

## **Appendix 2: Notes on Briefing Packet by Center for Food Safety Staff Scientist, William Freese**

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 in at least two sections: phenotypic characterization and food and feed safety.

FDA concedes on the first page of the phenotypic characterization section that the data sets supplied by AquaBounty do not permit determination of the actual rate of abnormalities or "adverse outcomes" in GE salmon (p. 21). Similar observations are made repeatedly throughout this section. A few examples follow:

"...the high rate of removal of early-life stage fry (e.g. fry or smolts)" means that "the adult fish in the study may not reflect the nature or incidence of abnormalities of the initial population." (p. 26)

"... culling practices were not documented in the study report ... For the safety study, it is not known whether culling was comparable for all four study groups"<sup>1</sup> (p. 27)

"... there is some uncertainty regarding the likelihood or incidence of abnormalities of AquaAdvantage Salmon under commercial rearing conditions." (p. 31)

"... there was extensive culling at the ABT PEI facility which was often done on what is described as on an *ad hoc* basis .... Typically, no data were collected on fish culled as excess inventory, therefore, morbidity and malformation information are not available for these fish. If the culled eggs or fry were from crosses exhibiting high occurrences of malformations, morbidity and/or mortality .... this would tend to skew the population of the fish remaining in the facility after culling towards one with lower occurrence of these parameters. Thus, data collected on later life stages in this facility may be biased to some extent, with the bias potentially increasing with the age of the fish." (p. 33)

AquaBounty's failure to document its culling practices leaves us completely ignorant as to the true rate of abnormalities in the company's GE salmon. The incidence of abnormalities is important information, both for animal health and for food safety, as discussed further below.

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<sup>1</sup> That is, the two GE salmon and two non-GE salmon control groups. This means that all of the abnormal GE fish could have been culled and so not tested; and any abnormal non-GE control salmon left uncultured.

A related and partially overlapping problem is the small number of salmon selected for the “animal safety study,” which appears to be too small to deliver meaningful results as to the phenotypic range and health status of the much larger population of fish this study is supposed to represent.

Historical data demonstrate the existence of one to several thousand AquaBounty GE salmon and hundreds of non-GE salmon in most years from 2003 to 2007, broken down into diploid and triploid, for four groups (Table 4, p. 28). In a poorly described and largely undocumented, multi-stage selection process, AquaBounty eventually chose 48 salmon for its animal safety study to represent the much larger numbers of fish under its control.

We are first informed of a “pre-enrollment qualification” process whereby a certain unspecified number of salmon were “pre-enrolled” (selected) to form a pool of “study fish” (p. 24). We are given no information on the provenance of these study fish, what precisely qualified them or disqualified other fish for “pre-enrollment,” or even the time period over which this process took place. However, the pre-enrolled fish were apparently observed at four time points (also unspecified) and all found to exhibit normal behavior and be in good health. One naturally wonders about the size, behavioral and health status of the fish that were not selected for “pre-enrollment.” This represents a first and undocumented selection process whereby abnormal GE fish could have been excluded from further consideration.

These study fish are presumably equivalent to the 400-800 “candidate fish” referred to subsequently (p. 24). These candidate fish comprised 100-200 fish in each of four groups: diploid GE, triploid GE, diploid conventional and triploid conventional, the latter two groups as conventional comparator or control fish. It is not clear why we are given such broad ranges for the number of candidate fish in each group and overall, since precise numbers are presented later in Table 3 (645 fish overall, from 97 to 194 in each of the four groups). The fact that triploid GE salmon have the lowest incidence of malformations (10.2%) is quite surprising, given the known tendency for both genetic engineering of growth hormone constructs (Devlin et al 1995) and triploidy to induce malformations in salmon, and suggests that AquaBounty selectively culled abnormal fish from this group prior to and in the completely undocumented “pre-enrollment” phase. FDA too is concerned by this possibility, noting that AquaBounty reported a variety of different culling procedures it used, but without specifying which procedures were used with which groups or when:

“...According to ABT, in some cases, the non-GE lower-mode siblings of a cross are culled; alternatively, a predetermined number of fish are netted out and culled, or smaller or fish with irregularities are culled. ***For the safety study, it is not known whether culling was comparable for all four study groups.***” (p. 27, emphasis added).

In other words, AquaBounty used whatever culling procedures it chose to use, in some cases selectively culling fish with irregularities, and **perhaps employing different culling criteria for the four different study groups**, and did this without documentation.

Table 3 (p. 27) presents the second stage in this process: “pre-qualification selection.” Fish showing clear signs of morbidity were apparently excluded at this stage (pp. 24-25). Approximately half of the candidate fish in each group were selected. The rationale for this step in the selection process is not presented. Although AquaBounty claims that “determination of inclusion or exclusion was made by random selection” (Table 3, caption), the exclusion of all moderately deformed (rank 3) salmon from the GE diploid and GE triploid groups would be highly unlikely under random selection. None of the eight moderately deformed GE salmon present in the GE diploid (four) or GE triploid (four) pools were included. On probability grounds, this gives rise to suspicion that biased selection was perhaps practiced at this second stage of the process.

Finally, AquaBounty selected 48 salmon from the “pre-qualified” group of 325 salmon, 12 in each of four groups, with 6 males and 6 females in each group: GE diploid, GE triploid, conventional diploid, and conventional triploid (size-matched to the GE groups) (p. 24). Note from Table 3 that by this point in the selection process, there are no salmon in either of the GE pools with any malformation beyond “slight” (rank 2); these have all been culled at some prior stage of the process. Moderately and severely malformed GE salmon could have been predominant. We have no way of knowing. An additional twelve salmon – age- rather than size-matched to the GE salmon – are included as a satellite control group (p. 24), for a total of 60 fish.

These 60 fish were then subjected to a number of tests and measurements. One question that immediately arises is whether the small groups of 12 and 6 are large enough to detect with any sensitivity adverse effects of genetic engineering, the inserted growth hormone gene, or triploidy.

According to AquaBounty, the answer is no. Table 6 (p. 37) presents data on deformities in the study fish, broken down by group. Jaw erosions were identified in 4 of 12 fish in the GE diploid group, but in none of the other groups, including the three control groups. Thus, AquaBounty diploid GE salmon had a 33% incidence of jaw erosions, vs. 0% for the control groups. This is a very strong and clear difference, but according to AquaBounty, “the effect of small sample size” makes the finding uninterpretable.<sup>2</sup>

In other words, AquaBounty is implying that the animal safety study that the company itself designed has too few animals to permit a statistically significant difference to be found between a 33% incidence of a particular deformity in one treatment group (GE diploid salmon) vs. a 0% incidence of this deformity in all three control groups.

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<sup>2</sup> “The effect of small sample size has been suggested by ABT as a limitation on the interpretation of jaw erosions” (p. 40).

This of course begs the question of why AquaBounty would design a study in which a strong, clear difference between treatment (GE) and control groups is uninterpretable. The obvious answer is this: to avoid being forced to explain it as an adverse effect of the genetic engineering process or inserted genetic construct.

FDA finds a similar flaw in AquaBounty's allergenicity assessment. FDA has "notable concerns" with "the *overall study design*:" (p. 100, emphasis in original), one of which was overly small sample size. For the allergenicity assessment, AquaBounty selected only 18 salmon, six in each of three groups (GE diploid, GE triploid and conventional diploid). FDA also criticizes AquaBounty's failure to control for sex or maturity. A third criticism offered by FDA is that AquaBounty "unblinded" the identities of the 18 samples from the 18 sample, contrary to protocol. This introduced the possibility of bias in the outcomes of the fluorescent enzymatic immunoassay (FEIA) and Western blot analyses.

Despite the numerous deficiencies in AquaBounty's data and flawed study designs, including intentional use of sample sizes that are too small to deliver interpretable data, and FDA's acknowledgement of these deficiencies, FDA then proceeds to analyze the data as if it represented something real. There is no scientifically defensible justification for proceeding in this manner.

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.