I. INTRODUCTION

Alphaject 2-2 emulsion for injection is a bivalent, adjuvanted inactivated bacterial and viral vaccine containing inactivated *Aeromonas salmonicida* and *Infectious pancreatic necrosis virus* (IPNv). It is intended for administration to Atlantic salmon of 39g weight (0.1ml dose) by intraperitoneal injection for prevention of furunculosis, caused by *Aeromonas salmonicida*, and reduction in clinical signs and mortality caused by IPNv.

Furunculosis was a major disease in the UK salmon industry and has been successfully treated by vaccination policies since the advent of oil adjuvanted vaccines in the early 1990s. There is a recognised problem with IPNv infection in Scotland. It causes mortality in fry and fingerlings and Atlantic salmon post smolting. It is characterised by behavioural changes (erratic swimming, sluggishness, loss of appetite), gross external and internal lesions and histopathological changes. IPN virus is a birnavirus with a worldwide distribution. The disease was first recognised in the USA in 1941 but IPN virus was not isolated until 1957. The Sp serotype has been consistently identified with serious disease outbreaks in fresh and seawater.

The recommended dosage is 0.1 ml per fish weighing a minimum of 39 g. The vaccine is intended for administration by intraperitoneal *(i.p.)* injection. The fish should be anaesthetised prior to injection. Vaccinate at water temperatures from 1 °C – 18 °C and preferably below 15 °C. The vaccination equipment should be disinfected before use. The vaccine should be left to slowly reach 15 °C – 20 °C by keeping it at room temperature and should be well shaken prior to use. To reduce the risk of adverse reactions, it is important to deposit the entire dose in the abdominal cavity. The injection needle used should have appropriate diameter, and length to penetrate the abdominal wall by 1 - 2 mm. The entire needle should be inserted into the midline about one, to one and a half pelvic fin lengths anterior to the base of the pelvic fin.

II. QUALITY ASPECTS

Product Development and Composition

Alphaject 2-2 emulsion for injection has been developed for active immunisation of atlantic salmon to prevent mortality of the disease caused by *Aeromonas salmonicida* (furunculosis) and to reduce mortality of the disease caused by IPNV (infectious pancreatic necrosis).

Active Substance

	Names of ingredients	Quantity per dose
Active substances	Inactivated *A.salmonicida* (AL2017)	RPS* ≥ 80 *relative percentage survival
	Inactivated IPNV	RP** 1.5-4.8 ** Relative potency vs reference vaccine

Other Substances

	Names of ingredients
Constituents of the adjuvant	Paraffin, light liquid
Constituents of the excipient	Macrogolglycerol hydroxystearate Sorbitan oleate

Packaging Materials

The vaccine is packaged in half litre, sterile UVO injection bags of multi layer plastic foil with ethylene vinyl acetate (EVA) as the contact layer. The giving port is closed by a rubber stopper covered with an aluminium seal with a plastic lid. The particulars of the containers and the controls performed are provided and conform to the regulation.

Manufacture of the Finished Product

The product is manufactured fully in accordance with the principle of good manufacturing practice from licensed manufacturing sites.

Production of the antigens
The antigens are cultured separately by conventional manufacturing techniques and inactivated with formaldehyde.

Blending of the final product
The finished product is prepared by mixing and standardising the content of the antigens before emulsification with the adjuvant.

Control of Starting Materials

Starting materials of non-biological origin used in production comply with the relevant Ph. Eur. Monographs or in-house specifications.

Biological starting materials used are in compliance with the relevant Ph. Eur. monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the Ph. Eur., any deviation was adequately justified.

The master and working seeds have been produced according to the seed Lot System as described in the relevant guideline.

Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

Finished Product Quality Control

The results of the analysis of various batches have demonstrated that a product can be produced which consistently meets the agreed specification.

Stability of the Product

Active substance
Data have been provided to demonstrate that before blending, antigen bulk may safely be stored for the relevant time period under refrigerated conditions

Finished Product
The company provided data on three commercial scale batches and on pilot batches. These data showed that the product was stable for 12 months at temperatures between and 2 °C and 8 °C.

In-Use

It is intended that once opened, the product will all be used during the same day. This effectively equates to an in-use shelf life of 8 hours. Data presented by the company have indicated that the product will be stable for this length of time in use.

CONCLUSIONS ON QUALITY

The data provided by the company included satisfactory descriptions of the production and quality control procedures. The in-process and finished product tests ensure an efficacious, safe and consistent product. The stability data provided show that a shelf life of 1 year and in-use shelf life of 8 hours is justified for the product when stored between 2 °C and 8 °C, protected from light.

III. SAFETY ASPECTS

The Applicant has presented short and long term safety in Atlantic salmon using a batch of maximum antigen content. Data is provided to comply with the Ph.Eur. monograph for Furunculosis with three batches at double maximum dose in at least 50 fish.. The use of the vaccine in fish intended for broodstock has been contra-indicated as no suitable reproductive safety data have been presented. The SPC warnings are adequately supported.

Laboratory Tests

A GLP safety study included three ALPHA JECT 2-2 batches having the maximum limit of potency. For each batch 52 fish of less than the recommended minimum weight were vaccinated with a double dose. No abnormal behaviour or mortality was recorded in the observation period of 21 days post vaccination, and the side effects were recorded to be acceptable for the three batches. The study concluded that ALPHA JECT 2-2 containing maximum potency is safe to use for fish with less than minimum weight.

Additional supportive GLP studies have also been submitted.

Reproductive performance:
No investigation of effect on reproductive performance was conducted. This is acceptable since the SPC carries the warning.

Field Trials

The applicant has conducted field studies to examine the safety of one dose of ALPHA JECT 2-2 and to investigate efficacy. Atlantic salmon with documented history of no disease were included in the study. The test group consisted of 600 fish and the control group of 1000 fish. They were vaccinated with 0.1 ml of ALPHA JECT 2-2 or 0.1 ml saline (controls) Fish were individually weighed at vaccination and growth and side effects were evaluated 3, 6 and 12 months after vaccination. Mortality was recorded daily.
Fish were released to seawater approximately 16 weeks post vaccination. During the first 21 days after vaccination no vaccinates died. The cumulative mortality both over the freshwater and over the seawater period was low for vaccinated and controls. There was little difference between groups or cages. The side effects were acceptable at all 3 time points. Some long term reduction in weight gain in vaccinated fish could be seen.
This study supported the long term safety over 12 months of a batch of vaccine of maximum antigen content for *A.salmonicida* and medium range for IPNv in fish of minimum recommended weight at vaccination.

CONCLUSIONS ON SAFETY AND RESIDUES

Conclusions on User Safety
A user risk assessment indicates that the main risk for the people administering the product is the possibility of accidentally injecting themselves. The possible outcome of injecting a product containing mineral oil is well documented and a detailed warning on the appropriate action to take is included in the summary of product characteristics and on the information provided with the product.

Conclusions on Consumer Safety
None of the ingredients of the Alpha Ject 2-2 emulsion for injection are such as would cause unacceptable residues in treated fishes. There is no need for a withdrawal period and no consumer safety concerns.

Conclusions on Environmental Safety
The Applicant has conducted a phase I assessment and concluded that there is no significant risk to the environment.

V. EFFICACY ASPECTS

The Applicant has provided data to support an onset of immunity of 600 degree days from vaccination. The protection against *Aeromonas salmonicida* lasts for at least 12 months post vaccination. Protection against IPNv has been demonstrated for up to two and a half months.

The SPC claim "Prevent mortality of the disease caused by *Aeromonas salmonicida* (furunculosis) and reduce mortality of the disease caused by IPNv (infectious pancreatic necrosis) in Atlantic salmon of a minimum weight of 39 g" is fully supported by the data provided.

Laboratory Tests

The efficacy of the product has been demonstrated in laboratory studies in accordance with the relevant requirements which supported the short term efficacy with regard to prevention of mortality caused by *Aeromonas salmonicida.* In the study, one test group of 200 and two test groups of 70 Atlantic salmon were vaccinated with 3 batches of the product and a control group of 200 fish were vaccinated with saline. Five weeks post vaccination 100 of the 200 fish group and 35 of the 70 fish groups were challenged with *Aeromonas salmonicida*. The challenge was conducted in a separate tank for the group containing 100 fish. Repeat challenge of the two groups of 35 fish was conducted 7 weeks post vaccination, due to low control mortality in the first challenge. All dead fish in the vaccinated groups were examined and 15 % of the dead fish in the control group. The RPS values were above Ph. Eur. requirement for all three batches.
The test addresses Ph. Eur. compliance.

Onset of immunity

Aeromonas salmonicida
Another study was conducted in Atlantic salmon of less than the minimum recommended weight, free of disease and unvaccinated. Two groups of 500 fish were vaccinated with 0.1ml ALPHA JECT 2-2 and 1500 with 0.1 ml saline as control. Six weeks after vaccination 100 fish from each of the test groups and the control group were challenged with *Aeromonas salmonicida* and divided into duplicate tanks. The challenge was followed by 21 days observation. All dead vaccinated fish and 30 % of dead controls were examined for bacteriology. *Aeromonas salmonicida* was isolated from all examined dead fish post challenge. There were no significant differences between the two groups over 21 days. The study supports the protection against *Aeromonas salmonicida* challenge 6 weeks post vaccination in fish of less than the minimum recommended weight. The study supports Ph. Eur. compliance and establishes an onset of immunity of 504 degree days.

IPNV

In this study Atlantic salmon parr with less than the recommended minimum weight was vaccinated intraperitoneally with 0.1 ml of six different vaccine batches of ALPHA JECT 2-2. A control group was injected with 0.1 ml saline.

After vaccination the fish were smoltified. Bath challenge was conducted 10 weeks after vaccination, at approx. 10ºC. Mortality was recorded daily from the day of challenge to termination of the experiment 60 days post challenge. The mortality was confirmed to be IPNv specific by agglutination.

The results in this study show that ALPHA JECT 2-2 vaccines containing IPNv antigens within the specification of the vaccine induce specific protection in vaccinated Atlantic salmon following an IPNv challenge. The results also show that the onset of immunity is 596 degree days for the fish vaccinated with IPNv in this study.

Duration of immunity

Aeromonas salmonicida
This study was conducted to evaluate the efficacy against *Aeromonas salmonicida* in Atlantic salmon (*Salmo salar*) 6 and 12 months after vaccination with ALPHA JECT 2-2. The fish were of less than the recommended minimum weight at vaccination and free from IPNv and antibodies to *Aeromonas salmonicida.* Three groups were used, two vaccinated with different batches of ALPHA JECT 2-2 and one with saline. Fish were vaccinated intraperitoneally with 0.1ml vaccine. The fish were challenged with *Aeromonas salmonicida* at 6 months and 12 months post vaccination. 35 fish were challenged for each group at 6 months and approximately 100 fish in each group at 12 months. All dead vaccinated fish and 30 % of the dead control fish were examined bacteriologically. Blood was sampled from 10 fish prior to vaccination and from 10 fish per group 3, 6 and 12 months after vaccination. Sera were tested for antibodies to *Aeromonas salmonicida*. RPS values for both batches of vaccine at 6 months and 12 months showed that both batches gave full protection against the disease caused by *Aeromonas salmonicida*. Antibody levels increased up to 12 months post vaccination. The study concluded that ALPHA JECT 2-2 with minimum antigen content induced protection against furunculosis when challenged at 6 and 12 months post vaccination. This study could not demonstrate duration of protection for the IPNv component due to difficulties in challenging larger fish with IPNv.

IPNv
Duration of protection has been demonstrated up to two and a half months in trials performed with a vaccine containing IPNv and additional antigens to those found in ALPHA JECT 2-2.

Field Trials

Efficacy is presented in 3 field trials:

In the first study healthy Atlantic salmon with documented history of no disease were selected at the time of vaccination. The fish had been kept in the hatchery since hatching and were acclimatised before vaccination. The test group consisted of 600 fish and the control group of 1000 fish. At vaccination the fish had the recommended minimum weight. They were vaccinated with 0.1 ml of ALPHA JECT 2-2 or 0.1 ml saline (controls). Mortality was recorded daily per tank during the freshwater stage and every other day in the seawater stage. Fish were evaluated 3, 6 and 12 months post vaccination. The cumulative mortality both over the freshwater period and over the seawater period was low for the vaccinated and control fish. There was no significant difference between groups or cages. At the end of the trial, 12 months post vaccination, there was a deficit of fish which was considered to be due to attacks by cormorants.
The fish were free of antibodies against *Aeromonas salmonicida* prior to vaccination. Antibody levels increased up to 12 months post vaccination. No antibody response was seen in the control group until 12 months post vaccination. The antibody level found in the control group 12 months post vaccination is attributed to contact with *Aeromonas salmonicida*. This study supported the efficacy of the product.

The second study included healthy Atlantic salmon with a documented history of no disease. The test group consisted of 300 fish and the control group of 500 fish. At vaccination the average weight was less than the minimum recommended weight. They were vaccinated with 0.1 ml of ALPHA JECT 2-2 or 0.1 ml saline (controls).

The fish were distributed equally in two tanks. Mortality was recorded daily per tank and every 2nd day when in seawater. Fish were evaluated 3, 6 and 12 months post vaccination. The cumulative mortality over the freshwater period was low. The total cumulative mortality in the seawater period was somewhat higher but, there was little difference between groups or cages. The higher mortality in the seawater period is associated with attacks by cormorants.
Fish were free from antibodies to *Aeromonas salmonicida* prior to vaccination. Antibody titres were detectable at 3 months post vaccination and high at 6 months post vaccination whereas controls were still negative. At 12 months post vaccination the antibody titres were still at a level indicating good protection.

A third study provides a field survey of the efficacy of ALPHA JECT 2-2 post-vaccination of Atlantic salmon (*Salmo salar*) under UK field conditions. Data from 4 farming sites are presented to demonstrate that the vaccine reduces mortality in Atlantic salmon attributable to IPN. This report includes a population of approximately 3.7 million fish, 2.2 million of which were vaccinated with ALPHA JECT 2-2. Efficacy was evaluated as mortality of fish vaccinated with this vaccine compared with fish vaccinated with a negative control vaccine (ALPHA JECT 1200). At some sites efficacy was evaluated against historical data. In all cases fish were vaccinated with the recommended dose of the respective products i.e. 0.1 ml of ALPHA JECT 2-2 and 0.2 ml for ALPHA JECT 1200.

At site one no IPN mortality occurred. The site was only 700 meters from Site two.

Site two, suffered heavy post-transfer mortality and had a prolonged clinical IPN outbreak. The mortality in the ALPHA JECT 2-2 vaccinated fish was significantly lower than for ALPHA JECT 1200 vaccinated. It was not possible to accurately differentiate post-transfer mortality from IPN mortality.

At site three and four, year class of 2004 vaccinated with ALPHA JECT 1200 and year class of 2006 vaccinated with ALPHA JECT 2-2 were compared. At these sites mortality in the 2006 generation of fish vaccinated with ALPHA JECT 2-2 was very much lower than mortality in the previous generation that were stocked in 2004 and vaccinated with ALPHA JECT 1200.

CONCLUSIONS ON EFFICACY ASPECTS

The Applicant has provided data to support an onset of immunity of 600 degree days (504 and 596 days respectively for *Aeromonas salmonicida* and IPNv). The protection against *Aeromonas salmonicida* lasts for at least 12 months post vaccination. Protection against IPNv has been demonstrated for up to two and a half months.
The SPC claim “Prevent mortality of the disease caused by *Aeromonas salmonicida* (furunculosis) and reduce mortality of the disease caused by IPNv (infectious pancreatic necrosis) in Atlantic salmon of a minimum weight of 39 g” is fully supported by the data provided.

PART V. OVERALL CONCLUSION ON THE PRODUCT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the benefit risk profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Product Information Database of the Veterinary Medicines Directorate website.

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The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

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