Environmental risk assessment of genetically modified sterile VIRGIN® salmon for use in research trials in aquaculture sea-cages

Norwegian Scientific Committee for Food and Environment

Innspill fra molekylærgruppen

VKM concludes that the applicant has provided information demonstrating that the modified (edited) sequences resulted in intended changes at the gene expression level for the F0 VIRGIN rescued fish (parents of the VIRGIN F1) and GCF fish. However, it is important to note that the GCF fish are not offspring of the F0 VIRGIN rescued fish, i.e., the GCF fish are produced by a direct CRISPR-Cas9 mediated modification. On the contrary, the 303 F1 VIRGIN fish are produced by crossing rescued F0 VIRGIN male and female fish, i.e., they inherited a loss-of-function mutation in the *dnd* gene from their parents (F0 VIRGIN rescued fish). Therefore, the *dnd* gene expression level data provided in the application is not from fish equivalent to the 303 F1 VIRGIN fish, as stated in the application version 3.

The applicant also analysed 28 dnd-KO (loss-of-function) salmon, sectioned the gonads and found that they all lacked germ cells, and also lacked expression of the germ cell marker *vasa*. Based on the assumption that all 303 VIRGIN salmon have proven double alellic loss-of-function mutations, they all are supposedly sterile. The *dnd* genotype variants of the 28 dissected dnd-KO fish were not provided in the application. Nor is it stated whether these 28 dnd-KO fish are offspring of F0 VIRGIN rescued fish or produced via direct CRISPR knock-out.

Information on the 28 dissected dnd-KO regarding *dnd* genotype variants, *dnd* mRNA expression levels, method of production, i.e., whether they are offspring of F0 VIRGIN rescued fish or directly produced by CRISPR-Cas9 mediated modification (as the GCF), is missing. If the 28 dnd-KO fish are offspring of F0 VIRGIN rescued fish, this would greatly facilitate the risk assessment of the 303 F1 VIRGIN fish.

Further, information regarding how the different indels (Appendix 1, Excel-file) affect the reading frame of the *dnd*-gene in the 303 VIRGIN fish, as presented in the supplementary table S4 in Güralp et al (Güralp et al 2020), should also be provided. Also, a description of the exon-intron structure of *dnd* and the domains of the expressed unmodified protein and their functions is not included in the application. Such information would help to substantiate that the indels in question abolish the biological activity in *dnd* knockouts. Information on location of indels from the sequence analyses of the 303 F1 VIRGIN fish should therefore also be included.

Innspill fra ERA-gruppen

In the application, the control group is described as "We use siblings of the VIRGIN fish that do not have mutations in the dead end gene (see appendix for overview of fish)". Does this mean that the 485 control (wild-type) individuals are from the same crosses as the 303 double knockout homozygotes? If so, is it possible to compare family size among the 303 double KO fish and the 485 wt fish with the data at hand?