ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

United Kingdom
Veterinary Medicines Directorate
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NATIONAL PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Winvil 3 Micro Emulsion for Injection for Atlantic Salmon

Date created: 6th October 2014

MODULE 1

PRODUCT SUMMARY

Name, strength and pharmaceutical form	Winvil 3 Micro Emulsion for Injection for Atlantic Salmon
Applicant	Elanco Europe Ltd Lilly House Priestley Road Basingstoke Hampshire RG24 9NL
Active substance(s)	Inactivated *Aeromonas salmonicida* subspecies *salmonicida* Inactivated *Moritella viscosa* Infectious pancreatic necrosis virus (IPNV) Serotype A_2
ATC Vetcode	QI10AL0
Target species	Atlantic salmon (*Salmo salar*)
Indication for use	For the active immunisation of Atlantic salmon *(Salmo salar)* to reduce mortality due to infection with *Aeromonas salmonicida* (furunculosis). The onset of immunity to *A. salmonicida* occurs at 446 degree days (mean water temperature °C multiplied by the number of holding days) following vaccination. For the active immunisation of Atlantic salmon to reduce mortality due to infection with Infectious Pancreatic Necrosis Virus (IPNV). The onset of immunity to IPNV occurs at 625 degree days following vaccination. For the active immunisation of Atlantic salmon to reduce mortality due to infection with *M. viscosa* (winter ulcer disease). The onset of immunity to *M. viscosa* occurs at 286 degree days following vaccination. The duration of immunity to *A. salmonicida, M. viscosa* and IPNV is not known. This is a Provisional Marketing Authorisation. A full set of supporting efficacy data is not available for this product. In particular, protection against specific clinical signs such as

	ulceration associated with *M. viscosa* has not been demonstrated. Until 2,000 dd (approximately 5 months) after vaccination, fish vaccinated with this product may develop abdominal lesions that range from slight to major, and pigmentation of the viscera and fillet/abdominal wall that may range from none to moderate. Visceral granulomas may occur in < 1% of vaccinated fish.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Veterinary Medicines Directorate website (www.vmd.defra.gov.uk)

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Exceptional provisional application in accordance with the Veterinary Medicines Regulations.

I. SCIENTIFIC OVERVIEW

Winvil 3 micro emulsion for injection is a trivalent viral and bacterial inactivated oil-adjuvanted vaccine for use in Atlantic salmon. The viral component is against Infectious Pancreatic Necrosis (IPNV) and the bacterial components are against *Aeromonas salmonicida* and *Moritella viscosa*.

Winvil 3 has a Provisional Marketing Authorisation (PMA) as a full set of efficacy data are not available for this product. This type of Marketing Authorisation is usually applied for and issued in order to address an urgent situation e.g. new disease where there is no fully authorised product in the UK to prevent the condition. Due to the nature of the use of such drugs, a full dossier meeting all requirements for the marketing of the drug is not required, provided that the safety, quality and efficacy of the product can be supported by available data, and a favourable benefit/risk profile is determined. There is a requirement for Periodic Safety Update Reports (PSURs) to be submitted for annual reassessment of this product. Moreover, results of any additional studies must be submitted to the Veterinary Medicines Directorate (VMD).

Whilst other products are available in the UK containing the IPNV and *Aeromonas* components there are no vaccines for the treatment of winter sore disease, for which *M. viscosa* is considered the principal aetiological agent. Therefore the application for a PMA is justified.

The product is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market. It has been shown that the product can be safely used in the target species; the slight reactions observed are indicated in the SPC[1].

The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC. The efficacy of the product was demonstrated according to the claims made in the SPC. The overall benefit/risk analysis is in favour of granting a marketing authorisation.

[1] SPC – Summary of Product Characteristics

II. QUALITY ASPECTS

A. Composition

The product contains inactivated *Aeromonas salmonicida* subspecies *salmonicida*, inactivated *Moritella viscosa* and inactivated Infectious Pancreatic Necrosis Virus (IPNV) Serotype A_2 as active substances. The excipients are mineral oil, polyoxyethylene sorbitan monooleate, sorbitan sesquioleate, phosphate buffered saline and residual formaldehyde.

The container/closure system consists of a 500 ml single tube intravenous bag with a plastic screw cap closure and a plastic clamp off device together with a fluid transfer tubing. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the adjuvant, vaccine strain and inactivating agent are justified. The inactivation process and the detection limit of the control of inactivation are correctly validated. The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site. IPNV antigen is developed by preparing and inoculating Chinook Salmon Embryo (CHSE) cell cultures and incubating the cells until signs of the disease are evident. The virus is then harvested and inactivated by adding formalin (formaldehyde solution), then the solution is homogenised and concentrated. The *Aeromonas salmonicida* component is produced by growing the cultures in tryptic soy broth (TSB) which are then used to form an inoculum and production cultures for fermentation. The fermented cells are then harvested and inactivated using formalin prior to concentration. The *Moritella viscosa* component is produced by growing the cultures in Vibrio + 2% NaCl broth production medium. A fermenter is inoculated with the seed culture and following incubation the production culture is harvested and inactivated using formalin.

The adjuvant is prepared by mixing the mineral oil with sorbitan sesquiolate which is subsequently sterilised. The final formulation is made by mixing the IPNV antigen with the sterile phosphate buffered saline (PBS) before adding part of the sterilised polyoxyethylene sorbitan monooleate to form a homogenous solution. The *Aeromonas salmonicida* antigen is then added to a mixture of the *Moritella viscosa* antigen and the remaining polyoxyethylene sorbitan monooleate, again forming a homogenous solution. The IPNV solution is then slowly added to the prepared antigen and subsequently the bacterin solution is added and the final solution is thoroughly mixed. The solution is emulsified and filled in the final containers under aseptic conditions. Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

The active substances are inactivated *Aeromonas salmonicida*, inactivated *Moritella viscosa* and inactivated IPNV, of which only *Aeromonas salmonicida* is described in a pharmacopoeia. In-house specifications have been provided for each of the active substances. The active substances are manufactured in accordance with the principles of good manufacturing practice.

Biological starting materials used are in compliance with the relevant European Pharmacopoeia (Ph. Eur.), Monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the relevant guidelines; 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.

All excipients are manufactured in accordance with their respective monographs. Certificates of analysis have been provided.

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

Scientific data and certificates of suitability issued by the EDQM have been provided and 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.

E. Control tests during production

The tests performed during production are described and the results of 4 consecutive pilot scale batches, conforming to the specifications, are provided.

F. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements; any deviation from these requirements is justified. The tests included are for identification and potency of the active substances, residual formaldehyde, fill volume, emulsion viscosity and conductivity, miscibility, safety, specific gravity and sterility.

The demonstration of the batch to batch consistency is based on the results of 4 consecutive batches produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

G. Stability

Very limited stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions. This is acceptable for a PMA and a provisional shelf life of 12 months has been determined.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

Shelf life

- Shelf life of the finished product as packaged for sale: 12 months.
- Shelf life after first opening the immediate packaging: use within 10 hours of opening, remaining vaccine should be discarded at end of use.

Special precautions for storage

- Store and transport refrigerated (2°C - 8°C).
- Do not freeze.
- Keep in the original container to protect from light.

III. SAFETY ASSESSMENT

Winvil micro 3 is a water-in-oil emulsion containing formalin inactivated cultures of *Aeromonas salmonicida* (strain As27), *Moritella viscosa* (strain Vvi1), and Infectious Pancreatic Necrosis Virus (IPNV). The *Aeromonas salmonicida* and IPNV antigens and adjuvants are already contained in the authorised vaccine Birnagen Forte.

The product is recommended for use in Atlantic salmon from 43.7 g against furunculosis, winter ulcer disease and IPN. The onset of immunity to *A. salmonicida* occurs at 446 degree days, at 625 degree days for IPNV and at 286 degree days for *M. viscosa.* The duration of immunity is not known.

The product has a provisional marketing authorisation therefore limited safety data were assessed. The batches used for testing contained inactivated *Aeromonas salmonicida* (strain As27) RPS_{END} ≥80%[2], inactivated *Moritella viscosa* (strain Vvi1) RPS_{END} ≥83% and inactivated Infectious Pancreatic Necrosis Virus (IPNV) RP ≥2.0[3] ELISA.

Laboratory trials

The safety of the administration of Winvil 3 Micro in the target animal is demonstrated in safety studies. Two studies were conducted to evaluate the safety of this vaccine in the target animal species: one study was a controlled laboratory study to evaluate safety following a double dose of vaccine; and the other was a field study to evaluate safety following a single dose of vaccine.

The safety of a double dose of vaccine was evaluated in healthy salmon. Either a double dose of vaccine containing maximum antigen input or a saline control was administered as a 0.1 ml dose via intraperitoneal (IP) injection under general anaesthesia. Fish were monitored at least once daily for 21 days at 10°C. A minimum of 10 fish from each group were euthanised and examined for

[2] RPS – Relative Percent Survival
[3] RP - A unit of Relative Potency compared to a reference vaccine

evidence of local or systemic. There were no mortalities following vaccination and no abnormal local or systemic reactions were observed during examination. In the group that received the test product abnormal swimming behaviour and a loss of appetite were noted.

A single dose safety study was conducted at one site under field conditions. Healthy salmon received one of three treatments: either 0.05 ml of the test vaccine (containing maximum antigen input), 0.1 ml oil adjuvant only (negative control) -or 0.1 ml of Birnagen Forte (positive control) all via IP injection under general anaesthesia. Half of the vaccinated fish were sampled at 1500 degree days post-vaccination and the remaining fish at 2000 degree days post-vaccination. Internal adhesions were scored using the Speilberg scoring system. Four months after vaccination the adhesion scores were found to be low for fish vaccinated with the test product, however longer term safety is unknown. Higher adhesion scores were seen in fish vaccinated with the positive control product.

The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and standard procedures were applied albeit not GLP. It was concluded that the short term safety of the product is acceptable based on the data submitted. The following warnings are included on the SPC:

- Fish may take up to 11 days to return to normal feeding. Side effects following intra-peritoneal administration of oil-adjuvanted vaccines may occur within several months following seawater transfer.
- Fish vaccinated with this product may present visceral adhesions within the peritoneal cavity, melanin pigmentation in parietal abdominal muscle or in rare cases granulomas among the viscera. Adhesions may be minor, connect various organs to the abdominal wall and leave an opaque peritoneum after being removed in up to 22% of vaccinated fish. Parietal melanin pigmentation may occur in <1 % of vaccinated fish and be moderately extensive yet readily removed and contain small areas that penetrate beyond the muscle surface. Visceral granulomas may occur in <1 % of vaccinated fish.

As the vaccine is not intended to be administered on more than one occasion, studies to assess the repeat administration of one dose were not performed and this is accepted. No investigation of effect on reproductive performance was conducted therefore the following warning will be included on the SPC ‘Do not use in fish selected for broodstock’.

There are no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny therefore a specific study was not carried out. Relevant warnings and precautions are included on the SPC and product literature.

The vaccine is inactivated and thus the specific tests to be performed for live vaccines are not applicable. All the components of the vaccine are either of biological origin or are allowable excipients according to Table 1 of the Annex to Commission Regulation (EU) No 37/2010 which indicates no MRLs are required or are considered out of scope (Regulation (EC) No 470/2009) when used as a veterinary medicinal product. Based on this information a withdrawal period of zero degree days is accepted.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

- No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.

Field studies

A field study was conducted for Winvil 3 Micro Emulsion for Injection for Atlantic Salmon. However this was limited in terms of the duration of the study as it did not cover the entire Atlantic Salmon production cycle. This is acceptable for a PMA. A further long-term study was conducted post-authorisation.

User Safety

Appropriate studies were approved:

Ensure that the method of restraint, handling and administration, e.g. by the use of guarded needles, minimises the risk of accidental injection/self-injection.

To the user:
This product contains mineral oil. Accidental injection/self-injection may result in severe pain and swelling, particularly if injected into a joint or finger, and in rare cases could result in the loss of the affected finger if prompt medical attention is not given.

If you are accidentally injected with this product, seek prompt medical advice even if only a very small amount is injected and take the package leaflet with you.
If pain persists for more than 12 hours after medical examination, seek medical advice again. Personal protection like gloves and guarded needles should be used when handling the veterinary product.

To the physician:
This product contains mineral oil. Even if small amounts have been injected, accidental injection with this product can cause intense swelling, which may, for example, result in ischaemic necrosis and even the loss of a digit. Expert, PROMPT, surgical attention is required and may necessitate early incision and irrigation of the injected area, especially where there is involvement of finger pulp or tendon.

Ecotoxicity

The applicant provided a Phase I environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required. The assessment concluded that as the vaccine is indicated for parenteral administration, at a rate of 0.05 ml per fish on one occasion, there is minimal risk of environmental contamination. The components of the vaccine are either of biological origin, inert or commonly used chemical excipients present at very low concentrations. It is highly unlikely that large amount of the product will be disposed of. It was concluded that the risk to the environment is negligible and precautions listed on the product literature are adequate to ensure safety to the environment when the product is used as directed.

IV CLINICAL ASSESSMENT (EFFICACY)

Clinical Studies

Winvil 3 has a PMA and therefore a full set of efficacy data are not available for this product. The vaccine can be accepted as a PMA due to the lack of available vaccines against winter ulcer disease in salmon and the economic importance and welfare implications of this disease.

Winvil micro 3 is a water-in-oil emulsion containing formalin-inactivated cultures of *Aeromonas salmonicida* (strain As27), *Moritella viscosa* (strain Vvi1), and Infectious pancreatic necrosis virus (IPNV).

Laboratory Trials

The efficacy of the product has been demonstrated in laboratory studies. Studies demonstrating the onset of immunity for each of the pathogens have been presented. No data on the duration of immunity are available.

Aeromonas salmonicida

A study was conducted in accordance with the Ph. Eur. monograph for inactivated oil-adjuvanted furunculosis vaccines to confirm the onset of immunity for *A. salmonicida* using the test vaccine at minimum antigen input. Two hundred fish free from *A. salmonicida* were used. The fish were divided into two groups and received either 0.05 ml of vaccine or 0.05 ml of saline administered via IP injection under general anaesthesia. After 420 – 446 degree days post-vaccination the fish were challenged with, live challenge organism, via IP injection.

Fish were divided into two tanks, each containing 50 vaccinates and 50 controls. Fish were monitored for 21 days post-challenge, tanks were inspected for mortalities and all dead fish were necropsied, with kidneys cultured on Brain Heart Infusion (BHI) agar plates. According to the monograph the relative percent survival (RPS_{60}) following challenge in vaccinated fish must be ≥80%, when 60% of control fish have died as a result of infection with the challenge organism.

The results of the study showed that vaccinate mortality was no greater than 20% following testing with 4 batches of the vaccine. Control mortality was no less than 72%. For the vaccine batch with the lowest As27 input (205 µg/dose),

and challenge given 446 degree days after vaccination, the vaccinate mortality was 18% and the control was 81%. This resulted in a RPS_{60} of 87.5%. The lowest RPS_{60} calculated across the 4 batches was 80.0%.

It was concluded that the test vaccine fulfilled the efficacy requirement of the monograph. Following challenge with *A. salmonicida* at 420 – 446 degree days post-vaccination a good RPS_{60}, of >80%, was demonstrated. As only mortality was assessed this will form the basis of the indication and the onset of immunity is determined as 446 degree days.

Moritella viscosa
A study to determine the onset of immunity for *M. viscosa* using vaccine at minimum antigen input was provided. Two hundred fish free from *M. viscosa* were used. The fish were divided into two groups and received either 0.05 ml of vaccine or 0.05 ml of saline administered via IP injection under general anaesthesia. After 280 - 329 degree days post-vaccination the fish were challenged with live challenge organism via IP injection.

Fish were divided into four tanks, each tank had 25 vaccinates and 25 control animals. Fish were monitored for 21 days post-challenge, tanks were inspected for mortalities and all dead fish were necropsied. Any fish with a pale or haemorrhaged liver, splenomegaly and/or kidney necrosis were recorded as specific losses. Efficacy of the vaccine could be proven if ≤20% of vaccinates died and a RPS_{END} of 71.4% is achieved; 70-98% must have died due to specific mortality.

The results showed that vaccinate mortality was never >14% of the 4 batches tested whilst control mortality was >70% in all cases. The fish given the vaccine with the lowest antigen input (6.0×10^5), received challenge 286 dd post-vaccination, had a vaccinate mortality of 10% whilst the control mortality was 70%. The RPS was calculated to be 85.7%. The lowest RPS calculated from the 4 batches of vaccine trialled was 80%.
It was concluded that the test vaccine fulfilled the pre-defined efficacy requirement. Following challenge with *M. viscosa* at 280 – 329 degree days post-vaccination a good RPS_{END}, of >83%, was demonstrated. As only mortality was assessed this will form the basis of the indication and the onset of immunity is determined as 286 degree days.

Infectious Pancreatic Necrosis Virus
A study to determine the onset of immunity for IPNV using vaccine at minimum antigen input was provided. In total 1250 fish free from IPNV were used. One thousand fish were divided into 5 groups; 4 groups received 0.05 ml of vaccine and the fifth group was given 0.05 ml of saline. Vaccine or saline was administered via IP injection. After 625 degree days post-vaccination the fish were challenged by means of cohabitational infection. Trojan fish, from the same stock as the vaccinated and control fish, had been infected with live IPNV and were introduced to the tanks.

Fish were divided into two tanks, containing both vaccinated and control animals. Fish were monitored for 36 days post-challenge, tanks were inspected for mortalities and a representative selection of dead fish was necropsied, gross pathological signs were recorded. Head kidney samples were tested by ELISA

and some by PCR, to confirm if the IPN virus was present. Efficacy of the vaccine was determined by a statistically significant difference in survival in favor of the vaccinates compared to the controls.

The results showed that the percent mortality for vaccinated fish in one tank was <50% and in the second tank was 55.1 – 68.8%, whilst for controls the total mortality was 64% and 82.7% respectively. From the data the PRS_{60} was calculated for each of the 4 vaccinate groups for both tanks. For the first tank the RPS_{60} was 32.7 – 40.1% and for the second tank 47.9 – 64.7%.

Statistical analysis was performed to compare the control and vaccinate survival rates. The analysis demonstrated a statistically significant higher survival rate ($P<0.01$) for the vaccinated group compared to the control group.

It was concluded that there was a statistically significant decrease in mortality following IPNV challenge in fish vaccinated with the test product compared to controls. As only mortality was assessed this will form the basis of the indication and the onset of immunity is determined as 625 degree days.

Field Trials

A field study was conducted for Winvil 3 Micro Emulsion for Injection for Atlantic Salmon. However this was limited in terms of the duration of the study as it did not cover the entire Atlantic Salmon production cycle. This is acceptable for a PMA. A further long-term study was conducted post-authorisation.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

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.

MODULE 4

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.

(www.gov.uk/check-animal-medicine-licensed)

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.

(www.gov.uk/check-animal-medicine-licensed)