

Comments of Consumers Union on Genetically Engineered Salmon, Food and Drug  
Administration Docket No. FDA-201034-N-0001,  
Veterinary Medicine Advisory Committee Meeting

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Consumers Union<sup>1</sup> (CU) welcomes the opportunity to comment on FDA's appraisal of some of the safety issues associated with the AquAdvantage Salmon which has been genetically engineered with a growth hormone to reach mature size more quickly.

### Summary

This assessment of a genetically engineered (GE) salmon is the first evaluation of a GE animal and will set a precedent for future approvals of GE animals. FDA should be especially cognizant of the scientific quality of the data and the rigor of the analysis needed to do a proper safety assessment of GE animals in this case. Unfortunately, the evidence of FDA's evaluation of the AquAdvantage salmon suggests that FDA has set the bar very low. There is sloppy science, small sample sizes (only 6 fish per group for the allergenicity study), questionable practices (manipulating IGF-1 data), and woefully inadequate analysis (a conclusion of growth hormone levels in the flesh, despite having no data at all on growth hormone levels due to use of insensitive test methodology). This analysis does not conform to FDA standard for assessment of a New Animal Drug (NAD).

FDA requires New Animal Drugs to be shown to be safe for animals, humans and the environment. This has not been shown for the GE salmon. The data presented, although woefully incomplete, do raise a potential serious human health issue—that of increased allergenicity. If this product (GE animal) does increase the allergenic risk (e.g. an increase in allergenic potency), it should not be approved. Data from a mere 6 salmon (e.g. GE triploids) is neither sufficient nor rigorous enough to conclude that no problem exists.

Because FDA's assessment is inadequate, we are particularly concerned that this salmon may pose an increased risk of severe, even life-threatening allergic reactions to sensitive individuals. Instead of approving this product, FDA should be requiring studies

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<sup>1</sup> *Consumers Union, publisher of Consumer Reports, is an expert, independent nonprofit organization whose mission is to work for a fair, just, and safe marketplace for all consumers and to empower consumers to protect themselves. To achieve this mission, we test, inform, and protect. To maintain our independence and impartiality, Consumers Union accepts no outside advertising, no free test samples, and has no agenda other than the interests of consumers. Consumers Union supports itself through the sale of our information products and services, individual contributions, and a few noncommercial grants. Over 7 million people subscribe to Consumer Report or Consumer Reports online.*

with data from many more engineered fish, not the tiny sample of six fish on which it currently bases its conclusions. Unfortunately, even the data from those six fish raises concerns, especially given the data on six GE diploid fish that were ignored.

Bottom line, this safety assessment is insufficient and needs to be redone with all the needed data. Commissioner Hamburg last week honored Dr. Frances Kelsey for her stand that prevented thalidomide from harming American children many years ago. FDA needs to bring Dr. Kelsey's spirit to this assessment and focus on rigorous science with a view toward protecting public health.

### **Phenotypic characterization**

One of the risk/hazard questions FDA asks for the GE salmon is whether any direct or indirect toxicity effects of the AquAdvantage genetic construct can be detected in the GE salmon's phenotype (physical appearance or characteristic) compared to a non-engineered comparator. FDA evaluated 11 characteristics: a) general husbandry conditions; b) specific conditions at Prince Edward Island (PEI) facility; c) general health observations; d) feed consumption and weight gain; e) overall mortality and morbidity; f) physical exams; g) clinical pathology assessments; h) necropsies; i) disease resistance; j) smoltification and seawater survival; k) other phenotypic characteristics.

#### *Husbandry and rearing conditions (a and b)*

A fundamental problem with all the phenotypic characterization data, and indeed all the nutritional and food safety assessment data, is that they all come from GE Salmon raised in the PEI facility, not at the facility in Panama. FDA admits that the culture/husbandry conditions at the facility in Panama will likely differ significantly from the conditions at the PEI facility with unknown effect on the GE salmon's phenotype but then concludes that it has no concerns with the different culture conditions: "the culture (e.g., water temperature, pH, alkalinity, etc.) were likely to be significantly different from the facility at PEI as a result of differences in, among others, water surface, facility design, and environmental factors due to geographic location. . . . the effect of the difference between the PEI and Panama facilities, especially temperature, on the resulting AquAdvantage phenotype is unknown. Conclusion: The husbandry and rearing conditions at the PEI and Panama facilities do no present concerns with respect to animal health."

We do not understand how FDA can conclude, in the absence of any data on the phenotype of GE salmon raised at the Panama facility, that there are no animal health concerns with GE salmon raised at the Panama facility. This lack of data is highly problematic as the GE salmon that consumers will be exposed to will be those grown at the Panama facility. FDA appears willing to conclude that there are no animal or human safety problems from AquAdvantage salmon raised in Panama based on ***no data at all*** from fish raised in Panama even as they admit that the effect of the different culture and

rearing conditions on the phenotype of the GE salmon is unknown. This should be unacceptable for a GE animal approval, as it appears to violate the NAD regulations.

FDA is regulating the GE salmon as a New Animal Drug, with the NAD being the genetic (e.g. rDNA) construct itself. Thus, the husbandry and rearing conditions of GE fish into which the genetic construct has been inserted would constitute the production process. Under the NAD provisions of the Federal Food Drug and Cosmetics Act (FFDCA), a NAD is granted for a specific production process; if a company changes the production process for a NAD, the company must submit data to the FDA to show that such a change does not have an effect on the safety or efficacy of the NAD, i.e. the FDA does not assume that drugs made with different production processes are equivalent and requires data to show they are equivalent. Since the husbandry/rearing conditions differ between Panama and PEI—the former being in the tropics, the latter in the temperate zone—this means that the production process (e.g. husbandry/rearing conditions) differs as well, and FDA should require Aqua Bounty to submit data showing that it does not impact the safety of the NAD. FDA should insist, for example, that the rearing conditions in Panama do not increase the levels or potency of allergenic proteins in the salmon.

**FDA must demand data on GE salmon produced under the same (e.g. husbandry and rearing) conditions as will be used to produce the GE salmon which consumers will consume. FDA should not approve the AquAdvantage Salmon until Aqua Bounty presents such data.**

#### *General Health observations*

The controlled, blinded study on the general health and behavior associated with the AquAdvantage salmon examined fish from the PEI facility at four separate times “following pre-enrollment qualification of the fish from each study group.” The study made assessments for “feeding activity, behavior, posture, and position in the water column, coloration, observation of any external lesions, morbidity, mortality, and any other abnormal clinical signs” and concluded that “AquAdvantage Salmon show no general health or behavioral abnormalities relative to comparator fish.”<sup>2</sup> However, FDA does not specify what “pre-enrollment qualification(s)” were used to select fish into the study group. In addition, they provide no data at all on any of the assessed traits. With no data and no knowledge of the criteria used to select the fish used in this study, there is no way to verify FDA’s conclusion, and no way to make any independent conclusion about the validity of such a conclusion.

#### **Food Safety Assessment**

The primary risk question for the food safety assessment was whether there were any direct and indirect effects associated with eating edible products from the GE salmon. The assessment narrowly defines direct food consumption effects as “those associated

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<sup>2</sup> Pg. 24 in FDA. 2010. VMAC Briefing Packet AquAdvantage Salmon.

with exposure to the Chinook salmon growth hormone in food from AquAdvantage Salmon.”<sup>3</sup> Thus, the direct food consumption effects are identified as alterations in levels of hormones associated with the somatotropic growth, including IGF-1 and the allergenicity of the gene expression product. The indirect effects, defined as those “that can be attributed to the rDNA construct or its gene product perturbing the physiology of the animal,” looked at were alterations in the composition of edible tissues and alterations in the endogenous allergenicity of edible tissues.

We believe that the food safety assessment too narrowly defines direct and indirect effects, uses small sample sizes, appears to manipulate data, and employs crude scientific measures to come to the conclusion that the triploid GE Salmon are as safe to eat as non-engineered salmon. We disagree with the conclusion of this assessment on the grounds that the data are too superficial and of insufficient scientific quality to warrant approval. We think that FDA should require more data, especially on the growth hormone (including IGF-1) and allergenicity and more data using more sophisticated scientific methods—such as molecular biological and biochemical techniques to analyse potential changes at the level of gene transcription and message translation DNA—on indirect effects.

### **Direct effects**

#### **Growth hormones and IGF-1**

The food safety assessment concludes that “No biologically relevant difference were detected in the levels of the gene product (the Chinook salmon growth hormone), or any endogenous metabolite or substance . . . impacted by the hormone.”<sup>4</sup> But this conclusion is based on sloppy science and deficient data.

On the growth hormone question, two different studies were evaluated and both were deficient. The first was a peer-reviewed study published in 1992 that reported on growth hormone levels in the AquAdvantage salmon.<sup>5</sup> The sample size was very small; only 5 GE salmon, 7 non-GE siblings and 5 control fish. The plasma levels of growth hormone in the GE salmon (39.9 ng/ml) was 41% higher than that of their non-GE siblings (28.2 ng/ml) control fish and 95% higher than the control fish, but neither difference was statistically significant, due to the extremely small sample size. Indeed, the sample size was so small, a doubling in growth hormone level in the GE salmon compared to control fish is not statistically significant.

Another drawback of this study is the fact that the fish evaluated were very small in size; the GE salmon averaged only 47.3 grams, while the non-GE siblings and controls averaged only 9.48 grams and 10.4 grams, respectively. Evaluating growth hormone levels in GE salmon that weigh less than 2 ounces is not the same as evaluating the levels

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<sup>3</sup> Pg. 62. Ibid

<sup>4</sup> Pg. 61. Ibid

<sup>5</sup> Du, SJ, Gong, A, Fletcher, GL, Schears, MA, King, MJ, Idler, DR and CL He. 1992. Growth enhancement in transgenic Atlantic salmon by the use of an “all fish” chimeric growth hormone gene construct. *Nature Biotechnology*, 1: 176-

in fish that have reached marketable size. People don't eat salmon that weigh 2 ounces. FDA should have dismissed this study as irrelevant to the question of the direct food consumption risk of the GE salmon, due to the small weight of the fish.

The second study looked at a number of hormones, including growth hormone and IGF-1 in GE salmon (both diploid and triploid), non-engineered counterparts raised at PEI facility and in non-salmon from another commercial fish farm. This study was a significant improvement over the 1992 study, as it looked at market size fish, used a larger sample size (30 GE salmon, 33 sponsor controls and 10 farmed controls), and looked at hormone levels in the skin and muscle rather than plasma. Unfortunately, however, the sensitivity of the test methods used was woefully inadequate. The lower limit of quantification for growth hormone in tissue (e.g. muscle and skin) was 10.4 ng/gm (see Table 15 in VMAC briefing packet). This limit of detection was so high that growth hormone was detected in *none* of the 73 samples tested. In spite of being unable to detect growth hormone in any of the fish tested, the FDA concludes, "No biologically relevant difference were detected in the levels of the gene product (the Chinook salmon growth hormone)." <sup>6</sup> This is not a scientifically valid statement. How can FDA conclude that there are no biologically relevant differences in growth hormone levels between GE and non-GE salmon when the study uses a methodology that cannot detect growth hormone in these fish? This would be like the police using a radar gun that cannot detect speeds below 120 mph and concluding that there is no "relevant difference" in the speed of cars versus bicycles.

The fish have been engineered to produce growth hormone throughout the year, rather than just for 3 months, so one would expect higher growth hormone levels in the GE salmon compared to controls. Other studies with Coho salmon engineered with a growth hormone (GH) gene found that "Plasma GH concentrations was approximately 2-fold higher in transgenic than non-transgenic salmon." <sup>7</sup> Tests do appear to exist which can detect growth hormone in fish at the 1 – 10 nanogram level. However, there appear to be no data, either in this food safety assessment or in the published literature, on the level of growth hormone in the flesh of AquAdvantage salmon. Without such basic information how can the FDA credibly maintain that this is an adequate food safety assessment? It can certainly not conclude that there is no difference between the AquAdvantage salmon and controls.

The data on IGF-1 are almost as problematic as the data on growth hormone levels. IGF-1 is a hormone that has been associated with increased risk of a number of cancers, especially prostate <sup>8</sup>, breast <sup>9</sup>, colorectal <sup>10</sup>, and lung <sup>11</sup>. The limit of quantification

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<sup>6</sup> Pg. 61 in FDA. 2010. Op Cit

<sup>7</sup> Pg. 27 in Raven, PA, Uh, M, Sakhrani, D, Beckman, BR, Cooper, K, Pinter, J, Leder, EH, Silverstein, J and RH Devlin. 2008. Endocrine effects of growth hormone overexpression in transgenic coho salmon. *General and Comparative Endocrinology*, 159: 26-37.

<sup>8</sup> Chan, J.M., Stampfer, M.J., Giovannucci, E., Gann, P.H., Ma, J., Wilkinson, P., Hennekens, C.H. and M. Pollack. 1998a. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science*, 279: 563-566.

for IGF-1 in tissue (skin and muscle) was 3.27 ng/g (see Table 15, VMAC briefing packet). Out of the 73 samples tested (30 GE salmon, 33 sponsor controls and 10 farmed controls), only 17—11 sponsor controls and 6 GE salmon—had detectable levels of IGF-1 (e.g. greater than 3.27 ng/g). Of the fish with detectable levels of IGF-1, the average IGF-1 level in the engineered fish was 40% higher than in the sponsor controls (10.26 ng/g and 7.34 ng/g, respectively) (Table 15 in VMAC briefing packet).

All 6 of the GE salmon in which there were detectable levels of IGF-1 were diploid fish, not the triploid fish that may be approved for consumption. The FDA assessment then looks more carefully at the IGF-1 data in a separate Table entitled “IGF-1 Levels in Mature Diploid Salmon” (see Table 16, VMAC briefing packet), where individual results are listed. However, Table 16 proceeds to analyze the IGF-1 data in a manner that has the effect of making it appear that there is no real increase in the IGF-1 levels in GE salmon compared to the sponsor controls. In Table 15 we see that there were 11 detectable IGF-1 values for the sponsor control fish and 6 values for the GE salmon. In Table 16, however, there are now 7 values for the sponsor controls and 7 values for the diploid GE salmon. Since there were only 6 detectable levels of IGF-1 in GE salmon, FDA decided to add one of the samples that was below the limit of quantification and then used that limit, 3.27 ng/g, as the value for the seventh sample. Adding this seventh sample to the GE salmon reduces the average IGF-1 level from 10.26 ng/g (see Table 15) to 9.26 ng/g (see Table 16). There was no reason why FDA had to add this seventh sample. Although there were detectable IGF-1 levels in 11 of the sponsor control fish, only 7 of those values are included in Table 16. It appears that FDA dropped the 4 lowest values of IGF-1 levels in sponsor control fish. Table 15 lists the minimum and maximum values for IGF-1 in sponsor control fish as 3.56 ng/g and 12.24 ng/g, respectively, while in Table 16, the minimum and maximum values were now 6.19 ng/g and 12.24 ng/g, respectively. Dropping the four lowest IGF-1 values caused the average IGF-1 level in the sponsor control fish to increase from 7.34 ng/g to 8.89 ng/g, allowing FDA to state “there did not appear to be a statistically significant difference between the mean IGF-1 level for the GE and non-GE salmon.”<sup>12</sup> Such a manipulation of data—adding a lower number for the GE salmon, thereby reducing its average level, while deleting 4 low IGF-1 levels for the non-GE salmon, thereby increasing its average level—is scientifically unsound.

In spite of saying that there did not appear to be a statistically significant difference between the mean IGF-1 levels in GE and non-GE salmon, FDA notes that the range of values for the GE salmon was more than 10% higher, and so they do a margin of exposure (MOE) assessment to show that the level of IGF-1 is not a problem. They first start by noting that IGF-1 levels are closely linked to growth hormone levels and that

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<sup>10</sup> Ma, J., Pollack, M.N., Giovannucci, E., Chan, J.M., Tao, T., Hennekens, C.H. and M.J. Stampfer. 1999. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-1 and IGF-binding protein-3. *Journal of the National Cancer Institute*, 91: 620-625.

<sup>11</sup> Yu, H., Spitz, M.R., Mistry, J., Gu, J., Hong, W.K. and X. Wu. 1999. Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. *Journal of the National Cancer Institute*, 91: 151-156.

<sup>12</sup> Pg. 69 in FDA. 2010. Op Cit

IGF-1 may pose a hazard for humans: IGF-1 “has been considered as potential hazard for human consumption following increased growth hormone levels in food producing animals,”<sup>13</sup> (a reference to the issue of IGF-1 levels in milk from cows treated with a recombinant bovine growth hormone, aka rbGH).

FDA performs a conservative MOE assessment by making various assumptions. First, they assume that the average daily consumption of non-tuna finfish is the value for the 95<sup>th</sup> percentile eater (e.g. person who eats more non-tuna finfish than 95% of the population), or 300 g. Second, they assume that all the salmon consumed has IGF-1 levels equal to the highest level found in the GE salmon, or 18.43 ng/g. Third, for “natural background” levels of IGF-1 level in humans, they look at the most sensitive population, teenage boys, and use that value. The MOE analysis shows that the MOE for the mature diploid GE salmon is 330, i.e. amount of IGF-1 in serum of teenage boys is 330 times the level of IGF-1 found in the GE salmon they might eat, while the MOE for non-engineered salmon is 508.

Given an MOE of 330, the FDA concludes that the amount of IGF-1 consumed in salmon is so low that it couldn't possibly increase serum levels of IGF-1. This is very similar to the argument that FDA made for the safety of rbGH. FDA calculated that the amount of IGF-1 consumed in milk from cows treated with rbGH is roughly 0.08% of the amount of IGF-1 created in the body every day. FDA concluded that even if all the IGF-1 survived digestion, the IGF-1 in milk couldn't increase serum levels of IGF-1. However, a paper published in 2002 shows this argument may be incorrect. A team of scientists at Brigham and Women's Hospital and Harvard Medical School in Boston, lead by Dr. Michelle Holmes, used data from a large, long-term (25 years) study of more than 1,000 nurses who record their diets carefully and who were then watched for changes in health. The study found that higher serum levels of IGF-I were found in the women who consumed the most dairy products and noted that other studies had found a link between increased dairy intake and increased serum IGF-I levels. As the study noted: “Our most consistent dietary finding was the positive association of IGF-I levels with total dairy and milk intake. . . . Two other studies have supported an effect of milk intake on IGF-I levels. A randomized trial of 204 men and women where the intervention was to encourage consumption of three servings/day of nonfat milk to affect bone remodeling found that the 101 subjects in the intervention group had a statistically significant 10% average increase in serum IGF-I levels, whereas the control group had no change in levels (Heaney et al., 1999). In addition, Ma *et al.* (2001) observed a positive association between intake of dairy food and IGF-I levels among 318 men enrolled in the Physicians' Health Study. . . . *These results raise the possibility that milk consumption could influence cancer risk by a mechanism involving IGF-I*”<sup>14</sup> *italics added.*

In an interview discussing this study, Dr. Michelle Holmes stated, “ ‘We concluded that greater milk consumption was associated with higher levels of IGF-1,’ said Holmes. *‘This association raises the possibility that diet could increase cancer risk by*

<sup>13</sup> Pp. 69-70. Ibid

<sup>14</sup> Pp. 859-860 in Holmes, M.D., Pollak, M.N., Willett, W.C., and S.E. Hankinson. 2002. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiology, Biomarkers & Prevention*, 11: 852-861.

*increasing levels of IGF-1 in the blood stream. However, more research must be done to determine whether milk consumption itself is directly linked to cancer risk'* <sup>15</sup> italics added.

In the case of milk, casein, the major protein in milk, has been found to protect IGF-1 from digestion.<sup>16</sup> The question remains whether any of the proteins in salmon muscle protect IGF-1 from digestion, and whether that might increase circulating levels of IGF-1 similar to that found for milk. More research would be needed to answer such a question.

The IGF-1 data from the second study are flawed as well. Given the insensitivity of the test for IGF-1 in muscle and skin, IGF-1 was not detected in any of the 10 farm controls and also was not detected in *any* of the triploid GE salmon. The safety assessment does not state how many of the 30 GE fish were triploids. Since it is the triploid GE salmon that are being considered for approval, we have the case that there are no data on IGF-1 levels in the GE salmon being considered for approval.

**Thus, for both growth hormone as well as IGF-1, there are no data on levels in the flesh of triploid GE salmon, because only insensitive tests were used to try and detect it. Given this lack of data on two of the identified potential hazards of this GE fish, rather than state that there are no problems, FDA should say that this study is of insufficient quality and needs to be redone using more sensitive test methods. In addition, prior to this GE salmon being approved, the company should provide data on the levels of growth hormone and IGF-1 in the muscle of triploid GE salmon that have been raised in Panama, not at the PEI facility. This is particularly important for IGF-1, a hormone linked to a number of cancers.**

## **Allergenicity**

The question of allergenicity of food derived for a GE organism can be divided into two parts: 1) the potential allergenicity of the newly expressed protein(s) in the GE food, and 2) any potential change in the endogenous allergenicity of the organism into the genetic construct was inserted, in other words, does the insertion of the growth hormone construct cause a change in the level of allergenic proteins normally found in Atlantic salmon. In the case of the GE salmon, FDA considered the potential allergenicity of the Chinook salmon growth hormone to be a direct food consumption hazard while any change to the endogenous allergenicity of Atlantic salmon is considered as an indirect food consumption hazard.

### *Potential allergenicity of Chinook growth hormone*

FDA refers to the Codex Alimentarius *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals*<sup>17</sup> and states that the three main components of testing for allergenicity of a newly expressed protein are: 1)

<sup>15</sup> At: [http://www.fass.org/FASStrack/news\\_item.asp?news\\_id=689](http://www.fass.org/FASStrack/news_item.asp?news_id=689)

<sup>16</sup> Kimura, T., Murakawa, Y., Ohno, M., Ohtani, S. and K. Higaki. 1997. Gastrointestinal absorption of recombinant human insulin-like growth factor-I in rats. *The Journal of Pharmacology and Experimental Therapeutics*, 283: 611-618.

<sup>17</sup> Available at: [http://www.codexalimentarius.net/web/standard\\_list.do?lang=en](http://www.codexalimentarius.net/web/standard_list.do?lang=en)



allergenicity of the gene source, 2) structural similarity to know allergens, and 3) resistance to proteolytic digestion.

In terms of the allergenicity of gene source, FDA notes that finfish are one of the eight major allergenic foods in the US. Since Chinook salmon is a finfish, FDA made the conservative assumption that the Chinook growth hormone was a putative salmon allergen. FDA also notes that individuals allergic to Chinook salmon would also be likely allergic to Atlantic salmon and would avoid all salmon, including AquAdvantage salmon.

For structural similarity to known allergens, FDA refers to the Codex rDNA Animal Guidelines which gives recommendations on how to compare the structure of the gene product to that of know allergens in order to evaluate potential IgE cross-reactivity. FDA did two separate structural comparisons, as suggested by Codex. First, they considered that if the product of the inserted gene had a greater than 35% identity in a segment of 80 or more amino acids, it would be considered a suspect allergen. Second, they searched stepwise contiguous identical amino acids segments as they may represent linear IgE-binding epitopes.

True food or environmental allergens trigger an IgE antibody response in an allergic individual. The focus on epitopes is a crucial one since the immune system cannot recognize the whole structure of a macromolecule, such as a protein or glycoprotein, but can only smaller sections called determinants or epitopes. The caveat to this is that the immunological behavior of an epitope can be affected by the whole structure of the macromolecule. In principal, two types of epitopes exist: linear (or continuous) epitopes based directly on the primary protein structure (e.g. amino acid sequence) and conformational (or discontinuous) epitopes based on the (3-dimensional) surface area of a molecule formed by discontinuous sections of the primary protein structure. Epitopes can be fairly small.

Searches of a couple of allergen databases revealed no amino acid sequence identities of greater than 35% in segments of 80 amino acids. For the stepwise contiguous identical amino acid segments search, FDA looked for matches of eight or more contiguous amino acids with any entries in the two allergen databases.

We believe that the use of 8 contiguous amino acids as a screen is not sensitive enough. The problem of the eight amino acid match approach (EAAM-approach) been succinctly described by Dr. Wolf-Mienhard Becker in his paper, "Sequence homology and allergen structure," written for the 2001 Joint WHO/FAO Expert Consultation on the Allergenicity of Genetically Modified Foods. Dr. Becker notes that the use of the EAAM-approach "leads to the insight that conformational epitopes and contiguous parts of these epitopes after denaturation, and epitopes made up by sugar residues, are not identifiable by this procedure. Apart from the result [that] identified linear epitopes of peanut or cod fish only consist of 6 or 4 contiguous amino acid residues which are essential for IgE binding. Thus the EAAM-approach would fail. The conclusion from

that is that the EAAM-approach even including only six contiguous amino acids can only identify potential allergenic components but not rule them out.<sup>18</sup> Indeed, both the Joint WHO/FAO Expert Consultation on the Allergenicity of Genetically Modified Foods<sup>19</sup> and the US Environmental Protection Agency's Scientific Advisory Panel<sup>20</sup> have recommended using identity of 6 or 4 identical contiguous amino acids rather than 8. **We urge FDA to require the company to redo the contiguous amino acid approach and redo the search using identity of 6 or more contiguous amino acids.**

For resistance to proteolytic digestion, FDA decided not to require such a test, reasoning that “there is no scientific rationale to suggest an altered resistance to pepsin [degradation] when the protein is expressed in Atlantic salmon rather than Chinook salmon.” However, perhaps as a result of insertional mutagenesis or some other unintended effect, there could be some form of posttranslational modification of the Chinook salmon when expressed in Atlantic salmon that could affect proteolytic digestion. FDA should have required such a test.

## **Indirect effect**

### *Endogenous Allergenicity of Atlantic salmon*

The question investigated was whether the edible tissue from GE salmon is more allergenic than non-GE Atlantic salmon. FDA has stated that a potential indirect food consumption hazard could be that the insertion of the AquaAdvantage construct could alter the endogenous levels of allergens in their GE salmon due to insertional mutagenesis. To investigate this potential increase in endogenous allergenicity, AquaBounty had two studies performed: one involving human sera from people with salmon allergies, the other looking for qualitative changes in the major salmon allergen parvalbumin (*Sal sI*). FDA seriously criticized both studies.

The human sera study involved examining the potential quantitative and qualitative changes in allergens in salmon muscle and skin from market-size diploid and triploid GE salmon and non-GE diploid salmon and was performed by IBT Reference Labs.

IBT developed an inhibition assay to determine the relative allergenic potency (RP) of the individual salmon samples/extracts based on the ImmunoCAP system. The inhibition assay involved taking soluble salmon extracts and exposing them to highly salmon-reactive IgE pooled sera from people with highly salmon-reactive IgE to see how

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<sup>18</sup> Becker, W. M. 2001. Topic 4: Sequence Homology and Allergen Structure. Working Paper Biotech 01/06 for the Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, January 22-25, 2001. Rome, Italy.

<sup>19</sup> At: <ftp://ftp.fao.org/docrep/fao/007/y0820e/y0820e00.pdf>

<sup>20</sup> SAP 2000a. Session II - Mammalian Toxicity Assessment Guidelines for Protein Plant Pesticides. FIFRA Scientific Advisory Panel Report No. 2000-03B, September 28, 2000. At: <http://www.epa.gov/scipoly/sap/meetings/2000/june/finbtmamtox.pdf>

much the salmon extracts inhibited binding of such salmon-reactive IgE. Relative allergenic potency was estimated using percent inhibition of pooled non-GE salmon controls. IBT then normalized the average RP values using the mean RP value for the sponsor control fish. The resulting normalized RP values for the control fish, diploid GE, and triploid GE fish were 1.00, 1.52 and 1.20, respectively. AquaBounty set its acceptability criteria for RP values based on FDA's Guidance for Reviewers: Potency Limits for Standardized Dust Mite and Grass Allergen Vaccines, which set limits of 0.5-2.0 RP. AquaBounty then concluded that both diploid and triploid GE fish fall within the bounds of an equivalent response vs. control fish, meaning there was no meaningful increase in allergenic potential.

FDA noted a number of problems with this study. The first problem was the very small sample size. There were only six fish in each group for a total of 18 fish, making it hard to find statistically significant differences. Another problem was that AquaBounty unblinded the identities of the samples sent to IBT for testing to facilitate the use of the control samples in further analyses. Perhaps the most serious problem was that the normalized RP values. These RP values were calculated using a pooled extract from all 6 non-GE diploid samples. Use of such a pooled extract of non-GE diploid samples confounded any direct comparison of allergenic potency of GE vs. non-GE diploid since the samples were not independent. Finally, FDA did not think the use of RP values for standardized dust mite and grass allergenic vaccine lots relevant to this food safety study.

FDA requested all the data from AquaBounty and IBT so they could do an alternative analysis. FDA did their own analysis and came up with mean allergenic potency values for each salmon extract. Their analysis found that the mean allergenic potencies of the GE diploid (3.37) and triploid (2.64) salmon were 52% higher and 20% higher than the mean allergenic potency of the non-GE diploid controls (2.21). A statistical analysis found that the 52% increase in mean allergenic potency of the GE diploid salmon compared to the non-GE control was highly statistically significant ( $p < .001$ ), while the allergenic potency of the GE triploid salmon was not statistically significant. The FDA concluded that the "triploid ABT salmon pose no additional risk than control Atlantic salmon. Insufficient data and information were available from which to draw a conclusion regarding possible additional allergenic risk posed by diploid ABT salmon."<sup>21</sup>

We strongly disagree with FDA's conclusion from these data. This human sera study, even with a very small sample size, found a highly statistically significant increase in mean allergenic potency of GE diploid salmon compared to non-GE controls. This means that the act of genetic engineering did lead to an increase in allergenic potency, at least based on this test. The fact that the increased allergenic potency of the GE triploid salmon was not statistically significant could be due to the small sample size. **Rather than say triploid GE salmon pose not additional risk compared to control Atlantic salmon, we feel that FDA should have required further study of this allergy question using larger samples sizes. To base a conclusion of no additional risk on exactly six engineered fish, when those data themselves suggest a possible problem, is not**

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<sup>21</sup> Pg. 106 in FDA 2010. Op cit

**responsible science or responsible risk assessment. FDA owes it to the thousands of Americans who are allergic to finfish to demand more data on the allergenicity of these engineered salmon from AquaBounty.**

Finally, to determine if there were any qualitative changes in the major salmon allergen parvalbumin (*Sal s1*) due to insertional mutagenesis of the growth hormone construct, the salmon extracts were analyzed using SDS-PAGE and Western blotting. AquaBounty Technologies “concluded that both ABT salmon and non-GE Atlantic salmon express one predominant isoform of parvalbumin; therefore, there is no qualitative difference between parvalbumin expressed in ABT salmon and control Atlantic salmon.”<sup>22</sup>

FDA felt that the technical flaws in this study—lack of appropriate controls, experimental conditions that preclude detection of more than one band per FSFH lane, and poor quality of the Western blots—were so serious that “no reliable conclusions can be drawn from this study regarding parvalbumin in ABT salmon vs. non-GE control.”<sup>23</sup> Rather than require another study, FDA simply conclude that triploid GE salmon pose no additional allergenic risk than control Atlantic salmon.

We are deeply concerned by FDA’s conclusions on allergenicity using the Western blot and feel that FDA should require further analysis. Western blot analysis is a relatively crude tool as it will only tell you the relative length of a protein or its 3D-structure of the protein. Western blots cannot detect posttranslational processing, such as changed glycosylation patterns. Changed posttranslational processing can dramatically change the potential allergenicity/immunogenicity of a protein. A key example of this was work done by Australian and US scientists investigating the immunogenicity of a bean  $\alpha$ -amylase inhibitor ( $\alpha$ AI) that was genetically engineered into a pea. Analysis of the amino acid sequence of the  $\alpha$ -amylase inhibitor in the bean and pea found that they had the same amino acid sequence. In spite of the same amino acid sequence, the authors “demonstrated in mice that consumption of the modified  $\alpha$ AI and not the native form predisposed to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the modified  $\alpha$ AI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these proteins. Thus, transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.”<sup>24</sup> Again, the Western blot analysis as used by ABT would not have detected such a change in this protein. The Australian authors did a more sophisticated analysis of the proteins; in addition to a Western blot they also used MALDI-TOF (Matrix-assisted laser desorption/ionization-time-of-flight) mass spectrometry which was able to detect subtle differences in the  $\alpha$ AI produced in the bean compared to the transgenic form found in the pea. Given that there was a change in the immunogenicity of the  $\alpha$ AI in these two

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<sup>22</sup> Pg. 104 Ibid

<sup>23</sup> Pg. 105 Ibid

<sup>24</sup> Pg. 9023 in Prescott, VE, Campbell, Moore, A, Mattes, J, Rothenberg, ME, Foster, PS, Higgins, TJV and SP Hogan. 2005. Transgenic expression of bean  $\alpha$ -amylase inhibitor in peas results in altered structure and immunogenicity. *J of Ag. and Food Chemistry*, 50: 9023-9030.

different legumes, and that the transgenic  $\alpha$ AI in the pea had an adjuvant effect (e.g. increased the immunogenicity of other proteins), it's possible that the movement of the Chinook growth hormone gene into salmon could possibly result in some subtle changes which could lead to increased allergenicity of the GE fish, such as observed in the GE diploid salmon. **We urge FDA to require ABT to redo the Western blot analysis and also use MALD-TOF mass spectrometry to determine if there were any subtle changes to the growth hormone gene inserted into Atlantic salmon.**

### *Composition of edible tissue*

To look for potential indirect effect associated with the AquAdvantage salmon, ABT decided to evaluate compositional differences between GE salmon and non-GE Atlantic salmon, with a look at proximate, vitamin, mineral, amino acid or fatty acid compositions of edible tissues.

One interesting part of this analysis involved various fatty acids. Although there were a number of statistically significant differences in specific fatty acids, FDA concluded that "ABT salmon are not materially different from other Atlantic salmon with respect to omega-3 and omega-6 fatty acid levels and the ratio of omega-3 to omega-6 fatty acids."<sup>25</sup> While this statement is true, it is misleading. Looking at Table 28, we see that the ratio of omega-3 to omega-6 fatty acids is 10.4 for wild caught fish, 4.1 or 3.9 for farmed salmon, and 3.6 for the ABT salmon. Note that the GE salmon had the lowest omega-3 to omega-6 ratio and that the ratio for wild fish was almost three times larger. Thus, it does appear that, in terms of the ratio of omega-3 to omega-6 fatty acids, GE salmon fare worse than wild fish and slightly worse than farmed salmon. It is important to have a balance of omega-3 and omega-6 (another essential fatty acid) in the diet. Omega-3 fatty acids help reduce inflammation, and most omega-6 fatty acids tend to promote inflammation. The typical American diet tends to contain 14 - 25 times more omega-6 fatty acids than omega-3 fatty acids. Thus, consuming foods that have a higher ratio of omega-3 to omega-6 fatty acids can help reduce inflammation.

We disagree with FDA about their narrow focus on what constitutes an indirect effect of GE. FDA considered that only alterations of endogenous allergenicity and composition of edible tissue were appropriate indirect effects to investigate. We think that there can be a range of indirect or unexpected effects that could lead to potential adverse health effects.

For example, the previously mentioned 2005 Australian study on transgenic peas found that the genetic engineering process unexpectedly turned a protein that is relatively "safe" into one that causes adverse health effects and increased the potential for adverse effects in other proteins<sup>26</sup>. A group of Australian scientists looked at the transfer of a

<sup>25</sup> Pg. 96 in FDA. 2010. Op cit

<sup>26</sup> Prescott, VE, Campbell, PM, Moore, A, Mattes, J, Rothenberg, ME, Foster, PS, Higgins, TJV and SP Hogan. 2005. Transgenic expression of bean  $\alpha$ -amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agricultural and Food Chemistry*, 53: 9023-9030.

gene from beans into peas. The gene codes for a protein,  $\alpha$ -amylase inhibitor ( $\alpha$ AI), that confers resistance to certain weevil pests. The  $\alpha$ AI in raw beans inhibits the action of amylase, an enzyme that degrades starch. So  $\alpha$ AI in raw beans can cause gastrointestinal problems in humans. When beans are cooked, the  $\alpha$ AI is easily digested and causes no problems. However, when the gene for  $\alpha$ AI was inserted into peas, the resultant protein had the same amino acid sequence as the bean  $\alpha$ AI, yet the structure of the protein had been subtly altered (through a process called post-translational processing), causing an immunological reaction in mice fed the transgenic peas, but not in mice fed normal beans. The adverse/immunological reaction to the transgenic pea  $\alpha$ AI was not mitigated by boiling the peas. The mice fed transgenic peas, in addition to developing an immunological reaction to the pea  $\alpha$ AI, also developed an immunological reaction to a number of proteins normally found in peas; mice fed these same proteins from non-engineered peas developed a far smaller immunological response, thus demonstrating that the transgenic pea  $\alpha$ AI acts as an adjuvant to increase the immunogenicity of native pea proteins.

This new study involving  $\alpha$ AI is extremely important. This study found that moving the same gene between two relatively closely related plants (common beans and peas) can result in a protein that, although it contains the exact same amino acid sequence, is relatively safe in the donor plant (common beans), but is potentially harmful in the recipient plant (peas) and can increase the potential hazardousness of other proteins found in peas. These are all clearly unintended and unexpected effects that clearly result in an adverse health effect.

A paper reviewing the food safety issues associated with genetically engineered crops listed a range of documented unintended effects and concluded that “The development and validation of new profiling methods such as DNA microarray technology, proteomics, and metabolomics for the identification and characterization of unintended effects, which may occur as a result of the genetic modification, is recommended.”<sup>27</sup>

**We urge FDA to use the newer more sophisticated molecular and biochemical methods such DNA microarray technology, proteomics, and metabolomics for the identification and characterization of indirect effects.**

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<sup>27</sup> Kuiper, HA, Kleter, GA, Notebom, HPJM and EJ Kok. 2001. Assessment of food safety issues related to genetically modified foods. *The Plant Journal*, 27(6): 503-528.