

**UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
THE CENTER FOR VETERINARY BIOLOGICS**

Environmental Assessment

For Issuance of a Permit for Distribution and Sale of an Imported Infectious Hematopoietic Necrosis Virus Vaccine, DNA

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Prepared by:
United States Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
The Center for Veterinary Biologics

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I. Proposed Action

APHIS is considering granting a permit for distribution and sale in the United States of an Infectious Hematopoietic Necrosis Virus Vaccine, DNA, for use in healthy salmonids weighing at least 30 grams (1.058 ounce). Novartis Animal Health US, Inc., Larchwood, Iowa, has requested authorization to provide the vaccine as an aid in the prevention of disease caused by infectious hematopoietic necrosis virus (IHNV) in salmon and trout.

Under the provisions of the Virus-Serum-Toxin Act of 1913, as amended in 1985, the USDA must ensure that veterinary biologics are pure, safe, potent, and efficacious and not worthless, contaminated, dangerous, or harmful. Biological products produced in other countries may be imported into the United States for research and evaluation, transit shipment, or general sale and distribution, in accordance with Title 9, Code of Federal Regulations, Part 104. Products imported under a Permit for Distribution and Sale must meet the same requirements as those required for licensed (domestically produced) products. Accordingly, APHIS has conducted a risk analysis of this product and has concluded that the safety risks to animals, public health, and the environment are low. A copy of the firm's risk analysis with confidential business information redacted is available online and by request, as indicated in the *Federal Register* Notice announcing the proposed issuance of a permit. The APHIS risk analysis covers the firm's document, but also includes review of other studies and tests conducted by the firm, the confirmatory testing completed by APHIS personnel on the Master Seed to confirm the vaccine's purity, identity, and expression of immunogenic protein, and results of literary and regulatory review regarding this and similar previously tested or licensed products.

II. Background

IHNV causes an acute rhabdovirus infection of salmonids resulting in high mortality among fry and fingerlings (young or small fish) at water temperatures less than 12°C (53.6°F). IHNV is not considered to be pathogenic for people. In this vaccine, only the glycoprotein (G) gene of IHNV is present, expressing the surface protein of the virus envelope. It is not associated with virulence and does not encode a toxin, enzyme, or allergen.

The confirmatory testing at the Center for Veterinary Biologics (CVB) laboratory for the Master Seed (MS) bacterial host containing the recombinant plasmid was satisfactory. CVB laboratory characterization of the MS included identity testing, sequence analysis, gene expression, and purity testing.

The vaccine made from the plasmid DNA is licensed in Canada. Aqua Health Ltd., a division of Novartis Animal Health Canada, Inc., developed the vaccine (trade name

APEX-IHN™). It has been commercially available in Canada since 2005 and has been used in field trials there since 2003.

The imported IHNV vaccine to be used under permit for distribution and sale is a replication-incompetent plasmid which has been genetically modified to contain an immunogenic gene. The route of administration for this vaccine is intramuscular, given by trained personnel in the area immediately anterior and lateral to the dorsal fin.

Plasmid DNA has been used previously to express foreign genes for vaccine development. Examples of USDA-licensed plasmid DNA vaccines include West Nile Virus Vaccine licensed in 2005, and Canine Melanoma Vaccine licensed in 2006. No safety issues have arisen from the use of these plasmid DNA vaccines.

III. Need for the Proposed Action

There are no infectious hematopoietic necrosis virus vaccines approved for use in fish. A replication-incompetent DNA vaccine can potentially be both safe and efficacious, inducing a protective immune response (Kurath, *et al.* 2006; Garver, *et al.* 2005).

IV. Areas of Concerns

The three areas of concern to APHIS are: 1) animal safety, 2) public health, and 3) environmental safety. APHIS has conducted its own risk analysis to assess whether risks are associated with the proposal to issue a permit for this imported vaccine. The safety characteristics of the vaccine have been thoroughly evaluated. The conclusions derived from the risk analysis for each of the areas of concern are summarized below.

A. Animal Safety

The risk to animals is low.

- In a contained safety study conducted in Maine, nine hundred salmon weighing between 10 and 20 grams were vaccinated. Six buckets of 25 fish each were netted and placed into each of 6 holding tanks. After a 31-day acclimation period, the 150 fish from each tank were anaesthetized and randomly assigned to be injected intramuscularly with either saline control or one of two serials. The salmon were observed for 21 days after vaccination. There were no adverse events attributable to vaccination.
- There is a long history of safe use in salmonids in Canada. The firm reports that from 2003 to 2010, nearly 53 million fish in Canada were vaccinated without significant adverse effects.
- The vaccine is not a risk for target or non-target species because it cannot replicate in eukaryotic cells, is not infectious, and is highly purified. Reversion to virulence and shedding are not safety concerns applicable to this DNA vaccine. Should inadvertent ingestion occur, the DNA is readily degraded without negative effect to the host or non-target species.

- Regarding any potential for integration, it has been estimated that the probability of a DNA molecule to integrate into the chromosome of muscle cells after intramuscular injection is at least 3000x less than the natural mutation rate (Martin, *et al.* 1999; Ledwith, *et al.* 2000).
- In persistence studies conducted by the firm, within fourteen days following a 2x dose vaccination, no plasmid DNA was detected in major tissues. The tissues tested included the liver, spleen, head, kidney, gonad, and gut lumen. In muscle tissue at the site of injection, the copy number of plasmid (1.8×10^{12}) decreased greatly so that by day 251, only 0.01% of the plasmid remained. A 1x dose resulted in a 2.5-fold less copy number at day 252, or a 2.0×10^{-9} % reduction in copy number, a negligible amount.

B. Public Health

The risk to public health is likewise low.

- The plasmid has no virulence-associated genes, and no known mobility or integration motifs which would allow integration into the human genome.
- Only qualified personnel, knowledgeable in the art of inoculation and prevention of accidental exposure, will be administering the DNA vaccine. Training, experience, and the use of finger guards attached to the repeating syringe greatly decrease the probability of human exposure.
- Although the safety of this specific vaccine has not been evaluated in humans and is therefore unknown, accidental human injection is not expected to cause adverse effects. Many doses of various DNA vaccines using a plasmid vector have been administered to animals, including dogs, mice, guinea pigs, and horses (Grosenbaugh, *et al.* 2011; Martin, *et al.* 1999; Ledwith, *et al.* 2000; Manam, *et al.* 2000; Davis, *et al.* 2001). No significant adverse events have been reported for animals receiving those vaccines or the humans administering them. Regarding this IHNV vaccine, there was one case of human self-injection in 2009 which resulted in no negative impact to the patient.
- Numerous DNA vaccines have entered human clinical trials (Liu & Ulmer, 2005). There has been no evidence of autoimmunity, immunological tolerance, or integration in these various trials. The dose for fish (10 µg) of this vaccine is much lower than the dose of similar plasmid DNA vaccines used in human clinical trials (10x-30x dose) with no adverse events.
- Minute quantities of plasmid DNA in muscle tissue at the injection site were detected in the firm's persistence studies, out to day 728 post-vaccination, past the time of marketing. However, it is known that plasmid DNA is rapidly degraded in the stomach by gastric acid, and in the duodenum by pancreatic and bile secretions (Maturin & Curtiss, 1977) and represents an infinitesimal amount relative to salmon genomic DNA that is routinely consumed.

C. Environmental Safety

The risks to the environment are low.

- The risks to environmental safety are low, given that the vaccine is based on highly purified and well-characterized plasmid DNA that does not replicate in vaccinated animals.
- The vaccine, which has no adjuvant, is administered to salmonids at the hatchery in artificial tank-based rearing systems.
- In the unlikely event the vaccine is released in the environment, the plasmid will not be maintained without kanamycin selection pressure, an antibiotic not used in agricultural or human health applications. The plasmid construct cannot readily infect bacteria without prior lab-based chemical or physical treatment of the bacteria to generate cells competent to take up plasmids.
- The plasmid is unstable in the environment and exposure to nucleases, pH variations, oxidation, and/or other denaturing conditions will result in degradation of the DNA.

The information above provides support to the claims made regarding the safety of the IHNV vaccine to animals, public health, and the environment. There are no apparent substantial issues with adverse environmental impacts concerning use of this vaccine.

V. Alternatives

Two alternatives were considered. The only alternative considered other than the preferred action alternative is not to approve the proposed permit for distribution and sale, the “no action” alternative. We have considered the applicants’ goals in light of the agency’s public interest and responsibilities and any potential environmental impact. Based upon the results of our risk analysis and the potential applications for this vaccine in disease control, APHIS adopts the alternative that the proposed permit be approved.

VI. Conclusion

Based upon the risk analysis documented in this EA, APHIS has determined that implementation of the proposal would not significantly affect the quality of the human environment and that the preparation of an Environmental Impact Statement is not required (Finding of No Significant Impact).

References

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**Proposed Issuance of a Permit for Distribution and Sale of an Imported
Infectious Hematopoietic Necrosis Virus Vaccine, DNA**

Novartis Animal Health US, Inc.

February 2014

**Environmental Assessment and
Finding of No Significant Impact**

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has considered the environmental effects associated with a proposal to issue a permit for distribution and sale of an imported Infectious Hematopoietic Necrosis Virus Vaccine, DNA, for use in salmonids. Aqua Health Ltd., a division of Novartis Animal Health Canada, Inc., developed the vaccine (trade name APEX-IHN™) which is licensed for use in Canada. The Permittee for receipt, sale, and distribution in the United States is Novartis Animal Health US, Inc. We have analyzed the potential impacts on animal safety, public health, and environmental safety and we have prepared an Environmental Assessment and conducted a risk assessment that presents the conclusion of our analysis. As a result, APHIS has determined that implementation of the proposal would not significantly affect the quality of the human environment and that the preparation of an Environmental Impact Statement is not required (**Finding of No Significant Impact**).

Recommendations:

- (1) The Environmental Assessment has been provided to the public via publication of a Notice in the *Federal Register*. Risk should be communicated to the public when identified.
- (2) Any adverse or unexpected results should be communicated to the proper authorities immediately.


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Date