DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Silver Spring, MD 20993

NOV 19 2015

Wenonah Hauter
Executive Director
Food & Water Watch
1616 P Street, NW
Suite 300
Washington, DC 20036

Re: Docket # FDA-2015-P-1094

Dear Ms. Hauter:

This letter responds to the citizen petition concerning AquaBounty Technologies' (ABT) AquAdvantage Salmon that you submitted to the Food and Drug Administration (FDA or the agency) on April 2, 2015 (Petition). The citizen petition requests that FDA review "ABT's New Animal Drug Application ('NADA') for AquAdvantage [S]almon under the [Federal Food, Drug, and Cosmetic Act's (FD&C Act)] food additive provisions and deem the AquAdvantage [S]almon a food additive…."; review "the AquAdvantage [S]almon's components, including its gene expression product (the 'GEP') of the recombinant DNA ('rDNA') construct, and deem them food additives under the" FD&C Act; "[r]ender the GEP of the AquAdvantage [S]almon an added substance under the [FD&C] Act's adulteration provisions"; "[m]ake a finding that neither the AquAdvantage [S]almon, nor the GEP used to create it, is generally recognized as safe ('GRAS') for human consumption"; and promulgate regulations that state that (1) AquAdvantage Salmon and all components of such salmon are food additives that require an approved food additive petition before they may be safely used and (2) AquAdvantage Salmon "along with all of the components within such salmon are deemed adulterated" and are prohibited from use in human food under 21 CFR 189.1 because "evidence demonstrates that AquAdvantage [S]almon's GEP presents a public health risk and it has not been shown by adequate scientific data to be safe for use in human food." Petition at 3-4, 7.

As explained below, FDA is denying your requests under 21 CFR 10.30(e)(3) because the rDNA construct as integrated in the DNA of AquAdvantage Salmon is intended for use as a new animal drug and, therefore, under the FD&C Act, it cannot also be a food additive that FDA may regulate under the FD&C Act's food additive provisions. In addition, the GEP is not an added "poisonous or deleterious substance which may render" food derived from AquAdvantage Salmon "injurious to health" under § 402(a)(1) of the FD&C Act. As part of FDA's food safety review of the new animal drug application, we evaluated anything "formed in or on food" from AquAdvantage Salmon as a result of the integration of the rDNA construct; this includes the gene expression product. 21 U.S.C. 360b(d)(2)(A); 21 CFR 500.82(b) (an animal drug residue is "any compound present in edible tissues of the target animal which results from use of the sponsored compound, including the sponsored compound, its metabolites, and any other substance formed in or on food because of the sponsored compound's use."); Guidance for Industry (GFI) 187, Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs at 25 ("risk issues involved in determining food and feed safety can be divided into two overall categories. The

first addresses whether there is any direct toxicity, including allergenicity, via food or feed consumption of the expression product of the article.").[1] FDA has approved the new animal drug application for AquAdvantage Salmon, and found food derived from AquAdvantage Salmon safe for human consumption under § 512 of the FD&C Act. For this reason, FDA declines to promulgate a regulation under 21 CFR Part 189 prohibiting AquAdvantage Salmon from use in human food.

I. The ABT rDNA Construct is a New Animal Drug

The definition of a drug, in section 201(g) of the FD&C Act, includes "articles (other than food) intended to affect the structure or any function of the body of man or other animals." The definition of "new animal drug" in section 201(v) of the FD&C Act includes that it is a drug intended for use in animals that is not generally recognized as safe and effective for use under the conditions prescribed, recommended, or suggested in the drug's labeling, and that has not been used to a material extent or for a material time.

In AquAdvantage Salmon, the α-form of the *opAFP-GHc2* rDNA construct (ABT construct) is inserted in the DNA of Atlantic salmon at the α-locus in order to affect the structure or function of the Atlantic salmon so that significantly more of such salmon grow to at least 100 grams within 2,700 ^{0}C days than their non-GE comparators. The construct is thus intended to affect the structure or function of the salmon and, as a result, it meets the FD&C Act definition of a drug.[2] Because the construct is not generally recognized as safe for this intended use and has not been used to a material extent or for a material time, it is a new animal drug as defined under section 201(v)(2) (21 U.S.C. 321(v)(2)) of the FD&C Act.[3]

Generally under the Act, a new animal drug is "deemed unsafe" unless FDA has approved a new animal drug application (NADA) for that particular use, unless the drug is only for investigational use and conforms to specified exemptions for such use under an Investigational New Animal Drug exemption (21 USC 360b(a)(1), (a)(3)), or unless the drug is used in conformance with regulations promulgated under sections 512(a)(4) or (5) of the Act (21 U.S.C. 360b(a)(4) or (5)). The ABT rDNA construct, therefore, requires FDA review as a new animal drug and approval where, as here, the approval standards have been met.[4]

---

[1] The GEP is also relevant to other elements of the NADA review, e.g. target animal safety ("[w]ith regard to health of the GE animal, including the target animal safety requirements of 21 CFR 514.1(b)(8), we recommend that you submit data regarding whether the rDNA construct or its expression product(s) cause any direct or indirect toxicity." GFI 187 at 23.

[2] Indeed, in your petition, you acknowledge that "the GEP is *intended to affect* the characteristics of AquAdvantage [S]almon...." Petition at 17.

[3] Your petition asks that FDA "[m]ake a finding that neither the AquAdvantage [S]almon, nor the GEP used to create it, is generally recognized as safe ("GRAS") for human consumption." Petition at 2. As stated above, FDA did not consider the ABT construct to be GRAS, but considered it to be a new animal drug requiring FDA approval.

[4] The data and information a sponsor must submit to demonstrate that the new animal drug that is the subject of an application meets the FD&C Act standard of approval is set forth in section 512(b)(1) of the FD&C Act (21 U.S.C. 360b(b)(1)). As described in this section, the burden is on the sponsor to provide this information and to prove that its new animal drug meets this approval standard. Thus, contrary to your assertion (Petition at 29), ABT was required to provide the data and information demonstrating that its product meets the standard of approval, including the standard for food safety. In FDA's review, as described in this letter and in the FOI Summary (see FOI Summary, Section IX), the agency found that the data and information ABT provided supported a finding that the new animal drug that is the subject of ABT's AquAdvantage Salmon application met this standard.

II. Because the ABT rDNA Construct is a New Animal Drug It Cannot be a Food Additive

The definition of a food additive in section 201(s) of the FD&C Act includes that it is "any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food....except that such term does not include... a new animal drug...." Because the ABT rDNA construct as used in AquAdvantage Salmon meets the definition of a new animal drug as discussed above, it cannot also be a food additive when used for the same intended use.

In your citizen petition you maintain that a substance can be both a new animal drug and a food additive because "[t]here is no indication that Congress intended [section 201(s)(5) of the FD&C Act] to mean that simply because part of a food's production process involves a new animal drug, the Agency cannot review other parts involving food additives;" and because FDA has in the past treated "substances as both new animal drugs and food additives depending on how the substances are used." For the reasons set forth below, we do not agree with these arguments.[5]

A. Legislative History

The legislative history of the FD&C Act indicates Congress's intent that a substance intended for a use that meets the FD&C Act definition of a new animal drug must be regulated solely under § 512 of the FD&C Act as a new animal drug and not under both § 512 as a new animal drug and § 409 as a food additive.

Prior to enactment of the Animal Drug Amendments of 1968 (ADA), animal drugs intended for use in food-producing species were regulated as both drugs, under § 505 of the FD&C Act, and as food additives under § 409 of the FD&C Act. S.R. 1308, 90th Cong., 2d Sess., 2 (1968). The drug approval process evaluated the safety of the animal drug to the target animal under § 505. The food additive approval process evaluated the human food safety of the animal drug and "any substance formed in or on food as a result of" use of the animal drug under § 409. *See* Withdrawal of Approval of the New Animal Drug Application for Enrofloxacin in Poultry; Commissioner Decision (Enrofloxacin Decision), Docket No. 2000N-1571 at 94, *citing* H.R. Rep. No. 90-2168, at 2 (1968); *Hearing before the Subcomm. on Health of the Comm. on Labor and Public Welfare on S. 1600 and H.R. 3639*, 90th Cong. 92-96 (1968). The ADA consolidated the authorities in §§ 505 and 409 as they relate to animal drugs in new § 512 of the FD&C Act. Pub. L. No. 90-399, § 101(b) (1968). When Congress enacted new § 512, it "created a category of drugs called 'new animal drugs' (defined in § 201(w) of the [FD&C Act]) and excluded them from the definition of 'new drug' at §201(p) and from the definition of 'food additive' at §201(s). Because §512

---

[5] In addition, your petition asserts that a "[f]inding that the AquAdvantage [S]almon should be prohibited as an unsafe food additive aligns with the agency's current guidelines." Petition at 7. Specifically, you assert that "from its first guidance issued on the subject of bioengineered foods in 1992, the Agency has said that genetically engineered foods would fall under its food additive provisions." Petition at 7-8. Your assertion is incorrect; this policy statement, as its title indicates, applies only to foods derived from new plant varieties developed through genetic engineering. While you note this distinction (see Petition at 8, fn. 15), the fact that the food additive regulation in 21 CFR 170.30(f)(2) does not make such a distinction has no bearing on the scope of the policy statement or its contents, as you allege in your petition. In its 1992 Policy, the Agency merely references the regulation at 21 CFR 170.30(f) in support of its position, noting that existing regulations indicate "*it might be appropriate in some circumstances* to review the GRAS (and implicitly food additive) status of foods or substances of natural biological origin that have a history of safe use but which subsequently have had "significant alteration by breeding and selection." 57 FR 22984, 22990.

contained the applicable language from both § 409 and § 505, after 1968, FDA continued to apply the same two approval standards (safe and effective as a drug, and safe as a food additive) to new animal drugs as it had before 1968." Enrofloxacin Decision, at 94-95.

This history demonstrates that Congress intended to eliminate a dual regulatory scheme for new animal drugs that clearly would have required approval as both a drug and a food additive of substances that, like the ABT rDNA construct, are new animal drugs. Accordingly, in its regulation of the ABT rDNA construct, FDA has applied both the drug and food additive approval standards that Congress consolidated in § 512 of the FD&C Act and has issued a single NADA approval under that provision.[6]

While your petition maintains that FDA could regulate a substance as both a drug and a food additive where "part of a food's production process involves a new animal drug" and "other parts" involve food additives, it is not the case here that part of the process involves a new animal drug and "other parts" involve a food additive. The ABT rDNA construct is intended to affect the structure or function of salmon and, as explained above, it is therefore a drug. The safety of any substance that is "formed in or on food" derived from AquAdvantage Salmon as a result of use of this drug, including its gene expression product, is reviewed as part of the new animal drug approval and not separately under the food additive provisions.[7] *See* § 512(d)(2)(A) of the FD&C Act. Moreover, in reviewing the AquAdvantage Salmon NADA, FDA applied the same standard (reasonable certainty of no harm) in determining the safety of food derived from AquAdvantage Salmon that would apply in reviewing a food additive. See section II A 2 of this letter.

B. The Same Substance Cannot be a New Animal Drug and a Food Additive When Used for the Same Intended Use

In your petition you maintain that FDA has in the past treated "substances as both new animal drugs and food additives depending upon how the substances are used." Petition at 11. We do not dispute this; in fact, a critical element of the definition of a drug is that it is "intended for use" in one of the ways set forth in § 201(g)(1) of the FD&C Act. A substance that is intended for a use that will affect the structure or function of an animal is a drug. If that same substance is intended for a different use—one that is not encompassed by the FD&C Act drug definition—then it would

---

[6] "In the Animal Drug Amendments of 1968 (1968 Amendments), Congress streamlined the process by combining parts of § 505 and § 409 into a new section, § 512 . . . Because § 512 contained the necessary language from both § 409 and § 505, after 1968, FDA continued to apply the same two approval standards (safe and effective as a drug, and safe as a food additive) to new animal drugs as it had before 1968." *See* Withdrawal of Approval of the New Animal Drug Application for Enrofloxacin in Poultry; Commissioner Decision (Enrofloxacin Decision), Docket No. 2000N-1571 at 94–95, *citing* H.R. Rep. No. 90-2168, at 1, 3 (1968); *Hearing before the Subcomm. on Health of the Comm. on Labor and Public Welfare on S. 1600 and H.R. 3639*, 90th Cong. 92-96 (1968).

[7] Section 512(d)(2)(A) of the FD&C Act states, in relevant part, that "in determining whether such drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling thereof, the Secretary shall consider, among other relevant factors, (A) the probable consumption of such drug and of any substance formed in or on food because of the use of such drug, (B) the cumulative effect on man or animal of such drug, taking into account any chemically or pharmacologically related substance, (C) safety factors which in the opinion of experts qualified by scientific training and experience to evaluate the safety of such drugs, are appropriate for the use of animal experimentation data, and (D) whether the conditions for use prescribed, recommended, or suggested in the proposed labeling are certain to be followed in practice."

"Safe" or "safety" is defined for food additives at 21 CFR 570.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." As explained above, this same standard is applicable to the food safety review of new animal drugs.

not be a drug. A case you cite in your citizen petition, *U.S. v. Naremco*, 553 F.2d 1138 (8th Cir. 1977), illustrates this concept well. Petition at 11-12. *Naremco* concerned the use of gentian violet as the active ingredient in two different products for two different intended uses: as a drug in GV-Eleven Medicated, which was intended for use "to treat internal fungal diseases in poultry," and as a food additive in GV-Eleven Mold Inhibitor, which was intended for use "to prevent fungal growth in poultry feed." *Id.* at 1141. However, the court did not hold in *Naremco* that gentian violet, when intended for a *single* use that meets the FD&C Act drug definition, could be both a new animal drug and a food additive.[8]

Similarly, the Import Alert you cite illustrates this same concept.[9] The Import Alert stated that evening primrose oil may be sold if "(1) when sold as a drug, it is considered a new drug and the responsible person holds an approved new drug application or (2) when sold as a food, it is considered a food additive, and prior to marketing a food additive petition is submitted to FDA…and is approved by the FDA." *U.S. v. 45/194 Kg. Drums of Pure Vegetable Oil*, 961 F2d 808 (9th Cir. 1992), 810 fn 1, *citing* Import Alert 66-04.[10] The language used in the Import Alert indicates that evening primrose oil could have separate uses as *either* a drug *or* a food additive depending upon how the product(s) is marketed. It does not indicate that evening primrose oil, when intended for a single use, could be both a drug and a food additive simultaneously.[11]

III. The ABT rDNA Construct and its GEP are not "Poisonous or Deleterious" Substances Which May Render Food Derived from AquAdvantage Salmon Injurious to Health

Under § 402(a)(1) of the FD&C Act, a food is adulterated if it "bears or contains any poisonous or deleterious substance which may render it injurious to health…." You maintain that FDA "must

---

[8] Your characterization of the court's decision as "holding that gentian violet is considered an animal drug when ingested for short periods of time and a food additive when consumed on a daily basis" (Petition at 11-12) is incorrect. There is no discussion in the opinion of the significance of the period of time over which the substance is ingested. Rather, it is the intended use, in one case to treat disease in an animal and in the other to prevent mold growth in feed, which determined that GV-Eleven Medicated was a drug and GV-Eleven Mold Inhibitor was a feed additive.

[9] Your citizen petition cites *U.S. v. 45/194 Kg. Drums of Pure Vegetable Oil*, 961 F2d 808 (9th Cir. 1992) as supporting the theory that the same substance can be a food and a drug; however, we note that the decision itself does not concern this issue. It is the Import Alert, cited in footnote 1 of the decision, that is relevant to that issue. Moreover, the Import Alert concerned a human drug, not an animal drug.

[10] Import Alert 66-04 is no longer in effect.

[11] Your petition also cites 21 CFR 558.15, which states that "[t]he Commissioner of Food and Drugs will propose to revoke currently approved subtherapeutic…uses in animal feed of antibiotic and sulfonamide drugs whether granted by approval of new animal drug applications, master files and/or antibiotic or food additive regulations…." You contend that this language demonstrates that "the Agency believes it has the authority to regulate antibiotic and sulfonamide drugs as new animal drugs as well as food additives and that these categories are not mutually exclusive." Petition at 12. However, you failed to read this rule in light of the legislative history of § 512 of the FD&C Act as explained above. The regulation you cite was originally proposed on February 1, 1972 (37 FR 2444), less than four years after Congress consolidated the drug and food additive authorities for new animal drugs in § 512, and it was finalized not long afterwards on April 20, 1973 (38 FR 9811). At that time, there were many new animal drugs that had been approved as both drugs under § 505 and as food additives under § 409 prior to enactment of the ADA and enactment of § 512. If the agency had only referred in its statement to withdrawal of antibiotics approved as new animal drugs, the statement would not have encompassed the many antibiotics for use in animal feed at the time that had been approved as drugs and food additives under the old authorities or had been permitted to be marketed under a veterinary master file. Thus, when read in light of this history, the reference to antibiotics approved as food additives makes abundant sense as a reference to the pre-ADA dual approval status of new animal drugs for use in animal feed.

find the GEP to be an added substance…because it is a poisonous or deleterious substance that is artificially inserted into Atlantic salmon, possibly rendering the salmon injurious to health." Petition at 18. FDA has approved the ABT rDNA construct and its GEP in an NADA approval under § 512 of the FD&C Act. As part of this approval, FDA determined that the construct and its GEP are safe for consumption pursuant to the requirements of § 512(d)(2). See Freedom of Information (FOI) Summary, Section IX. The GEP is, therefore, not a "poisonous or deleterious substance which may render it injurious to health." Below we address your criticisms of the FDA animal drug review process generally and our findings with respect to specific components of AquAdvantage Salmon.

A. General Approach to Review

Your petition contends that FDA's new animal drug review process for AquAdvantage Salmon has been deficient in several respects. While you do not explicitly state so, we presume that you contend that as a result of these deficiencies, FDA has not adequately found AquAdvantage Salmon to be safe for consumption.[12] As explained below, this argument is without merit.

1. Adequate and Well-Controlled Studies

You state in your petition that "ABT's studies submitted to CVM were neither adequate nor well-controlled as required by the Agency's own new animal drug regulations." Petition at 32, citing 21 CFR 514.117(a). The regulation you cite, concerns studies "required to establish, by substantial evidence, that a new animal drug is effective….[and] may also be relied upon to support target animal safety." The evidentiary standard under this regulation does not apply to studies conducted to demonstrate food safety. "The primary purposes of conducting adequate and well controlled studies of a new animal drug is to distinguish the effect of the new animal drug from other influences…." 21 CFR 514.117(a). The requirements established in the "adequate and well controlled" regulations are designed for clinical studies, in which different groups of animals, treated and untreated, are observed to determine the effect of the drug. These types of studies are used to demonstrate effectiveness and sometimes target animal safety, however, food safety studies are not clinical studies. Moreover, the "adequate and well controlled" standard concerns how an effectiveness study is conducted as opposed to what the study must demonstrate (i.e. effectiveness). There is no corollary standard in a regulation concerning how a food safety study must be conducted in order to demonstrate food meets the "reasonable certainty of no harm" standard. Nevertheless, we address your assertions concerning the conduct of food safety studies and explain why the studies support a finding of "reasonable certainty of no harm" in the remainder of section III below.

Your petition asserts that the Agency's evaluation included "analyses of male and female and diploid and triploid salmon," rather than just AquAdvantage Salmon which are triploid and all-female. Petition at 32. FDA believes the sponsor's study[13] on composition, which included male and female, diploid and triploid Atlantic salmon (see FOI Summary Section IX C 2 a i) made appropriate comparisons in order to provide a scientifically robust assessment of whether transgenic male or female Atlantic salmon (TX) have important compositional differences, not only

---

[12] Your petition asserts that these deficiencies point to a need to regulate AquAdvantage Salmon under the food additive provisions of the FD&C Act, however, as explained earlier, FDA cannot regulate the ABT gene construct or its GEP as a food additive because it is a new animal drug. Petition at 29-41.

[13] *A Single-Blind, Comparator-Controlled, Quantitative Analysis of the Composition of Muscle Skin from Diploid and Triploid Atlantic Salmon (Salmo salar) Modified Transgenically with the AquAdvantage Gene Cassette (opAFP-GHc2). Covance Laboratories Inc., Wisconsin. Covance Study Identification 7352-100. Study Report AAS-HFS-001. Report Dated 22 January 2003.*

from the sponsor control (SC) (Atlantic salmon of the family from which AquAdvantage is derived) but also Atlantic salmon that is currently available as food (FC or farmed control i.e., Atlantic salmon purchased from fish farms that sell their harvests to commercial markets).

FDA provided its scientific rationale for the analysis of the entirety of the composition data in the FOI Summary:

> Characteristics of individual fish, e.g., sex or season of harvest (time of catch), may have an impact on their composition. The comparisons of interest are between TX, SC, and FC salmon with consideration of ploidy. If, in general, the relative differences among TX, SC, and FC salmon are the same for both ploidies, then ploidy is not a consideration and comparisons among groups can be made ignoring ploidy. Variability among fish within groups is considered when making the comparisons and inclusion of fish with different characteristics broadens the inference.
> FOI Summary, Section IX C 2 a.

2. Use of "Substantial Equivalence" Test

Your petition asserts that CVM used a "substantial equivalence test" to evaluate the safety of AquAdvantage Salmon, and that "[t]his concept is not a proper method for evaluating the safety of foods." You contend that the Codex Alimentarius Commission guidelines (Codex) support your assertion. Petition at 29-30. Your assertions are incorrect: FDA did more than a comparison between food from AquAdvantage Salmon and food from farm-raised Atlantic salmon; FDA assessed any identified differences for their impact on food safety. In addition, your citation overlooks key Codex text.

In the FOI Summary and Veterinary Medicine Advisory Committee Briefing Packet,[14] (Briefing Packet) FDA does not describe or define its evaluation of food from AquAdvantage Salmon[15] as a "substantial equivalence test." Instead, FDA states that safe or safety is defined for food additives at 21 CFR 570.3(i) to mean that there is

---

[14] FDA, Veterinary Medicine Advisory Committee Briefing Packet (September 20, 2010), http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf.

[15] The agency uses the following nomenclature when referring to GE salmon:

> **AquaBounty Technologies salmon (ABT salmon)** are any GE Atlantic salmon from the E0-1α lineage irrespective of ploidy, zygosity, or gender (i.e., the set of salmon that includes diploid GE salmon that may be used as broodstock, as well as AquAdvantage Salmon or other triploid GE salmon).
>
> **AquAdvantage Salmon (AAS)** are the triploid, hemizygous, all-female Atlantic salmon from the E0-1α lineage GE Atlantic salmon subject to this application. They are a subset of ABT salmon.
>
> **GH transgenic Atlantic salmon or GH genetically engineered Atlantic Salmon** are GE Atlantic salmon that contain a growth hormone (GH) construct, but whose specific lineage is unknown.
>
> ***opAFP-GHc2 construct*** refers to the α-form of the *opAFP-GHc2* recombinant DNA construct inserted into Atlantic salmon at the α-locus (i.e., the regulated article), to produce ABT salmon, including those diploid animals that serve as broodstock.

> a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.

This same standard is applicable to the food safety review of new animal drugs. See fn 6.

FDA further described its evaluation in the FOI Summary:

> The primary risk question considered in FDA's evaluation was whether there were any risks of direct or indirect effects associated with the consumption of edible products derived from AAS. The conclusions of this assessment are provided in the context of food safety. Accordingly, the most appropriate way in which to consider the primary risk question is to determine whether there is any difference between food from AAS and other Atlantic salmon, and whether food from AAS is as safe as food from other Atlantic salmon. To this end, FDA conducted a weight-of-evidence evaluation[16] of the data and information provided in support of a food safety assessment.
> FOI Summary, Section IX A.

"Substantial equivalence" is a commonly misunderstood concept, often construed to be a food safety standard, an entire food safety assessment, or the conclusion of a comparative assessment, with a new food being "substantially equivalent" to a comparator food as the end of the assessment, with no further consideration of the safety of any differences. Given the context, this is how FDA interprets your use of the term "substantial equivalence test" in your petition.

FDA notes that the Codex Alimentarius Commission (Codex) defines substantial equivalence as just the first step of a comparative safety assessment, different from what you imply in your petition:[17]

> The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart. It aids in the identification of potential food safety and nutritional issues and *is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA animals*. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.
> CAC/GL 68-2008 at paragraph 14.

FDA's determination of "whether there is any difference between food from AAS and other Atlantic salmon" and "whether food from AAS is as safe as food from other Atlantic salmon" (FOI Summary, Section IX A) may be interpreted to constitute substantial equivalence as defined by

---

[16] Throughout its review, CVM considered the relevance and quality of various data and information based on factors such as what species was studied, study design quality, etc., and weighted its review accordingly. We refer to this as a weight-of-evidence evaluation.

[17] Codex Alimentarius Commission (Codex) (2008) Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals. CAC/GL 68-2008.

Codex and an evaluation of the safety of any identified differences relative to the safety of Atlantic salmon, respectively, an approach consistent with Codex.[18]

FDA's food safety assessment included an evaluation of AquAdvantage Salmon relative to Atlantic salmon to identify differences including an assessment of the newly expressed protein Chinook salmon growth hormone and hormones that may potentially be affected by expression of the Chinook salmon growth hormone, proximates, amino acids, fatty acids, vitamins, minerals, and endogenous allergens, as well as an evaluation of the safety of any identified differences. These differences include Chinook salmon growth hormone as well as hormones that may potentially be impacted by its expression, and Vitamin B6. FDA then evaluated the safety of Chinook salmon growth hormone for consumption, and performed margin of exposure assessments for IGF1 and Vitamin B6. FDA concluded that none of these actual or potential differences pose additional toxicological or nutritional risks to consumers. FOI Summary, Section IX.

In summary, FDA did not merely identify differences between AquAdvantage Salmon and farm-raised non-GE Atlantic salmon and declare AquAdvantage Salmon "substantially equivalent" to its comparator, using a "substantial equivalence test" inconsistent with Codex as you assert in your petition. Petition at 29-30. Instead, consistent with the safety assessment strategy recommended by Codex, FDA first identified the differences between AquAdvantage Salmon and farm-raised non-GE Atlantic salmon, and then assessed the safety of those differences. For these reasons, your criticism of FDA's safety assessment as solely a "substantial equivalence test" that is inconsistent with Codex is mistaken.

3. Use of Le Curieux-Belfond et al. Review, and Related Issues

Your petition cites the review by Le Curieux-Belfond et al. (2009), which recommends that a number of studies, in addition to the ones evaluated by FDA, be conducted on aquatic GE animals intended for food use. This includes "a complete chemical analysis of the various nutrient groups and also of the pollutants potentially accumulated in the animal," "all the modification or unexpected results due to random insertion, generated by the transgene," and "sub-chronic and chronic series of tests of toxicity." Petition at 30-31.

FDA's food safety assessment involved an evaluation of direct and indirect effects of the insertion of the rDNA construct, including an assessment of the safety of the Chinook salmon growth hormone (the protein intended to be produced by the AquAdvantage construct) as well as the hormones that may potentially be affected by its expression, and a comprehensive compositional analysis. FOI Summary, Section IX. None of these assessments indicated any cause for concern regarding food safety.

Further, no scientific rationale was provided by Le Curieux-Belfond et al. to support the hypothesis that AquAdvantage Salmon accumulates toxic substances at a greater rate than non-GE farm-raised Atlantic salmon such that additional studies of the type suggested by the article would be warranted from a safety perspective. FDA requires that all seafood that it regulates, including all farm-raised Atlantic salmon, whether non-GE or AquAdvantage Salmon, meet all existing applicable food safety standards, including with regard to levels of toxic substances.[19]

---

[18] Codex, CAC/GL 68-2008 at paragraph 14 ("The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.").

[19] We note also that FDA's review of the data on the clinical chemistry, which includes tests of liver function (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and bilirubin), indicates

4. Animal Feeding Studies

Your petition also cites Le Curieux-Belfond et al. (2009) as well as the FDA Redbook[20] as stating that whole food feeding tests, referred to as "sub-chronic and chronic series of tests of toxicity," are necessary. Petition at 9, 30-31. In addition, you assert that compliance with the Codex requires the use of toxicological studies. Petition at 8-9. FDA historically has rejected the *a priori* use of whole food animal feeding studies to assess food safety and has articulated its reasoning, as follows, in a published peer-reviewed journal authored by Agency officials:[21]

> However, animal tests on whole foods, which are complex mixtures, present problems that are not associated with traditional animal toxicology tests designed to assess the safety of single chemicals. Potential toxicants are likely to occur at very low concentrations in the whole food, and the tests may therefore be inadequately sensitive to detect toxicants. Efforts to increase the amount of whole food ingested by the test animals in order to increase the sensitivity and attempt to establish a traditional margin of safety (for example, a 100-fold safety factor) may not always be possible. When tests are contemplated, careful attention should be paid to test protocol, taking into account issues such as nutritional balance and sensitivity.
> Kessler et al. at 1832.

Further, FDA's approach is consistent with that of Codex:

> Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, and based on the experience of assessing the safety of whole foods, a more focused approach is required for the safety assessment of food derived from animals, including recombinant-DNA animals. This has been addressed by the development of a multidisciplinary approach for assessing safety, which takes into account both intended and unintended changes that may occur in the animal or in the food products derived from it, using the concept of substantial equivalence.
> CAC/GL 68-2008 at paragraph 13.

---

that there are no biologically relevant differences in those functional tests between AquAdvantage Salmon, which are triploid, and the diploid Atlantic salmon currently available for food consumption. As the liver is the site of detoxification of most xenobiotics (sometimes referred to as "pollutants"), not only is the *a priori* assumption that there is a difference in the ability of AAS and non-GE, farm-raised Atlantic salmon to detoxify pollutants incorrect, but there is good empirical evidence to indicate that the major organ of detoxification appears to be functionally indistinguishable between GE and non-GE salmon, which suggests that ABT salmon would be able to detoxify (and excrete) pollutants to the same degree that non-GE Atlantic salmon would. (See FOI Summary, Appendix 1).

[20] FDA, Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 1993, Chapter VII C. We note that the Redbook applies to foods derived from plants, not animals, and that the final, more recent version of the Redbook (2000) does not include the chapter you cite from the 1993 draft Redbook on "Foods and Food Additives Developed by Biotechnology." See http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf. You have attached the most recent, final version of the Redbook to your petition as Attachment G and include a page from the earlier, draft Redbook without noting that the page you attach from the 1993 Redbook does not exist in the current version. Furthermore, you fail to note that the 1993 draft Redbook pointed to the same complications of whole food animal feeding studies that we note above and in footnote 19 ("animal tests on whole foods, which are complex mixtures, present problems that are not associated with traditional animal toxicology tests…." Redbook (1993) Chapter VII C, citing Kessler et al. (1992)).

[21] Kessler, D.A., Taylor, M.R., Maryanski, J.H., Flamm, E.L. and L.S. Kahl (1992) The Safety of Foods Developed by Biotechnology. *Science*, 256:1747.

Your use of Codex to support your assertion is a misinterpretation of Codex text.

More recently, Bartholomaeus et al. (2013)[22] have issued a comprehensive review of the performance and utility of whole food toxicity studies on GE crops, referred to as GM crops in this publication (whose basic principles transfer to whole food toxicity studies on food from GE animals). Among other findings, they concluded that:

> No whole food toxicity study was identified that convincingly demonstrated toxicological concern or that called into question the adequacy, sufficiency, and reliability of safety assessment based on [crop] molecular characterization, transgene source, agronomic characteristics, and/or compositional analysis of the GM crop and its near isogenic line.....Thus, based on the comparative robustness and reliability of compositional and agronomic considerations and on the absence of any scientific basis for a significant potential for *de novo* generation of toxicologically significant compositional alternations as a sole result of transgene insertion, the conclusion of this review is that whole food animal toxicity studies are unnecessary and scientifically unjustifiable.

You rely on Le Curieux-Belfond et al. to support the necessity of numerous toxicological studies to demonstrate the safety of food from AquAdvantage Salmon, in order to "consider all the modifications or unexpected results, due to random insert, generated by the transgene," to "check all the possible side effects of an unknown product," and to "identify any risk, including unexpected ones." Petition at 30-31.

FDA's food safety assessment included information and conclusions drawn from prior steps of the AquAdvantage Salmon evaluation, as well as data and information evaluated for the identity, composition, level(s) of expression product from the o*pAFP-GHc2* construct, and other potential downstream hazards that may be influenced by the expression product, and allergenicity. This evaluation meets FDA's statutory and regulatory requirements for demonstrating food safety (21 U.S.C. 360b(d)(2), 21 CFR 514.1(b)(8)), as described by GFI 187, and is consistent with the Codex Alimentarius Commission's *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals* (CAC, 2008). Safe or safety is defined for food additives at 21 CFR 570.3(i) to mean that there is "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance." This same standard is applicable to the food safety review of new animal drugs. See fn 6.

We note that FDA's food safety assessment did consider changes and unexpected results, due to the random insertion of the rDNA construct. These include risks of indirect effects associated with the consumption of edible products derived from AquAdvantage Salmon, including a comprehensive compositional analysis and evaluation of endogenous allergenicity. FOI Summary, Section IX C 2.

---

[22] Bartholomeaus, A., W. Parrott, G. Bondy, and K. Walker (2013) The use of whole food animal studies in the safety assessment of genetically modified crops: Limitations and recommendations. *Cri. Rev. Toxicol.*, 43(S2): 1-24 available at http://informahealthcare.com/doi/pdfplus/10.3109/10408444.2013.842955

In addition, we note that Codex states the following:

> The safety assessment of food derived from recombinant-DNA animals involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information is necessary in order to assess unintended effects, because no one individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health.
> CAC/GL 68-2008 at paragraph 18.

Therefore, your citation of the necessity of whole food toxicological studies as postulated by Le Curieux-Belfond et al., and other claims that whole food animal feeding toxicological tests are necessary in the safety assessment of foods derived from GE animals and consistent with Codex guidelines are unsubstantiated and mistaken, respectively. The quotes of Le Curieux-Belfond et al. cited in your petition in essence state that whole food feeding toxicology studies should be performed routinely for genetically engineered fish, irrespective of the specific modification, the animal's phenotype, and the types of analysis routinely performed for a food safety assessment consistent with GFI 187 and the Codex, without referencing any scientific publications to support this position. Although in a separate section of their review, Le Curieux-Belfond et al. cite a number of scientific publications to support some whole food toxicological studies, we note that these publications were not provided along with your petition, and so the Agency was not obligated to consider them[23]. Nevertheless, we note that the opinions expressed in some of these publications differ from the internationally agreed-upon food safety assessment approach of Codex,[24] and some were not relevant to food from AquAdvantage Salmon.[25] Based on the data and information relevant to the safety of food from AquAdvantage Salmon before the Agency, most of which have been made available publicly in the Briefing Packet, and the Codex approach to the safety assessment of food from GE animals, there are no data or forms of information that would suggest food from AquAdvantage Salmon poses a risk different from or greater than that of non-GE farm-raised Atlantic salmon. Therefore, no additional testing is warranted.

---

[23] 21 CFR 10.20(c)(1) states "Information referred to or relied upon in a submission is to be included in full and may not be incorporated by reference, unless previously submitted in the same proceeding.
(1) A copy of an article or other reference or source cited must be included, except where the reference or source is:(i) A reported Federal court case;(ii) A Federal law or regulation;(iii) An FDA document that is routinely publicly available; or (iv) A recognized medical or scientific textbook that is readily available to the agency."
Further, under 10.20(c), failure to comply with these requirements will result in exclusion from consideration of any portion that fails to comply.

[24] For example, Le Curieux-Belfond et al. cite The Royal Society of Canada (2001) *Elements of Precaution: Recommendations for the Regulations of Food Biotechnology in Canada* that discusses a number of additional types of studies for the safety assessment of genetically engineered organisms, mainly plants, that go far beyond the safety assessment framework articulated in Codex.

[25] For example, Le Curieux-Belfond et al. cite Vecchio, L., Cisterna, B, Malatesta, M., Martin, T.E. and M. Biggioera (2004) Ultrastructural analysis of testes from mice fed on genetically modified soybean. *Eur. J. Histochem.* 48(4), 448-454. Le Curieux-Belfond et al. state, "A diet containing genetically modified soybean also showed some effects on mouse testis (Vecchio et al., 2004), may be due to the traces of contained herbicide to which the soybean was tolerant." We note that effects seen in whole food feeding toxicology studies due to the presence of a pesticide are not relevant to the safety of food from ABT Salmon.

A. IGF1 and Other Hormones

1. GEP and its Relation to IGF1

You contend that the GEP (Chinook salmon growth hormone) "is artificially added to Atlantic salmon, likely increasing the level of IGF-1 and altering other hormone levels, possibly rendering AquAdvantage [S]almon injurious to health."[26] Petition at 18.

You state the "sponsor" study found that "AquAdvantage [S]almon had a 41.5% and 94.6% greater mean plasma concentration of growth hormone than non-GE siblings and control salmon, respectively," but that "FDA discounted these differences because they were not statistically significant, although it is clear that this is due to lack of statistical power of the study due to small samples sizes, as an independent statistician has noted." Petition at 24; see also Petition at 37-38. We understand you to be referring to data and information from the publication by Du et al. (1992), which is not a sponsor study.[27]

The Du et al. study reported body weight and plasma levels of hormones in non-GE Atlantic salmon and in growth hormone (GH) transgenic Atlantic salmon that were derived from the same parental animals from which the EO-1α lineage eventually was derived. As stated in the FOI Summary, FDA considered this publication because it provides a framework for the identification and characterization of potential hazards that may be found in AquAdvantage Salmon. It does not, however, provide useful *conclusions* regarding hazards of AquAdvantage Salmon. The GH transgenic Atlantic salmon used in this study were derived from the same parental animals from which the AquAdvantage Salmon lineage was eventually derived; therefore, the GH Atlantic salmon were distantly related to, but were not, AquAdvantage Salmon or other ABT salmon. See FOI Summary, Section IX C 1 a. In addition, the hormone levels measured in this study were taken from the plasma of small (less than 100 g) fish. Plasma hormone levels in such small Atlantic salmon would not be representative of hormone levels present in the muscle/skin of market size Atlantic salmon that would be consumed both because of the small size of the salmon and because plasma hormone levels are not necessarily equivalent to hormone levels in muscle/skin (i.e. the parts of the salmon that are consumed). Therefore, it did not provide reliable conclusions regarding any hazards that AquAdvantage Salmon might present. FOI Summary, Section IX C 1 a.

Further investigation conducted by the sponsor[28] on ABT salmon and control Atlantic salmon showed that ABT salmon did not have statistically different concentrations in muscle/skin of estradiol, testosterone, 11-ketotestosterone, T3, or T4 when compared to sponsor control fish. Growth hormone was below the limit of quantitation in all samples whether GE or non-GE. Even if there had been an increase in salmon growth hormone in ABT salmon, fish growth hormone does not bind to mammalian growth hormone receptors, nor does it have any biological activity in mammalian systems. FOI Summary, Section IX C 1 a.

---

[26] Specifically, your petition asserts that elevated levels of IGF1 are "linked to breast, prostrate [sic], lung, and colorectal cancers." Petition at 25. You further cite other adverse health effects as including early puberty, increased reproductive aging and reduced lifespan in rodents. Id.

[27] Du, S.J., A. Gong, G.L. Fletcher, M.A. Schears, M.J. King, D.R. Idler, and C.L. He (1992) Growth Enhancement in Transgenic Atlantic Salmon by the Use of an "All Fish" Chimeric Growth Hormone Gene Construct. *Nature Biotechnol.* 1:176.

[28] *Determination of IGF1, GH, T3, T4, 11-Keto Testosterone, Testosterone, and Estradiol in Salmon Tissue. CTBR Bio-Research Inc. Canada. Project Number 42361. Study Report AAS-HFS-001. Report dated 26 July 2004.*

Based on the information presented above, your comment regarding the difference in plasma growth hormone levels and the statistical power of the study is not relevant to the levels of GH in AquAdvantage Salmon, and does not provide any meaningful information regarding the safety of ABT salmon, including AquAdvantage Salmon, or FDA's safety determination.

With respect to IGF1 in muscle/skin of market-size ABT salmon and control Atlantic salmon, initial evaluation of the results of the sponsor's study[29] suggested that there may have been an increase in the level of IGF1 in the ABT salmon compared to sponsor control fish. A further evaluation of the data showed that the most apparent potential differences were between the mature diploid sponsor control and the mature diploid ABT salmon. Specifically, one mature diploid ABT salmon exhibited an increased level of IGF1 in comparison to mature diploid comparator Atlantic salmon. CVM conducted a margin of exposure (MOE) assessment in order to determine whether the observed differences are biologically relevant. For purposes of this assessment, CVM considered whether exposure to IGF1 at the maximum concentration identified in the one outlier mature diploid salmon fell within the range of daily exposures or was different from those of the sponsor control fish such that the difference is expected to result in an adverse outcome. The assessment determined the MOE for dietary consumption of IGF1 in a 50 kg teenager,[30] assuming all the non-tuna fish that that teenager ate were ABT diploid salmon with the high outlier value of IGF1 (see FOI Summary, Table 29). CVM determined that the resulting maximum estimated level of consumed IGF1, assuming that IGF1 was present at the maximum concentration from the mature diploid ABT salmon, is 1,220 µg/3.7 µg/day yielding a 330-fold MOE, which corresponded to approximately 0.003 of the total serum burden. The incremental increase in IGF1 exposure from the maximum estimated mature diploid ABT salmon intake relative to that from the study comparator yielded only 1.2 µg per day or 0.001 of the total serum burden. Therefore, IGF1 from ABT salmon poses no additional risk compared with non-GE Atlantic salmon. FOI Summary, Section IX C 1 a iii, Food Safety Review, August 24, 2010.

2. Salmon That Were the Basis for FDA's IGF1 Conclusions

You are critical of FDA's conclusions on IGF1 because you say they "were based on a comparison of diploid GE and non-GE salmon, possibly including male and female fish." However, we in fact conducted the additional analyses of IGF1 in the subset of *mature diploid* ABT and control Atlantic salmon and the MOE assessment out of an abundance of caution.

FDA notes that the levels of hormones present in muscle/skin tissue were determined for 30 ABT salmon, 33 sponsor control, and 10 farm-raised salmon. Only 6 ABT salmon out of 30 exhibited IGF1 levels that were above the assay's limit of quantitation (i.e., 3.47 ng/g tissue), all of which were mature *diploid* ABT salmon. FOI Summary, Tables 26-27. For all *triploid* ABT salmon, including AquAdvantage Salmon, the levels of IGF1 were below the assay's limit of quantitation in muscle/skin tissue, which is so low that it is not a safety concern. FOI Summary, Tables 25-26. Consequently, we find your criticism not relevant to IGF1 levels in AquAdvantage Salmon, and further find it does not provide any meaningful information regarding the food safety of AquAdvantage Salmon or FDA's food safety determination given the above discussion.

---

[29] Id.

[30] CVM chose teenaged boys as the most "sensitive" population based on their biological sensitivity to the effects of IGF1 due to their rapid growth and development, and their tendency to consume adult portions of food despite a lower body weight. See FOI Summary, Section IX C 1 a iii and fn 23.

3. Toxicological Studies and IGF1

You state that ABT "has not conducted one toxicological study that investigates the health effects of consuming salmon with artificially elevated levels of hormones, including IGF[1]." Petition at 25.

We have addressed the issue of the value of whole food toxicological studies above, in Section A 4 of this letter. That, combined with the discussion of the lack of a demonstrable increase in hormone levels in AquAdvantage Salmon intended for the food supply support FDA's conclusion that such testing is unnecessary.

4. Number of Data Points on Hormone Levels

Your petition states that "most of the company's studies failed to capture sufficient data points on hormone levels, meaning already small sample sizes were even further diminished." Petition at 38.

First, FDA notes that the sponsor conducted only one study on hormone levels in AquAdvantage Salmon.[31] The publication by Du et al. (1992) is not a sponsor study nor is it a study of AquAdvantage Salmon. In its review, FDA evaluated the sponsor study on hormones, in which samples were analyzed from a total of 73 salmon, consisting of 10 farm-raised control, 33 sponsor control, and 30 ABT salmon. See FOI Summary, Section IX C 1 a ii. All 73 salmon were tested for each hormone, but only those with detectable levels of the hormone in muscle/skin tissue are listed in Table 26 in the FOI Summary. Although a number of samples fell below a given assay's limit of detection, sample size remained constant. Thus, the number of data points listed in Table 26 does not reflect "small samples sizes" as you suggest, but instead reflects that not all of the 73 salmon tested had levels of hormones that were sufficient to be detected.

5. IGF1 in Diploid Salmon

You claim that "AquAdvantage salmon manifested a 39.8% greater level [of IGF1] in diploid salmon than the control group." Petition at 24. This assertion is inaccurate. In FDA's analysis of IGF1 levels in mature diploid ABT salmon and sponsor control farm-raised Atlantic salmon, there was only a small difference in IGF1 levels and it was not statistically significant. FOI Summary, Table 27. Because IGF1 has been considered a potential hazard, FDA performed additional analyses including an MOE assessment using conservative, health protective assumptions. FDA concluded that even if the expression of IGF1 was present at the highest levels measured, and even if expected high consumers of salmon ate no non-tuna fish but ABT salmon containing this likely upper bound level of IGF1, the margin of exposure to this endogenous component of food would be well within levels of exposure from other dietary sources of IGF1, and poses no additional risk. See Section 1, above, Section K 3 below (explaining that high consumers are teenaged boys who eat no non-tuna fish but ABT salmon with this upper bound level of IGF1), and FOI Summary, Section IX C 1 a iii.

---

[31] *Determination of IGF1, GH, T3, T4, 11-Keto Testosterone, Testosterone, and Estradiol in Salmon Tissue. CTBR Bio-Research Inc. Canada. Project Number 42361. Study Report AAS-HFS-001. Report dated 26 July 2004.*

6. Exposure to IGF1 in Milk

You cite the Hansen paper (1997) (fn 97) as stating that IGF1 binds to casein, and the Kimura et al. study (1997) as stating that IGF1 is absorbed from the gastrointestinal (GI) tract into the bloodstream (fn 97) so that "many consumers are already ingesting higher levels of IGF-1 through the milk they drink." Petition at 26. Unlike cow's milk, salmon muscle/skin does not contain casein which may bind to IGF1 and potentially protect IGF1 from undergoing degradation in the GI tract. In the MOE assessment of IGF1, FDA did not assume that IGF1 from ABT salmon would be broken down in the GI tract, and therefore would not be absorbed. Instead we assumed total intake is equivalent to body burden (i.e., all of the IGF1 that was consumed was absorbed), a conservative and health protective approach that would likely overestimate the IGF1 consumed and absorbed. Consequently, your comments regarding the Hansen paper and Kimura et al. study are not relevant to FDA's MOE and do not call into question the safety of exposure to IGF1 from consumption of ABT salmon. Casein, a protein in milk that can bind to and protect IGF1 from degradation in the GI tract is not present in salmon muscle/skin. In its MOE assessment, FDA assumed that all IGF1 consumed (total intake) from ABT salmon was not degraded in the GI tract and that it was absorbed.[32]

B. Allergenicity

1. Levels of Allergens and New Allergens

You allege that AquAdvantage Salmon may "cause elevated levels of, or entirely new, allergens after insertion of the GEP" because "allergenicity levels found even from the small sample sizes provided by ABT were considered statistically significant in diploid salmon with the GE construct, which will compose up to five percent of the AquAdvantage [S]almon that consumers eat." Petition at 25-26. Specifically in your petition, you state that "the allergenic potency for both triploid and diploid AquAdvantage salmon was on average 19.5 and 52.5% higher, respectively, than non-GE salmon comparators", and that "the difference observed in triploid AquAdvantage [S]almon was not found to be statistically significant" but in question due to small sample sizes. You also express concerns regarding the allergenicity of the Chinook salmon growth hormone expressed in ABT salmon due to "unpredicted consequences from manipulation of genes." Petition at 26-27.

In assessing the allergenicity of food from AquAdvantage Salmon, we used an approach that is consistent with that recommended by Codex: evaluating the allergenic potential of the new protein, Chinook salmon growth hormone, as well as potential changes in the allergenic potency of Atlantic salmon. FDA's assessment found that triploid ABT salmon pose no additional allergenic risk than comparator Atlantic salmon, and the sponsor-submitted study was considered adequate to evaluate the potential increased allergenicity of the GE salmon.[33] FOI Summary, Sections IX C 1 b and IX C 2 b ii. With respect to allergenicity, there are two considerations: (a) the potential allergenicity of the newly expressed protein present in the food; and (b) the endogenous allergenicity of food from the recipient organism.

a) Potential Allergenicity of Newly Expressed Protein

With respect to the first consideration, potential allergenicity of the expression product, Chinook salmon growth hormone, FDA considered whether the presence of the Chinook salmon growth

---

[32] Id.

[33] Because AquAdvantage Salmon are an all-female hemizygous subset of triploid ABT salmon (that include male triploid salmon), the conclusions for the triploid ABT salmon also apply to AquAdvantage Salmon.

hormone in Atlantic salmon would pose a new risk of food allergy to consumers, given that Atlantic salmon are fish, which are one of the eight major food allergens in the U.S. (FALPA, 2004; Hefle et al., 1996).

The Codex Alimentarius Commission's Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals (CAC, 2008) describes a conservative (health protective) approach to determining whether a newly expressed protein present in a food from an rDNA organism is likely to pose an allergenic risk. This assessment strategy includes the following three main components:

- allergenicity of the gene source;
- structural similarity to known allergens; and
- resistance to proteolytic degradation (2008).

In the case of ABT salmon, the introduced growth hormone gene was isolated from Chinook salmon, which are fish, and fish are one of the eight major food allergens in the U.S. (FALPA, 2004; Hefle et al., 1996). FDA made the conservative (health protective) assumption that the transferred Chinook growth hormone was a putative salmon allergen. It is important to note, however, that individuals allergic to Chinook salmon also would be likely allergic to Atlantic salmon. Because salmon present a hazard to salmon-allergic individuals, salmon-allergic individuals will likely avoid consumption of all salmon, including AquAdvantage Salmon. See FOI Summary Section IX C 1b i.

To evaluate potential IgE cross-reactivity of the Chinook salmon growth hormone with known allergens, FDA conducted searches of the AllergenOnLine database and the Structural Database of Allergenic Proteins using deduced peptide sequences from GenBank and search criteria consistent with guidance provided by the Codex rDNA Animal Guideline. These searches revealed no amino acid sequence identities of greater than 35% in segments of 80 or more contiguous amino acids with any entries in either database. In addition, there were no matches of eight or more contiguous amino acids with any entries in either database. Therefore, these search results indicate that Chinook salmon growth hormone does not have significant structural similarity to known allergens. FOI Summary, Section IX C 1b ii.

With respect to resistance to proteolytic degradation, the newly expressed protein in ABT salmon is the native Chinook salmon growth hormone. There is no scientific rationale to suggest an altered resistance to pepsin when the Chinook salmon growth hormone is expressed in Atlantic salmon rather than Chinook salmon, therefore, FDA found the pepsin resistance assay to be unnecessary. FOI Summary, Section IX C 1b iii.

FDA concluded that the expression of Chinook salmon growth hormone in ABT salmon, including AquAdvantage Salmon, does not present a new risk of allergic reaction to salmon-allergic individuals and is unlikely to cause allergic cross-reactions.

b) Endogenous Allergenicity

With respect to endogenous allergenicity, FDA evaluated whether the edible tissue from ABT salmon is more allergenic than the non-GE comparator[34]. FDA concluded that triploid salmon pose

[34] *Sponsor Study: A Comparator-Controlled Immunochemical Study of the Allergenic Potency of Muscle-Skin from Diploid and Triploid Atlantic Salmon (Salmo salar) Modified Transgenically with the AquAdvantage*

no additional allergenic risk than control Atlantic salmon. There was no evidence of a statistically significant difference between the mean allergenic potency for sponsor control diploid salmon compared to the triploid GE salmon, including AquAdvantage Salmon. FOI Summary, Section IX C 2 b. Given that salmon is often consumed as one individual fish fillet per serving rather than a mixture of many fish, we also considered the allergen level in individual fish in addition to group means. FOI Summary, Section IX C 2 b iii.

In addition, we note that individuals allergic to salmon are already likely to avoid all salmon. CVM consulted Dr. Dean Metcalfe, Chief, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, on general scientific matters related to endogenous allergens in foods known to be allergenic. With respect to transferring a gene from one species in an allergenic food group (e.g. fin fish) to another closely-related species, Dr. Metcalfe observed that for people who know they are allergic to fin fish, there would be no new risk as they are already likely practicing food avoidance. With respect to the endogenous allergenicity of fin fish, although different species of fin fish show up to 100-fold differences in the level of the major allergen, there is no apparent public health impact because individuals who are allergic to one species of fin fish generally avoid consuming all species of fin fish. Thus, small changes in the levels of endogenous allergens would likely have little or no public health impact. In Dr. Metcalfe's opinion, increases in endogenous allergen levels of less than five-fold in this setting would not be expected to result in an adverse effect on public health. See FOI Summary, Section IX C 2 b v and Appendix 2. Use of a five-fold increase in the level of endogenous allergens in an allergenic food could serve as a signal that an additional evaluation for possible public health impact would be warranted, not because a five-fold increase would cause a public health problem, but because it would provide a useful "flag" to investigate whether one would result. For this reason, small changes in the levels of endogenous allergens in finfish[35] would likely have little or no public health impact.

To the extent there is a concern regarding the potential presence of diploid ABT salmon in the AquAdvantage population, we identified no novel food safety concerns that would be encountered were diploid ABT salmon consumed, because as noted above, people who are allergic to salmon are already likely to avoid all salmon regardless of whether it is genetically engineered. FOI Summary, Section IX C. We note that although study data showed one outlier diploid salmon that had an elevated level of measured relative allergenic potency (FOI Summary, Section IX C 2 b), the difference was not sufficiently elevated to reach the five-fold level that Dr. Metcalfe identified as an appropriate signal for further inquiry. In fact, the difference was not even a two-fold increase. Out of an abundance of caution, however, the agency judged that it would want additional data and information to draw a conclusion on the relative allergenic potency of diploid ABT salmon. In addition, we note that under the conditions established in the approved application, ABT must follow manufacturing specifications to ensure to the greatest extent possible that AquAdvantage Salmon are an all triploid population.[36]

Gene Construct opAFP-GHc2). Testing Facility: IBT Reference Laboratory. Kansas. Study Report AAS-HFS-003. Report dated 22 March 2006.

[35] ABT salmon are finfish. For this reason, FDA believes that based on all the factors discussed above, small changes in the levels of endogenous allergens in ABT salmon would likely have little or no public health impact.

[36] We note that during validation testing, the lowest effectiveness observed for triploidization in an individual batch of eggs was 98.9% and the mean was 99.8%. See FOI Summary, Section VIII B 3 f.

2. Parvalbumin

You state that "FDA acknowledges its lack of sufficient data to make conclusions regarding the AquAdvantage [S]almon's content of parvalbumin, a major allergen." Petition at 27.

We have not acknowledged a lack of sufficient data to make conclusions regarding parvalbumin in AquAdvantage Salmon. In our review, we noted that we could not draw conclusions from the Western blot analyses for parvalbumin. FOI Summary, Section IX C 2 b iv and Briefing Packet at 104-105. We note, however, that the fluorescent enzymatic immunoassay (FEIA) used to determine the relative allergenic potency of muscle-skin extracts from ABT salmon compared with extracts from sponsor control non-GE salmon detected parvalbumin along with other salmon allergens. Therefore the parvalbumin Western blot analyses were not necessary to draw conclusions regarding the allergenic potency of ABT salmon relative to non-GE farm-raised Atlantic salmon because we were able to rely on the FEIA to provide a more comprehensive measure of parvalbumin and all other salmon allergens. FOI Summary, Section IX C 2 b iii and iv.

3. Conduct of Allergenicity Studies

Your petition is critical of studies ABT conducted on allergenicity, citing the departure of the principal investigator, unblinding of samples, and six samples per group with the number of female triploid salmon unknown as factors affecting the reliability and validity of the studies. Petition at 27. You do not specify how you believe these study limitations impacted the data or the conclusions that FDA has drawn from the data. Nevertheless, we address the study limitations below.

In our review, we noted the limitations of the studies that you cited in your comment. FOI Summary, Section IX 2 b and Briefing Packet XII C 2 b and c. As stated above, the parvalbumin Westerns blots were so badly flawed that we rejected them; that is, we did not draw conclusions from or rely upon these results. Briefing Packet, Section VII C 2 b. FDA determined that the departure of the principal investigator had no impact on the current study. Staff changes on studies occur commonly without compromising the results of the experiment. Moreover, the new principal investigator prepared a new set of individual allergen extracts and prepared a de novo potency analysis. In its review, FDA evaluated these new data, rather than previously generated data. Regarding the second criticism, blinding is often incorporated in study designs to avoid unintentional bias on the part of the investigators. With respect to blinding, when dealing with objective data (e.g., measurements of effects made by machines, calculations according to *a priori* established equations), as with this study, the probability of introducing bias is low. On the other hand, when results contain a subjective component (i.e., assessment of the appearance of test subjects, grading of adverse outcomes along a continuum) the loss of sample blinding (i.e., unmasking the identity of the samples) may introduce bias. A common place for bias to be introduced is in the interpretation of results; we note that FDA disagreed with the sponsor's evaluation of the raw analytical results, and reanalyzed the data based on the analytical results. See FOI Summary, Section IX 2 b iii. FDA found them to be sufficiently robust to demonstrate the allergenic potency of triploid ABT salmon is not significantly different from that of sponsor control diploid salmon. Consequently, FDA concluded that triploid ABT salmon pose no additional allergenic risk than non-GE Atlantic salmon. Further, it is important to note that in the evaluation of the overall allergenicity of food from AquAdvantage Salmon, we were not determining whether AquAdvantage Salmon are allergenic, but rather, whether they differ in allergenicity from or pose any additional allergenic risk as compared to non-GE, farm-raised Atlantic salmon. FOI Summary, Section IX C 2 b. For a discussion of sample sizes, see Section K 2 and Appendix A of this letter.

4. Toxicological Studies for Allergens

You allege that toxicological studies are necessary for addressing "the serious potential risk(s) of elevated levels of…allergens." Petition at 27.

FDA disagrees regarding the utility of animal testing for allergens in food. As FDA has previously explained, "[a]nimal models are widely used in toxicology testing to assess the potential effect of a substance in humans. However . . . no appropriate animal model systems have been developed or validated to test potential human allergenicity. Although animals can be induced to become allergic to foods and proteins, there is no animal model that can differentiate between those foods that are commonly allergenic in humans and those that are not, or between allergenic and non-allergenic proteins in an individual food. Further, there are no data indicating that the level of sensitivities in animals reflect those seen in humans, that animals respond in the same manner as humans to modification of allergenic proteins caused by processing, or that animals respond in the same manner as humans to matrix effects. Therefore, we do not recommend the use of animal testing as an independent indicator of either the absence of allergenic protein or of whether an ingredient will cause an allergic response that poses a risk to human health."[37]

C. Vitamin B6

You state in your petition that "despite the extremely small sample size…there was a statistically significant difference in Vitamin B6" for diploid GE salmon." Petition at 15.[38]

For the sponsor study[39] on compositional analyses, which included analysis of levels of vitamin B6, samples were run from a total of 73 salmon, including 10 farm-raised control, 33 sponsor control, and 30 ABT salmon. See FOI Summary, Section IX C a i. FDA's analysis did find that concentrations of vitamin B6 were slightly elevated in diploid ABT salmon. CVM evaluated the biological relevance of this statistical difference using a MOE assessment, which indicated that, even if the highest level of vitamin B6 observed in the diploid ABT salmon were present in all ABT salmon, which include AquAdvantage Salmon, it would still be well within (and approximately 50 fold lower than) the upper bound recommended daily intake for vitamin B6 and, therefore, there is no additional food consumption hazard with regard to vitamin B6 compared with consumption of non-GE Atlantic salmon. See FOI Summary, Section IX C 2 a iii (b) (ii).

D. Omega-3 and Omega-6 Fatty Acids

Your petition also states that "Omega-3 to Omega-6 levels were . . . decreased in GE salmon." Petition at 15.

---

[37] FDA, "Guidance for Industry: Food Allergen Labeling Exemption Petitions and Notifications" at 18, available at http://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM395660.pdf.

[38] To support this assertion, you cite to Table 27 of the Briefing Packet, which reflects MOE estimates and does not provide information about the number of samples analyzed. Petition at fn 48. As noted above, the sponsor study on compositional analyses included samples from 73 salmon, which is not an "extremely small" sample size.

[39] *A Single-Blind, Comparator-Controlled, Quantitative Analysis of the Composition of Muscle Skin from Diploid and Triploid Atlantic Salmon (Salmo salar) Modified Transgenically with the AquAdvantage Gene Cassette (opAFP-GHc2). Covance Laboratories Inc., Wisconsin. Covance Study Identification 7352-100. Study Report AAS-HFS-001. Report Dated 22 January 2003.*

This assertion is in error. FDA's examination of omega-3/omega-6 ratios showed that they were virtually identical across the GE and control groups, and are similar to the ratios found in scientific literature for farm-raised Atlantic salmon (which constitute almost the entirety of the consumption of Atlantic salmon in the United States).[40] Wild caught Atlantic salmon have a higher omega-3/omega-6 fatty acid ratio than farm-raised Atlantic salmon, primarily due to the difference in their diets, but the appropriate comparator for farm-raised ABT salmon, including AquAdvantage Salmon, is farm-raised Atlantic salmon. FOI Summary, Section IX C 2 a iii (b) (iii) and Table 39, which corresponds to Table 28 of the Briefing Packet.

E. Other Aspects of Composition

Your petition asserts that "AquAdvantage [S]almon showed statistically significant differences in other aspects of its nutritional composition, including protein values (albumin, globulin, total protein, albumin:globulin ratio), calcium, cholesterol, phosphorous, total bilirubin, aspartate aminotransferase, and glucose." Petition at 15.

This statement is factually incorrect. It confuses clinical blood chemistry values with levels of nutrients found in muscle and skin. The data referred to in this statement are presented in the Phenotypic Characterization Review section of the FOI Summary (Section VII) and not the Food and Feed Safety Section (IX). The former is an evaluation of the health of the ABT salmon compared with appropriate farm-raised comparators (e.g., similar to blood tests that people receive at their annual physicals), while the latter analyzes the composition of edible tissues (i.e., muscle and skin).

F. ABT Salmon Health-Related Issues

Your petition asserts that AquAdvantage Salmon are more susceptible to *A. salmonicida*, and that the use of antibiotics to treat furunculosis, the disease caused by *A. salmonicida* "has already lead [sic] to the emergence of antibiotic-resistance [sic] bacteria, which may exacerbate public health issues related to hard-to-treat bacterial infections in humans." Petition at 22-23 (citations omitted).

Your assertion is based upon a statement from a Canadian Department of Fisheries and Oceans (DFO) draft risk assessment (2013). Petition at n. 75. The AquAdvantage Salmon facility in Canada has not had any incidence of this disease, however. Moreover, the most recent Fish Health Certificates DFO issued list *A. salmonicida* as "not detected." See AquAdvantage Environmental Assessment, Section 5.4.2. There is thus no evidence of increased incidence of *A. salmonicida* in AquAdvantage Salmon or of use of antibiotics to treat such an infection. Additionally, your petition cited the Canadian draft-in-revision version of the Environmental and Indirect Human Health Risk Assessment of the AquAdvantage Salmon (July 2, 2013). The final published version November 2013 states that, with respect to this issue, "[t]here 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 *A. salmonicida* (causative agent for furunculosis). Based on Fish Health Certificate data, fish disease risk at the AquaBounty facility in PEI is "well managed."[41] Additionally, the final, peer-reviewed report does not include the conclusion you cite from the draft report that AAS is "more susceptible" to *A.*

---

[40] See Seafood Health Facts (2014), available at http://seafoodhealthfacts.org/pdf/seafood-choices-salmon.pdf.

[41] Canadian Science Advisory Secretariat Summary of the Environmental and Indirect Human Health Risk Assessment of AquAdvantage Salmon, http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ScR-RS/2013/2013_023-eng.html.

salmonidica. See attachment from unreleased final report, *Environmental and Indirect Human Health Risk Assessment of the AquAdvantage Salmon*. Given that all farm-raised salmon are susceptible to *A. salmonicida*,[42] that there has been no incidence of *A. salmonicida* at the PEI facility, and that there has, consequently, been no use of antibiotics to treat this disease in AquAdvantage Salmon, there is no food safety issue concerning *A. salmonicida* in AquAdvantage Salmon.

G. "Culling" of Fish

Your petition alleges that key studies were biased because of improper "culling" of fish, indicating likely animal health issues in AquAdvantage Salmon due to the gene expression product. Petition at 35.

During the initial phase of review, FDA stated that as with all data sets, there are some uncertainties. See Briefing Packet at 21.[43] At the time FDA performed its preliminary review, which was released in the briefing packet for the Veterinary Medicine Advisory Committee meeting, the primary area of uncertainty was in determining the actual rate of adverse outcomes in grow-out facilities, as the process of selecting animals for the initial sponsor study, which has been referred to as "culling," may have influenced the apparent rate of abnormalities observed. Because of concerns that the culling procedures for the initial study may not have reflected typical aquaculture procedures and may have obscured adverse outcomes, FDA requested and received from the sponsor additional information regarding culling practices, the health of the ABT fish populations at the grow-out facilities, and the potential role that culling could have had in masking adverse outcomes.

These data and information submitted to the agency since the preliminary review are found in the FOI Summary, Section VII B 2, and Tables 8-13 and include information on morbidity and mortality from more than 150,000 ABT salmon and approximately 9,000 non-GE Atlantic salmon (from both the PEI and Panama facilities). These new data did not reveal any new abnormalities or altered rates of abnormalities beyond those identified in the initial study, and did not indicate any bias in the initial study's estimation of (i.e., did not mask) rates of morphologic abnormalities, mortality, or morbidity. In addition, FDA directed the sponsor to collect data from the Panamanian grow-out facility to be used as part of a surveillance program in the durability plan[44] (see FOI Summary, Genotypic and Phenotypic Durability Plan, Section VI), and as the basis for determining unexpected and serious adverse events in the post-approval record keeping and reporting requirements (see Letter of Approval, including Appendix A).

FDA's review of the additional data and information submitted since the preliminary review (see FOI Summary, Section VII), strengthened the agency's conclusions regarding the phenotype of

---

[42] *A. salmonicida* "has been recognized as a pathogen of fish for over 100 years." It "is one of the most studied fish pathogens, because of its widespread distribution, diverse host range and economically devastating impact on cultivated fish, particularly the *Salmonids."* OIE Diagnostic Manual for Aquatic Animal Diseases, OIE (World Organisation for Animal Health), Paris, France.

[43] FDA, Veterinary Medicine Advisory Committee Briefing Packet (September 20, 2010), available at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf.

[44] The genotypic and phenotypic durability *plans* ensure that the genotype and the phenotype of the GE animals in commerce (continued production) remain equivalent to those that were characterized in the durability assessment. GFI 187 recommends developing a sampling plan. The durability plan also involves post-approval record keeping and reporting requirements to identify any serious or unexpected adverse experiences (21 CFR 514.80).

ABT salmon, including AAS, by addressing these uncertainties, and indicated that the culling did not mask any adverse outcomes.

H. Data From PEI v. Panama

Your petition states that ABT's analysis failed to evaluate the GE salmon under the conditions in which it will be produced, because ABT's data collection took place at the PEI facility and not the grow-out facility in Panama.[45]

Because fish husbandry conditions, particularly those that affect water quality, can affect fish health and phenotype (e.g., morbidity, mortality and stress-related parameters), included in the assessment of phenotype was a consideration of husbandry conditions. ABT and comparator[46] salmon were cultured at PEI under standard conditions for the freshwater (hatchery and smolt production) phase of salmon aquaculture.[47]

With respect to the health of AquAdvantage Salmon, as measured by the incidence of morbidity, morphological abnormalities, and mortality, ABT conducted further studies at the PEI facility to determine whether there were any differences between the grow-out and breeding facilities. Once husbandry conditions were established (e.g., water quality, temperature, and oxygen concentration were appropriately regulated) results indicated that rates of mortality, culling, and irregularity (as depicted with rank scores) were generally low, and were within the same range as, or below, those observed in earlier year-classes of ABT salmon reared at the PEI facility. There were no consistent differences in mortality and morbidity between ABT salmon, including AAS, and non-GE comparator Atlantic salmon in the animal safety study, the large-scale historical retrospective data evaluation, or results obtained on subsequent year-classes of fish reared at the PEI and Panama facilities. See also subsection J of this document. Uncertainties regarding differences in animal health between the Canada and Panama facilities were significantly reduced, with approximately equal survival and animal health in both locations once husbandry conditions were established. FOI Summary, Section VII B 2 d. Therefore, your assertion that there were no data collected at the

---

[45] You also allege that the growth rate advantage of AquAdvantage Salmon is dramatically diminished in the Panamanian facility compared to the PEI facility. Petition at 32-34. We note that whether AquAdvantage Salmon achieves the claimed growth rate advantage is unrelated to the requests you make in your citizen petition, i.e. that FDA regulate AquAdvantage Salmon under the FD&C Act food additive provisions, and that FDA find the GEP a poisonous and deleterious added substance. We also note that the differences that are relevant to FDA's food safety determinations are the differences between GE salmon and non-GE comparator salmon. Any potential compositional differences between GE salmon raised at the PEI and Panama facilities would not identify areas of potential food safety concern for GE salmon that are not also present for non-GE salmon.
You also stated that the growth rate advantage is diminished at the PEI facility. ABT indicated that there were differences in growth rate between salmon at the PEI and Panama facilities and attributed those differences to the quality of the feed that the AAS were receiving ("after attaining approximately 100 g in size, all salmon reared at the AquaBounty Panama facility are fed locally manufactured salmon feed, which although it is adequate, does not deliver the same growth performance as higher quality (more expensive) imported feeds."). All Other Information (AOI) Review, attached to the Memorandum Recommending Approval. Nonetheless, the AAS raised in Panama still met the claim established by the sponsor when compared to diploid non-GE salmon. AOI Review, attached to the Memorandum Recommending Approval.

[46] Throughout this section, unless otherwise specified, the term "comparator" refers to non-GE farm-raised Atlantic salmon of a similar, but not identical, genetic background as ABT salmon, including, depending on the study, both diploid and triploid fish.

[47] Once they reach smolt size, Atlantic salmon are normally transferred to seawater and reared to market size in open water net pens, however, the entire lifecycle of ABT salmon, including grow-out to market size, occurs in contained freshwater facilities.

grow-out facility is incorrect, and your hypothesis that there might be "potentially dramatic differences" (Petition at 35) is not borne out.

I. Veterinary Medicine Advisory Committee Meeting

Your petition states that at the September 2010 meeting, the members of CVM's Veterinary Medicine Advisory Committee (VMAC) described FDA's approach as "lacking in rigor and poorly designed," among other criticisms (Petition at 22, 23), and gave FDA "suggestions for redoing its science to correct the lack of rigor… flaws in the FDA's statistical analysis, and ABT's imprecise measurements." Petition at fn. 89.

The purpose of the VMAC meeting was to obtain input regarding 1) the strengths and weaknesses of data presented in the VMAC briefing packet and 2) four specific questions presented to the VMAC. During the course of the meeting, time was allotted for conversation among the Committee members and interactions with the FDA staff engaged in this matter. The purpose of this discussion was to have an open exchange that would contribute to the VMAC Chairman's Report (VMAC Report),[48] with which the VMAC members would concur. The comments you cite are "cherry-picked" from the transcript of this discussion and in fact do not represent the VMAC's final opinions, as presented in the VMAC report.

FDA considered these final opinions in the VMAC report, but did not rely on the report in reaching its conclusions on the safety and effectiveness of the rDNA construct that is the subject of the NADA approval or the environmental impacts that would result from the approval for the reasons discussed below. Advisory committees provide recommendations to the Agency on matters brought before them for consideration, but FDA makes final approval decisions.[49]

In the VMAC report, the Committee agreed with FDA that there was some uncertainty associated with the culling practices. As described in Section H above, FDA requested and the sponsor provided a significant amount of data to address the uncertainties associated with the culling practices and the difference in rates of abnormalities between the Prince Edward Island (PEI) and Panama facilities. These data and information are found in the FOI Summary, Section VII B 2 and Tables 8-13, and include information on morbidity and mortality from more than 150,000 ABT salmon and approximately 9,000 non-GE Atlantic salmon from both the PEI and Panama facilities. These new data did not reveal any new abnormalities beyond those identified in the initial study, and did not indicate any bias in the initial study's estimation of rates of morphologic abnormalities, mortality, or morbidity. In addition, the conditions of approval for this NADA require detailed record-keeping and reporting of adverse outcomes including morbidity, mortality, and morphological abnormalities to ensure that ABT salmon, including AquAdvantage Salmon, are as healthy as other non-GE farm-raised Atlantic salmon. See Letter of Approval, Appendix A. Therefore, we feel this particular concern as articulated in the VMAC report has been adequately addressed.

---

[48] Chairman's Report, VMAC Meeting: September 20, 2010, available at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM230467.pdf.)

[49] FDA, "Guidance for Industry: Advisory Committees: Implementing Section 120 of the Food and Drug Administration Modernization Act of 1997," available at http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079765.pdf. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079765.pdf.

The VMAC report made a further suggestion that an analysis of fluctuating asymmetry could be used to assess differences between AquAdvantage Salmon and non-GE Atlantic salmon. The agency considered this recommendation, but determined that although fluctuating asymmetry studies can be useful in assessing the developmental phenotypes of any animal, there was sufficient information without such studies for FDA to conclude that the AquAdvantage Salmon NADA demonstrated target animal safety. Moreover, FDA does not believe that this type of information would have added significantly to its ability to reach a decision regarding target animal safety for AquAdvantage Salmon. This is primarily because the differences between AquAdvantage Salmon and non-GE Atlantic salmon noted in the data were minimal and the agency does not believe they represent a safety concern. See FOI Summary, Section VII B 2. Nevertheless, in order to monitor rates of morphological abnormalities, the approval includes detailed post-market record-keeping and reporting requirements as referenced above. See Approval Letter, Appendix A.

With respect to food safety, overall, the VMAC Chair's report stated "[t]he committee deemed the studies selected to evaluate this question to be overall appropriate and a large number of test results established similarities and equivalence between AquAdvantage Salmon and Atlantic salmon [non-GE Atlantic salmon]."

Finally, the VMAC report indicated that the Committee found evidence in support of the claim of faster growth time (as set forth in the product definition) made for AquAdvantage Salmon.[50] Therefore, the VMAC report, which represents the final views of the committee as a whole, does not reflect that FDA's assessment was lacking in rigor or was based on poorly designed studies. The VMAC report largely agreed with FDA's approach and offered a helpful suggestion that we followed,[51] and others with which we disagreed for the aforementioned reasons.

J. Statistical Analysis

1. Study Design and Analysis

Your petition asserts that the statistical analyses performed by FDA are of "very limited value" and cites a comment by Dr. Elizabeth Colantuani, who reviewed VMAC briefing materials in conjunction with David Love, and Tim Schwab to assert that FDA's statistical analyses are deficient in several respects. Petition at 37-39 and Attachment GG. These arguments are incorrect for the reasons discussed below.

FDA notes that there may be many appropriate ways to perform statistical analyses. For assessing the safety of AquAdvantage Salmon, FDA performed its analyses by choosing an approach it deemed appropriate for the particular analysis to identify those statistical differences, if any, that the agency would further evaluate to decide whether the differences were biologically relevant (i.e., whether the differences impact safety). Although other statisticians might have used different approaches to identify differences warranting further analysis, FDA's approach identified

---

[50] In the case of the AquAdvantage Salmon NADA, the claim (as part of the production definition) is the following: "*Significantly more AquAdvantage Salmon grow to at least 100 g within 2,700 ^{0}C-days than their comparators.*"

[51] The VMAC report suggested that FDA request additional data to address uncertainty in determining the actual rate of adverse outcomes in grow-out facilities. The agency requested and received from the sponsor additional information regarding culling practices, the health of ABT fish populations at the grow-out facilities, and the potential role that culling could have had in masking adverse outcomes. (See FOI Summary Section VII B 2, and Tables 8-13 for additional data FDA received).

differences that warranted further analysis to determine biological relevance with respect to safety, which is the agency's concern. The agency's responses to specific assertions are described more fully in the responses below.

2. Sample Size

You point to a comment by Colantuani that, based on the results presented at the VMAC meeting, asserts that larger sample sizes are needed in order for the study to have sufficient statistical power. Petition at 38.

In particular, you cite this comment for the opinion that "far larger sample sizes (20 fish in each experimental group) were needed in order for the study to have sufficient statistical power to allow a conclusion that the measured differences in allergenic potency between GE Salmon and non-GE Salmon were statistically significant (assuming 80% power, 5% type-1 error rate, and the measured sample and population's magnitude of effect are equal. 27 fish per group would be required to achieve 90% power). FDA only had data from 6 fish per group." Id.

Statistical significance, in and of itself, however, is insufficient to determine whether there are any biologically relevant differences that could pose public health concerns with respect to either toxicological, allergenic, or nutritional risk. There can be differences that are statistically significant but that nevertheless have no impact on the health of those who consume the salmon, i.e. they are not biologically relevant. With respect to allergenicity, the biologically relevant difference that is important to public health/food safety is not whether food from AquAdvantage Salmon is allergenic; as a fish, salmon is one of the eight major food allergens in the U.S. FALPA, 2004. The relevant public health/food safety question is whether ABT salmon differ in allergenicity from or pose any additional allergenic risk compared to non-GE, farm-raised Atlantic salmon. FOI Summary, Section IX C 2 b.

Further, FDA disagrees that larger samples sizes were needed in order to determine whether the measured differences in allergenic potency between GE salmon and non-GE salmon were statistically significant. We note that estimation of sample size is a prospective exercise, and should not be determined after the fact in the manner suggested by Colantuani. Sample size selection should be part of study planning along with consideration of other key study parameters, with sample size related to the estimated difference (See Appendix A). Assuming the same variance, desired power and level of significance, the smaller the difference of interest, the larger the estimated sample size needed; conversely, the larger the difference of interest, the smaller the sample size needed.

Regarding potential differences in allergenic potency, the Agency was interested in large differences that would have biological relevance (see Section III C 1 b of this letter and FOI Summary, Section IX C 2 b iii, Figure 5) between AquAdvantage Salmon and farm-raised non-GE Atlantic salmon, which would correspond to small sample sizes. A sample size calculation is generally performed prior to study conduct to determine how big the study should be to have a reasonable chance of detecting an effect of a specified magnitude. Therefore, the Agency cannot comment on the power analysis for the study that was conducted. Any retrospective analysis of what the power of that study may have been would not be reliable.[52]

---

[52] Zumbo, B.D. and Hubley, A.M. (1998). A note on misconceptions concerning prospective and retrospective power. *The Statistician.* 47-2. 385-388; Hoenig, J.M. and Heisey, D.M. (2001). The abuse of power: The pervasive fallacy of power calculations for data analysis. *The American Statistician.* 55-1. 19-24.

To address Dr. Colantuani's assertion that a larger sample size was needed, we will examine below what the power of a future study might be. To estimate the power of a future study, a researcher needs to make assumptions about the variance of the measurements, as well as the magnitude of the difference that the study is expected to detect. The estimated variability from the previous study, which has a residual variance of 0.23, is a useful starting point. If a difference in allergenic potency of 2.0 is biologically relevant (a highly conservative assumption given that the five-fold difference Dr. Metcalfe identified as the appropriate signal for further inquiry would correspond to a difference in allergenic potency of 8), and the assumption regarding a variance of 0.23 in a future study is correct, a study with six fish in each of two groups will have over 99% power to detect a difference, using a t-test at 5% level of significance. In order to get a more conservative estimate of power, the researcher can assume higher variability, for example by doubling the assumed variance to 0.46. With double the variance, the study will still have over 99% power to detect a mean difference as small as 2. In other words, if the variability were double what the researcher thought it would be, a study of this size would still reliably detect a difference. Thus, with the knowledge obtained from the preceding study, were a second study to be conducted, under the assumptions stated, a sample size of six would be more than adequate to detect a biologically relevant difference. Our review of Coluntuani's comments and our sample size calculations for a future study (described above) lead us to conclude that if the sponsor were to conduct the study again, it would not be necessary to alter the number of animals used.

3. Analysis of IGF1

You further cite Colantuani's comment as concluding that "sample sizes of 31 fish in each experimental group were needed in order for the study to have sufficient statistical power to allow a conclusion that the measured differences in IGF-1 between GE Salmon and non-GE Salmon were statistically significant (assuming 80% power, 5% type-I error rate, and the measured sample and population's magnitude of effect are equal. To achieve 90% power, the study would need to include 41 fish per group)." Petition at 38; Exhibit GG.

Although statistical analyses may help to focus attention on a particular difference, statistical significance, in and of itself, is insufficient to determine whether there are any biologically relevant differences that could pose public health concerns. There could be differences that are statistically significant but that nevertheless have no impact on the health of those who consume the salmon, i.e., they are not biologically relevant. Because the agency is concerned with potential food consumption risks, the agency examined whether the difference in IGF1 levels between non-GE comparator Atlantic salmon and the one outlier diploid GE salmon could result in a safety concern if consumed at the maximum likelihood estimate by the highest consumers of salmon (i.e., a more biologically based approach). Out of an abundance of caution, we assumed that the outlier level represented all the AAS that would be consumed and conducted a "margin of exposure"[53] assessment assuming that the most sensitive individuals (i.e., teenaged boys, based on their biological sensitivity to the effects of IGF1 due to their rapid growth and development, and their tendency to consume adult portions of food despite a lower body weight) consumed diploid ABT salmon with the highest outlier IGF1 levels. The MOE for dietary consumption of Atlantic salmon

[53] This type of analysis is referred to as a "margin of exposure" (MOE) analysis, and is a standard methodology to determine whether a risk is present. In the current approach the MOE compared teenaged boys' total serum burden of IGF1 to the hypothetical daily exposure due to consumption of Atlantic salmon (non-GE) or ABT salmon with IGF1 concentrations like those in the outlier ABT salmon in the sponsor study. *Determination of IGF1, GH, T3, T4, 11-Keto Testosterone, Testosterone, and Estradiol in Salmon Tissue. CTBR Bio-Research Inc. Canada. Project Number 42361. Study Report AAS-HFS-001. Report dated 26 July 2004.*

(non-GE), assuming daily non-tuna consumption was entirely of ABT salmon at the outlier levels, is 1,220 µg/day/2.4 µg/day. This provides a 508-fold margin of exposure, equivalent to approximately two one-thousandths (0.002) of the total serum burden. The MOE for dietary consumption of IGF1, assuming that IGF1 was present at the maximum concentration recorded from the mature diploid ABT salmon outlier is 1,220 µg/day/3.7 µg/day. This provides a 330-fold margin of exposure, which corresponds to approximately 0.003 (3 parts in 1000) of the total serum burden of IGF1. The calculation of the incremental increase in IGF1 exposure from the maximum estimated GE salmon intake relative to IGF1 exposure from the non-GE study comparator yielded only 1.2 µg per day or 0.001 of the total serum burden. The apparent difference in IGF1 in mature diploid ABT salmon compared to sponsor control non-GE salmon was small, and FDA determined it to be of no biological relevance because the margin of exposure to this endogenous component of food would be well within levels of exposure from other dietary sources of IGF1, and poses no additional risk. Thus, any statistical differences in populations become irrelevant in light of this alternative, biologically-based approach to assess any potential safety concern for IGF1 exposure from ABT salmon. As noted elsewhere in this document, diploid ABT salmon are not the subject of the approved application, and ABT must adhere to stringent manufacturing requirements to ensure that triploidy is maintained at a very high level. See Section III C 1 b, FOI Summary, Section IX C 1 a iii.

4. Analysis in Peer Reviewed Study

Colantuani cited as another example of concerns regarding FDA's analyses data presented in a Table titled "Body Weight and Plasma Concentrations of Growth Hormone and T3." Petition at Exhibit GG, citing Briefing Packet, Table 13 (FOI Summary, Table 24).

The data Colantuani cites are derived from a peer-reviewed publication (Du et al. (1992)). As stated in Section III B 1 of this document, and the FOI Summary (Section IX C 1 a), FDA considered this peer-reviewed publication because it provides a framework for the identification and characterization of potential hazards that may be found in AquAdvantage Salmon. As stated above (see B.1. and FOI Summary, Section IX C 1 a) the Du et al. study reported body weight and plasma levels of hormones in non-GE Atlantic salmon and in growth hormone (GH) transgenic Atlantic salmon that were derived from the same parental animals from which the EO-1α lineage eventually was derived, however, they were not AquAdvantage Salmon. The salmon in the Du et al. study also were small (< 100 g) and therefore not the size of salmon ordinarily consumed. In addition, the measurements were taken as plasma hormone levels, which are not necessarily equivalent to hormone levels in muscle/skin i.e. the parts of the salmon that are consumed. As stated in the FOI Summary, FDA considered this publication because it provides a framework for the identification and characterization of potential hazards that may be found in AquAdvantage Salmon. It does not, however, provide useful *conclusions* regarding hazards of market-sized AquAdvantage Salmon. We did not conduct statistical analyses of this study; instead, we reported the *authors'* data and *their* analyses. Therefore, although this peer-reviewed study provided information about potential hazards that may be present in AquAdvantage Salmon, it was not a determination of hormone levels in market-size ABT salmon and control Atlantic salmon as the sponsor study was and, therefore, it did not provide reliable conclusions regarding any hazards that AquAdvantage Salmon might present as the result of statistically significant differences (or not) among the groups of salmon. Colantuani's criticism of the data from this study is thus not relevant to FDA's analyses.

5. Equivalence Versus Difference Testing

Colantuoni stated that the hypotheses tested for the allergenicity variable should have been either a test of equivalence or non-inferiority, depending on the question asked by the agency (i.e., whether

AquAdvantage Salmon "are more favorable in terms of allergenicity," or whether AquAdvantage Salmon "are at least not worse or the same as the sponsor controls in terms of allergenicity."). Petition at Attachment GG.

We note that statistical analyses are a useful tool in determining differences between or among groups of individuals or populations. As discussed above (see subsection K 3), statistical significance is not always the critical factor in making decisions regarding safety. Rather than take either of the approaches posited by Colantuani, FDA focused on the public health ramifications of any statistically significant differences in relative allergenicity between the groups of salmon evaluated, given that salmon is an allergenic food. FDA analyzed allergenic potency using analysis of variance (ANOVA) with type included in the statistical model as a fixed effect as an initial tool in performing its overall analysis of public health risk. FOI Summary, Section IX C 2 b iii. Given that salmon is often consumed as one individual fish fillet per serving rather than a mixture of many fish, we also considered the allergen level in individual fish in addition to group means. FOI Summary, Section IX C 2 b iii. Initial evaluation of the results suggested that there may be an increase in the relative allergenic potency in the GE diploid salmon compared to sponsor control salmon. There was no evidence of a statistically significant difference between the mean allergenic potency for sponsor control diploid fish compared to the triploid GE fish. A statistically significant difference existed between the mean allergenic potency for sponsor control diploid fish compared to the diploid GE fish. Table 41 of the FOI Summary summarizes the statistics for the mean allergenic potency per group. The agency concluded that the allergenic potency of triploid ABT salmon is not significantly different from that of sponsor control diploid salmon.

More importantly, however, with respect to biological relevance of potential statistical difference, the agency asked whether public health would be endangered if an individual were to consume a diploid ABT salmon (the group that showed a statistically significant difference from Atlantic salmon that the public consumes now). As part of concluding our review of the application for the approval of AquAdvantage Salmon, we consulted Dr. Dean Metcalfe, Chief, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health on general scientific matters related to endogenous allergens in foods known to be allergenic. FOI Summary, Section IX C 2 b v and Appendix 2.

In this consultation, Dr. Metcalfe noted that the level of allergen at which individuals experience allergic reactions is largely variable for all major allergenic food groups, including fin fish, and therefore a threshold has not yet been established for an allergic reaction to any of the major allergenic foods. He also noted that the major "treatment" for a food allergy is avoidance of the food causing the allergic reaction.

With respect to transferring a gene from one species in an allergenic food group (e.g. fin fish) to another closely-related species, Dr. Metcalfe observed that for people who know they are allergic to fin fish, there would be no new risk as they are already likely practicing food avoidance. With respect to the endogenous allergenicity of fin fish, although different species of fin fish show up to 100-fold differences in the level of the major allergen, there is no apparent public health impact because individuals who are allergic to one species of fin fish generally avoid consuming all species of fin fish. Thus, small changes in the levels of endogenous allergens would likely have little or no public health impact in the context of allergenicity. FOI Summary, Section IX C 2 b v and Appendix 2. Use of a five-fold increase in the level of endogenous allergens in an allergenic food could serve as a signal that an additional evaluation for possible public health impact would be warranted, not because a five-fold increase would cause a public health problem, but because it would provide a useful "flag" to investigate whether one would result. In Dr. Metcalfe's opinion,

increases in endogenous allergen levels of less than five-fold in this setting would not be expected to result in an adverse effect on public health.[54]

For this and the reasons described above, FDA concludes that the statistical analyses it employed are adequate to the issues presented, and has made the appropriate, public health protective decisions, based on biological relevance to food safety regarding the relative allergenicity of AAS and other ABT fish.

IV. Conclusion

As explained above, FDA regulates AquAdvantage Salmon as a new animal drug because the ABT rDNA construct as integrated in the DNA of AquAdvantage Salmon is intended to affect the structure and function of the body of an animal and the construct is not generally recognized as safe for this intended use and has not been used to a material extent or for a material time. FDA cannot regulate an article that is intended for use as a new animal drug as both a new animal drug and a food additive. Accordingly, FDA is denying your request to deem AquAdvantage Salmon's components, including its GEP, as food additives under 21 CFR 10.30(e)(3). In addition, FDA has concluded that AAS is safe for human consumption under § 512 of the FD&C Act. Accordingly, we are also denying your requests to find food derived from AquAdvantage Salmon to be adulterated under the FD&C Act.

Sincerely,

Leslie Kux
Associate Commissioner for Policy

[54] As mentioned previously in this document, we note that although study data showed one outlier diploid salmon that had an elevated level of measured relative allergenic potency (FOI Summary, Section IX C 2 b), the difference was not sufficiently elevated to reach the five-fold level that Dr. Metcalfe identified as an appropriate signal for further inquiry. In fact, the difference was not even a two-fold increase. See supra, Section III C 1 b. Out of an abundance of caution, however, the agency judged that it would want additional data and information to draw a conclusion on the relative allergenic potency of diploid ABT salmon. Furthermore, because salmon present a hazard to salmon-allergic individuals, salmon allergic individuals will likely avoid consumption of all salmon, including AquAdvantage Salmon. In addition, we note that under the conditions established in the approved application, ABT must follow manufacturing specifications to ensure to the greatest extent possible that AquAdvantage Salmon are an all triploid population. See supra, fn 36.

Appendix A: Statisticians' further response to sample size

In general, estimation of sample size is a prospective exercise. It is dependent on many things, including biological parameters, measurement factors such as accuracy and precision, and the design of the study being conducted. In general, FDA receives data from the sponsor, and develops an analysis plan based on the study report and data submitted by the sponsor and conducts its own analyses, and in the case of the studies being referenced in your petition, did not have input into the study design.
Sample size estimation is dependent on availability, accuracy, and precision of the values needed in the estimation, which are very dependent on knowledge of the experimental material, the design of the study being conducted, and the variables and parameters being tested. If a researcher wished to perform additional experimental studies comparing ABT and comparator non-GE salmon, the results mentioned in your petition would be useful for planning purposes, but they would not guarantee that the future study would provide the same or different results.

Additional points to be considered when estimating sample size, and to which various portions of your Petition allude, especially Exhibit GG, include the following issues, followed immediately by our response:

- *Study design*: Sample size estimation also depends on the study design and assumptions about the experimental material. Using the same estimated difference and variance, a study including three groups will require fewer subjects that a study including only two groups. Additionally, in the absence of contradictory evidence, it is often assumed that for most variables, the variability for each group is not substantively different.
- *Selection of a variable*: In most scientific studies, data are collected for multiple variables. It is common to estimate a sample size, which will then be used for all data collected. That is, one would enroll a specified number of animals in a study and collect all data from all enrolled animals rather than collect data from a different number of animals depending on the variable of interest. Therefore, one could estimate a sample size for a selected variable, and this sample size would be too large for some variables and too small for other variables to meet arbitrary power targets.
- *Estimates of variability*: Estimates of variability by its nature are highly variable and the estimated variability seen in one study is unlikely to be replicated in another study.
- *Estimated difference*: Generally, a clinical, biological, or economical difference is identified that is of importance to the researcher. Assuming the same variance, desired power, and level of significance, the smaller the difference of interest, the larger the estimated sample size needed. Specification of a zero difference is the worst case scenario because it maximizes the estimated sample size.

Given the above considerations in sample size calculation, it is important to provide the underlying assumptions and specific set of conditions along with the estimated sample size. For example, one would identify the specific variable of interest, type of study, the number of groups in the study, the estimated variability and the difference used in the estimate. A sample size estimate may say: "In a three group study making pair-wise comparisons of mean [variable-of-interest] between a control and each of two groups in the presence of a significant F-test (alpha = 0.05) a study with at least [X] animals should provide 80% power to detect a difference of [specified-difference] (alpha = 0.05) if the study realizes [variability-specified]." Alpha is the chance of concluding that a difference exists when there is no such difference. A smaller alpha reduces the chance of concluding a non-existent difference. . Power is the chance of concluding that a difference exists when the difference truly exists. (Steel, Robert G D and Torrie, James H. *Principles and procedures of statistics*. New York: McGraw, 1960). Generally, after a study is completed, we find

that we have over- or under-estimated a key component, the study can be conducted differently, or our understanding of the underlying science has changed. It is very likely that the next iteration of calculations will provide a different sample size for the next study.

Therefore, attributing lack of statistical significance to a lack of power, after the study is completed, as your petition implies, may not be correct. After a study is completed, if the specified hypothesis comparing means is found to be significant (alpha = 0.05), the conclusion is drawn that evidence of a statistically significant difference in means exists and a scientific interpretation of the importance of the observed difference follows. If the specified hypothesis comparing means is found not to be significant (alpha = 0.05), the conclusion is drawn that there is no evidence of a statistically significant difference in means based on this study. The conclusion of "no statistical evidence" of a difference rather than the conclusion that the means are not different is important. The conclusion of "no statistical evidence" does not imply that the study was poorly designed or underpowered. Caution should be used with attributing the observance of lack of significance to a single cause. Not detecting the expected difference can occur because study difficulties, e.g., enrollment of animals, the realized variability was larger than expected, or the differences in means was smaller than planned and may not be relevant.