copy of the opAFP-GHc2 integrant at a single locus that was confirmed to be stably integrated by PCR and Southern blots;
2. Hazard through toxicity/pathogenicity: The methods used to produce the notified living organism do not raise any indirect human health concerns. Neither of the source organisms from which the inserted genetic material was derived (the Chinook Salmon and Ocean Pout) are known to produce toxins, nor are the inserted genetic material or expressed growth hormone associated with any toxicity or pathogenicity in humans;
3. Hazard through human zoonoses: While there are reported cases of zoonotic infections associated with fish, particularly for immunocompromised individuals, there are no reported cases attributed to either the notified organism or the wild-type salmon;
4. Hazard through allergenicity: Data from allergenicity testing submitted previously in 2013 did not indicate any increases in allergenic potential compared to non-transgenic counterparts, and the sequence of the inserted transgene or any potentially expressed proteins from the constructs do not match any known allergens or toxins; this conclusion is also supported by the Health Canada's novel food decision of 2016; and
5. There have been no apparent adverse indirect human health effects reported by staff of the Bay Fortune facility after more than 20 years of operation.

Human Health Hazard considerations from the Problem Formulation for the Risk Assessments of the AquAdvantage® Salmon (for reference):

Table 1: Considerations for hazard severity (human health).

HAZARD	CONSIDERATIONS
High	• Effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal. • Prophylactic treatments are not available or are of limited benefit. • High potential for community level effects.
Medium	• Effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. • Some potential for community level effects.
Low	• **No effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals.** • **Effective prophylactic treatments are available.** • **No potential for community level effects.**

Uncertainty related to indirect human health hazard assessment

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 2. Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organism. Adequate information was also provided by the notifier describing in good detail the methods used to genetically modify the notified line including the sources of the genetic materials and the stability of the resulting genotypes and phenotypes. Sequence analyses of the inserted genetic material did not match any known toxins or allergens and no reports in the scientific literature were found for adverse effected attributed to the inserted material in humans.

Table 2. Categorization of uncertainty related indirect human health hazard.

Description	Uncertainty Ranking
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 The potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.	Negligible
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.**	**Low**
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.	Moderate
Significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).	High

While there were no reports of adverse human health effects directly associated with the notified organism, surrogate information from the literature on other transgenic fish species appear to

indicate the potential for an increase in susceptibility of human pathogens (Jhingan et al. 2003; Kim et al. 2013). However, cases of zoonotic infections from fish are rare and most often associated with immunocompromised individuals (Boylan 2011). While it is not known if there is an increase in allergen expression in AAS, allergic responses are likely in susceptible individuals with existing fish allergies.

The indirect human health hazard of AAS is considered to be low with low uncertainty. The uncertainty is considered low since much of the information on human health effects are based on reports from surrogate organisms due to the lack of experimental data on AAS, specifically regarding pathogen susceptibility.

EXPOSURE ASSESSMENT

SOURCES OF EXPOSURE

The notifier intends to expand manufacturing capabilities to produce on an annual basis up to 250 metric tonnes (MT) of AAS fish using contained land-based facilities at Rollo Bay and Bay Fortune in the Prince Edward Island.

With the addition of the Rollo Bay facility to the existing Bay Fortune facility, the notifier hopes to produce enough eggs to satisfy requirements for the production sites in Panama, Indiana and Rollo Bay. During the risk assessment, the company expressed its intent to manufacture and sell diploid non-transgenic Atlantic Salmon eggs to external parties.

The main source of human exposure is expected to be from the manufacture of AAS eggs and the production of different age-classes of AAS including fry, smolts, juveniles, adult broodstock and the 400 g to 5 kg market weight grow-out fish. The physical state of the manufactured product will be largely eggs and killed fish. However, there may be occupational exposure to milt used to fertilize the eggs and direct contact with live fish. Furthermore, if the production of non-transgenic fish for external parties occurs along-side transgenic fish, the general population could potentially be exposed to AAS because of an operational containment failure due to human error leading to accidental shipment of transgenic eggs to customers.

MANUFACTURE IN CANADA

The notifier intends to expand manufacturing capabilities to produce AAS using contained land-based facilities at Rollo Bay and Bay Fortune in the Prince Edward Island. The Rollo Bay site is located in a predominantly agricultural area of approximately 28 hectares bound by Route 307 (Bear River Road), a north-south highway that connects Highway 2 (Veteran’s Memorial Highway) and Highway 16 (Northside Highway). The site is in eastern PEI (Kings County) and is approximately 1.5 km north of the closest coastal waters. The site is about 7 km northwest of Souris, PEI (estimated population: 1,232), which is approximately 78 km northeast of the provincial capital of Charlottetown (estimated population: 38,174).

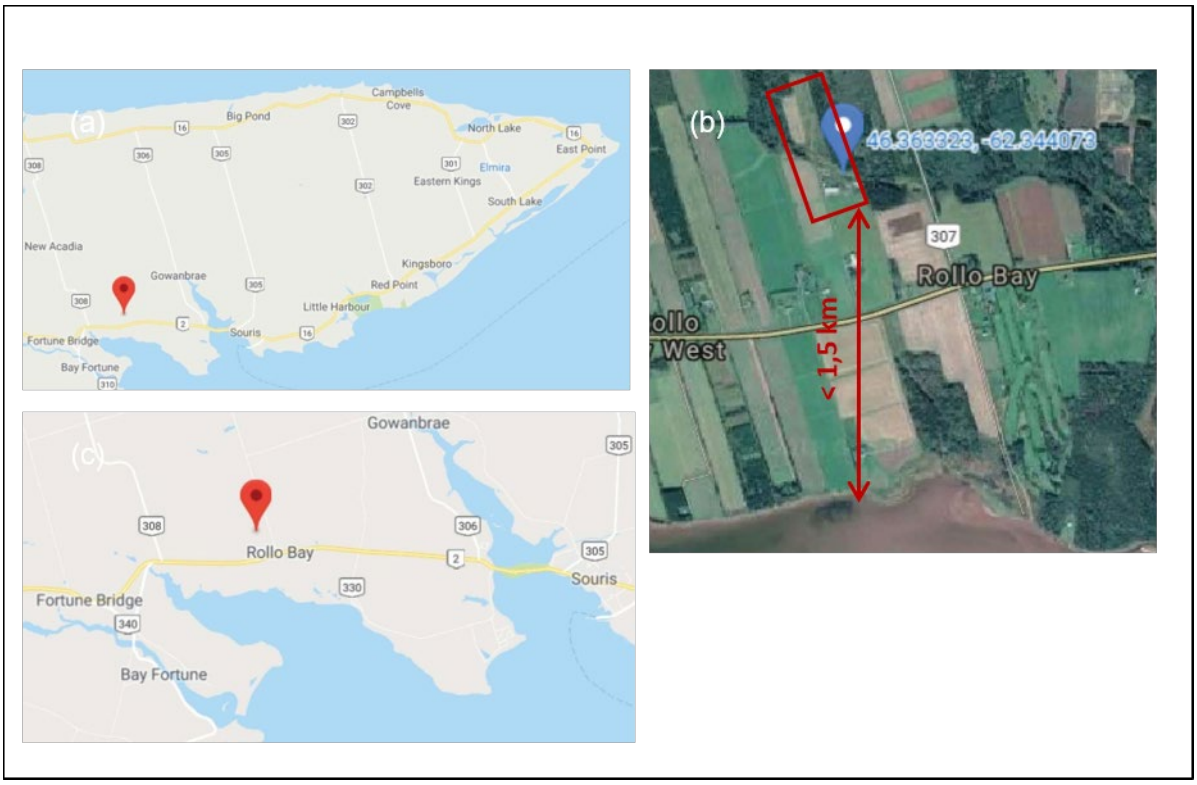

Figure 1. Maps (Google, 2018) showing (a) Eastern PE, (b) Rollo Bay, and (c) Site of manufacture at Rollo Bay.

Just like the current operations at Bay Fortune facility, the manufacture and production of AAS at Rollo Bay site and will be confined to land-based, freshwater facilities, each of which have multiple and redundant means of containment designed to prevent the release of AAS into the Canadian environment. Greater detail regarding containment measures has been provided by the company for the expressed purpose of the current risk assessment and review, but is identified as confidential business information and is not included in this report. All three buildings at Rollo Bay will be constructed and equipped with systems appropriate for the climate and are expected to withstand high winds and weight of snow in winter months. During the winter months, snow load on the roof will be monitored and snow removed as needed. No trees will be located adjacent to the buildings to prevent damage from falling limbs or trees in the event of a tropical storm or hurricane.

Multiple systems will be in place to monitor site security, prevent unapproved intrusion, avoid inadvertent escape of fish and prevent loss of operational capacity. The aquaculture buildings will be equipped with independent back-up generators that will meet power requirements in the event of an electrical outage. Culture tanks in all buildings will be monitored continuously for water level, dissolved-oxygen levels, pH, temperature, carbon dioxide and ozone.

Operationally, human exposure could arise from the:

- manufacture of AAS eyed-eggs, EO-1α broodstock eggs and non-transgenic eggs;
- grow-out of AAS from first feeder fry to juvenile fish of approximately 400 g until about 5 kg market weight;
- purging, harvesting and slaughter of market weight AAS fish for delivery to off-site processing facilities;

- production of the two lines of fish used to produce the AAS, the homozygous EO-1α neomales and the non-transgenic females derived from the Saint John River strain that was the original source of the EO-1α line of salmon; and
- activities involving third parties that may arise if production of non-transgenic fish for sale is to occur along-side that of transgenic fish at the Rollo Bay facility.

According to the notifier, the Rollo Bay operations will be managed according to established SOPs based on the successful operations (over 20 years) at notifier's facilities at Bay Fortune and in Panama. It is indicated in the information provided that, AquaBounty Canada (ABC) will ensure the same level of proficiency and quality control is in place at the Rollo Bay facility. Staff will be trained in all fish handling procedures related to their responsibilities, will be supplied with the equipment required to operate the facilities in a secure manner, will understand and follow the SOPs in place for all activities, and supporting documentation will be maintained. SOPs for the Rollo Bay facilities are being developed with SOPs currently in use at the Bay Fortune broodstock facility and the Panama grow-out facility as templates. The SOPs for Rollo Bay will be modified based on experiences in current operations and to address the site-specific operational conditions and equipment present at the Rollo Bay facility.

INTRODUCTION OF THE ORGANISM

According to the notifier, the mode of action underlying the principal benefit of the AAS is the transient acceleration of growth during early life that is produced by a stably integrated and predictably heritable GH transgene. As such, the only intended current use of the notified living organism is commercial aquaculture production in land-based contained facilities to capitalize on the accelerated growth rate of the AAS fish. The objective is not for introduction into the Canadian environment but to use the Rollo Bay facility for the purpose of keeping AAS broodstock to be used to manufacture AAS eggs and to grow AAS for commercial sale of killed whole AAS to processors in the United States and Canada.

AquaBounty Technologies (ABT) has also established a 1500 MT commercial grow-out facility in the United States. That facility is located in Albany and is operated by AquaBounty Farms – Indiana (ABF-IN). On April 27, 2018, ABT received approval from FDA to operate the Indiana facility for grow-out of AAS for human consumption. Eyed-eggs produced by ABC at its facilities in Rollo Bay and Bay Fortune in Prince Edward Island will be sold to AquaBounty Panama (ABP), ABF-IN, and used by ABC for grow-out at the new facility in Rollo Bay, PEI.

Since these entities are wholly-owned and operated by ABT, the Sponsor will exercise singular and direct control over all aspects of manufacture and production involving live animals. No vendors have been identified for the AAS that will be produced in Canada or Indiana. At harvest, AAS will be harvested and sold into existing seafood-distribution channels for processing, export, and retail sale. ABP currently sells whole, killed AAS to multiple processors in Panama for export as fillets. ABF-IN and ABC will sell killed, whole AAS to processors in the United States and Canada, respectively.

ENVIRONMENTAL FATE

The climate at the Rollo Bay site is generally damp, with average annual rainfall of 87 cm and average annual snowfall of 340 cm; the average temperature is -7°C in January and 19°C in July. Average minimum and maximum daily temperatures by-month for Charlottetown have ranged from -16.6 to 13.5°C and -3.3 to 23.2°C, respectively, over the past 30 years.

Effluent from the site enters a brook before leaving the property. The brook will be hospitable to salmonids at all life-stages for the time when the site is in operation. The brook is part of the

Rollo Bay watershed and travels about 1.5 km before entering the Northumberland Strait, a saline body of water.

Not much is known on the potential for survival of the different life stages of GH transgenic fish and subsequent performance in the wild. While sex-reversal, domestication and growth hormone transgenesis may impact fitness, they have not been demonstrated to prevent survival and reproduction. Consequently, considering the fact that the wild type Atlantic Salmon is known to survive environmental conditions in the areas in close proximity to the intended manufacturing site at Rollo Bay, PEI, all life-stages of the notified living organism (from embryo to adult) are likely to survive in the Canadian environment if released here.

OTHER POTENTIAL USES

The notifier states that the only intended current use of AAS is commercial aquaculture production in land-based contained facilities in Canada, United States (proposed) and Panama. To date, manufacture and production of AAS has been confined to the notifier's land-based freshwater facilities in Prince Edward Island and Panama. In the United States, no life stage of AAS may be raised in ocean net pens under the New Animal Drug Application approval granted by the USFDA in 2015. Use in research and development is possible such as for example in testing of DNA-based vaccinations in fish (US Patent 5780448A). There may also be other unknown potential uses, if production of non-transgenic fish for sale occurs along-side that of transgenic fish. However, these uses are difficult to identify in this assessment due to lack of information on activities involving third parties.

EXPOSURE CHARACTERIZATION

Risks from workplace exposure to the notified strain are not considered in this assessment[1].

The human exposure potential of the AquAdvantage® Salmon is assessed to be low because:

1. The main source of human exposure to the notified organism is expected to be from the manufacture of up to 250 MT of AAS fish per year in contained land-based facilities at Rollo Bay and Bay Fortune in Prince Edward Island, each of which have multiple and redundant means of containment designed to prevent the release of AAS into the Canadian environment;
2. There is no intentional release of AAS fish into the environment and the physical state of the manufactured products (eggs, different age-classes of AAS including fry, smolts, juveniles, adult broodstock, killed fish and milt to be used to fertilize the eggs) is not expected to lead to increased human exposure;
3. Human exposure to AAS is expected to be further reduced through the use of operational controls, including procedures for operating the redundant layers of containment, documentation, reporting of containment breaches, staff training, and other site-specific SOPs, which have been developed based on experiences in current operations at Bay Fortune (broodstock and hatchery) and in Panama (grow-out);

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

4. Since there is no intentional environmental release of AAS fish from the facility and the fact that only market weight fish leaving the facility will be harvested and killed before release from the facility, the likelihood of human exposure to live AAS will be greatly minimized;
5. While there are uncertainties associated with the expected fitness of AAS fish in natural environments, conditions may be favourable for survival and dispersal of AAS fish if released into the freshwater brook that runs through the Rollo Bay facility and potentially result in human exposure;
6. Physical and operational measures in place at Rollo Bay and Bay Fortune facilities including multiple containment barriers, wastewater treatment, and the processing of solid waste are expected to decrease the likelihood of human exposure to the notified AAS fish; and
7. Apart from the use in research, there are no other foreseeable potential uses of AAS outside containment if the Rollo Bay facility is used to produce fish only for internal uses. However, there may be unknown potential uses if production of non-transgenic fish for sale to third parties is to occur along-side that of transgenic fish at the Rollo Bay facility.

Considerations used to characterize the likelihood of human exposure to AAS are presented in Table 3.

Uncertainty related to indirect human health exposure assessment

The ranking of uncertainty associated with the indirect human health exposure assessment is presented in Table 4. Although adequate information was provided by the notifier on the sources of exposure and measures in place at the two land-based facilities, conditions may be favourable for survival and dispersal of AAS fish if released into the freshwater brook that leads to the Northumberland Strait. Since the notified fish is not for intentional environmental release, uncertainty on potential human exposure may only arise as a result of accidental or unintended releases of AAS fish. Available information in the scientific literature indicates a potential for survival of these fish in the Canadian environment. Therefore, because of limited information on exposure scenarios in the Canadian environment, the human exposure to the notified organisms is considered low with low uncertainty.

Table 3: Exposure considerations (humans).

EXPOSURE	CONSIDERATIONS
High	• The release quantity, duration and/or frequency are high. • The organism is likely to survive, persist, disperse proliferate and become established in the environment. • Dispersal or transport to other environmental compartments is likely. • The nature of release makes it likely that susceptible humans or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem. • In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible humans.
Medium	• It is released into the environment, but quantity, duration and/or frequency of release is moderate. • It may persist in the environment, but in low numbers. • The potential for dispersal/transport is limited. • The nature of release is such that some susceptible humans may be exposed and/or exposure will be of intermittent frequency and/or short duration. • In relation to exposed humans, routes of exposure are not expected to favour toxic, zoonotic or other adverse effects.
Low	• **It is used in containment (no intentional release).** • **The nature of release and/or the biology of the organism are expected to contain the organism such that susceptible populations or ecosystems are not exposed.** • **Low quantity, duration and frequency of release of organisms that are not expected to survive, persist, disperse or proliferate in the environment where released.**

Table 4: Uncertainty ranking associated with the indirect human health exposure.

Available Information	Uncertainty Ranking
High quality data on the organism, the sources of human exposure and the factors influencing human exposure to the organism. Evidence of low variability.	Negligible
High quality data on relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism or valid surrogate. Evidence of variability.	**Low**
Limited data on the organism, relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism.	Moderate
Significant knowledge gaps. Significant reliance on expert opinion.	High

RISK CHARACTERIZATION

NOTIFIED USE

In this assessment, risk is characterized according to a paradigm embedded in section 64 of CEPA 1999 that a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard, and on what we can predict about exposure from the notified use.

AAS is a genetically modified Atlantic Salmon designed to reach market size faster than conventional salmon. The faster growth is the result of the introduction of a transgene construct containing a growth hormone gene from a Chinook Salmon and the regulatory sequences of an antifreeze protein gene from an Ocean Pout. The notified organism will be grown to market size in a land-based contained facility in Rollo Bay, Prince Edward Island.

Although there are reported cases of zoonotic infections from exposure to fish, there are no reported cases attributed directly as a result of environmental exposure to Atlantic Salmon in the scientific literature. There are no reported cases of allergenicity associated with salmon growth hormone nor is the overall allergenic potential of the AAS expected to be any different from non-transgenic counterparts. The inserted transgene and the methods used to modify the notified organism do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low potential exposure, and the effective containment procedures implemented at the land-based facilities, the human health risk associated with the use of the AquAdvantage® Salmon for commercial aquaculture production in land-based, contained facilities in Rollo Bay, PEI is assessed to be low.

RISK ASSESSMENT CONCLUSION

There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from commercial aquaculture production in land-based, contained facilities in Rollo Bay, PEI. This risk to human health associated with the AquAdvantage® Salmon is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

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