Appendix A (amended on April 26, 2018)

This document highlights certain provisions specified in the approved application[1] (NADA 141-454) for use of the new animal drug described below, as well as applicable post-market requirements for records and reports of adverse events and other experiences. This document is not meant to be an all-inclusive list of the provisions specified in the approved application or the applicable post-market requirements.

The approval is for a single copy of the α-form of the *opAFP-GHc2* recombinant DNA (rDNA) construct at the α-locus in the EO-1α lineage of triploid hemizygous, all-female Atlantic salmon (*Salmo salar*) known as AquAdvantage Salmon (AAS) under the conditions of use specified in the application. This rDNA construct at this specific site in the genome is the new animal drug ("the article") that is the subject of the new animal drug application (NADA) approval. For ease of reference, this Appendix will refer to "AAS," or "the article," or "the product" when referring to the drug.

The applicant, as defined in 21 CFR 514.3, is AquaBounty Technologies (ABT) of Maynard, Massachusetts. Should another firm acquire the application or otherwise become a holder of the application, that firm will be responsible for meeting all applicable requirements of FD&C Act.

I. Conditions established in the approval

Section 512 of the FD&C Act [21 U.S.C. 360b(a)(1)] requires that there be in effect an approved NADA for use of a new animal drug, except as specified, and that the drug, its labeling, and its use conform to the approved application. This approval is limited to the α-form of the *opAFP-GHc2* recombinant DNA construct at the α-locus in the EO-1 α lineage of triploid hemizygous, all-female Atlantic salmon (*Salmo salar*) under the conditions of use specified in the application. ABT must notify us of any change to the conditions established in the approval, including those identified below, in accordance with 21 C.F.R. 514.8. Any change to the conditions may require the submission of a supplemental new animal drug application. If a change is made prior to our approval of a required supplemental new animal drug application, the change will result in the article, in this case, AAS, being considered an unsafe new animal drug and, therefore, adulterated within the meaning of the FD&C Act. 21 U.S.C. § 360b; 21 U.S.C. 351(a)(5). Further, any food that bears or contains the article, if its use does not conform to the approved application, will be considered adulterated for bearing or containing an unsafe new animal drug. 21 U.S.C. § 342(a)(2)(C)(ii).

We remind ABT of the following conditions of use specified in the approved application:

A. Manufacturing Methods, Facilities, and Controls

a. The approved application specifies the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of AAS that are necessary to preserve the identity, strength, quality, and purity of AAS or its components, including special precautions used in ABT's operations.[2] 21 U.S.C. 360b(b)(1)(D); 21 C.F.R. 514.1(b)(5). ABT must notify us of any change to

[1] For purposes of this document, reference to the "approved application," "application" or "approval" encompasses the approval of both the original (November 19, 2015) NADA and supplemental (April 26, 2018) NADA 141-454.

[2] As described in Guidance for Industry (GFI) 187, Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs, "manufacturing methods" for GE animals should encompass the method

these conditions, beyond the variations provided for in the application, and submit a supplemental application, as specified in 21 C.F.R. 514.8. More specifically, ABT will submit a supplemental application prior to making changes as specified in 21 C.F.R. 514.8 and 21 C.F.R 514.1(b)(10) and described in the approved application, including changes in production, facilities, equipment, or SOPs except for changes in feed, changes in water temperature, or other minor changes for which ABT has contacted FDA in advance, and FDA has agreed that prior approval of a supplemental NADA is not necessary. As described in the approved application, ABT will contact CVM to determine whether and how to prepare any such applications.

b. ABT will submit the Minor Changes and Stability Report as described in 21 CFR 514.8 annually to FDA Center for Veterinary Medicine, Office of New Animal Drug Evaluation. This report will include, but not be limited to
    1. Results of testing under the Product Durability Plan of ABT Salmon[3] and Triploid Eyed-Eggs that are within specifications, and
    2. Descriptions of any changes made in the reporting period to manufacturing methods, processes, facilities, equipment, or SOPs used in the production of ABT Salmon or AAS Salmon that are not the subject of a supplement.

1. Facilities

The approved application specifies that only the following facilities will be used for manufacturing, processing, and packing AAS or its components[4] i.e., producing, raising, and harvesting AAS: (1) ABT's land-based, contained broodstock facility on Prince Edward Island, Canada, where triploid eyed-eggs will be produced (PEI facility), as described in the application; (2) ABT's land-based, contained grow-out facility in Panama, where fish hatched from these triploid eyed-eggs will be grown to maturity and harvested (Panama facility), and (3) ABT's land-based, contained grow-out facility in Indiana, where fish hatched from these triploid eyed-eggs will be grown to maturity and harvested (Indiana facility), as described in the application . The application specifies that ABT will maintain the integrity, including security, of the PEI, Panama, and Indiana facilities. The approval does not authorize the production or grow-out of AAS in facilities in the United States other than the PEI, Panama, and Indiana facilities specified in the application.

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by which the rDNA construct was introduced into the initial GE animal, the breeding strategy used to produce the lineage progenitor, and full characterization of the article and insertion site(s) once stabilized genomically, including the copy number and orientation of the rDNA construct. In addition, "manufacturing methods" for GE animals should include requirements for analytical controls for the finished product, in this case, the nature and type of growth conditions, including the presence of physical containment so as to ensure the safety, identity, quality, and purity of AAS, and a stability program that should include information demonstrating the durability of the genotype and phenotype.

[3] ABT salmon are any genetically engineered Atlantic salmon from the e E0-1α lineage irrespective of ploidy, zygosity, or gender (i.e., the set of salmon that includes diploid genetically engineered salmon that may be used as broodstock, as well as AquAdvantage Salmon or other triploid genetically engineered salmon.

[4] Components of AAS include all materials and precursors that are required to produce the final marketed product (i.e., AAS that meets the product definition). These include, but are not limited to, the various DNA fragments composing the final rDNA construct, as well as the ABT and other Atlantic salmon that are precursors of AAS.

2. Physical Containment

The approved application specifies that ocean net pens are not authorized for any use of any life stage or ploidy of genetically engineered (GE) Atlantic salmon leading to the production of AAS. In addition, the application describes specific methods and controls used for maintaining physical containment of AAS in freshwater culture systems (i.e., land-based tanks) at the PEI, Panama, and Indiana facilities. These methods and controls include maintaining dissolved oxygen content, temperature, water flow rate, and stocking density within tanks, and adequately identifying, by labeling, all tanks containing either the diploid broodstock containing the *opAFP-GHc2* rDNA construct or AAS.

3. Breeding, Testing, Production and Lot Release of Triploid Eyed-Eggs

The approved application specifies methods and controls for breeding, testing, production and lot release of triploid eyed-eggs necessary to ensure the identity, strength, quality, and purity of AAS and any ABT salmon used in producing AAS including the following:

a. ABT will derive the all-female gynogen population (homozygous female gynogen population) via gynogenesis, which will be masculinized to "neomale" status (homozygous neomale broodstock) to produce milt as described in the application.

b. ABT will test the resulting homozygous neomale broodstock fish via PCR and Southern Blot to confirm the presence of the *opAFP-GHc2* construct, its copy number, and insertion site for the first three production cycles after approval, as described in the application. ABT must report results from these studies as part of the periodic drug event reporting process as described in Section II B below.

    i. If no out-of-specification results occur during the first three-year cycle, ABT will discontinue testing homozygous neomale broodstock, but will continue testing the homozygous broodstock source females as described in the application.

    ii. If testing indicates that the *opAFP-GHc2* construct is not durable (i.e., there are changes in copy number, orientation, location, etc.), ABT will consult with FDA to determine next steps to develop procedures for the regeneration of the EO-1 α lineage, which may include filing a supplemental NADA. To facilitate regeneration of the line, ABT will maintain cryogenically preserved milt at two distinct locations.

c. ABT will use milt from the homozygous neomale broodstock to fertilize eggs from female non-genetically modified (wild type) Atlantic salmon, producing an all-female population of fertilized eggs that are hemizygous for the *opAFP-GHc2* construct.

d. ABT will subject these fertilized eggs to pressure shock treatment, resulting in triploid eggs with one copy of the *opAFP-GHc2* construct (i.e., triploid AAS eyed-eggs.) ABT will assess triploidy by flow cytometry as described in the application. Release specifications in the application have been set to assure that at least 95% of AAS eyed-eggs are triploid, based on sampling as described in the application.

i. In the event that a sample from a discrete lot does not meet the criteria specified in the application on first testing, ABT will not release the lot for shipment until a second, larger sample from the same lot, when analyzed with the first sampling, meets specifications. If the results of the second sample when analyzed in combination with the first do not meet specifications, the entire lot will be destroyed.

ii. ABT will keep records of all testing, and of any lot destruction, and method of disposal.

iii. If lots are out of specification (OOS), ABT will investigate to determine the cause, keep records of the investigation, and report the OOS results and their likely cause, as described in Section II A 1 below.

4. Shipment from PEI Facility to Panama and Indiana Facilities

The approved application specifies methods and controls for shipment of the AAS eyed-eggs from the PEI facility to the Panama and Indiana facilities, including the following:

a. ABT will ship only triploid AAS eyed-eggs produced in the PEI facility, as described in the application, to the Panama and Indiana facilities, and such eggs will be the only source of AAS.

b. ABT will keep records of the number of triploid eyed-eggs shipped from the PEI facility to the Panama and Indiana facilities, as well as the number of eyed-eggs received at the Panama and Indiana facilities.

c. ABT will identify all vessels (e.g., tanks, incubation trays) holding AAS using appropriate labeling. ABT will ship eyed-eggs meeting the release criteria for triploidy from the PEI facility in a hard-plastic insulated cooler containing alternating trays of eggs and wet ice; the cooler will be bound with packing straps and further secured in a heavy cardboard shipper container, as specified in the approved application.

d. All packages containing AAS eyed-eggs will be in the control of ABT staff during transit from the PEI facility to the airport (Canada) and to the Panama and Indiana facilities from the airport (Panama, Indiana); control of such packages from airport (Canada) to airport (Panama, Indiana) will be transferred to a freight-forwarder by prearrangement.

B. New Animal Drug Labeling

The approved application includes copies of the agreed upon labeling for AAS eyed-eggs identifying the product name, indication, warnings, directions for use, and animal safety data (see 21 CFR 514.1(b)(3)). In addition, the approved application provides other conditions regarding the labeling of AAS eyed-eggs, including the following:

a. ABT will affix the FDA-approved bilingual (English and Spanish) Product Label printed on tear- and water-resistant paper to both the egg crate and shipping container.

    i. The label will show the product name, and will provide information on the product identity, claim, limitations on use, warnings, and handling instructions of immediate importance to the end-user.

    ii. The label will identify the shipment as "Eggs and Fry" (in the event that some eyed eggs may hatch in transit) and "Not for Resale".

b. The Product Label will also bear the following warnings:

    i. Rear only in a physically-contained freshwater culture facility as specified in an FDA approved application;

    ii. Must not be reared in conventional sea cages or net-pens;

    iii. Dispose of morbid or dead fish in a manner consistent with local regulations.

c. ABT will inform FDA of any changes to the label or labelling, with the exception of typographic or design changes which may be reported as specified in 21 CFR 514.8(c)(3)(ii), to determine whether prior approval of a supplemental NADA is required prior to implementing the change approval.

C. Registration and Listing

The approved application specifies that ABT will register the PEI, Panama, and Indiana facilities with the FDA as drug establishments in FDA's Electronic Animal Drug Registration and Listing System. In addition, the application specifies that ABT will list the *opAFP-GHc2* construct in FDA's Electronic Animal Drug Registration and Listing System.

D. Inspections

The approved application specifies that ABT will allow FDA officers and employees to enter and inspect the PEI, Panama, and Indiana facilities; inspections at these facilities may extend to all equipment, finished and unfinished materials (e.g., gametes, broodstock, and all life stages of AAS), containers (including hatching trays and land-based tanks), and labeling, as well as all documents that bear on whether AAS is produced, (i.e., manufactured), processed (i.e., raised or harvested), or packed for shipment at the facilities may be adulterated, misbranded, or otherwise in violation of the FD&C Act.

In addition, the application specifies that ABT will provide the FDA/Center for Veterinary Medicine/Office of Surveillance and Compliance/Division of Surveillance with copies of documents related to inspections or regulatory actions taken with respect to the PEI, Panama, or Indiana facilities by any regulatory authorities within thirty (30) days of receipt of those documents if no deficiencies or negative actions are taken, and within three (3) days of receipt of those documents in the event of a deficiency or negative regulatory action.

II. Post-Market Records and Reports of Adverse Events and Other Experiences

ABT must establish and maintain indexed and completed files containing full records of all information pertinent to safety or effectiveness that has not been previously submitted as part of ABT's application in accordance with 21 CFR 514.80. In addition, ABT must submit to FDA reports of data, studies, and other information concerning experience with the article, as specified in the approved application, or required by 21 CFR 514.80.

To help clarify certain record and reporting keeping requirements, we have summarized the key requirements below. Nonetheless, ABT should consult 21 CFR 514.80 and related FDA guidance (available at: http://www.fda.gov/animalveterinary/safetyhealth/reportaproblem/ucm212682.htm) to ensure that ABT is complying with all applicable recordkeeping and reporting requirements.

A. Adverse Events, Product and Manufacturing Defects, and Other Experiences

ABT must maintain records and submit reports pertaining to adverse events, product and manufacturing defects that may result in serious adverse drug events, or other experiences, including:

1. Information pertaining to product and manufacturing (production) defects that may result in serious adverse events (e.g., genotypic or phenotypic durability), in accordance with 21 CFR 514.80(b)(1), including information on the failure to meet specifications for AAS triploid eggs, but excluding breaches of physical containment, which are addressed in Section II C below.

2. Serious, unexpected adverse events in all life stages of diploid ABT salmon intended to be used as broodstock or AAS at the PEI, Panama, or Indiana facility, in accordance with 21 CFR 514.80(b)(2), including mortality, morbidity, and/or gross morphological changes in AAS that exceed the rates or severity described in product labeling, and any presumptive or confirmed disease in ABT salmon intended to be used as broodstock or AAS.

3. Adverse events in all life stages of diploid ABT salmon intended to be used as broodstock or AAS and product/manufacturing defects not previously reported under 21 CFR 514.80(b)(1) and (2), including events that are not serious or unexpected (as established by the ranges of such events listed on product labeling) at the PEI, Panama, or Indiana facility, in accordance with 21 CFR 514.80(b)(4)(iv).

B. Periodic Drug Experience Report

ABT must maintain records and submit periodic drug experience reports, in accordance with 21 CFR 514.80(b)(4), including information on the following:

1. Results of testing ABT salmon intended to be used as broodstock for genotypic durability;
2. Summary of health status, mortality, morbidity and/or gross morphological changes in ABT salmon intended to be used as broodstock;
3. Results of testing fertilized eggs for triploidy;

4. Labeling accompanying shipment of triploid eyed-eggs;
5. Quantity of triploid eyed-eggs shipped from Canada to Panama and Indiana;
6. Triploid eyed-eggs received by the Panama and Indiana facilities from Canada;
7. Non-clinical laboratory studies and clinical data not previously reported for ABT salmon including AAS;
8. Summary of health status, mortality, morbidity and/or gross morphological changes in ABT salmon including AAS salmon;
9. Adverse experiences observed in ABT salmon including AAS;
10. Summary reports of increased frequency of adverse experiences in ABT salmon including AAS;
11. Number of AAS harvested from the Panama and Indiana facilities;
12. Minor breaches of physical containment that do not result in ABT salmon including AAS entering uncontained waters, which are further described in Section II C 4 below.

C. Special Drug Experience Report

ABT must maintain records and submit special drug experience reports (21 CFR 514.80(b)(5)) for breaches in physical containment, as follows:

1. In the event of a significant breach or failure of physical containment such that any life stage of ABT salmon, including AAS, is likely to be found in uncontained waters, ABT must report the event via telephone within one (1) working day to both CVM Division of Compliance[5] and the appropriate FDA field office, with a written report within three (3) working days describing the breach or failure, its causes, and the number and ploidy of escaped fish on Form FDA 1932 to both CVM and the appropriate FDA field office (i.e., a three-day NADA/ANADA field alert report (21 CFR 514.80(b)(1)).

2. In the event of a security breach where ABT cannot reasonably account for the contained presence of all ABT salmon, including AAS, ABT must report the event via telephone within one (1) working day to both CVM Division of Compliance and the appropriate FDA field office, with a written report within three (3) working days describing the breach or failure, its causes, and the number and ploidy of escaped fish on Form FDA 1932 to both CVM and the appropriate FDA Field Office (i.e., a three-day NADA/ANADA field alert report (21 CFR 514.80(b)(1)).

3. In the event of any other failure that impacts operability of physical containment equipment (e.g., due to the result of weather or lack of maintenance of the facilities and equipment) where there was no known escape or release of fish into uncontained waters, ABT must report the event within three (3) working days, describing the breach or failure and its causes on Form FDA 1932 to both CVM and the appropriate FDA field office (i.e., a three-day NADA/ANADA field alert report (21 CFR 514.80(b)(1)).

4. In the event of a minor breach of physical containment that does not result in ABT salmon, including AAS, entering uncontained waters (e.g., escapes of fish from land-based tanks to

[5] As identified by CVM.

the floor or ground), ABT must report the breach in periodic drug experience reports (21 CFR 514.80(b)(4)).

D. Record Retention

ABT must retain records and reports of all information required under 21 CFR 514.80 for a period of five (5) years after the date of submission (21 CFR 514.80(e)).

ABT must maintain records of all investigational research for a period of at least two years following the approval as described in 21 CFR 511.1(b).

E. Additional Requirements

In addition to the requirements listed above, ABT is responsible for meeting all requirements of the FD&C Act and its implementing regulations that apply to any of its products.