Preliminary assessment of the ZKBS
of the available documents regarding an application
by the company Aqua Bounty Technologies for approval of
genetically modified salmon (brand name AquAdvantage) in the U.S.
in respect of potential environmental risks

During Federal Minister Aigner’s visit to the ZKBS, the drafting of a statement on an application for approval of genetically modified salmon in the U.S. was agreed upon in the meeting of 9 November 2010. With reference to this agreement, the Federal Ministry of Food, Agriculture and Consumer Protection sent the ZKBS a link to the documents to be reviewed by letter of 16 November 2010, asking to specifically address the environmental impact that would result from the possible release of genetically modified salmon.

The document provided does not constitute the complete application materials submitted by the applicant to the competent U.S. authority within the approval procedure. Instead, it includes an expert review by the competent U.S. regulatory authority (FDA, 2010b) assessing the genetically modified salmon requested to be approved.

The ZKBS therefore can only make a preliminary assessment of the application for approval based on the information given in the expert review by the competent U.S. regulatory authority. The ZKBS thus does not have all the data required for an environmental risk assessment. A verification of the available information and enquiries to complete the data are also not possible. Due to these shortcomings, the ZKBS is unable to conclusively assess the possibility of unintentional release of the genetically modified salmon and to fully ascertain and assess potential adverse effects on the environment. The ZKBS would also like to point out that the following preliminary assessment solely addresses potential environmental risks resulting from the approval of genetically modified salmon, but does not cover the aspect of food safety.

1. Background

The company Aqua Bounty Technologies submitted the original application for approval of genetically modified salmon to the U.S. Food and Drug Administration (FDA) as early as 1995. In the past, the FDA delayed making a decision because it was long unclear what criteria should be used to assess the safety of genetically modified animals intended for human consumption. When definitive regulatory guidelines for genetically modified animals came into force in early 2009 (FDA 2009), the FDA resumed the approval process. In August 2010, the FDA released an expert review (FDA, 2010b) concluding that AquAdvantage salmon and the food produced from it does not differ in the composition of its ingredients from conventional salmon and the products made from it and that genetically modified salmon is safe to consume. Moreover, the FDA concluded that any potential risk to wild salmon is negligible because of the intended combination of numerous safety precautions (FDA, 2010b).

To present the results of their safety assessment and receive feedback from experts, the FDA hosted a two-day public hearing with the authority’s competent advisory committee (Veterinary Medicine Advisory Committee, VMAC) (website with materials: http://www.fda.gov/Adviso-
ryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm201810.htm). In addition to speakers of the FDA, other scientific experts, representatives of the company *Aqua Bounty Technologies* as well as environmental and consumer organisations were given a chance to speak. According to the available information, the majority of the experts believed that the *AquAdvantage* salmon combined with the intended safety precautions does not pose a risk to the environment and human health (minutes of the meeting at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM230471.pdf. and http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM230469.pdf.). However, concerns were expressed about several studies being based on an insufficient body of data, which is why further investigations were recommended. The representatives of consumer organisations, in particular, expressed concerns, arguing that the genetically modified salmon had not been sufficiently examined for the risk of allergies.

2. **Atlantic salmon**

Atlantic salmon (*Salmo salar*) belongs to the genus *Salmo* in the family *Salmonidae* within the order *Salmoniformes*, which is part of the class of bony fish (*Osteichthyes*). Like all salmon, Atlantic salmon is a migratory fish with a complex life cycle. Being anadromous (freshwater-spawning) fish, sexually mature Atlantic salmon ascend from the Atlantic Ocean, where they spend most of their lives, far into European and North American rivers to spawn in the gravel beds of their headwaters. Each salmon returns to the same river in which it hatched. The eggs (roe) laid by the female salmon are fertilised by the semen (milt) which the male sprays on them. Only about 9-20% of the fertilised eggs survive the next winter, with the young fish hatching in April of the following year. The young salmon usually remain in their native rivers for two to three years before migrating downstream into the Atlantic. After spending one or several winters in the open sea, the fish become sexually mature and return to their birthplace to spawn themselves the next autumn. Unlike salmon of the genus *Oncorhynchus*, which includes Pacific salmon such as Chinook salmon, Atlantic salmon is iteroparous, meaning it is able to reproduce more than once in its lifetime. However, the migration and the spawning act are not only dangerous due to the presence of many predators, but are also extremely tiring, causing many salmon to die from exhaustion or related illnesses. The majority of Atlantic salmon dies after mating once without being able to return to the open sea. Some of the salmon survive and mate a second time, with very few being able to reproduce three or more times in their lives (FDA, 2010a).

Atlantic salmon is a very popular food fish for which there is correspondingly strong consumer demand. Commercial salmon farming became established in the 1970s, with aquaculture currently being a growing global market. Today’s largest aquaculture producers of Atlantic salmon are mainly Norway and Chile (FDA, 2010a).

3. **Genotypic and phenotypic traits of genetically modified salmon**

*AquAdvantage* salmon is genetically modified Atlantic salmon that shows accelerated growth due to the introduced modification.

The introduced recombinant DNA (rDNA) construct (opAFP-GHc2) contains the cDNA of the gene for a growth hormone (GH) from Chinook salmon (*Oncorhynchus tshawytscha*), which is consumed by humans, like all salmon. The GH-encoding cDNA is controlled by the regulatory nucleotide sequences (promoter and terminator) of an anti-frost-protein (AFP)-encoding gene
from ocean pout, *Zoarces americanus* (syn. *Macrozoarces americanus*), another edible type
of fish adapted to cold ocean regions.

The genetically modified female founder animal (E0-1 *founder*), from which the *AquAdvantage*
line was derived, was created by microinjection of the linearised opAFP-GHc2 construct into
fertilised *Salmo salar* eggs. Based on this mosaic E0-1 *founder*, two rapidly growing, genetically
modified F1 progeny were selected for further breeding. These F1 progeny had two in-
dependently segregating insertions: a functional α-form and a non-functional β-form. The
breeding of six subsequent generations (F2-F7) has led to the establishment of the *AquAdvantage*
salmon line (E0-1α), which contains a single copy (hemizygous) of the α-form of the
insert (E0-1α *locus*). No endogenous gene was interrupted by integrating the rDNA construct.
Moreover, stable inheritance of the E0-1α *locus* across several generations was confirmed.

The expression of the introduced gene for a growth hormone from Chinook salmon under the
control of the AFP promoter from *Zoarces americanus* causes the *AquAdvantage* salmon to
grow faster than the wild type of Atlantic salmon. The AFP promoter derived from a type of fish
adapted to cold sea regions enables the genetically modified salmon to produce growth hor-
mone at low water temperatures and therefore throughout the year, unlike wild-type salmon.
As a result, the genetically modified salmon reach their slaughter weight after only about 16 to
18 months. Atlantic salmon usually needs about three years to reach this weight.

4. Production conditions

The *AquAdvantage* salmon is planned to be farmed in land-based aquaculture. The production
of the fertilised eggs is to take place on Canada’s Prince Edward Island (PEI) in the Gulf of
Saint Lawrence. The fertilised eggs are then to be transported by air freight to Panama, where
the fish are to be raised in the highlands. The transport to and from the airport is carried out in
safe and labelled boxes overland by car or truck. Salmon that are ready to be slaughtered are
killed at the production facility in the Panamanian highlands, then taken to the processing busi-
nesses in Panama and ultimately shipped to the U.S. as a ready-to-serve product.

5. Assessment

5.1. Definition of risk

A risk can be defined as the product of a potential hazard and the probability of that hazard
actually occurring.

Harm to the environment caused by the escape of genetically modified animals may result
from effects at genetic, evolutionary and ecological level.

Regarding *AquAdvantage* salmon, the assessment of whether the genetically modified salmon
poses a risk to the environment focuses on the probability of the occurrence of various events
that could result in the genetically modified salmon entering the environment and on any po-
tentially related harm.

5.2. Potential risks

As shown in Fig. 1, the risk assessment is primarily concerned with the probability of the ge-
etically modified organism (GMO) accidentally entering the environment. The critical factor in
the risk assessment is the probability of the GMO becoming established in the community,
because this may have an impact on the affected resources in the accessible environment(s) due to direct or indirect effects.

Figure 1: Conceptual model of hazard identification/risk assessment (according to the National Research Council of the National Academies, NRC, 2002 and FDA, 2010b)

Against this background, the following questions are of primary importance in the risk assessment of *AquAdvantage* salmon:

1. What is the probability of the *AquAdvantage* salmon escaping from the production facilities when using the safety precautions planned by Aqua Bounty Technologies?
   - This issue is addressed in chapter 5.3.1.

2. In the event of an escape, what is the probability of the *AquAdvantage* salmon surviving and spreading in the affected environment(s)?
   - This issue is addressed in chapter 5.3.2.

3. What is the probability of the escaped *AquAdvantage* salmon reproducing and establishing itself in the affected environment(s)?
   - This issue is addressed in chapter 5.3.3.

4. What possible consequences could result from an escape of *AquAdvantage* salmon for the affected environment(s)?
   - This subject is addressed in chapter 5.4.
5.3. Containment measures

Various containment measures are taken to achieve the highest possible level of safety. The intended safety precautions are a combination of technical/mechanical, geographical/geophysical as well as biological/reproductive containment measures.

5.3.1. Technical/mechanical barriers

Several independent technical barriers have been integrated into the water systems at both the facility on PEI where the fertilised eggs are produced and the facility in the Panamanian highlands where the genetically modified fish are raised. All flow systems are thus fitted with at least three mechanical barriers.

All areas of the production facility on PEI are equipped with at least three independent types of technical containment, which includes locking the animals in covered tanks and providing redundancy in checking and filtering water outlets that may be accessible to the fish. Since genetically modified fish capable of reproduction (fish providing eggs and sperm) are kept on PEI and the geographical/geophysical conditions do not rule out the survival of any potentially released adult fish, technical/mechanical barriers constitute the only containment measures for these animals. According to the expert review by the FDA (FDA, 2010b), the Canadian Department of Fisheries and Oceans, DFO, (http://www.dfo-mpo.gc.ca/) inspected the facility as the competent government authority in 1996 and 2001, classifying it on both occasions as being “as escape-proof as can reasonably be expected”. During the current examination, the FDA requested a more recent report from the DFO. In response, the FDA reviewer was informed that the facility is now no longer monitored by the DFO, with regulatory control having been taken over by the Canadian government body Environment Canada (http://www.ec.gc.ca/).

At the Panamanian grow-out facility, the fry as well as the juvenile salmon are kept in small tanks and the older fish in large tanks until they are ready to be slaughtered. Altogether, there are at least eleven technical barriers placed in succession between the fry tanks and the local river. Additionally, nets prevent the fish from being removed from the tanks, either actively by predators or passively in the event of overflow. Whether the protective measures are sufficient in the event of tanks bursting is not entirely clear.

Overall, the probability of the genetically modified salmon escaping from the production facilities is significantly reduced throughout all stages of life by the numerous successively placed and redundant technical measures.

5.3.2. Geographical/Geophysical conditions

The environmental conditions in the geographical areas of the production facilities on PEI and in Panama provide additional containment of possibly escaping animals, because the prevailing conditions are adverse to the survival, growth and reproduction of the genetically modified salmon and/or their individual development stages.

The survival of escaped fertilised eggs, which are usually generated at the production facility in November and December, and genetically modified salmon in early development stages on PEI is limited due to the high salt content (~ 2.1 %) and the low temperatures (below 0 °C in the winter months) in the nearby river mouth or the Gulf of Saint Lawrence.

There is only unclear information available on whether the Atlantic salmon in the region of the production facility is native to PEI and capable of reproduction, so it must be assumed that any
released salmon in the region can theoretically survive. This applies particularly to the various genetically modified founder animals capable of reproduction that are kept on PEI as part of the production process of fertilised *AquAdvantage* salmon eggs.

In Panama, the survival of the salmon at any stage of life is limited due to the high temperatures (≥ 25 °C) in the lower course of the drainage area.

In summary, the geographical/geophysical conditions in the respective aquatic environments surrounding the facilities on PEI and in Panama present a considerable obstacle to the possible survival and spread of individual development stages of *AquAdvantage* salmon at other locations in the event of an escape.

5.3.3. Biological/reproductive containment

The production process is designed to produce all-female *AquAdvantage* salmon populations. To do so, androgen-induced sex reversal is used to convert female *AquAdvantage* salmon homozygous for E0-1α into so-called “neomales”. These are animals that have a female genotype and produce sperm (milt). Fertilising the roe of true wild-type females with this sperm produces all-female progeny hemizygous for E0-1α.

The *AquAdvantage* salmon eggs intended for grow-out are subjected to pressure treatment shortly after fertilisation to induce triploidy. The results of a validation study indicated 99.8% effectiveness, with the lowest effectiveness found for an individual batch of eggs being 98.9%. Routine analyses are designed to ensure that all egg batches contain at least 95% triploid eggs (FDA, 2010b). However, there is not yet any practical experience with this on an industrial scale. The induced triploidy causes the salmon to be infertile, i.e. triploid salmon are sterile and are not capable of reproduction.

The salmon populations used for production consist almost exclusively of triploid females so that any potentially escaping salmon capable of surviving in the local conditions are not able to reproduce and become permanently established in the local environment.

5.4 Possible environmental consequences of an escape of genetically modified fish

Environmental effects of the escape of genetically modified fish are generally difficult to predict because they largely depend on the fish species, the genetic modification and the receiving environment. An assessment of potential environmental damage caused by the escape of *AquAdvantage* salmon is not possible for the ZKBS because the necessary documents have not been made available to the ZKBS.

So far, no genetically modified fish have escaped into the environment, which is why the assumptions are based on studies of conventional fish species that escaped from fish farms, or on laboratory tests with genetically modified fish. Due to the complexity of aquatic ecosystems and the multitude of combinations of fish species and genetic modifications, only an example-based, incomplete description of possible environmental effects can be provided.

In general, the escape of genetically modified fish into the environment can be expected to have effects on the gene pool of a species (loss of genetic diversity), the population of a species (intraspecific competition) and a community (interspecific competition).

The three possible effects on genetic diversity that are most likely to occur as a result of escaping fish are (a) extinction, e.g. due to increased mortality of progeny or reduced fitness of a population, (b) hybridisation, which may lead to behavioural changes or reduced fitness and
(c) loss of local adaptation of populations, which may lead to reduced adaptation to environmental changes, for example (Cowx et al., 2010).

Possible effects on the population of a species, including changes in the ecosystems, are conceivable as a result of interspecific and intraspecific interactions (Cowx et al., 2010). Possible mechanisms described in scientific literature include (a) a change in predation behaviour, (b) an expansion in the prey spectrum due to increasing body size and (c) a reduced ability of non-genetically modified fish to compete with genetically modified fish. The latter may be due to factors such as increased energy expenditure in defending territories or foraging, displacement from protective structures in connection with increased predation risk as well as displacement from the accustomed habitat due to more aggressive behaviour of the genetically modified fish. The escape of genetically modified fish could also lead to changes in the habitat structure or nutrient cycle, thereby significantly affecting the aquatic environment. For example, herbivorous fish could contribute to changes in the density of aquatic plants, thereby changing the oxygen content in waters or depleting the resources of other organisms or taking away their hiding places. Increased consumption of aquatic plants could also lead to the release of additional nutrients, resulting in changes in the communities (e.g. due to increased plankton growth) or water quality.

6. Conclusion

Various safety precautions are intended to prevent damage to the environment caused by the escape of genetically modified salmon. Technical containment measures are taken in order to prevent the escape of the salmon from the production facilities. If any fish were to escape into the environment despite these measures, the geographical/geophysical conditions in the area of the production facilities are expected to hinder the survival and spread of these animals. If any escaped AquAdvantage salmon were to survive in the affected environment despite the prevailing adverse conditions, the intended biological/reproductive containment measures are expected to prevent the survival and spread of these animals.

As stated above, a risk can be defined as the product of a potential hazard and the probability of that hazard actually occurring. Although none of the presented containment measures are presumed to be 100% effective by themselves, the intended combination of safety precautions in this case provides a significantly higher level of effectiveness than the respective individual measures. This minimises the probability of potential harm caused by the persistence and propagation of the fish outside the facilities. The occurrence of significant environmental effects on a global scale – resulting from the requested approval of AquAdvantage salmon in the U.S. by the company Aqua Bounty Technologies – is considered to be highly unlikely, provided that all intended containment measures are effective. In this connection, the reliability of the operator of the facilities is deemed to be indispensable by the ZKBS. Furthermore, continuous government monitoring by the competent regulatory authorities is considered to be especially important in ensuring the safety of the facilities and the consistent implementation of the individual safety measures.

The unintentional release of genetically modified salmon due to natural disasters, human error or criminal activities ultimately cannot be ruled out. In this connection, the ZKBS would like to point out that it is currently not possible to make a general statement regarding the environmental effects that would result from an accidental escape of genetically modified salmon. Potential effects are not only affected by the quantity (number) and quality (e.g. age, development stage, percentage of fertile animals) of the escaped fish, but largely depend on the receiving environment and its prevailing, multi-factorial conditions. Whether the escaping fish
would have increased or reduced fitness in the receiving environments than wild salmon cannot be predicted specifically and in general.

References

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