Environmental Assessment
for Field Testing
Mannheimia Haemolytica - Pasteurella Multocida Vaccine, Avirulent Live Culture

I. Proposed Action

APHIS is considering granting authorization to ship an unlicensed Mannheimia Haemolytica – Pasteurella Multocida Vaccine, Avirulent Live Culture, for field safety testing. Schering-Plough Animal Health Corporation, Elkhorn, Nebraska, has requested authorization to conduct field tests under conditions of husbandry that are typical of the commercial cattle industry in the United States.

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. Accordingly, APHIS has conducted a risk analysis and has concluded that the safety risks to animals, public health, and the environment are low. A copy of the risk analysis with confidential business information removed is available upon request.

II. Background

Bovine respiratory disease (BRD) in the form of 'shipping fever' (SF) pneumonia is a significant cause of morbidity (approaching 35%) and mortality (5-10%) in North American cattle feedlots, resulting in major economic loss to the industry.[1] Although a number of factors and multiple pathogens are involved in the disease complex, *M. haemolytica* is the primary microorganism isolated from SF calves with pneumonia. *P. multocida* is less frequently isolated but is an important and common contributor to disease.

To prevent SF, it is important to reduce stressors that contribute to its development, such as prolonged transport, poor nutrition, excessive heat, and crowding. Vaccination against the agents involved is recommended at 2-3 weeks before transport and can be repeated on arrival at the feedlot. Vaccines for the SF bacterial pathogens generally include bacterins and subunits (toxoids). A live vaccine can potentially improve protection against disease by stimulating both cell-mediated and humoral immune response in vaccinated animals.

The vaccine under consideration consists of two genetically modified live bacterial strains. The *M. haemolytica* strain is characterized as biotype A, serotype 1 (A1). It was derived from a field isolate that was attenuated by gene deletion. The *P. multocida* strain is biotype A, serotype 3 (A3) and was also derived from a field isolate and attenuated by deletion.

The resulting avirulent live vaccine is for use in healthy cattle as an aid in the prevention and/or reduction of pneumonic lesions associated with bovine pneumonic pasteurellosis, commonly known as shipping fever. The proposed field safety test will be conducted in

at least three different geographical locations according to instructions on the product circular. The potential for escape and dispersal of the experimental vaccine from the proposed release sites is low. The personnel who will conduct the study are experienced in cattle health management.

III. Need for the Proposed Action

This experimental vaccine represents an attempt by Schering-Plough Animal Health Corp. to produce an efficacious and safe vaccine against SF that protects cattle with one dose administered subcutaneously.

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 a risk analysis to assess whether risks are associated with the proposal to field test this experimental vaccine. The safety characteristics of this 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. The two strains were safety tested separately and then together in a final product formulation.

Regarding the *M. haemolytica* deletant strain:

1. It was found to be safe in calves following subcutaneous or intranasal administration of a single dose. For the recommended subcutaneous route, there was no shedding, no dissemination from the injection site, and no spreading to nonvaccinated calves. Calves vaccinated intranasally, however, can shed the organism and spread it to other calves by nose-to-nose contact. The intranasal route will not be used in the field trials.

2. It was found to be stable genetically and phenotypically at Master Seed (MS)+20 *in vitro* passages and MS+5 *in vivo* passes in calves following intranasal administration.

3. Virulence of the deletant strain in non-target animals (sheep, mice) was much reduced compared to that of the parent strain. Safety following intranasal administration to sheep, the most susceptible non-target species, was tested. The organism was not detected in the sheep 14 days after inoculation and no evidence of clinical disease due to the deletant strain was observed.

Regarding the *P. multocida* deletant strain:

1. It was found to be safe for use in calves following subcutaneous administration of a single typical field dose.

2. When given at higher doses (not to be used in the proposed field trials), it caused some undesirable adverse reactions such as fever, lethargy, rapid breathing, and large injection site reactions. The calves recovered rapidly from the clinical signs without treatment but still had visible injection site reactions 21 days after vaccination. There was no detectable shedding or spread to other susceptible calves. At 7 and 21 days, the strain was isolated from the injection site and pre-scapular lymph nodes (not the blood, nasal passages, or lung).

3. In the host animal reversion-to-virulence study, the deletant strain was given intranasally rather than subcutaneously, to optimize potential replication. Minimal lung lesions were observed in the first and last passages only. Such lesions did not occur with the recommended subcutaneous route (see 2. above). The deletant strain given intranasally was not isolated from lung tissue in any of the calves.

4. The deletant strain was found to be genetically and phenotypically stable at MS+5 *in vivo* and MS+20 *in vitro* passage levels, as determined by PCR analysis of the deletion and by phenotypic testing.

5. The deletant strain is susceptible to commonly used antibiotics for cattle.

6. Safety of the deletant strain following intranasal administration to sheep, rats, rabbits, and chickens was demonstrated by the absence of clinical signs and no isolation of the *P. multocida* deletant strain. These species are known to be susceptible to *P. multocida*.

7. Intraperitoneal administration of the strain to mice to determine the LD_{50} indicated that the deletant strain was 400 times less virulent in mice than the parent strain.

B. Public Health

The risk to public health is low. There are no indications that special safety measures should be taken to conduct this study. Human exposure will be limited to the qualified personnel administering the vaccine and the people handling the cattle.

Regarding the *M. haemolytica* A1 deletant strain, its host range is essentially restricted to ruminants. Accidental human exposure, e.g. by injection, is not expected to cause adverse effects.

Regarding the *P. multocida* A3 deletant strain, cattle isolates of *P. multocida* are not generally implicated in human infections. Moreover, this attenuated strain is not pathogenic to otherwise susceptible non-target animals and does not contain any genetic material not already present in the environment. Accidental human injection is not expected to cause adverse effects.

Although the safety of this experimental vaccine containing the two attenuated strains has not been specifically evaluated in humans, and is therefore unknown, no safety hazards to the public health are expected.

C. Environmental Safety

The risks to the environment are low. Both parental bacteria are present in the environment.

Regarding the *M. haemolytica* deletant strain:

1. *M. haemolytica* wildtype strains have wide distribution and are readily found in ruminants. The deletant strain has reduced virulence, no plasmids or mobile chromosomal elements, and is expected to be much less of an environmental threat than the naturally occurring field strains.

2. The stability of the deletant strain at various temperatures and conditions was determined to be similar to that of the parent strain.

3. The deletant strain was found to be cleared from the site of injection within 2 weeks following vaccination and is more sensitive to a wider array of antibiotics than current circulating field isolates.

Regarding the *P. multocida* deletant strain:

1. *P. multocida* is a commensal organism of the respiratory and digestive tract of many domestic and wild animals, and is common to the nasopharynx of healthy cattle.

2. The survival of the deletant strain was found to be similar to the parental organism, which is not normally a long period of time in the natural environment. At cooler temperatures and following drying of the reconstituted vaccine, there is some survival.

Regarding the dual-fraction vaccine containing both attenuated strains:

1. There are no apparent substantial issues with adverse environmental impacts concerning this vaccine. The potential for escape and dispersal of the experimental vaccine is very low because the rate of shedding of the vaccine in both target and susceptible non-target species is very low. Also, its ability to survive in adverse conditions of temperature and drying is limited.

2. The firm reported that for calves vaccinated subcutaneously with the dual-fraction vaccine using single dose, 10-fold overdose, or 2-dose at 21-day interval regimens, transient elevations in rectal temperatures and local reactions at the injection site were observed. They believed this to be consistent with administration of a Gram-negative bacterial vaccine. The extent of these reactions can be better determined in the proposed field trials.

V. Alternatives

Two alternatives were considered. The only alternative considered, other than the preferred action alternative, is not to approve the proposed field tests, 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 field tests be approved.

VI. Conclusion

Based upon the risk analysis documented in this Environmental Assessment, 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

1. Baker, J.C. 1998. Respiratory Diseases of Cattle. pp. 1068-1080. In: The Merck Veterinary Manual, Eighth Edition. S.E. Aiello, (Ed), Merck & Co., Inc., Philadelphia.