CENTER FOR FOOD SAFETY

CENTER FOR FOOD SAFETY & INTERNATIONAL CENTER FOR TECHNOLOGY ASSESSMENT

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September 16, 2010

Aleta Sindelar
Office of the Director
Center for Veterinary Medicine
Food and Drug Administration

Cc: President Barak Obama
Secretary Kathleen Sebelius, U.S. Department of Health and Human Services
Dr. Margaret A. Hamburg, Commissioner, U.S. Food and Drug Administration
Dr. Jane Lubchenco, Administrator, National Oceanic and Atmospheric Administration

Dear Members of the Veterinary Medicine Advisory Committee:

RE: VMAC Review of the FDA staff proposal that AquBounty's AquAdvantage GE Salmon be approved for human food consumption

First, let me note that both the Center for Food Safety and the International Center of Technology Assessment do not think that the principles and procedures described in Guidance 187 - Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs are adequate for the oversight and regulation of GE animals. We submitted comments to the docket related to that Guidance. Please note that we believe that this 2008 guidance misapplies the statute, and that the pathway created violates the FFDCA and is an improper and unlawful way to regulate GE animals, including the salmon. Look back at our 2008 comments for more detail on that. I have attached those comments for your examination. Despite the fact that we believe that this process is unlawful, we are submitting comments nonetheless.

We believe that the members of this VMAC committee have an impossible job in that the framework that you are being asked to work, namely the fiction that a whole animal is essentially to be assessed as though it were a drug, is not an intellectually honest approach to the assessment of the animals.

We believe that the FDA should have developed a more fulsome novel foods approach that included adequate feeding trials assessing this animal as a human and animal food.

When we finally received these papers from the FDA only 10 days ago, the most striking thing was how little data the company had produced over the last 15 years. Either that, or the company and the FDA have decided not to present other data that are less supportive of the approval of this animal.

The next most startling thing was the remarkably small sample size of most of the studies. The larger studies are mostly cobbled together collections of data from various years that exclude important facts such as why were so many animals culled in ways that do not appear to be random as would be required by a good scientific review?

In short, the FDA's assessment of AquaBounty's genetically engineered growth hormone salmon is seriously flawed. Most basically, the assessment is based on unreliable, potentially biased data, making it impossible to come to any scientifically based conclusions. FDA repeatedly acknowledges the potential for bias in AquaBounty's data , but refuses to draw the obvious and unavoidable conclusion: that scientific evaluation of the GE salmon is impossible until scientifically sound data are collected and presented.
Unreliable and potentially biased AquaBounty data sets are noted by FDA especially in two sections: phenotypic characterization and food and feed safety

I used to study the development of Chinese fisheries and fisheries research. The Chinese, for those of you who don't study fisheries were the first researchers who learned to use hormones to spawn carp and the first to clone fish. Most of the significant Chinese studies I reviewed included around 500 animals, so the small sample size of the AquaBounty studies stands out for me. Of course, the Chinese often start with smaller sample sizes, but they then go to larger studies. The studies in these papers are a good start, but I do not think that they should be considered adequate for the review of the first food from a genetically engineered animal. Rather than the FDA requesting follow up studies after approval to make up for the inadequacy of the studies presented here, the FDA should insist that these studies be done before the fish is approved.

Many of you supervise graduate students; I would hope that you would insist that your students redo studies that are as statistically flawed as many of the studies in this document. I urge you to ask yourself if you would really not require the student to do a better job before you sign off on their work, because that is what you are being asked to do today. Is this really the best work that US scientists can do on this subject? The FDA seems to think so, but your job is to review this work dispassionately, don't just pass the student, make sure that all the research has been done properly. I don't think that you can look at this set of studies and say that adequate work has been done.

Let me highlight what we think are just some of the most troubling parts:

Analysis of the EO-1α transgene insertion site of AquAdvantage Salmon is incomplete and therefore inadequate[i]

Yaskowiak *et al*. (2006) have sequenced the EO-1α transgene and 1136bp of upstream flanking sequence and 730bp of downstream flanking sequence from AquAdvantage Salmon. However they have not identified and sequenced the original insertion site from wild-type Atlantic salmon. Therefore, nothing meaningful can be said about the extent of genomic damage at the insertion site and the fundamental question, *Has insertion of the EO-1α transgene disrupted or deleted any important functional sequences in AquAdvantage salmon?,* remains unanswered.

The company has failed to prove that the EO-1α transgene they have characterized is the sequence responsible for the growth phenotype of AquAdvantage Salmon.

Yaskowiak *et al*. (2006) and the FDA assessment (p.16) claim, "*AquAdvantage* Salmon currently used for production contain a single well-characterized copy of the construct at the α-locus." However, their experiments are unable to rule out the possibility that one or more copies of the Chinook salmon growth hormone DNA are located at a site genetically linked to the EO-1α transgene. This is because their Southern blot analysis was carried out using probes to a fragment of 5' regulatory sequence and 3' regulatory sequence and not to the Chinook salmon growth hormone coding sequence itself (Yaskowiak *et al.* (2006), Figure 1 and discussion p. 471). Consequently, it is not possible to determine whether additional intact and functional copies of the Chinook growth hormone coding sequence are present in the *AquAdvantage* genome, at a site genetically linked to the EO-1α transgene.

AN INCOMPLETE DISCUSSION OF THE POSSIBLE DISRUPTION OF OTHER GENES BY THE LOSS OF DNA AT THE INSERTION SITE [ii]

The discussion of the insertion of the sequence data notes that a part of the 35 base repeat regions was lost and assumes that because repeated regions are "non-essential" and that since the insertion site was not a protein coding region, that the loss of the chromosomal DNA is unlikely to adversely affect the fish.At least in human genome research, repeats are no longer considered 'junk' DNA. Repeats may have important functions in the regulation of gene expression and higher order genome structure and repeat DNA present in coding sequences can be involved in disease. There is no discussion of the role of repeated regions in the evolution of salmonids and no discussion of the role of non-coding DNA is controlling protein producing genes.[iii]

THE CHIMERICAL GENE CONSTRUCT INSERTED INTO THE AQUADVANTAGE SALMON SHOULD NOT BE EQUATED WITH NATURAL CHINOOK SALMON OR OCEAN POUT DNA. [iv]

While both Chinook salmon and ocean pout DNA have been consumed by people, the particular AquAdvantage construct that depends on fusing both genes together and using synthetic DNA and a plasmid[v]. This chimerical gene construct has never been eaten by people before and should not be conflated with natural DNA found in the Atlantic salmon, Chinook salmon, or eelpouts. Even the chimerical gene as it was inserted into the animal was rearranged such that a portion of the far 5' non-coding regions of the insert was displaced to 3' end of the insert. [vi] Indeed, the novelty of this construct is why these hearings are being held. The inadequate food safety review discussed later, makes it hard to claim that there are no food consumptions risks.

POOR SAMPLING IN THE RESEARCH ON ADVERSE OUTCOMES AND MORPHOLOGY:[vii]

The small sample size is this study, the lack of data on the selection procedures, and the fact that fish with obvious morbidly were excluded from the study makes it nearly impossible to determine the likelihood of adverse outcomes when the fish are grown commercially. It is not clear how the fish in the study were selected from the already small groups of "candidate" fish (100-200 fish in each group). Moreover, the fish in various groups were harvested at different times of the year.

The FDA itself admits that there are design errors in this study of only 48 fish (Only 12 of which are the GE fish intended for human consumption.)

The study had a design limitation that could potentially affect the results and thus our interpretation. The design limitation results from the high rate of removal of early-life stage fish (e.g., fry or smolts). ABT indicated that this practice normally occurred at the ABT PEI hatchery at regular intervals because of space limitations. The net result is that the adult fish in the study may not reflect the nature or incidence of abnormalities of the initial population.[viii]

Still, the FDA concludes*:*

There are no adverse effects on size, body weight, or related parameters in AquAdvantage Salmon relative to comparator fish other than the effects expected from the introduction of the AquAdvantage construct.[ix]

Instead of requiring the company to gather more information on how the fish thrive in actual grow out conditions, such they would experience in commercial operations*, CVM has recommended a surveillance program as part of the durability plan (see Genotypic and Phenotypic Durability Plan, Section VI below), and will closely monitor post-market surveillance reports of adverse events*.[x]

HISTORICAL DATA PROVIDED BY ABT SHOULD NOT BE USED, NOT CLEAR HOW THEY WERE COMPILED AND MAY NOT BE COMPARABLE DATA SETS

The FDA itself details problems with the ABT historical data and described it to be of limited value.

ABT provided a summary of historical data addressing the health of AquAdvantage Salmon in several consecutive year-classes. Morphologic ranking data were reported for diploid and triploid fish of the 2003-2007 year-classes and are summarized in Table 4 below. These reports did not describe how these historical data were compiled or whether they included assessments of culled fish. Therefore, the inferential value of these data is limited and may be subject to the same concerns described previously for the safety study."[xi]

The FDA looked at data from years before 2003, but did not report on its observation for those years. The company claims to have data for 15 years of production, but this document used only data from five years. Another ABT table does use data from 2001&2002, but that is not a part of their analysis of morphological irregularities.[xii]

Moreover, it does not explain why the size of the year class is so highly variable, i.e. 1165 triploid GE fish in 2003, but only 38 in 2005 and 138 in 2007. Moreover, as the population of fish in the sample gets smaller, the percentage of favorable fish varies considerably, i.e. from 39 percent in 2003, the year 2005 has the lowest percentage of highly ranked fish, only 7.9 percent, and the most recent year presented has a 92.4% highest ranking. It is not clear what is actually happening to the fish given the lack of clear data about culling and the change in cultural techniques during the period. Having the other 10 years of missing data would help some, but only if the culling and sampling practices were available. Changing the ranking system in 2007 does not help either.[xiii]

ABT also provided a short white paper, prepared in response to a FDA request, addressing the occurrence and origin of morphological irregularities in salmonids and summarizing data presented in several other submissions on abnormalities found in AquAdvantage Salmon. The section of the white paper discussing the frequency and etiology of malformations in salmonids is less than a page long.

According to ABT's white paper, many factors and/or conditions have been associated with developmental abnormalities in salmon, including deficiencies in phosphorus and vitamin C, excess vitamin A, high or variable temperatures during early growth phases, exposures to certain drugs (e.g., oxytetracycline), contaminants in feeds (e.g., heavy metals, insecticides, PCBs), and some parasites.

NO DATA ON WHETHER AQUADVANTAGE SALMON REQUIRE LARGER AMOUNTS OF ANTIBIOTICS:

However, the reference to the white paper contains the one of only two references to antibiotic use with these animals. In the only other discussion of antibiotics, the company attributed low survival rates in both the ABT fish and the comparators primarily to fungi and opportunistic bacteria, and notes that offspring of both GE and non-GE crosses periodically required treatment with drugs such as formalin, chloramine-T and salt, but does not discuss if more drugs were required for the GE fish or not. One would have expected a more complete discussion of the antibiotics used with all of the ABT fish and their comparators. Without such data, it leaves open whether the GE fish are even as healthy as other triploid fish. The company routinely

attributes health and phenotypic problems to cultural conditions or triploidy, but failed to develop large enough samples to assess this.

The FDA again admits that there are problems with many aspects of the data presented, but rather than ask the company for better data before approving this fish for human consumption instead again points to its plan to monitor these issues AFTER approval, "*The Durability Plan includes monitoring, data collection, and reporting of abnormalities observed under commercial production and grow-out conditions to address this residual uncertainty*.[xiv]

Moreover, it unfortunately still finds the data satisfactory for its "weight of evidence" review. *Although this is not an adequate and well controlled study due to the variability of husbandry conditions, numbers of fish crosses, and long time course, this information is nonetheless considered as part of our weight-of-evidence evaluation, and contributes to our understanding of the effect of the AquAdvantage construct on the fish.* [xv]

MORE RESEARCH NEEDED ON WHY ABT SALMON HAVE JAW EROSIONS AND INFLAMED TISSUES

At least two significant findings from this inadequate study demand additional study before the AquAdvantage salmon is considered for approval:

1)The effect of the ABT construct on jaw erosion in the fish. The effect of small sample size has been suggested by ABT as a limitation on the interpretation of jaw erosions.xvi

2) An increased prevalence of focal inflammation in various tissue types in AquAdvantage Salmon has the strongest correlation with the presence of the AquAdvantage construct among the findings in this study. That these fish may have been immunocompromised as a result of seasonality or other factors confounds the interpretation of these findings. [xvii]

Again the FDA complained that the sample size is too small to measure rate of abnormalities that might be expected in commercial grow out facilities, but excuses the company from any requirement to produce the data before the approval of the fish by saying:

There is no practical way ABT could have generated the appropriate data without producing – and destroying – commercial lots of fish. Nonetheless, we believe that incorporating an appropriate surveillance/durability plan will provide sufficient data and information to the Agency to minimize this uncertainty.[xviii]

DOES REDUCED FITNESS IN THE WILD MEAN PROBLEMS IN FISH FARMS, TOO?

Another comment notes that the fitness of the fish may affect the survival of the GE fish in the wild, but does not discuss whether that means the fish will be compromised in the fish farm environment.

The FDA notes *With respect to the environmental safety evaluation, several phenotypic changes have been identified that are consistent with the presence of the AquAdvantage construct and appear to result in decreased fitness (e.g., increased oxygen requirements, decreased critical swimming speed, lower metabolic scope, etc.). These changes are expected to impact survival and establishment should any AquAdvantage Salmon escape from commercial production facilities.*[xix]

Again the FDA looks to its proposed post approval plan to answer questions that should be resolved before approval.

Post-approval monitoring and reporting as part of the Durability Plan is recommended to address uncertainties associated with the incidence of malformation rates in the early life-stages of AquAdvantage Salmon under commercial grow-out conditions. This monitoring should be conducted with several year-classes of salmon at the ABT grow-out facility in Panama. Additional data on malformations, morbidity and mortality in culled fish at the PEI brood stock facility would also decrease uncertainties.[xx]

NO DATA PRESENTED ON WHY NUCLEIC ACIDS IN THE AQUABOUNTY CONSTRUCT ARE GRAS

The FDA assumes that all DNA, however configured, is inherently safe and does not need to be addressed in a food safety review of salmon. Were this assumption true, we would have no need for Biosafety protocols or no need to worry about bioterrorists. This genetic construct is unique in that it is a chimera of three different DNA sources: the Chinook salmon growth hormone gene, the promoter DA sequence from the eelpout gene, and synthesized linking DNA. It is not clear that FDA is doing more than asserting a statement of belief when it says:

"Because nucleic acids, including DNA, are presumed to be Generally Recognized As Safe (FDA, 1992), there is no direct food consumption risk associated with exposure to the AquAdvantage construct itself."

Salmonid fishes have many repeated DNA sequences. Salmonids have twice as many copies of most genes as other vertebrates. It is into one of these repeated areas that the company has inserted its DNA construct. Again the assumption is that this construct will not cause problems. The great differences in the sexual expression in various salmonids suggest that genes in these repeated sequences can greatly affect sexual reproduction in these fish.[xxi] However, recent findings about the nature of the non-coding DNA sections of the genome suggests that the GRAS finding should require demonstration by the company supported by clear data, not simple assertion by FDA as though it were a fact.[xxii]

ALLERGENITY TESTS FLAWED, TOO.

One of the food safety concerns that was directly tested for by ABT was to determine whether their GE salmon was more allergenic that other farmed salmon. Again, the sample design and size limited the validity of the one study that the FDA found acceptable.

The FDA staff found that the number of samples per group was limited (only six fish). Fish were included regardless of size, sex, or maturity and as such may not be like the AquAdvantage Salmon that will be marketed. No farm raised salmon were included as a control. The principle investigator changed during the study. Samples were improperly un-blinded, leading to possible bias in both the fluorescent enzymatic immunoassay (FEIA) inhibition and Western blot analyses. These and other violations of the research protocol led the FDA to attempt its own re-analysis of the data. Even then the Western Blot data to determine if any qualitative changes occurred in the major salmon allergen parvalbumin (*Sal s1*), due to the insertion the AquAdvantage construct at the α- locus in ABT salmon was unacceptable and no conclusion could be drawn due to faulty data.

Nonetheless, the FDA concluded that triploid GE fish were acceptable, but diploid GE fish might pose allergy problems.[xxiii]

Still, the FDA warns ominously:

One potential indirect hazard that may result from the insertion of the AquAdvantage construct at the α- locus is a possible increase in the endogenous levels of allergens in ABT salmon due to insertional mutagenesis in a region of the genome that may act as a regulator of the expression of one or more of these proteins. Although the previous study attempted to address this point, its various technical deficiencies make it difficult to determine whether the allergenicity of salmon, or the prevalence of any known endogenous protein that has been implicated in allergic responses (i.e., parvalbumin) have changed, thereby somehow increasing the allergenicity of the fish.[xxiv]

ANIMAL FEED SAFETY DATA COMPLETELY LACKING

The use of the AquAdvantage Salmon as an animal feed was not examined at all. No trials were conducted on animals that might consume these fish as feed. Instead, the FDA simply assumes that it is safe for animal feed, despite the fact that some likely animals that might be fed these fish could consume large quantities of the fish, i.e. mink or other predatory fish. The FDA says: "*the evaluations and their corresponding data were generated from studies not specifically designed to examine animal feed safety.*"[xxv]

THE ENVIRONMENTAL IMPACT ASSESSMENT IS INADEQUATE

The Environmental Impact Assessment relies primarily on the company's assurance that physical containment at the Prince Edward Island breeding facility and the Panama grow out facility will work and assumes that the company will obtain essentially 100% sterility of the AquAdvantage triploid fish.

The FDA, to its credit, correctly notes that "the potential for accidental releases of AquAdvantage Salmon at the production and grow-out sites due to natural disasters has not been

explicitly addressed in the EA except that it is stated that 'no such natural disasters have occurred, or are known to occur, in proximity to the PEI and Panama facilities.'

The FDA staff point out correctly that PEI does, in fact, have major storm events; including hurricanes and strong winter storm. The facility is only 25 feet above sea level and 120 feet from a tidal river. Storm surge waves from hurricanes can exceed 35 feet and move a half a mile inland. This is not a location where the company can confidently assert that no disaster will ever befall the facility. The recent increase in the severity of both summer and winter storms in the Atlantic region combined with redrawing of flood maps by engineering authorities make it difficult to claim that severe weather cannot affect the facility. Both fertile fish and eggs may be able to escape the facility during such extreme events. The companies claim that salmon streams are silted up by local farming operations and other landfills should not be considered an effective physical deterrent as storms can also remove that silt and the PEI or Canadian government might decide to restore these former salmon streams.

The need for physical containment grows as the facility would be expanded to facilitate a higher production level of eggs. This is not addressed adequately in the study. While the study does address unauthorized releases by unauthorized persons, no where does the study address the possibility of intentional releases by authorized persons. As the company needs to hire more staff, they cannot assume that a staffer won't decide to release fish or eggs on their own.
The company has shown no quantitative data that demonstrates that is triploid eggs have been effectively sterilized. The company should be required to produce those data before the fish is approved, not after as suggested by the FDA.[xxvi]

The papers have conflicting information on whether the fish will or will not survive in salt water environments. A small study on page 42 shows that at least the diploid GE fish survive well in salt water and this is used to demonstrate the similarity of the GE fish to other Atlantic Salmon. On pg 123, it is argued that any fish that escaped the Prince Edward Island facility could not tolerate the higher salinity of the sea waters.

The lack of a good experimental design to test whether or not AquAdvantage salmon at all stages of their lives can tolerate salt water after being reared in fresh water opts for a default assumption that the fish can tolerate salt water if they escape. The paper agrees with this assumption.

"Freshwater-reared GE Coho and Chinook salmon containing growth hormone rDNA constructs apparently do not lose their ability to tolerate high levels of salinity, even as adults (Robert Devlin, Canadian Department of Fisheries and Oceans, personal communication, August 13, 2010). If this is also true for AquAdvantage Salmon because this tolerance is a secondary result of the added growth hormone gene, survival of adults and older life stages would not be expected to be compromised if escape occurred in PEI."[xxvii]

FDA MUST NOT APPROVE THE AQUADVANTAGE SALMON, DO ITS OWN REVIEW OF THE AQUABOUNTY DATA, REQUIRE AQUABOUNTY TO SUBMIT ADEQUATE DATA AND DO A COMPLETE ENVIRONMENTAL IMPACT STATEMENT

We find that the studies submitted by AquaBounty so flawed statistically that we recommend that FDA commission an independent statistician to analyze this and other aspects of AquaBounty's GE salmon safety study. If AquaBounty's own assessment – that it designed the animal safety study with too few animals to permit interpretation of findings – is accurate, then ALL the animal safety study data must be dismissed as uninterpretable. This would mean of course that any conclusions drawn from such data have no scientific merit. The statistical evaluation should apply not only to the animal safety study, but to all data in the Briefing Packet, including historical data and those provided for the food and feed safety section. Any decision on AquaBounty's GE salmon should be postponed pending this statistical analysis.

Likewise, we believe that the environmental assessment presented by AquaBounty is a wholly inadequate review for this fish that the company has announced that it wishes to grow near US population centers. The stipulation that the FDA tries to place on the approval, namely that the eggs be raised in Canada and the fish raised in Panama ignores the easy transfer of a material like eggs in the international market. A full environmental impact statement should be required before the approval of the fish.

Sincerely,

Jaydee Hanson
Senior Policy Analyst for Cloning and Genetically Engineered Animals.
Center for Food Safety &

Policy Director
International Center for Technology Assessment

[i] Please see attached Appendix 1 for a longer discussion of these points
[ii] FDA briefing packet to VMAC, p. 16

[iii] See for example, Teri A. Manolio, Francis S. Collins, et al. "Finding the missing heritability of complex diseases" Nature 461, 747-753 (8 October 2009)

[iv] FDA-VMAC, p.17

[v] P. 10 notes "Recombinant DNA inserts from three sources were used for the final construct. These sources included regulatory sequences from ocean pout, the growth hormone coding region from Chinook salmon and small synthetic linkers to aid in assembly of the inserts and plasmid."
[vi] Ibid, p. 18 "This analysis identified a rearrangement compared with the original construct (Panel A Figure 2). The rearrangement displaced a portion of the far 5' non-coding regions of the insert to the 3' end of the insert (Panel B Figure 2)."

[vii] Please see our longer discussion of these points in Appendix 2.
[viii] P.26
[ix] P.26
[x] P.19
[xi] P.27
[xii] P.33 See Table 5: Average Survival from the 2001-2006 Year-Classes.
[xiii] See data presented on p.28 & 29.
[xiv] P.33
[xv] P.40
[xvi] P.40
[xvii] P.41
[xviii] P.45
[xix] P.45
[xx] P.47
[xxi] See for example, Stephen H. Forbes, et al, "One of two growth hormone genes in coho salmon is sex linked,"Proc. Nati. Acad. Sci. USA Vol. 91, pp. 1628-1631, March 1994
[xxii] P.63
[xxiii] P. 100-104
[xxiv] P.106
[xxv] P. 109
[xxvi] P.128
[xxvii] P.129