



Name of company	AquaBounty Technologies, Inc.	
First day of assessment period	2018/07/28	
Last day of assessment period	2019/03/24	
Identity	EO-1 α salmon including the triploid, hemizygous, all-female line; Trade name: AquaAdvantage [®] salmon refers only to the triploid, hemizygous, all-female line; Explicit biological name: Atlantic salmon (<i>Salmo salar</i> L.) bearing a single copy of the α -form of the opAFP-GHc2 recombinant deoxyribonucleic acid construct at the α -locus in the EO-1 α lineage.	
Organism type	Fish	
Use	Commercial aquaculture in a land-based facility	
Anticipated quantity	250 metric tonnes to be produced annually	
Assessment level of concern	Human Health Hazard	Low for toxicity/pathogenicity Low for human zoonoses Low for allergenicity
	Human Exposure	Low
	Environmental Hazard	<ul style="list-style-type: none"> • Negligible through environmental toxicity • Negligible through horizontal gene transfer • Negligible to biogeochemical cycling • Low as a vector of disease • Low to habitat • Moderate to biodiversity • Moderate through interspecific hybridization • High through intraspecific hybridization • High through trophic interactions
	Environmental Exposure	Low
Assessment conclusion under section 64 of the <i>Canadian Environmental Protection Act, 1999</i>	Not toxic	
Category	Not eligible for listing on the Domestic Substances List	
Recommended Action	Significant New Activity notice	
Waiver	Requested under paragraph 106(8)(b) of the <i>Canadian Environmental Protection Act, 1999</i> for information element 5(a) of Schedule 5 of the <i>New Substances Notification Regulations (Organisms)</i>	



Synopsis: The EO-1 α salmon was notified for use in a commercial, land-based, aquaculture facility in Rollo Bay, Prince Edward Island. EO-1 α salmon are all fish that derive from EO-1 φ and are part of the EO-1 α lineage of Atlantic salmon (including AquAdvantage[®] salmon) that contain the integrated, α -form of the opAFP-GHc2 construct. A detailed description of containment measures was provided by the notifier in 2013 (New Substance Notification 16528) and as additional information submitted for this notification. A waiver of the information requirement for item 5(a) of Schedule 5 of the *New Substances Notification Regulations (Organisms)* was accepted under paragraph 106(8)(b) of the *Canadian Environmental Protection Act, 1999*, based on the sufficiency of the proposed containment measures. Despite the negligible to high range of hazard potential for the nine ecological endpoints, the risk to the environment associated with EO-1 α does not meet the criteria in paragraphs 64(a) or (b) of the *Canadian Environmental Protection Act, 1999* due to the low potential for exposure. A significant new activity notice is recommended to require that containment measures are maintained as described and significant new activities are appropriately reported and assessed. Similarly, assessment of available information does not suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from use in commercial aquaculture and as such EO-1 α salmon do not meet the criteria in paragraph 64(c) of the *Canadian Environmental Protection Act, 1999*.

EO-1 α and AquAdvantage[®] Salmon: background information

EO-1 α salmon is an Atlantic Salmon (*Salmo salar*) containing a single insert of the opAFP-GHc2 transgene at the EO-1 α locus while AquAdvantage[®] salmon is a triploid ($\geq 98.5\%$), all-female subset of the EO-1 α lineage. The opAFP-GHc2 gene construct consists of a chinook salmon (*Oncorhynchus tshawytscha*) growth hormone gene under the control of an ocean pout (*Macrozoarces americanus*) anti-freeze protein promoter. The target phenotypic difference between EO-1 α salmon and non-transgenic Atlantic salmon is a significant increase in growth rate, allowing it to reach market size twice as fast as the non-transgenic fish (18 vs 28-36 months).

Context

EO-1 α salmon was first notified for production of eyed-eggs at the company's contained land-based facility in Bay Fortune (New Substance Notification 16528). In August, 2013, Environment and Climate Change Canada and Health Canada rendered a decision on the EO-1 α salmon (New Substance Notification 16528 – not toxic under paragraph 64 of the *Canadian Environmental Protection Act, 1999*), for the growth of adult AquAdvantage[®] salmon at Bay Fortune, Prince Edward Island, based on science advice sought from the Department of Fisheries and Oceans (Department of Fisheries and Oceans, 2013).

On July 27, 2018 a notification under the *New Substances Notification Regulations (Organisms)* was submitted to Environment and Climate Change Canada for the production of EO-1 α salmon eggs and fish and commercial grow-out of EO-1 α salmon in a contained, land-based facility in Rollo Bay, Prince Edward Island. The notifier intends to expand their manufacturing capabilities to produce, on an annual basis, up to



12 million EO-1 α salmon eyed-eggs and up to 250 tonnes of AquAdvantage[®] salmon. 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 other countries and Rollo Bay, and produce fish for the food and feed market. In addition to EO-1 α salmon, the company will maintain a broodstock of non-transgenic St. John River domestic strain salmon that will be used as the source of non-transgenic Atlantic salmon eggs needed for the manufacture of EO-1 α Salmon. The company may also sell fertilized non-transgenic Atlantic salmon eggs to external parties.

Under a Memorandum of Understanding between the Department of Fisheries and Oceans, Environment and Climate Change Canada and Health Canada, the Department of Fisheries and Oceans conducted an environmental risk assessment with the goal of determining if EO-1 α salmon was toxic as defined under section 64 of the *Canadian Environmental Protection Act, 1999*, and Health Canada conducted an indirect human health risk assessment of the EO-1 α salmon. The services of the Canadian Science Advisory Secretariat were sought in the form of a peer review process for these risk assessments (science advice), the result of which was a Science Advisory Report (Department of Fisheries and Oceans, 2019), which was used to inform the *Canadian Environmental Protection Act, 1999* final risk assessment decision. The Science Advisory Report will be publically available on the Canadian Science Advisory Secretariat website.

Hazard assessment

The environmental hazard potential of the EO-1 α salmon, should it escape from containment, is assessed to range from negligible to high for various endpoints because:

- 1) **Hazard through environmental toxicity:** Growth hormone levels in EO-1 α salmon represent a negligible hazard to predators or scavengers. Despite expression of the opAFP-GHc2 transgene in EO-1 α salmon, growth hormone levels remain below experimental detection limits and no differences were reported for other measured hormones in the muscle-skin samples from commercial sized EO-1 α salmon compared to controls.
- 2) **Hazard through horizontal gene transfer:** There is a negligible hazard to the environment through horizontal gene transfer from EO-1 α salmon. The EO-1 α construct integrated in the EO-1 α salmon does not contain viral vectors, transposable elements, or other known factors that may increase the potential for deoxyribonucleic acid uptake by/mobility to prokaryotic organisms. Moreover, in the absence of co-transfer of regulatory elements, prokaryotes lack the promoters required for expression of eukaryotic genes;
- 3) **Hazard to biogeochemical cycling:** Given the limited role of Atlantic salmon on river nutrient cycling, escaped EO-1 α salmon are expected to have a negligible effect on the geochemical cycle in returning rivers;



- 4) **Hazard as a vector for disease:** EO-1 α salmon has a low hazard potential as a vector of disease. Information from the rearing facility indicates fish health is well managed. As well, the proposed recirculating system with UV and ozone treated water would decrease potential for pathogen release from the facility relative to a traditional net-pen aquaculture site. EO-1 α salmon have not shown any increased disease susceptibility or ability to act as a reservoir for pathogens, however some surrogate models of growth hormone transgenic fish (including Coho Salmon and zebrafish) have demonstrated impaired immunity, which could alter vector capability;
- 5) **Hazard to habitat(s):** Potential hazards (harm) to habitat from EO-1 α salmon are low. Reproductive behaviour of salmonids, including Atlantic Salmon, has been shown to influence habitat through ecosystem engineering and bioturbation including significantly disturbing the streambeds. While digging behaviour in EO-1 α females has not been examined, domestic Atlantic Salmon and growth hormone transgenic Coho Salmon have lower digging frequency than wild or hatchery fish;
- 6) **Hazard to biodiversity:** Overall, the potential for EO-1 α salmon to impact prey and competitor community dynamics through altered appetite, behaviour, and possible habitat use at different life stages if released outside of containment, could result in moderate hazard to biodiversity. Computer modelling of the effects of growth hormone transgenic Coho Salmon escapes in the Strait of Georgia, BC shows escaped fish could theoretically impact biomass of different groups when large numbers were released in repeat escape events, and effects depended on predicted diet of escaped fish. Wild Atlantic Salmon express growth hormone seasonally, with higher levels in the summer. EO-1 α salmon are expected to maintain high expression of growth hormone year round, which could result in increased feeding motivation and prey consumption relative to wild Atlantic Salmon in the winter.
- 7) **Hazard through intraspecific hybridization:** Potential for harm to wild populations of Atlantic Salmon from hybridization with EO-1 α salmon has not been specifically examined but is potentially high should the AquAdvantage[®] salmon escape containment. In model studies on hybridization of escaped domestic Atlantic Salmon with wild populations of Atlantic Salmon, impacts to wild populations were well understood and included lower fitness or increased straying of hybrid offspring, with long term consequences including alterations in life-history traits, decreased population productivity and decreased resilience to environmental changes;
- 8) **Hazard through interspecific hybridization:** Potential hazard through hybridization and introgression of EO-1 α salmon genes into other species of fish is moderate. The opAFP-GHc2 transgene is expressed in hybrids generated from EO-1 α salmon and Brown Trout crosses; it cannot be determined if EO-1 α -Brown Trout hybrids could pose greater harm to native species through interspecies hybridization than domestic Atlantic Salmon through this pathway; and
- 9) **Hazard through trophic interactions:** Some trophic interactions (freshwater competition and predation) have high potential to harm wild native populations should the AquAdvantage[®] salmon escape containment; trophic interactions as prey are negligible and are supported by the Canadian Food Inspection Agency novel feed decision of 2016.



Section 5(a) of the *New Substances Notification Regulations (Organisms)* Schedule 5 requires that a notifier provide data on the invasiveness of higher organisms. In this case, evaluators and the Canadian Science Advisory Secretariat determined there was insufficient data to reach a conclusion on the invasiveness of EO-1 α salmon. Consequently, the notifier requested that this requirement be waived. A waiver may be granted under 106 (8)(b) of the *Canadian Environmental Protection Act, 1999*, which states that if “a living organism is to be used for a prescribed purpose or manufactured at a location where, in the opinion of the Ministers, the person requesting the waiver is able to contain the living organism so as to satisfactorily protect the environment and human health.” Given the containment measures in place for manufacture of EO-1 α salmon, the waiver request was accepted.

The human health 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 that was confirmed to be stably integrated at a single locus by polymerase chain reaction 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 Atlantic 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) The notifier states that there have been no apparent adverse indirect human health effects reported by staff of the Bay Fortune facility during 20 years of operation.

Hazards related to organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System¹.

¹ A determination of whether one or more of the criteria of section 64 of the *Canadian Environmental Protection Act, 1999* are met is based upon 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 the *Canadian Environmental Protection Act, 1999* is not relevant to, nor does it preclude, an assessment against the criteria in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use.



Exposure assessment

Science advice provided to Environment and Climate Change Canada through the Canadian Science Advisory Secretariat considered two exposure scenarios: a) one in which non-transgenic eggs were sold to outside parties and b) one in which no such sales occurred. Scenario a) could, through human error or poor quality assurance/quality control, result in inadvertent mixing of transgenic and non-transgenic eggs and potential release from containment of the AquAdvantage® salmon; scenario b) would eliminate this possibility. Robust quality assurance/quality control would also reduce the probability of this accidental mixing. Subsequent to the conclusion of the Canadian Science Advisory Secretariat process, the notifier provided Environment and Climate Change Canada with additional information indicating that they may sell non-transgenic eggs to external parties, but provided additional detailed procedures designed to maintain separation of transgenic from non-transgenic eggs. The environmental exposure potential of the EO-1 α salmon is assessed to be low because:

- 1) Up to 12 million EO-1 α salmon eyed-eggs and up to 250 tonnes of sterile, EO-1 α salmon per year will be manufactured in contained land-based facilities at Rollo Bay in Prince Edward Island, which has multiple and redundant means of physical, chemical and operational containment designed to prevent the release of EO-1 α salmon into the Canadian environment (described in detail in Department of Fisheries and Oceans, 2019). All eggs intended for grow-out are subjected to pressure treatment that renders them triploid and therefore sterile, a biological containment measure that is an additional layer of containment to the existing physical, chemical and operational controls.
- 2) There is no intentional release of EO-1 α salmon (eggs, or any age-class of EO-1 α salmon including fry, smolts, juveniles, adult broodstock, killed fish or milt used to fertilize the eggs) into the environment;
- 3) Operational controls, including procedures for operating redundant layers of containment, documentation, reporting of containment breaches, staff training, and other site-specific Standard Operating Procedures, have been developed and adapted based on experiences in current operations at Bay Fortune (broodstock and hatchery) and in Panama (grow-out). No breaches of containment have been reported at these facilities;
- 4) Should there be an inadvertent release, conditions may be favourable for survival and dispersal of EO-1 α salmon if released into the drainage brook that runs through the Rollo Bay facility; however, they would need to survive in the drainage brook, migrate to and survive in marine ecosystems, migrate to spawning grounds of wild populations at the same time as wild fish, then successfully reproduce. The closest stream with wild Atlantic Salmon populations is within 50 km of the Rollo Bay facility. Given



that it is unlikely that all these conditions are present at the same time, it is therefore unlikely that any EO-1 α salmon would be able to mate with Wild Salmon; and

- 5) If the company decides to produce non-transgenic eggs for sale, operational procedures designed to prevent any accidental mixing of transgenic with non-transgenic egg batches² include:
 - a. temporal separation of egg production of the two types
 - b. physical separation of the two types of eggs
 - c. highly sensitive genetic testing procedures to validate egg genotypes and
 - d. clear labeling protocols.

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 occupational exposure from the manufacture of EO-1 α salmon eyed-eggs and up to 250 tonnes of market-weight EO-1 α salmon per year within contained land-based facilities at Rollo Bay and Bay Fortune in Prince Edward Island;
- 2) Since there is no intentional environmental release of EO-1 α salmon from the facility, containment measures in place are expected to prevent releases, and market-weight fish will be harvested and killed before leaving the facility, the general Canadian population is not expected to be exposed to live EO-1 α salmon; and
- 3) Should there be an inadvertent release, conditions may be favourable for survival and dispersal of EO-1 α salmon if released into the freshwater brook that runs through the Rollo Bay facility and human exposure could result through activities such as angling.

Other potential uses

Other potential uses or activities could include production of eggs and AquAdvantage[®] salmon outside of containment or by other Parties at other locations. Since these could result in different exposures, measures should be taken to ensure containment is maintained or, failing that, a new notification would be required. A significant new activity notice is recommended to require that containment measures are maintained as described and significant new activities are appropriately reported and assessed.

Risk Characterization

Despite the negligible to high range of potential hazard arising from different ecological endpoints, there is low potential for exposure, especially in light of additional measures for maintaining separation between

² See Appendix for greater detail on the measures proposed by the company.



transgenic and non-transgenic eggs. The environmental risk associated with AquAdvantage® salmon for use in commercial, contained, land-based aquaculture is assessed to be low.

Owing to the low potential hazard and the low potential exposure, the human health risk associated with the AquAdvantage® salmon for use in commercial, contained, land-based aquaculture is assessed to be low.

Risk Assessment Conclusion

AquAdvantage® salmon does not meet the criteria in paragraph 64(a) or (b) of the *Canadian Environmental Protection Act, 1999* due to the containment measures, both physical and biological, and other procedures in place at the Rollo Bay Facility. A significant new activity notice is recommended to require that any changes to containment procedures that would increase the likelihood of release of EO-1α salmon to the environment would require new information to be provided for assessment as part of a new notification.

The available information does not suggest a risk of adverse human health effects at exposure levels predicted for the general Canadian population. This risk to human health associated with the AquAdvantage® salmon does not meet the criteria in paragraph 64(c) of the *Canadian Environmental Protection Act, 1999* and no further action is recommended.

References

Department of Fisheries and Oceans. (2013). Summary of the environmental and indirect human health risk assessment of AquAdvantage® salmon. Canadian Science Advisory Secretariat Science Response 2013/023. 26 pp.

Department of Fisheries and Oceans. (2019). Environmental and Indirect Human Health Risk Assessments for the Manufacture and Grow-out of EO-1α Salmon, including the AquAdvantage® Salmon, at a Land-Based and Contained Facility near Rollo Bay, PEI. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2019/014.

Appendix

Quality Assurance/Quality Control measures proposed to be implemented by AquaBounty related to the production and sale of non-transgenic salmon eggs	
Temporal separation	When operations with transgenic fish begin in Rollo Bay, at times when non-transgenic eggs are to be produced, AquaBounty will spawn only non-transgenic fish.
Physical Separation	The Heath Stacks and Upwellers are separate physical units. Each tray and/or upweller is clearly labeled and each incubation unit – stack or upweller is physically separate. The incubation location of each lot of eggs is



	<p>predetermined; it is not possible for eggs in one Heath stack or upweller to be mixed with the eggs from a different upweller.</p> <p>AquaBounty further commits to incubating non-transgenic eggs in a separate facility from the facility used to incubate transgenic eggs when operations with transgenic fish begin at Rollo Bay, where “facility” is a designated area or location, which will be described within AquaBounty’s standard operating procedures, for production and/or housing of non-transgenic eggs. This could be an area within an AquaBounty facility, one of the existing facilities, or a new stand-alone facility.</p>
Genotype testing	<p>AquaBounty will develop a protocol for sampling non-transgenic eggs before they leave the facility. The protocol will be developed with sufficient statistical power to determine if milt from even a single transgenic neo-male was used to fertilize eggs.</p>
Labelling	<p>AquaBounty will modify all cross codes to include “AAS” in the code if the cross was made with transgenic fish or “NTG” in the cross-code for non-transgenic crosses. Moreover, AquaBounty will introduce an additional layer of protection in the form of color-coding labels (different color labels) to further distinguish between transgenic and non-transgenic eggs.</p>