# Transgenic salmon: a final leap to the grocery shelf?

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**Despite being caught up in regulatory proceedings for 15 years or more, AquAdvantage salmon, the first animal genetically engineered (GE) for food purposes, continues to raise concerns. Are any of these concerns scientifically justified?**



The tortuous passage of AquAdvantage salmon through the US regulatory system provides a stark reminder of the adage that sometimes it is good not to be first. A fast-growing transgenic fish containing a gene encoding Chinook salmon growth hormone under the control of an antifreeze protein promoter and terminator from ocean pout, AquAdvantage salmon has been subjected to one of the most prolonged, if not exhaustive, regulatory assessments in history. This process culminated last September with a meeting of the Veterinary Medicine Advisory Committee (VMAC) as well as a public hearing, together with the release of a comprehensive health and safety briefing and an environmental assessment package on the transgenic animal developed by AquaBounty Technologies of Waltham, Massachusetts. Despite VMAC's determination that AquAdvantage salmon is "as safe as food from conventional Atlantic salmon," critics continue to raise concerns relating to its allergenicity, levels of insulin-like growth factor 1 (IGF-1) and composition of polyunsaturated fatty acids as well as the potential impacts of the fish on the environment. In the following article, we briefly trace the twists and turns taken by AquAdvantage salmon through the US regulatory regime. We then address each of the remaining 'regulatory' concerns, which have been raised as justifications for delaying approval of this new animal drug.

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AquaBounty Technologies

If US regulators continue to dally, AquaBounty Technologies' transgenic salmon is unlikely to ever reach its market, let alone spawn a wave of new products.

## A long and tortuous journey

Unlike transgenic plants, GE food animals are regulated as drugs in the United States, and as such they must go through the US Food and Drug Administration (FDA) new animal drug approval process. This means that developers must show their product to be safe and effective as well as provide an assessment of its environmental impacts, under the requirements of the National Environmental Policy Act (NEPA).

When AquaBounty Technologies (then A/F Protein) initiated discussions with the FDA seeking regulatory guidance for development and approval of AquAdvantage salmon in 1993, no defined regulatory pathway existed for GE animals. Under the Coordinated Framework for Regulation of Biotechnology, a 1986 policy that called for the US Environmental Protection Agency (EPA), US Department of Agriculture (USDA) and FDA to regulate GE products using existing laws, USDA was the lead agency to regulate GE plants, but no regulatory path had been clearly set out for GE animals. The company petitioned for regulation under FDA because they thought the rigorous pathway for approval would help assuage public concerns regarding food from GE animals. Additionally, the FDA new animal drug approval route has a defined endpoint (that is, the product is either approved or it is not), rather than the ambiguous "no further questions" endpoint of the FDA's Center for Food Safety and Applied Nutrition GE plant food safety evaluations. A formal application for an investigative new

animal drug with intent to commercialize the AquAdvantage salmon was made on September 14, 1995. More than 15 years later, the application is still under regulatory review.

The FDA uses a hierarchical risk-based approach to assess GE animals and their edible products. As with plants, this approach is event-based, meaning that each time a GE animal is generated as the result of the insertion of a recombinant DNA construct at a new genomic location(s), that new event requires a separate evaluation within the confines of its limitations for use. In the seven-step regulatory process described by FDA[1], the agency examines the safety of the recombinant DNA (rDNA) construct to the animal, the safety of food from the animal and any environmental impacts posed (collectively the 'safety' issues), as well as the extent to which the performance claims made for the animal are met ('efficacy'). Molecular characterization of the rDNA construct determines whether it contains DNA sequences from viruses or other organisms that could pose health risks to the GE animal or to those eating the animal. Molecular characterization of the GE animal lineage determines whether the rDNA construct is stably inherited over multiple generations. Phenotypic characterization assesses whether the GE animals are healthy, whether they reach developmental milestones as non-GE animals do and whether they exhibit abnormalities. A durability assessment reviews the sponsor's plan to ensure that future GE animals of this line will be equivalent to those examined in the pre-approval review.

If the GE animal is intended as a source of food, as is the case with the AquAdvantage salmon, FDA assesses whether the composition of edible tissues differs and whether its products pose more of an allergenicity risk than non-GE counterparts. To meet the procedural requirements of the NEPA, FDA also requires the preparation of an environmental assessment of the animal and of conditions proposed for raising the GE animal as outlined in the product definition. The data requirements for demonstrating environmental safety focus upon the rDNA construct, host organism, production system, physical and biological confinement measures, and the receiving environment. Should the review indicate "no significant impact" (a legal term of art) under the proposed production conditions, the agency publishes a finding of no significant impact, also known as a FONSI. However, if substantial impacts to humans or the natural environment are indicated, a full environmental impact statement is required. In the final step, the sponsor data must support their claims for the GE animal, in this case that the AquAdvantage transgenic salmon grows faster than non-GE counterparts.

The AquAdvantage salmon application attempted to proactively mitigate environmental concerns by limiting the product definition to triploid, all-female, hemizygous transgenic Atlantic salmon produced at a single facility in Canada, and grown out in a fresh water, land-based culture facility in Panama. Both locations were inspected by FDA and featured simultaneous, multiple and redundant physical and geographical containment measures, effectively precluding concerns about the possibility of transgenic fish escape. And as an extra precaution, additional levels of biological containment were proposed, including the production of 100% female fish and triploidy induction with an average success rate of 99.8% (98.9–100%). All-female fish are unable to interbreed with each other, and triploidy results in sterility.

## Increased transparency

The FDA clarified its legal authority to regulate GE animals in a 2009 guidance[1] that was issued after considering the 28,000 public comments it received after the release of a 2008 draft version. Included in this final guidance was the FDA's stated intent to increase the transparency of its deliberations and actions by holding public advisory committee meeting hearings before approving any GE animal. And in an unprecedented move toward increased transparency, the FDA made the 171-page briefing package summarizing all of the health and safety data on the AquAdvantage salmon[2] and the 84-page environmental assessment[3] publicly available approximately two weeks before the public VMAC meeting in September 2010. This committee, appointed by the FDA and charged with providing scientific advice to the agency, consisted of independent veterinarians and scientists with expertise on the subject matter. The public release of the data package, done with the permission of the sponsor, was unprecedented for the FDA because new drug applications, be they for human or animal drugs, are subject to strong confidentiality provisions to protect trade secrets and confidential business information.

The unanimous conclusion of the FDA scientists after examining the AquAdvantage salmon data package was that the food "is as safe as food from conventional Atlantic salmon, and that there is a reasonable certainty of no harm from the consumption of food from this animal," and that there "is substantial, reliable information available in the environmental assessment document" to conclude that GE AquAdvantage salmon "are not expected to have a significant impact on the quality of the human environment (1) in the United States; (2) in foreign nations not involved in the action; or (3) on the global commons when raised and reared under the current conditions of physical, biological, and geographic/geophysical containment present at hatchery and grow-out facilities in Canada and Panama"[2]. Even so, this was not the unanimous conclusion of those who accessed the data package following its public release, citing several concerns.

## Endogenous allergens

One alarming claim asserted that the AquAdvantage salmon were more allergenic, with the AquAdvantage salmon having mean allergenic potencies 20% and 52% higher than non-GE counterparts (for example, http://www.organicconsumers.org/fish). The FDA did examine whether the AquAdvantage salmon posed an increased allergenicity risk. The Codex guidelines recommend determining whether the gene expression product of a transgene is homologous to known allergens[4]. This is an important test, and actually prevented the development of a GE soybean variety containing a known Brazil nut allergen[5]. This allergenicity determination was undertaken for the exogenous Chinook salmon growth hormone protein that is expressed in AquAdvantage salmon, and no homologies to known allergens were found. However, it was data from endogenous allergenicity testing that generated the "more allergenic" sound bite. Here the question being asked was whether AquAdvantage salmon have higher levels of endogenous fish allergens than nontransgenic Atlantic salmon. An experiment was performed to determine whether the "allergenic potency" of salmon extracts was higher in AquAdvantage salmon based on a human sera IgE inhibition binding assay. The FDA found several notable concerns with the study design and concluded there were insufficient data and information to draw a conclusion on the allergenic potency of AquAdvantage salmon.

It remains unclear whether any amount of data would have been able to answer the question. The reason being is that there is no consensus in the scientific and medical communities regarding the magnitude of the increase in endogenous allergens in an allergenic food that would present an additional risk to public health[6], especially given that individuals that are allergic to a particular food would likely avoid that food. While acknowledging this problem, the FDA still requested the sponsor to provide endogenous allergen data, stating "Regardless, in this part of our evaluation, we will look to see whether the GE animals are more allergenic—that is, *pose more of an allergic risk* [emphasis added by authors], than their non-GE counterparts"

(http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm222635.htm). This might seem a reasonable approach, but when it comes to data interpretation, or even designing the appropriate experiment, it raises the question: "What level of change would be (un)acceptable?"

Few studies have examined the natural variability of allergenicity that exists in traditional food sources (for example, different breeds of dairy cattle, species of fish and cultivars of nuts). It is known that natural variation exists in the allergenicity of available food crops due to differences in the genetics of commercial varieties[6] and interactions with the environment. In plants, there is wide variation in IgE binding to different varieties of the same species[7]. Apart from differences between varieties, natural variability in allergenicity can also occur due to harvest timing and storage conditions[8,9]. Up to a tenfold difference in allergenicity has even been reported between individual apples from within a single cultivar and harvest[10].

The major allergens responsible for cross-reactivity among distinct species of fish and amphibians are parvalbumins[11]. These proteins control calcium flow in the muscular sarcoplasm of the white meat, and parvalbumin is known to be the major allergen in the white muscle of Atlantic salmon[12]. The parvalbumin content of most commonly consumed fish species varies considerably and is also influenced by cooking method. In raw fish, parvalbumin levels vary significantly, with herring parvalbumin levels exceeding tuna levels by 100-fold[13]. This natural variation brings into question the scientific justification for performing experiments to determine whether GE fish have higher levels of endogenous allergens than their non-GE counterparts when we do not have, or require, analogous information on the fish we currently consume. In the absence of data on variation in non-GE Atlantic salmon populations and a validated approach to address the question of what level of change would be unacceptable[6], there is no way to evaluate whether biologically relevant differences exist in the levels of endogenous allergens in GE, or non-GE, fish.

## IGF-1

Another frightening food safety allegation was the suggestion that AquAdvantage salmon had "40% more IGF-1, a hormone linked to prostate, breast and colon cancers in humans" (for example, http://www.organicconsumers.org/fish). In fact, the data in the package showed there was no significant difference between the mean IGF-1 levels for the GE and non-GE diploid salmon (mean of 9.263 ng versus 8.892 ng per gram, respectively). IGF-1 data were only reported for 6 out of the 30 GE salmon analyzed because levels of the growth factor in the remaining 24 (80%) GE fish were below the assay limit of quantification (3.27 ng/g). Likewise, the majority of the control fish had IGF-1 levels below the detection limit of the assay. Because the range of IGF-1 values for the diploid AquAdvantage salmon exceeded that of the non-GE salmon by 10%, further analyses were triggered. This was justified as follows: "As part of the heuristic method applied to assessing data and information, our initial decision to begin assessing the biological relevance of any measurement began with determining whether that measurement exceeded the comparator range by 10% or more"[2]. There does not appear to be any scientific basis for selecting this 10% value as a trigger for additional investigation, nor is the biological relevance of this arbitrary value clear.

This abundance of caution in regard to nonsignificant differences in IGF-1 levels was picked up in a *New York Times* article, which stated "One issue that might attract some discussion at the public meetings is that the engineered salmon have slightly higher levels of insulin-like growth factor...a hormone related to growth hormone"[14]. This was followed by a discussion of the link between IGF-1 in the bloodstream and cancer, although the journalist did note that it is not clear how IGF-1 protein in food, which will presumably be digested in the gut, contributes to hormone levels in the blood. Despite this disclaimer and mention that FDA concluded that even if people ate a lot of the AquAdvantage salmon, it would not make a significant difference in the amount of the IGF-1 they would consume, critics maintained the transgenic fish is "more carcinogenic."

## Polyunsaturated fatty acid content

A food quality claim suggested that the GE AquAdvantage salmon were less nutritious, with the GE salmon having the lowest omega-3 to omega-6 ratio of any salmon[15]. Not unexpectedly, the farm-raised AquAdvantage salmon were not significantly different with regard to omega-3 and omega-6 fatty acid levels and the ratio of omega-3 to omega-6 fatty acids when compared with nontransgenic, farm-raised salmon fed the same diet. Despite statements to the contrary, the data showed that the AquAdvantage salmon had a marginally higher omega-3 to omega-6 ratio relative to nontransgenic controls. It might be asked why fatty acid data were requested of the sponsor in the first place, given that a growth phenotype would not normally be expected to alter the polyunsaturated fatty acid content of a fish. The rationale for looking at this was that consumers "might be concerned that consuming AquAdvantage salmon would lower their intake of omega-3 (ref. 2)."

Overall, the AquAdvantage salmon food safety studies do not suggest that the fast-growth phenotype is associated with any food safety concerns. On the basis of the available evidence, conclusions about inferior fatty acid content for the transgenic fish appear to have been based on isolated data points from the public briefing documents and irrespective of the fact that the complete context with proper comparators results in the reverse conclusion.

## Environmental concerns

The other main concern that was widely reported is that AquAdvantage fish would escape and cause the collapse of wild salmon populations. Environmental concerns are the most significant science-based concern associated with the development of GE animals, as detailed in a report of the National Research Council[16], which was prepared at the request of the FDA after the AquAdvantage salmon submission. The risk of harm from GE animals is the product of (i) harm, given exposure to the hazard (that is, the GE animal), and (ii) the probability of exposure[17,18]. And in this case, the probability of exposure given the 'Limitations for Use' associated with the AquAdvantage salmon product definition was seen to be extremely small due to the triple redundancy of simultaneous containment measures—that is, (i) land-based production with physical confinement barriers (screens), (ii) reproductive confinement measures resulting in 99% sterility and 100% female production stocks and (iii) thermally lethal lake and stream temperatures downstream from the proposed production facility in Panama—and high salinity of waters surrounding the Canadian location.

One of us (W.M.M.) has reviewed actual AquAdvantage salmon data collected by Moreau and colleagues[19] quantifying critical life history characteristics, such as relative viability and mating success of AquAdvantage salmon in multiple environments. Analysis of the data showed that none of the net fitness components[20] of AquAdvantage salmon were enhanced by expression of the transgene. As a result, the Trojan gene effect[21] would not be predicted to occur in the unlikely event AquAdvantage salmon did escape from confinement. Rather, selection over time would be expected to simply purge the transgene from any established population, suggesting a low probability of harm resulting from exposure to AquAdvantage salmon. Although W.M.M. presented this

information at the public meeting, it appears to have been largely ignored by those promulgating the Trojan gene effect.

The FDA has not yet made a decision as to whether to make a FONSI determination regarding the environmental assessment of the AquAdvantage salmon under the proposed Limitations for Use or whether it will require the preparation of a full environmental impact statement (EIS). A final decision will be made after comments from both the public and appropriate experts have been received and evaluated. NEPA regulations require the FDA's decision on whether to prepare a full EIS to take into account the degree to which the effects of its action are "likely to be highly controversial."

In February 2011, Friends of the Earth, Earthjustice, Greenpeace, Oceana, Ocean Conservancy, Pew Environment Group and the Union of Concerned Scientists sent a letter to FDA commissioner Margaret Hamburg, selecting excerpts from the public meetings to support their contention that there is a need for a comprehensive EIS that evaluates the full range of threats that stand to confront wild fish populations if AquAdvantage salmon are released into the natural marine environment. An EIS is typically hundreds (or even thousands) of pages in length, and must document all effects of the GE animal to the human environment[22]. This includes not only ecological effects, but also, according to various judicial interpretations, economic, social, cultural, historic and aesthetic effects.

We regard this as an ominous development for GE animals, as the requirement to develop a full EIS documenting all conceivable impacts to fulfill the requirements of NEPA has been successfully used in litigation to delay the planting of GE alfalfa and sugar beets. Indeed, it has been suggested that NEPA requirements are being used by some environmental groups (for example, the Center for Food Safety) as a legal approach to slow or prevent regulatory approvals of products to which they are opposed[22]. Requiring a full EIS would undoubtedly further delay approval of the AquAdvantage salmon and increase the regulatory cost.

Absent from the debate over the AquAdvantage salmon thus far has been any balancing discussion of the environmental risks associated with obtaining food from alternative sources of Atlantic salmon. In principle, there is no difference between the types of concerns and potential magnitude of the environmental risks associated with the escape of GE fish and those related to the annual escape of the millions of fish that are genetically divergent from native populations in other ways (for example, strains selected for enhanced growth, intraspecific crossbreeds and interspecific hybrids[23]). Selection for fast-growing fish using conventional breeding results in a shift in the allele-frequencies of many growth-associated genes. Farmed fish are known to have a fitness disadvantage, called a genetic load, in natural environments because domestication genes are only favorable in domestic environments. It is known that matings between escaped farmed salmon and wild native fish result in a "substantial risk of extinction for natural populations"[24]. Thus, the comparative risk of contained, sterile transgenic AquAdvantage salmon is likely to be no more than that of fertile, selectively bred, Atlantic salmon.

## Political hurdles

Less than two weeks after the public meeting that was intended to increase transparency, clarity and public confidence in the GE animal regulatory process, two separate letters—one from 11 US Senators mostly from the Pacific Northwest, and another from 29 members of the US House of Representatives—were sent to the FDA commissioner identifying a multitude of problems in the FDA's GE animal regulatory process, specifically citing the lack of transparency and opportunity for public participation. Ironically, the Senate letter included a suggestion that the "creation of a new genetically engineered species should not be treated as a new animal drug issue but undergo formal evaluation by FDA's Center for Food Safety and Applied Nutrition to review the product's potential health effect on humans" (http://begich.senate.gov/public/index.cfm/files/serve?File_id=0ebd87aa-3225-4ab3-a977-d484f270bbbf). This is the very regulatory path that was eschewed after more than a decade of deliberations and numerous opportunities for public input, in favor of an approach that concludes with a definitive approval or denial endpoint. The House letter also cited the "Trojan gene effect" to support the contention that any approval of GE salmon could represent a threat to the survival of native salmon populations.

And in what may prove to be the final blow to the AquAdvantage salmon's regulatory adventure, on June 16, the House agreed by a voice vote to approve a budget amendment that prohibits the FDA from spending any funds on the approval of a genetically engineered salmon—a measure that effectively bars GE salmon from reaching the market. This amendment was introduced by Rep. Don Young from Alaska, a state with a large wild-caught salmon industry. In offering the amendment, he argued that AquAdvantage salmon would compete with wild salmon in his state (http://www.cnsnews.com/news/article/house-moves-bar-genetically-modified-sal). The amendment was voted on by fewer than a dozen of the total 435 House members. In an accompanying press release Young stated, "Frankenfish is uncertain and unnecessary. Should it receive approval as an animal drug, it clears the path to introduce it into the food supply; my amendment cuts them off before they can get that far"[25]. US legislation must be approved by both the House and the Senate for it to become law, and the Senate has not yet voted on this issue.

## Perspective

The regulatory process associated with GE animals focuses on risks with little consideration of attendant benefits. And paradoxically, similar risks known to be engendered by conventionally bred animals (for example, fish selected to grow faster, outcompeting wild stocks) undergo no regulatory scrutiny; only GE animals trigger an extensive premarket review and NEPA requirement. Subjecting conventionally bred and GE animals to different regulatory standards is inconsistent from a scientific perspective and places an excessive regulatory burden on the development of GE technologies. Assessing potential risks in the absence of considering concomitant benefits and those risks associated with alternative food production systems gives disproportionate emphasis to the risk side of the GE food animal equation. Few, if any technologies could survive a risk-only analysis. Wild-caught fish deplete the oceanic stocks and do not present a long-term, ecologically sustainable solution to rising global fish demand. One of the benefits associated with the development of GE fish for aquaculture may well be in helping to reduce recognized pressure on wild fish populations[26].

The current regulatory approach in the United States, coupled with the unpredictable time frame, has stymied commercial investment in the development of GE animals for agricultural applications. The abuse of good-faith attempts to increase transparency and enable public participation in the GE animal regulatory process, coupled with political efforts to prohibit the FDA from regulating GE AquAdvantage salmon as it approaches the close of its protracted regulatory journey, are unlikely to have reassured potential investors. There is little benefit to society if attempts to increase public participation in the regulatory process are used as an opportunity to vilify technology. This outcome may jeopardize future access to improved genetic lines resulting from new technological developments (e.g., disease-resistant GE animals[27]), with negative consequences on food security and other broadly supported societal goals, including improved human and animal health.

**COMPETING FINANCIAL INTERESTS**
The authors declare no competing financial interests.

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