

Environmental Assessment

I. Proposed Action

APHIS is considering granting authorization to ship an unlicensed Marek's Disease - Newcastle Disease Vaccine, Serotypes 2 and 3, Live Virus, Live Marek's Disease Vector, for field testing. The vaccine is for the vaccination of chickens as an aid in the prevention of Newcastle disease and Marek's disease. Biomune Company, Inc., has requested authorization to conduct field trials to test the safety of this vaccine under conditions of husbandry that are typically employed in the commercial chicken industry in the US. If no substantial effects are observed on animal, human, or environmental factors, APHIS will consider licensure of this product without additional environmental documentation.

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

Infection of poultry by Newcastle disease virus can result in inapparent infections to disease with high mortality depending on the pathotype of the virus and age and susceptibility of the infected birds. All strains of Newcastle disease virus, however, are of the same serotype. Marek's disease infection of poultry may result in acute disease characterized by formation of lymphoid tumors in organs and high mortality, or a more chronic form of the disease typically resulting in paralysis due to lymphocyte accumulation in peripheral nerves.

The experimental vaccine being considered for use in the proposed field tests is a conventional veterinary biological product (a live, nonpathogenic serotype 2 Marek's disease vaccine) in combination with a live, nonpathogenic serotype 3 Marek's disease virus (herpesvirus of turkeys) which has been genetically modified to express antigens from Newcastle disease virus. Proposed locations for the field tests are commercial poultry houses in Arkansas, California, Delaware, Georgia, Nebraska, Pennsylvania, or Texas, USA. Up to 700,000 chickens maintained in poultry houses under normal husbandry conditions will be vaccinated subcutaneously at one day of age, or *in ovo* at 18 days of incubation, and monitored for adverse reactions or events.

III. Need for the Proposed Action

Newcastle disease (ND) and Marek's disease (MD) infections in poultry result in considerable economic losses to the industry in both morbidity and mortality of birds and condemnation of carcasses intended for food use. An effective and safe vaccine against these disease agents may have wide application under field conditions in the United States, and will reduce handling and stress of poultry by reducing the number of separate vaccinations required to provide protection against these diseases. Similar, separately licensed vaccines for these diseases have been

demonstrated to be safe and effective. It is expected that the data from these monitored field trials will confirm the safety of this vaccine for use in poultry and the environment in the US.

IV. Areas of Concern

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 in the United States. 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 poultry is low. Live virus vaccines for these diseases are licensed and widely used in the industry and have been demonstrated to be safe and effective.

The risk to non-target animal species is low. The known host range of the vaccine agents is limited to certain avian species.

(b)(4) (b)(4) have demonstrated that the vaccine is safe in these non-target animal species. Additionally, typical practices for use of the vaccine in chickens would normally preclude exposure of non-target species.

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 people in direct contact with the vaccinated chickens. The safety of this experimental vaccine in humans has not been evaluated, and is therefore unknown; however, no safety hazards to the public health are expected since the vaccine has been used safely in preliminary experiments in confined environments in the laboratory. Newcastle disease virus is known to cause occasional cases of mild conjunctivitis in occupationally exposed humans (certain poultry workers and laboratory personnel) exposed to significant quantities of live virus. This product consists of only selected DNA regions coding for ND antigens and not live ND virus, and is therefore expected to be safe. No adverse reactions were reported by laboratory personnel working with this vaccine in preliminary experiments, and these studies demonstrated that the vaccine agents are no more virulent than the naturally occurring wild type isolates.

C. Environmental Safety

The risk to the environment is low. No evidence of reversion to virulence or changes in genetic or phenotypic stability of the vaccine agents were found in serial backpassage studies in the laboratory. In experiments under confined laboratory conditions, the vaccine agents were not

observed to spread to non-inoculated chickens housed with vaccinated chickens. The vaccine agents were tested for their ability to survive in environmental material (poultry house litter) and could not be recovered more than 4 hours from the time of inoculation of the material. There are no expected adverse ecological events associated with the 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 field tests, the "no action" (denial, request more data) alternative. We have considered the applicants' goals in light of the agency's public interest and responsibilities and any potential environmental impact. The preliminary studies forming the basis for this decision have been reviewed, and the conclusions therein found to be reasonable. Previous approval of requests to test similar formulations have not resulted in environmental insult or adverse events. 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 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).

I. Introduction

A. Objective

1. Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live Marek's Disease Vector (unlicensed) was constructed from the agents listed below in the Research and Development Department, Biomune Company, Lenexa, KS. The recombinant will be combined with a conventional Marek's disease virus (MDV) serotype 2, SB1 vaccine strain resulting in Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed). If approved, the final vaccine will be made, tested and manufactured in the Manufacturing Department, Biomune Company, Lenexa, KS, U.S., Veterinary License No. 368. Biomune Company has level 2 animal containment facilities available for testing of live vectors.
2. The Regulated Biological Agent (RBA), designated rHVT/NDV, contains a turkey herpesvirus (HVT) backbone or vector component and expresses the (b)(4) gene of Newcastle disease virus (NDV).

B. Proposal

1. Species: Chickens, 18-day-old embryonating eggs and one-day-old or older
2. Proposed claim: Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed) is recommended for use in healthy one-day-old chicks or in 18 day-old embryonating eggs as an aid in the prevention of Newcastle disease and Marek's disease caused by very virulent Marek's disease
3. Geographic area: United States (all states)
4. Route of administration: *in ovo* to 18-day-old embryonating eggs and subcutaneous to one-day-old chicks
5. Brief description of the expected safety profile: The NDV (b)(4) will be placed (b)(4) in the HVT genome. Attenuation of the HVT vaccine strain has not been observed. Administration of the rHVT/NDV will cause a transient viremia in the chick that does not shed to contact chickens: Hatchability problems are not expected when administered *in ovo* and no adverse tissue reaction is expected at the site of inoculation when administered subcutaneously to one-day-old chicks.

Field safety studies conducted by Biomune Company, with the cooperation of four major broiler operations in distinct geographic areas of the U.S., demonstrated that the Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed), was safe for use under typical management conditions of the U.S. poultry industry. Two serials of vaccine were evaluated in a total of forty four thousand four hundred and seventy two (44,472) embryos by *in ovo* administration in 18 to 19 day-old embryos. These same two serials were evaluated in a total of thirty seven thousand six hundred (37,600) chickens at day of age by the SQ route. There were no adverse reactions in any of the vaccinated birds. Hatchability, overall performance and mortality were equivalent to that of the control group and normal farm management expectations. Results of these field safety studies demonstrated that the vaccine was safe for *in ovo* administration in 18 to 19 day-old embryos or SQ administration in day of age chicks.

II. Description of the Regulated Biological Agent Construction

A. The Backbone Biological Agent

1. Turkey herpesvirus is a double-stranded DNA virus in the *Herpesviridae* family and *Alphaherpesvirinae* subfamily. The genus is un-named but is referred to as Marek's disease-like viruses that are classified as MDV non-oncogenic, serotype 3. The parent strain has been used commercially to vaccinate chickens against Marek's disease since 1972 (Calnek and Witter, 1997). Turkey herpesvirus is the non-oncogenic, serotype 3 of MDV, which is classified in the Biosafety Level 1 category.
2. Physical Characteristics of the Backbone Agent

A flow diagram is provided in Figures 1 through 6.

(b)(4)

(b)(4)

(b)(4)

B. Donor Biological Agents and Donor DNA or Genes

1. The description of each donor biological agent is shown in Figures 2 through 6. (b)(4)

(b)(4)

- a. The NDV strain is (b)(4), which is a lentogenic strain (Nagai et al., 1980). The nucleotide sequences of the *HN*, *P*, *M* and *F* genes have been reported (Sato et al., 1987a and Sato et al., 1987b). (b)(4)

(b)(4)

(b)(4) Two of these vaccines were constructed in fowl poxvirus vectors (Newcastle Disease-Fowl Pox Vaccine, Live Fowl Pox Vector) and the third was constructed in an HVT vector (Marek's Disease-Newcastle Disease Vaccine, Serotypes 1 & 3, Live Marek's Disease Vector). (b)(4)

(b)(4)

(b)(4)

b.

(b)(4)

C. Construction and Characterization of the RBA

1. Diagrams for the construction of the RBA are provided as described below.

- a. The HVT (Backbone Biological Agent) genome is shown in Figure 1.

- b. (b)(4)
Figure 2.
- c. Plasmids used for construction of the RBA are described in Figures 2 through 6.
- d. (b)(4)
(b)(4) were used as the host cell for recombination of the homology plasmid (b)(4) (Figure 6).
- e. After transformation, virus successfully growing in (b)(4) were expanded in Recombinant Virus. (b)(4) virus was passed (b)(4) times to expand (b)(4)
2. Gene insertion was characterized by genetic detection described in II.C.3.a-c. (b)(4)
3. Physical Characterization of the Regulated Biological Agent
- a. (b)(4) are provided in Figure 6 as well as The (b)(4) product contains (b)(4) contains (b)(4) PCR product contains (b)(4) The sequence of these (b)(4) products was analyzed.
- b. (b)(4)
(b)(4) In one (b)(4) blot, a probe designed to bind to the *F* gene anneals to the 3.6-kb *SfiI-XbaI* (b)(4) In the second (b)(4)
- c. The two (b)(4) and the (b)(4) described in Figure 6 were used to test (b)(4)

- d. An electronic file providing the (b)(4) is provided.
4. Because the (b)(4) does not provide any new virulence factors to the (b)(4) is biologically the same as the parent virus, the recommended NIH/CDC biosafety level will be the same as the parent virus (BL1).
5. (b)(4) are the (b)(4) and the (b)(4) addition sites. (b)(4) and the enhancer region of the (b)(4) Vectors, are considered to be Biosafety Level 1 pathogens.

III. Biological Properties or Virulence for the Regulated Biological Agent used for Master Seed

A. Phenotypic Characteristics

1. The (b)(4) between (b)(4) is thought to be non-essential for HVT replication since recombinant virus replicates similar to the HVT parent strain *in vitro* and *in vivo*.
2. The RBA expresses the (b)(4), as demonstrated by a (b)(4). Briefly, (b)(4) After viral (b)(4) were visualized, the monolayer was fixed. The (b)(4) was incubated on the cell monolayer followed by incubation with (b)(4). Then, freshly prepared (b)(4) complexes from the (b)(4) were incubated on the monolayer. (b)(4) were observed after development with the (b)(4) which creates a (b)(4).
- (b)(4) was performed to detect the expression of the (b)(4) Antigen fo (b)(4) was prepared from a monolayer of (b)(4). The infected monolayers were harvested and centrifuged to (b)(4). The (b)(4)

antiserum was used to detect (b) 4 in the (b) 4
The (b) 4 band was observed at (b) 4 (See "Master Seed Virus
Testing of Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live
Marek's Disease Vector," which was approved by APHIS on September 3,
2003.)

B. Virulence Characteristics

1. There are no known virulence characteristics of the (b)(4) addition of the (b) 4 is not expected to be virulent for chickens.
2. The RBA does not contain any genetic elements or toxin genes that are known to be inherently virulent.

C. Virulence in Target and Non-Target Animals

- 1.

(b) 4

(b) 4

(b) 4

- 2.

(b) 4

(b) 4

(b) 4

D. Tissue Tropism

1. The tissue tropism of (b) 4 was determined and compared to the HVT parent strain.

a. Briefly, chickens were vaccinated with a (b) 4

(b) 4

(b) 4 Similarly, (b) 4 these same tissues at these time points. Based on these results, (b) 4

(b) 4

(b) 4

b. Safety was demonstrated in other (b) 4

(b) 4

2. Turkey herpesvirus is ubiquitous in domestic turkeys and is also ubiquitous in commercial chickens due to vaccine use since the 1970s. From the literature, HVT replicates in turkeys, but is non-oncogenic (b) 4 The host range of HVT has been defined by experimental infections of various birds, but has been better defined in tissue culture. Turkey herpesvirus is known to replicate in primary chicken, duck and quail cells as well as quail cell lines (b) 4

(b) 4

(b) 4

(b) 4

Also, investigators trying to replicate HVT in mammalian primary cultures and cell lines were unable to detect evidence of virus replication even after giving six to 10 blind passages (b) 4

(b) 4

E. Potential for Horizontal Gene Transfer

1. The chance of *in vivo* recombination between (b) 4
strains and other viruses was considered. (b) 4

(b) 4, recombination events with viruses (b) 4
(b) 4 in the cell cytoplasm are low due to the different locations of genetic material and the different types of genetic material. The chance of an (b) 4, but a remote possibility due to the (b) 4

2. The chance of an (b) 4 event between (b) 4 and (b) 4 virus are unknown because the occurrence of horizontal gene transfer between the various MDV serotypes is not known. Common vaccination practices in the United States involve the mixture of HVT (serotype 3), SB1 (serotype 2), and Rispens (serotype 1) vaccines. To date, no recombination events between any of these vaccines or with field MDV viruses (serotype 1) have been reported. One case of experimentally produced MDV serotype 2 recombination with MDV serotype 1 was reported but was not repeatable (b) 4. The chance of an *in vivo* recombination event is unknown, but a remote possibility due to the similar replication cycle of HVT and other MDV serotypes. No known physical and/or chemical factors are known to enhance the dispersal of rHVT/NDV in the environment.

F. Shed/Spread

1. Safety of vaccine transmission of rHVT/NDV from vaccinated chickens to non-vaccinated, contact chickens was evaluated by (1) transmission to non-inoculated, contact chickens when transmission was evaluated for the presence of HVT in the WBCs of contact controls and (2) comparing transmission of the above recombinant with the HVT parent strain.

(b) 4

neither adverse vaccine reactions nor clinical signs of MD or ND were observed. At various times during the three week period, vaccinates and non-vaccinated, contact controls were bled and WBCs were purified for virus isolation on CEF. At all time points, virus was isolated from the vaccinates, while virus was not isolated from the non-vaccinated, contact controls. Similar results were obtained in the HVT parent group. It was concluded that neither rHVT/NDV nor the HVT parent strain was transmissible. Therefore, rHVT/NDV is safe for use in chickens and poses no safety risk. These results are described in report # 304, entitled "Shed/Spread of Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live Marek's Disease Vector," which was approved by APHIS on September 3, 2003.

2. The transmissibility of the recombinant is thought to be similar to the (b) 4 (b) 4 parent strain. Reports in the literature for (b) 4 demonstrate that transmissibility of HVT is limited from chicken to chicken due to the limited virus present in the feather follicle epithelium (Cho, 1975; Zygraich and Huygelen, 1972).

G. Environmental Impact or Survivability

To establish environmental safety, rHVT/NDV was evaluated in the laboratory by determining the survival of rHVT/NDV in sterile, saturated shavings and sterile, saturated swabs at different temperatures and comparing its survivability to the HVT parent strain. Briefly, when virus in shavings was incubated at $25\pm 3^{\circ}\text{C}$, both rHVT/NDV and the HVT parent strain were isolated at only 0 and 2 hours post inoculation (hpi). At the $37\pm 3^{\circ}\text{C}$ incubation temperature rHVT/NDV was isolated from shavings at 0 and 2 hpi, while the HVT parent strain was isolated at 0, 2 and 4 hpi. In addition to evaluating the environmental stability with sterile shavings, sterile swabs were also evaluated. Briefly, at the $25\pm 3^{\circ}\text{C}$ incubation temperature, rHVT/NDV and the HVT parent strain were isolated from swabs at 0, 2 and 4 hpi. At the $37\pm 3^{\circ}\text{C}$ incubation temperature, rHVT/NDV was isolated from swabs at 0 and 2 hpi, while the HVT parent strain was isolated at 0, 2 and 4 hpi. Based on these studies, the ability of rHVT/NDV to survive in sterile saturated shavings and swabs was similar to the HVT parent strain. We conclude that the environmental stability of rHVT/NDV is four hours or less and is similar to the HVT parent strain. Therefore, the survivability of rHVT/NDV is low, which decreases the chance of dissemination and poses no safety risk. These results are described in report # 308, entitled "Environmental Stability of Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live Marek's Disease Vector," which was approved by APHIS on September 3, 2003.

REFERENCES

1. Boshart, M., Weber, F., Jahn, G., Dorsch-Hasler, K., Fleckenstein, B. and Schaffner, W. (1985) A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 41, 521-530.
2. Calnek, B.W. and Witter, R.L. (1997) Marek's Disease. In: B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds), *Diseases of Poultry*, 10th ed., pp. 369-413. Iowa State University Press, Ames, Iowa.
3. Cho, B.R. (1975) Horizontal transmission of Turkey Herpesvirus to chickens IV. Viral maturation in the feather follicle epithelium. *Avian Diseases* 19, 136-141.
4. Cowen, B.S. and Braune, M.O. (1988) The propagation of avian viruses in a continuous cell line (QT35) of Japanese quail origin. *Avian Diseases* 32, 282-297.
5. Griffin, B.E. (1981) Sequence and analysis of polyoma virus DNA. In: J. Tooze (Ed), *DNA Tumor viruses*, pp. 843-913. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
6. Hecket, R.A., Riva, J., Cook, S., McMillen, J. and Schwartz, R.D. (1996) Onset of protective immunity in chicks after vaccination with a recombinant Herpesvirus of Turkeys vaccine expressing Newcastle Disease virus fusion and hemagglutinin-neuraminidase antigens. *Avian Diseases* 40, 770-777.
7. Hirai, K., Yamada, M., Aral, Y., Kato, S. and Nii, S. (1990) Replicating Marek's disease virus (MDV) serotype 2 DNA with inserted MDV serotype 1 DNA sequences in a Marek's disease lymphoblastoid cell line MSB1-41C. *Archives of Virology* 114, 153-165.
8. Kost, T.A., Theodorakis, N. and Hughes, S.H. (1983) The nucleotide sequence of the chick cytoplasmic B-actin gene. *Nucleic Acids Research* 11, 8287-8301.
9. Lee, L.F. (1971) Large-scale production of Marek's Disease Virus. *Avian Diseases* 15, 565-571.
10. Lohse, P. and Arnold, H.H. (1988) The down-regulation of the chicken cytoplasmic B actin during myogenic differentiation does not require the gene promoter but involves the 3' end of the gene. *Nucleic Acids Research* 16, 2787-2803.
11. Meulemans, G., Halen, P. and Schyns, P. (1973) Susceptibility of mammalian and avian cell cultures to infection with cell-free turkey herpes virus. *Journal of Comparative Pathology* 83, 605-608.
12. Morgan, R.W., J. Gelb, Jr., Pope, C.R. and Sonderneijer, P.J.A. (1993) Efficacy in chickens of herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle Disease Virus: Onset of protection and effect of maternal antibodies. *Avian Diseases* 37, 1032-1040.
13. Morrison, T.G., Peeples, M.E. and McGinnes, L.W. (1987) Conformational change in a viral glycoprotein during maturation due to disulfide bond disruption. *Proceedings of the National Academy of Science* 84, 1020-1024.
14. Nagai, Y., Yoshida, T., Hamaguchi, M., Naruse, H., Iinuma, M., Maeno, K. and Matsumoto, T. (1980) The pathogenicity of Newcastle Disease Virus isolated from migrating and domestic ducks and the susceptibility of the viral glycoproteins to proteolytic cleavage. *Microbiolol. Immunol.* 24, 173-177.

15. Reddy, S.K., Sharma, J.M., Ahmad, J., Reddy, D.N., McMillen, J.K., Cook, S.M., Wild, M.A. and Schwartz, R.D. (1996) Protective efficacy of a recombinant herpesvirus of turkeys as an *in ovo* vaccine against Newcastle and Marek's diseases in specific-pathogen-free chickens. *Vaccine* 14, 469-477.
16. Samorek-Dziewanowska, E. (1977) Production of the high virus concentration of turkey herpesvirus (HVT strain FC-126) propagated in Japanese quail embryo fibroblast culture. Part I. *Bulletin of Veterinary Inst. Pulawy* 21, 10-16.
17. Sato, H., Oh-hira, M., Ishida N., Imamura, Y., Hattori, S. and Kawakita, M. (1987a) Molecular cloning and nucleotide sequence of P, M and F genes of Newcastle disease virus avirulent strain D26. *Virus Research* 7, 241-255.
18. Sato, H., Hattori, S., Ishida N., Imamura, Y. and Kawakita, M. (1987b) Nucleotide sequence of the hemagglutinin-neuraminidase gene of Newcastle disease virus avirulent strain D26: evidence for a longer coding region with a carboxyl terminal extension as compared to virulent strains. *Virus Research* 8, 217-232.
19. Sharma, J.M., Witter, R.L., Shramek, G., Wolfe, L.G., Burmester, B.R. and Deinhardt, F. (1972) Lack of pathogenicity of Marek's disease virus and herpesvirus of turkeys in marmoset monkeys. *Journal of the National Cancer Institute* 49, 1191-1197.
20. Witter, R.L., Nazerian, K. and Soloman, J.J. (1972) Studies on the *in vivo* replication of turkey herpesvirus. *Journal of the National Cancer Institute* 49, 1121-1129.
21. Witter, R.L. and Sharma, J.M. (1974) Transient infectivity and heterokaryon formation in hamster cell cultures inoculated with cell-associated stocks of Marek's disease virus and herpesvirus of turkeys. *Journal of the National Cancer Institute* 53, 1731-1742.
22. Yanagida, N., Yoshida, S., Nazerian, K. and Lee, L. (1993) Nucleotide and predicted amino acid sequences of Marek's disease virus homologues of herpes simplex virus major tegument proteins. *Journal of General Virology* 74, 1837-1845.
23. Zygraich, N. and Huygelen, C. (1972) Inoculation of one-day old chicks with different strains of turkey herpesvirus. II. Virus replication in tissues of inoculated animals. *Avian Diseases* 16, 793-798.

Biomune Co.
U.S. Vet. Lic. No. 368

Marek's Disease-Newcastle Disease Vaccine, Serotype 3,
Live Marek's Disease Vector

March 4, 2004
supersedes
February 24, 2004

(b) 4

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Addendum No. 1

FIELD SAFETY STUDIES FOR THE EVALUATION OF MAREK'S DISEASE- NEWCASTLE DISEASE VACCINE, SEROTYPES 2 & 3, LIVE VIRUS, LIVE MAREK'S DISEASE VECTOR, PRODUCT CODE 17H1.R1

OBJECTIVE

The purpose of the study is to confirm safety of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, under conditions of husbandry that are typically employed in commercial chicken production in the United States. Previously, laboratory studies have demonstrated the safety and efficacy of the vaccine.

MATERIALS AND METHODS

Cooperators Participating in Field Safety Evaluation. Three of the following seven locations will be selected to conduct the field safety trial.

Location 1:

Company -
City, State - (b) 4
Company Supervisor -
Number of chickens -
Number of doses of experimental vaccine -

Location 2:

Company -
City, State - (b) 4
Company Supervisor -
Number of chickens -
Number of doses of experimental vaccine -

Location 3:

Company -
City, State - (b) 4
Company Supervisor -
Number of chickens -
Number of doses of experimental vaccine -

Location 4:

Company –
City, State - (b) 4
Company Supervisor –
Number of chickens –
Number of doses of experimental vaccine –

Location 5:

Company –
City, State - (b) 4
Company Supervisor –
Number of chickens –
Number of doses of experimental vaccine –

Location 6:

Company –
City, State - (b) 4
Company Supervisor –
Number of chickens –
Number of doses of experimental vaccine –

Location 7:

Company –
City, State - (b) 4
Company Supervisor –
Number of chickens –
Number of doses of experimental vaccine –

Description of Site. Standard poultry houses for the rearing of chickens will be used. The chickens will be contained within the house by walls and chicken wire. Curtains may be raised and lowered during the day for cooling purposes. During the trial, the company's supervisor will restrict access to the house.

Biomune Participating Investigators. (b) 6, will supervise investigators and the study, which will be approved by the appropriate State Veterinarians and Veterinary Biologics, APHIS, USDA. (b) 6 the study monitor.

Vaccine. At least two of the three pre-licensing serials of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, will be used in these studies. The field trial participants will receive vaccine for "Experimental Use Only, Not For Sale" with directions for use, and accompanying sterile diluent.

Method of Vaccination. Chickens in paired houses will receive standard vaccinations according to the cooperating company's vaccination program. Before and during these trials, chickens in the test group will not receive vaccinations for Marek's Disease Virus (MDV) serotypes 2 or 3, or Newcastle Disease Virus (NDV). Chickens in each house will be vaccinated with one dose of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1. The vaccine will be administered *in ovo* in the hatchery in approximately half of the test sites and for the other half of the test sites the vaccine will be administered subcutaneously. Chickens will be housed under standard husbandry conditions. Vaccination records will be maintained for the duration of the study.

Morbidity Observations. The site manager will observe the chickens daily for 21 days post vaccination. Chickens will be observed for 1) adverse reactions and 2) clinical signs of Marek's Disease including paralysis, emaciation, blindness, weight loss, paleness, external gross lesions, and diarrhea; and Newcastle Disease including respiratory signs, neurological signs, and viscerotropic signs such as listlessness, weakness, and diarrhea.

Mortality Observations. Mortality records will be maintained for the duration of the study. Any adverse reactions will be recorded and reported immediately to the principal investigator (b) 6 and/or study monitor (b) 6. (b) 6 These chickens will be buried on site or disposed as per the routine practice of the farm.

Contingency plan in the case of adverse events. In the case of adverse events associated with administration of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, the company's supervisor must immediately inform the principal investigator (b) 6 (b) 6 and/or study monitor (b) 6. If the adverse event poses significant risk to other animals, the area will be placed under strict bio-security rules and posted "off limits." The USDA will be notified and the circumstances of the adverse event will be reviewed. If required, the trial may be terminated and strict biosafety procedures will be implemented until the flock is disposed, litter removed, and premises disinfected.

SUMMARY

Commercial chickens will be vaccinated *in ovo* or by the subcutaneous route with one dose of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, and safety will be evaluated during a 21-day observation period. After the 21-day observation period, data will be collected, summarized, and submitted to APHIS.

RISK ANALYSIS

**MAREK'S DISEASE-NEWCASTLE DISEASE VACCINE, SEROTYPES 2 & 3, LIVE
VIRUS, LIVE MAREK'S DISEASE VECTOR,
PRODUCT CODE 17H1.R1**

March 4, 2004
Supersedes
February 24, 2004

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(b) 6

BIOMUNE CO.
8906 ROSEHILL ROAD
LENEXA, KANSAS 66215

U.S. Veterinary License No. 368

I. Background

The etiologic agent of Newcastle disease (ND) in chickens and turkeys is Newcastle disease virus (NDV). Newcastle disease virus is a member of the family *Paramyxoviridae*, in the subfamily *Paramyxovirinae* and in the genus *Rubulavirus* (Alexander, 1997). Newcastle disease presents itself in many forms ranging from high mortality to an asymptomatic form. There are five recognized forms of ND: (1) viscerotropic velogenic Newcastle disease (VVND), (2) neurotropic velogenic Newcastle disease (NVND), (3) Beaudette's form, (4) Hitchner's form, and (5) an asymptomatic enteric form. Newcastle disease virus strains causing these disease forms are all in one serotype, but are classified into NDV pathotypes based on pathogenicity tests and tissues of virus isolation. Strains are classified as (1) velogenic (high-virulence), (2) mesogenic (moderate-virulence), (3) lentogenic (low-virulence) and (4) asymptomatic (Alexander, 1997). Both VVND strains and NVND strains are grouped in the velogenic pathotype (high-virulence). Both cause acute, lethal infections in all ages of chickens. Hemorrhagic lesions in the digestive tract characterize VVND strains, while neurological and respiratory signs characterize NVND strains. Beaudette's form is characterized by death in young chickens and usually involves strains in the mesogenic pathotype (moderate-virulence). Hitchner's form is characterized by mild respiratory infections usually caused by strains in the lentogenic pathotype (low-virulence), and many of these strains are used to prepare vaccines for use in young chickens. Asymptomatic enteric strains are usually isolated from the gut of chickens showing no disease (Alexander, 1997). Newcastle disease in turkeys and chickens is classified into the five disease forms described above, however clinical signs in turkeys are less severe than in chickens (Alexander, 1997).

In the United States poultry industry, VVND and NVND forms of ND are rare. The common form of the disease is the Beaudette's form, which causes respiratory infections in young chicks. The United States poultry industry has controlled ND using two strategies. The first strategy is to vaccinate hens to induce high maternal antibody levels to protect the chick through passive immunity. The second strategy is to vaccinate chicks with live NDV vaccines as maternal antibody levels decrease. This strategy involves multiple vaccinations, one at hatch and other vaccinations at various times within the first few weeks of life. On a flock basis it is difficult to predict when maternal antibodies wane, and as a result multiple vaccinations must be given during the first few weeks of life (Alexander, 1997). Another problem associated with live NDV vaccines is the potential for vaccine reactions causing mild respiratory disease.

Herpesvirus of Turkeys (HVT) is classified in the family *Herpesviridae* in the subfamily *Alphaherpesvirus*. The genus is un-named but is referred to as Marek's disease-like viruses that are classified as Marek's Disease Virus (MDV) non-oncogenic, serotype 3. Due to the large DNA genome, herpesviruses have been

evaluated for use as a viral vector carrying foreign gene(s). For poultry, HVT has been evaluated as a vector for various poultry viral diseases (Darteil, 1995; Morgan et al., 1993; Ross et al., 1993).

In an effort to produce a NDV vaccine that protects chicks early in life with one vaccination, Biomune Co. and Zeon Corporation have developed a recombinant vaccine in which the (b) 4 gene from a NDV (b) 4 strain was inserted into the HVT genome. The Master Seed Virus (MSV) for this vaccine is designated rHVT/NDV and is a component of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed).

II. Areas of Concern

APHIS has three areas of concern; (1) animal safety, (2) public health safety, and (3) environmental safety. Biomune Co. has conducted a risk analysis to assess whether there are risks associated with the proposed environmental release to the field of this vaccine in the United States. Following are the conclusions from the risk analysis for each of the areas of concern:

A. Animal Safety

The safety characteristics of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, which includes rHVT/NDV have been rigorously evaluated. Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 is a combination vaccine containing (1) a conventional MDV serotype 2 SB1 vaccine strain, and (2) a rHVT/NDV. The risk to animal safety associated with rHVT/NDV MSV is low. Upon acceptance of this risk analysis, we request authorization to conduct field safety trials with the combination vaccine Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, which contains rHVT/NDV.

The rHVT/NDV, a fraction of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 was demonstrated to be safe for use in chickens. Briefly, in accordance with 9 CFR 113.330(d)(2), except that 18-day-old embryos were vaccinated *in ovo*, a 10X dose of rHVT/NDV was administered. After a 21-day observation period, no adverse reactions or clinical signs of Marek's Disease (MD) or ND were observed. Therefore, this vaccine is safe for use in chickens and poses no safety risk. Also, to our knowledge, no adverse vaccine reactions have been reported in association with the HVT parent strain that is the source of most USDA licensed HVT vaccines used in the United States.

Safety of the rHVT/NDV MSV was demonstrated in accordance with 9 CFR 113.330(b). Briefly, the MSV was inoculated *in ovo* at a 10X dose, chicks were hatched, and observed for 120 days for clinical signs of MD or ND. As controls, a group of chickens were observed as negative controls and another group of chickens was inoculated with a MDV (b) 4 challenge strain, (b) 4. After 120 days, neither the negative control group nor the rHVT/NDV vaccinate group had grossly observable lesions of MD, while the (b) 4 inoculated group did have grossly observable lesions of MD. Also, weights of the rHVT/NDV and negative control groups were not statistically different from one another. Therefore, rHVT/NDV MSV is safe for use in chickens and poses no safety risk.

Safety of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, was also evaluated during an efficacy trial. Briefly, chickens were vaccinated with Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, at day of age. These chickens were held for four weeks to develop immunity before challenge. During this period, chickens were observed daily and no adverse vaccine reactions or clinical signs of MD or ND were observed. After this observation period, these chickens were challenged with NDV and this vaccine was efficacious. Therefore, this vaccine is efficacious and safe for use in chickens and poses no safety risk.

Reports in the literature for FC-126 demonstrate that transmissibility of HVT is limited from chicken to chicken due to the limited virus present in the feather follicle epithelium (Cho, 1975; Zygraich and Huygelen, 1972). Safety of vaccine transmission of rHVT/NDV from vaccinated chickens to non-vaccinated, contact chickens was evaluated by (1) transmission to non-inoculated, contact chickens when transmission was evaluated for the presence of HVT in the white blood cells (WBCs) of contact controls, and (2) comparing transmission of the above recombinant with the HVT parent strain. Briefly, chickens were vaccinated with a 10X dose of rHVT/NDV *in ovo*. At hatch, non-vaccinated, contact chickens were commingled with vaccinated chickens for three weeks. During this three-week period, neither adverse vaccine reactions nor clinical signs of MD or ND were observed. At various times during the three week period, vaccinates and non-vaccinated, contact controls were bled and WBCs were purified for virus isolation on chicken embryo fibroblasts (CEF). At all time points, virus was isolated from the vaccinates, while virus was not isolated from the non-vaccinated, contact controls. Similar results were obtained in the HVT parent group. It was concluded that neither rHVT/NDV nor the HVT parent strain was transmissible. Therefore, rHVT/NDV is safe for use in chickens and poses no safety risk.

To address the possible concern that the NDV gene insert into the HVT genome could cause variations to the HVT tropism, the tissue tropism of the rHVT/NDV was evaluated. Briefly, chickens were vaccinated with a 10X dose of rHVT/NDV or an equivalent amount of HVT parent strain and virus isolations were conducted on various tissues. Chickens vaccinated with rHVT/NDV showed no adverse vaccine reactions, no clinical signs of MD or ND through 21 days post inoculation (dpi), or gross lesions of MD or ND. On 10 and 21 dpi, rHVT/NDV was isolated from purified WBCs from the blood, spleen, thymus, and bursa. Similarly, the HVT parent strain was isolated from these same tissues at these time points. Based on these results, it was concluded that the tissue tropism of rHVT/NDV was similar to the HVT parent strain. Therefore, rHVT/NDV is safe for use in chickens and poses no safety risk.

Non-target animal safety studies were conducted with a 10X dose of rHVT/NDV in
(b) 4 From the literature, it was expected that HVT would replicate in turkeys since HVT was originally isolated from turkeys (Witter et al., 1970) and is ubiquitous in domestic turkeys. Herpesvirus of Turkeys is also known to replicate in turkeys, but is non-oncogenic (Witter et al., 1972; Calnek and Witter, 1997). From the literature, experimental infections of MDV (serotype 1) have shown quail and pheasants to be susceptible to infection (Calnek and Witter, 1997). The host range of HVT (serotype 3) is less defined than MDV by experimental infections of various birds, but has been better defined in tissue culture. Herpesvirus of Turkeys is known to replicate in primary chicken, duck, and quail cells, as well as quail cell lines (Cowen and Braune, 1988; Lee, 1971; Samorek-Dzieskanowska, 1977).

Safety was demonstrated in other avian species (b) 4
(b) 4 by: (1) inoculation with rHVT/NDV or the HVT parent strain, and (2) comparison of clinical signs, gross lesions, adverse vaccine reactions, and virus isolation between these two groups. Results demonstrated that other avian species inoculated with rHVT/NDV showed no clinical signs, no gross lesions, or adverse vaccine reactions. The rHVT/NDV was isolated from purified WBCs at each time point for five weeks post inoculation from all avian species. Also, identical results were obtained when avian species were inoculated with the parent HVT strain. Based on these results, it was demonstrated that the host range of rHVT/NDV is similar to the HVT parent strain. Therefore, rHVT/NDV is safe in these avian species and use in chickens poses no safety risk to other avian species.

Unsuccessful attempts to replicate HVT in several mammalian species such as
(b) 4 have been conducted (Calnek and Witter, 1997; Sharma et al., 1972). Also, investigators trying to replicate HVT in mammalian primary cultures and cell lines were unable to detect evidence of virus replication even after six to 10 blind passages (Meulemans et al., 1973; Witter and Sharma, 1974).

Safety was demonstrated in mammalian cell lines: (b) 4
(b) 4

(b) 4 These mammalian cell lines were inoculated with rHVT/NDV and passed five times. No cytopathic effects were observed in any cell line or any passage. Also, similar results were obtained when these species were inoculated with the parent HVT strain. Based on these results, it was concluded that the host range of rHVT/NDV was similar to the HVT parent strain. Therefore, rHVT/NDV is safe in these mammalian species and use in chickens poses no safety risk to mammalian species.

Other safety issues associated with (b) 4 such as genetic stability and purity were also addressed. (b) 4

(b) 4 No adverse vaccine reactions or clinical signs of MD or ND were observed during each passage or for 45 days in the last backpass group. The *in vivo* genetic stability of rHVT/NDV was confirmed using molecular tests to verify stability of the NDV. (b) 4

(b) 4 Briefly, (b) 4 analysis of DNA isolated from rHVT/NDV from the last backpass group verified the presence of the (b) 4 gene insert and verified that the gene insert was stable in the HVT genome. Once it was verified that the gene insert was stable in the HVT genome after backpassage, gene expression was confirmed by (b) 4 and a (b) 4 referred to as the (b) 4

To verify the *in vitro* stability of the (b) 4 of rHVT/NDV, MSV was passed five times *in vitro* resulting in MSV+5. Using the same molecular tests described above to verify stability of the gene insert (b) 4 and gene expression (b) 4 rHVT/NDV was confirmed to be genetically stable *in vitro*.

Purity of rHVT/NDV MSV was satisfactory in accordance with 9 CFR 113.27, 113.28, 113.30, 113.31, 113.34, 113.37, and 113.46. (b) 4

(b) 4

(b) 4 which was reported on the APHIS form 2008, which was submitted to APHIS on November 14, 2001.

B. Public Health Safety

The risk to public health is low for this vaccine. Human exposure will be limited to persons administering the vaccine or handling vaccinated chickens. Herpesvirus of Turkeys is known not to be of public health significance (Calnek and Witter, 1997) and no known infection in humans has been reported. We concluded that rHVT/NDV

is of no public health significance due to the narrow host range of herpesviruses to avian species (Calnek and Witter, 1997) and to the non-target animal safety studies described here within. Based on the confirmed narrow host range of rHVT/NDV, there are no expected safety concerns associated with human exposure.

C. Environmental Safety

The risk to the environment is low and there are no expected adverse ecological effects of rHVT/NDV on the environment. Exposure to non-target animals and dissemination of the vaccine into the environment will be restricted by its use in poultry houses.

For environmental safety, it was established that rHVT/NDV was not transmitted from vaccinated chickens to non-vaccinated chickens. As previously described, chickens were vaccinated *in ovo* with a 10X dose of rHVT/NDV. At hatch, non-vaccinated, contact chickens were commingled with vaccinated chickens for three weeks. During this three-week period, neither adverse vaccine reactions nor clinical signs of MD or ND were observed. At various times during the three week period, vaccinated and non-vaccinated, contact controls were bled and WBCs were purified for virus isolation on CEF. At all time points, virus was isolated from the vaccinates, while virus was not isolated from the non-vaccinated, contact controls. Similar results were obtained in the HVT parent group. It was concluded that neither rHVT/NDV nor the HVT parent strain was transmissible. Therefore, rHVT/NDV is safe for use in chickens and poses no safety risk.

(b) 4

(b) 4

(b) 4
rHVT/NDV is (b) 4 We conclude that the environmental stability of
and is similar to the parent HVT strain. Therefore,
the survivability of rHVT/NDV is low, which decreases the chance of dissemination
and poses no safety risk.

Also, the chance of *in vivo* recombination between rHVT/NDV and field NDV strains and other viruses was considered. Since HVT replicates its DNA in the cell nucleus, recombination events with viruses replicating RNA (i.e. NDV) in the cell cytoplasm (Alexander, 1997) are low due to the different locations of genetic material and the different types of genetic material. The chance of an *in vivo* recombination event is unknown, but a remote possibility due to the replication cycle of HVT.

The chance of an *in vivo* recombination event between rHVT/NDV and field MDV virus are unknown because the occurrence of horizontal gene transfer between the various MDV serotypes is not known. Common vaccination practices in the United States involve the mixture of HVT (serotype 3), SB1 (serotype 2), and Rispen's (serotype 1) vaccines. To date, no recombinations between any of these vaccines or with field MDV viruses (serotype 1) have been reported. One case of experimentally produced MDV serotype 2 recombination with MDV serotype 1 was reported but was not repeatable (Hirai et al., 1990). The chance of an *in vivo* recombination event is unknown, but a remote possibility due to the similar replication cycle of HVT and other MDV serotypes. No known physical and/or chemical factors are known to enhance the dispersal of rHVT/NDV in the environment.

III. Risk Characterization

A. Animal Safety

- | | |
|-------------------------------------------------------------------------------------------------|-------------|
| 1. Likelihood rating | Risk Rating |
| i. Low (L)= an adverse event is unlikely to occur | LL=1.00 |
| ii. Certain (C)= The rating is supported by direct scientific evidence | C=1.00 |
| 2. Consequence rating | |
| i. Low (CL)= The consequence, if the adverse event occurs is not severe | CL=1.00 |
| ii. Moderately certain (MC)= The rating is supported by direct and indirect scientific evidence | MC=0.75 |
| 3. Expected risk | |
| i. [(likelihood)x(degree of certainty)]X[(consequence)x(degree of certainty)]= Risk rating | |
| ii. [(1.00)x(1.00)]X[(1.00)x(0.75)]=0.75 | |
| 4. Risk rating | Low |

B. Public Health Safety

- | | |
|-------------------------------------------------------------------------------------------------|-------------|
| 1. Likelihood rating | Risk Rating |
| i. Low (L)= an adverse event is unlikely to occur | LL=1.00 |
| ii. Certain (C)= The rating is supported by direct scientific evidence | C=1.00 |
| 2. Consequence rating | |
| i. Low (CL)= The consequence, if the adverse event occurs is not severe | CL=1.00 |
| ii. Moderately certain (MC)= The rating is supported by direct and indirect scientific evidence | MC=0.75 |
| 3. Expected risk | |
| i. [(likelihood)x(degree of certainty)]X[(consequence)x(degree of certainty)]= Risk rating | |
| ii. [(1.00)x(1.00)]X[(1.00)x(0.75)]=0.75 | |
| 4. Risk rating | Low |

- C. Environmental Safety
1. Likelihood rating Risk Rating
 - i. Low (L)= an adverse event is unlikely to occur LL=1.00
 - ii. Certain (C)= The rating is supported by direct scientific evidence C=1.00
 2. Consequence rating CL=1.00
 - i. Low (CL)= The consequence, if the adverse event occurs is not severe
 - ii. Moderately certain (MC)= The rating is supported by direct and indirect scientific evidence MC=0.75
 3. Expected risk Low
 - i. $[(\text{likelihood}) \times (\text{degree of certainty})] \times [(\text{consequence}) \times (\text{degree of certainty})] = \text{Risk rating}$
 - ii. $[(1.00) \times (1.00)] \times [(1.00) \times (0.75)] = 0.75$
 4. Risk rating

IV. Conclusions

Based on this risk analysis, Biomune Co. concludes that the proposed field tests included in Addendum 1 will not result in a significant impact on animal safety, public health, or environmental safety. The risk rating for Recombinant Herpesvirus of Turkeys – Newcastle Disease Virus (rHVT/NDV) Master Seed is low. This recombinant virus with a low risk rating is combined with a conventional MDV serotype 2, SB1 vaccine strain, in the combination vaccine, Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1. The Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 was determined to be safe for use in chickens. Since the recombinant fraction of this combination vaccine has a low risk rating and the combination vaccine is safe for use in chickens following administration by either the *in ovo* route in the hatchery or the subcutaneous route, we request authorization to conduct field safety trials with the combination vaccine, Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1, upon acceptance from APHIS.

REFERENCES

1. Alexander, D.J. (1997) Newcastle Disease and other avian paramyxoviridae infections. In: B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds), Diseases of Poultry, 10th ed., pp. 541-569. Iowa State University Press, Ames, Iowa.
2. Calnek, B.W. and Witter, R.L. (1997) Marek's Disease. In: B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds), Diseases of Poultry, 10th ed., pp. 369-413. Iowa State University Press, Ames, Iowa.
3. Cho, B.R. (1975) Horizontal Transmission of Turkey Herpesvirus to chickens IV. Viral maturation in the feather follicle epithelium. Avian Diseases 19, 136-141.
4. Cowen, B.S. and Braune, M.O. (1988) The propagation of avian viruses in a continuous cell line (QT35) of Japanese quail origin. Avian Diseases 32, 282-297.
5. Dartel, R., Bublot, M., Laplace, E., Bouquet, J. F., Audonnet, J. C., Riviere, M. (1995) Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. Virology 211, 481-490.
6. Hirai, K., Yamada, M., Aral, Y., Kato, S. and Nii, S. (1990) Replicating Marek's disease virus (MDV) serotype 2 DNA with inserted MDV serotype 1 DNA sequences in a Marek's disease lymphoblastoid cell line MSB1-41C. Archives of Virology 114, 153-165.
7. Lee, L.F. (1971) Large-scale production of Marek's Disease Virus. Avian Diseases 15, 565-571.
8. Meulemans, G., Halen, P. and Schyns, P. (1973) Susceptibility of mammalian and avian cell cultures to infection with cell-free turkey herpes virus. Journal of Comparative Pathology 83, 605-608.
9. Morgan, R.W., J. Gelb, Jr., Pope, C.R. and Sonderneijer, P.J.A. (1993) Efficacy in chickens of herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle Disease Virus: Onset of protection and effect of maternal antibodies. Avian Diseases 37, 1032-1040.
10. Ross, L.J.N., Binns, M.M., Tyers, P., Pastorek, J., Zelnik, V. and Scott, S. (1993) Construction and properties of a turkey herpesvirus recombinant expressing the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. Journal of General Virology 74, 371-377.
11. Samorek-Dzickanowska, E. (1977) Production of the high virus concentration of turkey herpesvirus (HVT strain FC-126) propagated in Japanese quail embryo fibroblast culture. Part I. Bulletin of Veterinary Inst. Pulawy 21, 10-16.
12. Sharma, J.M., Witter, R.L., Shramek, G., Wolfe, L.G., Burmester, B.R. and Deinhardt, F. (1972) Lack of pathogenicity of Marek's disease virus and herpesvirus of turkeys in marmoset monkeys. Journal of the National Cancer Institute 49, 1191-1197.
13. Witter, R.L., Nazerian, K., Purchase, H.G. and Burgoyne, G.H. (1970) Isolation from turkeys of cell associated herpesvirus antigenically related to Marek's disease virus. American Journal of Veterinary Research 31, 525-538.
14. Witter, R.L., Nazerian, K. and Soloman, J.J. (1972) Studies on the *in vivo* replication of turkey herpesvirus. Journal of the National Cancer Institute 49, 1121-1129.

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Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3,
Live Virus, Live Marek's Disease Vector,
Product Code 17H1.R1

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15. Witter, R.L. and Sharma, J.M. (1974) Transient infectivity and heterokaryon formation in hamster cell cultures inoculated with cell-associated stocks of Marek's disease virus and herpesvirus of turkeys. *Journal of the National Cancer Institute* 53, 1731-1742.
16. Zygraich, N. and Huygelen, C. (1972) Inoculation of one-day old chicks with different strains of turkey herpesvirus. II. Virus replication in tissues of inoculated animals. *Avian Diseases* 16, 793-798.

Proposed Field Tests of an Experimental
Marek's Disease – Newcastle Disease Vaccine,
Serotypes 2 and 3, Live Virus, Live Marek's Disease Vector
Biomune Company, Inc.

Environmental Assessment and Finding of No Significant Impact

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 and the "no action" alternative associated with a proposal to field test and to license an experimental live vaccine for chickens manufactured by Biomune Company, Inc. The vaccine is a genetically modified Newcastle Disease, Marek's Disease Vaccine. The field tests may be conducted in the states of (b) 4

(b) 4 We have analyzed the potential impacts on animal safety, public health, and environmental safety and prepared an Environmental Assessment that represents the conclusions 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.

REPORT

**FIELD SAFETY STUDIES FOR THE EVALUATION OF MAREK'S DISEASE-
NEWCASTLE DISEASE VACCINE, SEROTYPES 2 & 3, LIVE VIRUS, LIVE MAREK'S
DISEASE VECTOR, PRODUCT CODE 17H1.R1**

November 29, 2006

BY

(b)(6)

**BIOMUNE COMPANY
8906 ROSEHILL ROAD
LENEXA, KANSAS 66215
U.S. VET. LIC. NO. 368**

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**FIELD SAFETY STUDIES FOR THE EVALUATION OF MAREK'S DISEASE-
NEWCASTLE DISEASE VACCINE, SEROTYPES 2 & 3, LIVE VIRUS, LIVE MAREK'S
DISEASE VECTOR, PRODUCT CODE 17H1.R1**

SUMMARY

Field safety in forty four thousand four hundred and seventy two (44,472) 18 to 19 day-old broiler embryos and thirty seven thousand six hundred (37,600) day of age chickens vaccinated with Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed) was demonstrated in three distinct geographical areas of the United States. There were no reports of adverse reactions in any of the vaccinated birds.

OBJECTIVE

The purpose of the study was to confirm the safety of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed) in commercial chickens reared under typical management conditions of the U.S. poultry industry in three geographical regions.

FIELD SAFETY TEST LOCATIONS AND PARTICIPATING FLOCKS

A. Vaccine: Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed), serial numbers

(b) 4

B. Chickens: Forty four thousand four hundred and seventy two (44,472) 18 to 19 day-old embryos were vaccinated by *in ovo* administration and a total of thirty seven thousand six hundred (37,600) day of age chickens were vaccinated by the subcutaneous (SQ) route. The 0.1 ml vaccine dose was administered *in ovo* and the 0.2 ml vaccine dose was administered subcutaneously under the skin of the neck according to routine Marek's disease vaccination procedures.

C. Field Safety Cooperators: Poultry producers in three distinct geographic locations evaluated vaccine safety. The locations were as follows:

1. (b) 4 One chicken house was designated for evaluating the vaccine by *in ovo* administration in 18 to 19 day-old embryos in a study initiated September 26, 2006. An additional house on the same farm served as the nonvaccinated control house. The vaccine serial number used in this operation was (b) 4 Nineteen thousand four hundred forty (19,440) embryos were vaccinated in this operation.

2. (b) 4 One chicken house was designated for evaluating the vaccine by *in ovo* administration in 18 to 19 day-old embryos in a study initiated September 18, 2006. An additional house on the same farm served as the nonvaccinated control house. The vaccine serial number used in this operation was (b) 4 Twenty five thousand and thirty two (25,032) embryos were vaccinated in this operation.

3. (b) 4 One chicken house was designated for evaluating the vaccine by SQ administration in day of age chickens in a study initiated October 3, 2006. An additional house served as the nonvaccinated control group. The vaccine serial number used in this operation was (b) 4 Nineteen thousand (19,000) chickens were vaccinated in this operation.
4. (b) 4 One chicken house was designated for evaluating the vaccine by SQ administration in day of age chickens in a study initiated October 10, 2006. An additional house served as the nonvaccinated control group. The vaccine serial number used in this operation was (b) 4 Eighteen thousand six hundred (18,600) chickens were vaccinated in this operation.

METHODS AND PROCEDURES FOR VACCINE EVALUATION

Vaccinating crews of the four major broiler operations utilized routine Marek's disease vaccination procedures for the field safety test evaluations of the Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed), in broiler birds. Embryos were vaccinated *in ovo* or day old chicks were vaccinated subcutaneously with a 0.1 ml or 0.2 ml dose of vaccine, respectively.

DATA COLLECTION, RECORD KEEPING AND SUMMARIZATION

Vaccinated chickens were observed daily beginning at day of age and ending at 21-days of age (22 days for the chicks vaccinated by the subcutaneous route). Flock producers and flock supervisors were responsible for day to day flock observations and recording daily mortality. (b) 4 Study Monitors, observed each flock during the post vaccination period. The observations that were recorded included adverse reactions, observations for clinical signs of Marek's disease virus and Newcastle disease virus, mortality and overall performance.

RESULTS

There were no adverse effects in hatchability of chickens following *in ovo* administration of the Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed). There were no adverse effects in chickens following SQ administration of the Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed). The flock supervisors reported that vaccinated and nonvaccinated chickens participating in the four studies remained in good general health. (b) 4 observed all flocks to be in good health following vaccination. In addition, there was no unusual mortality in any of the studies. A summary of hatchability, mortality and general post vaccination observations is provided in Table 1.

No significant differences were observed in hatchability between the vaccinates and the controls for either the (b) 4 Hatchability was normal based on farm management expectations as described in the attached letter from the company veterinarian. There was no significance difference in number of mortalities in the vaccinates and the controls during the observation period.

Table 1 - Results of Field Safety Studies of Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1.

Location	Vaccine Route	Vaccine Serial No./ Treatment Group	Hatchability No. hatched/ No. embryos set ¹ (%)	No. of Birds	Mortality		Observations
					Total No. of Deaths ²	% Total	
(b) 4	<i>In ovo</i>	(b) 4	16,900/19,440 (86.93%)	16,900	224	1.33%	No adverse vaccine reactions
		Control	16,000/17,585 (90.99%)	16,000	251	1.57%	No adverse vaccine reactions
(b) 4	<i>In ovo</i>	(b) 4	22,012/25,032 (87.94%)	22,012	244	1.11%	No adverse vaccine reactions
		Control	22,024/24,548 (89.72%)	22,024	291	1.32%	No adverse vaccine reactions
(b) 4	SQ ³	(b) 4	Not applicable	19,000	353	1.86%	No adverse vaccine reactions
		Control	Not applicable	19,000	226	1.19%	No adverse vaccine reactions
(b) 4	SQ	(b) 4	Not applicable	18,600	484	2.60%	No adverse vaccine reactions
		Control	Not applicable	18,600	533	2.87%	No adverse vaccine reactions

No. hatched/ No. embryos set¹ = For each test location, no significant difference in hatchability was observed between vaccinated and control groups.

Total No. of Deaths² = For each test location, no significant difference in mortality was observed between vaccinated and control groups.

SQ³ = subcutaneous

In summary, the field safety studies conducted by Biomune Company, with the cooperation of four major broiler operations in distinct geographic areas of the U.S., demonstrated that the Marek's Disease-Newcastle Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector, Product Code 17H1.R1 (unlicensed), was safe for use under typical management conditions of the U.S. poultry industry. Two serials of vaccine were evaluated in a total of forty four thousand four hundred and seventy two (44,472) embryos by *in ovo* administration in 18 to 19 day-old embryos. These same two serials were evaluated in a total of thirty seven thousand six hundred (37,600) chickens at day of age by the SQ route. There were no adverse reactions in any of the vaccinated birds. Hatchability, overall performance and mortality were equivalent to that of the control group and normal farm management expectations. Results of these field safety studies demonstrated that the vaccine was safe for *in ovo* administration in 18 to 19 day-old embryos or SQ administration in day of age chicks.

Biomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4

PRODUCT: HVT-NDV+SB1 Vaccine SERIAL NO: _____ (b)(4)

VACCINATION DATE: 9/26/06 VACCINATION ROUTE: In ovo

SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days

FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 19,440

DIRECTIONS

1. Fill form out in ink (pen)
2. On this form observe embryos/eggs until day of hatch, day 0 is day of vaccination. Record the bird observations after hatch on page 2 of this form.
3. Daily – record mortality
4. Daily – if embryos/eggs are normal, record “N” under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of “ditto” marks (“”) or a line with an arrow.

HATCHABILITY

	Number of Embryos/Chicks	DATE (m/d/ly)	OBSERVATIONS & COMMENTS	INITIALS
Vaccinated	19,440	9/26/06	N	(b) 6
Hatched	16,900	9/29/06	N	

Hatchability Rate for Vaccinates: 86.93%

CONTROL GROUP

Biomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4
PRODUCT: Merial HVT SERIAL NO: _____ (b) 4
VACCINATION DATE: 9/26/06 VACCINATION ROUTE: In ovo
SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days
FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 17,585

DIRECTIONS

1. Fill form out in ink (pen)
2. On this form observe embryos/eggs until day of hatch, day 0 is day of vaccination. Record the bird observations after hatch on page 2 of this form.
3. Daily – record mortality
4. Daily – if embryos/eggs are normal, record "N" under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks ("") or a line with an arrow.

HATCHABILITY

	Number of Embryos/Chicks	DATE (m/d/y)	OBSERVATIONS & COMMENTS	INITIALS
Vaccinated	17,585	9/26/06	N	
Hatched	16,000	9/28/06	N	

Hatchability Rate for Vaccinates: 90.99%

Pomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4

PRODUCT: HVT-NDV+SB1 Vaccine SERIAL NO: _____ (b)(4)

VACCINATION DATE: 9/26/04 VACCINATION ROUTE: In ovo

SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days

FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 19,440

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds through 21 days of age, day 0 is day of hatch.
3. Daily - record mortality
4. Daily - if birds are normal, record "N" under observations & comments
5. Daily - record your initials
6. Please complete each line. The USDA will not accept the use of "ditto" marks (") or a line with an arrow.

Day of Age	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	9-27	18	Date of Hatch	
1	9-29	15	N	
2	9-30	27	N	
3	10-1	28	N	
4	10-2	25	N	
5	10-3	14	N	
6	10-4	5	N	
7	10-5	9	N	
8	10-6	14	N	
9	10-7	6	N	
10	10-8	5	N	
11	10-9	4	N	
12	10-10	5	N	
13	10-11	5	N	
14	10-12	4	N	
15	10-13	6	N	
16	10-14	4	N	
17	10-15	6	N	
18	10-16	5	N	
19	10-17	12	N	
20	10-18	3	N	
21	10-19	4	N	

(b) 6

CONTROL GROUP
Biomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4
 PRODUCT: Meriel HVT SERIAL NO: _____ (b)(4)
 VACCINATION DATE: 9/26/06 VACCINATION ROUTE: In ovo
 SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days
 FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 17585

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds through 21 days of age, day 0 is day of hatch.
3. Daily – record mortality
4. Daily – if birds are normal, record "N" under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks ("") or a line with an arrow.

Day of Age	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	9-28	12	Date of Hatch	
1	9-29	10	N	
2	9-30	35	N	
3	10-1	31	N	
4	10-2	25	N	
5	10-3	29	N	
6	10-4	14	N	
7	10-5	12	N	
8	10-6	20	N	
9	10-7	8	N	
10	10-8	5	N	
11	10-9	4	N	
12	10-10	3	N	
13	10-11	1	N	
14	10-12	4	N	
15	10-13	5	N	
16	10-14	5	N	
17	10-15	5	N	
18	10-16	4	N	
19	10-17	12	N	
20	10-18	3	N	
21	10-19	4	N	

(b) 6

(b) 6

Biomune Company
8906 Rosehill Road
Lenexa, KS 66215

(B) (4),

Dear (b) 6

The variability in hatch results between the vaccinated and control groups of the field safety trial performed at (b) 4 on September 28, 2006, with the recombinant HVT/ND + SB-1 vaccine was a result of the breeder flock and not a detrimental effect of the vaccine. The 4% difference in number of chickens hatching between the vaccinated and control groups is due to the mix of eggs coming from two individual breeder houses on the same farm.

According to the breeder flock manager, problems involving a water shortage and excessive heat this summer on this farm have resulted in this flock experiencing decreased production and erratic hatches. It is not uncommon for this flock to have a 3% to 7% range in hatchability loss of broiler chickens hatching on consecutive hatching days. To illustrate the variability in hatch of this breeder flock which includes two houses, I have provided the per cent hatches, which are calculated as the number of chickens hatched divided by number of eggs set, for 9 recent consecutive hatch days including the date of the recombinant HVT/ND +SB-1 field safety study on September 28. These values are given below. Source flock wa (b)(4)

Friday, September 15 – 84.36%
Monday, September 18 – 90.74%
Thursday, September 21 – 87.28%
Monday, September 25 – 90.02%
Thursday, September 28 – 89.74%
Monday, October 2 – 82.12%
Thursday, October 5 – 87.31%
Tuesday, October 10 – 85.91%
Friday, October 13 – 86.28%

You may contact me by phone a (b) 6 if you have questions regarding this study.

(b) 4

(b) 4

Biomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4

PRODUCT: HVT-NDV+SB1 Vaccine SERIAL NO: _____ (b) 4

VACCINATION DATE: 9/18/06 VACCINATION ROUTE: In ovo

SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days

FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 25,032

DIRECTIONS

1. Fill form out in ink (pen)
2. On this form observe embryos/eggs until day of hatch, day 0 is day of vaccination. Record the bird observations after hatch on page 2 of this form.
3. Daily – record mortality
4. Daily – if embryos/eggs are normal, record “N” under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of “ditto” marks (“”) or a line with an arrow.

HATCHABILITY

	Number of Embryos/Chicks	DATE (m/d/y)	OBSERVATIONS & COMMENTS	INITIALS
Vaccinated	25,032	9/18/06	N	
Hatched	22,012	9/21/06	N	(b) 6

Hatchability Rate for Vaccinates: 87.94%

CONTROL GROUP

Biomune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4
PRODUCT: Merical HVT / Merical SBI SERIAL NO: _____ (b)(4)
VACCINATION DATE: 9/18/06 VACCINATION ROUTE: In ovo
SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days
FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 24,548

DIRECTIONS

1. Fill form out in ink (pen)
2. On this form observe embryos/eggs until day of hatch, day 0 is day of vaccination. Record the bird observations after hatch on page 2 of this form.
3. Daily – record mortality
4. Daily – if embryos/eggs are normal, record "N" under observations & comments
5. Daily – record your initials
6. Please complete each line. The USDA will not accept the use of "ditto" marks ("") or a line with an arrow.

HATCHABILITY

	Number of Embryos/Chicks	DATE (m/d/y)	OBSERVATIONS & COMMENTS	INITIALS
Vaccinated	24,548	9/18/06	N	(b) 6
Hatched	22,024	9/21/06	N	

Hatchability Rate for Vaccinates: 89.72%

B June@ Field Safety Study (In ovo Re)

NAME OF SITE & ADDRESS: _____

(b) 4

PRODUCT: HVT-NDV+SB1 Vaccine

SERIAL NO: _____

(b)(4)

VACCINATION DATE: 9/18/06

VACCINATION ROUTE: In ovo

SPECIES: CHICKEN

AGE OF EMBRYOS: _____

18 to 19 days

FLOCK ID: _____

(b) 4

NUMBER OF EMBRYOS VACCINATED: 25,032

Placed 22,012

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds through 21 days of age, day 0 is day of hatch.
3. Daily -- record mortality
4. Daily -- if birds are normal, record "N" under observations & comments
5. Daily -- record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks (") or a line with an arrow.

Day of Age	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	9/21	0	Date of Hatch	
1	9/22	16	N	
2	9/23	19	N	
3	9/24	15	N	
4	9/25	13	N	
5	9/26	16	N	
6	9/27	15	N	
7	9/28	9	N	
8	9/29	7	N	
9	9/30	3	N	
10	10/1	7	N	
11	10/2	4	N	
12	10/3	6	N	
13	10/4	22	NORMAL CULL BIRDS LAYING ON THEIR SIDE WERE NOT A LOT OF DOWN BIRDS <small>previously examined</small>	
14	10/5	12	N	
15	10/6	9	N	
16	10/7	13	N	
17	10/8	16	N	
18	10/9	10	N	
19	10/10	12	N	
20	10/11	4	N	
21	10/12	16	N	

(b) 6

(b) 4

CONTROL GROUP
Immune® Field Safety Study (In ovo Route)

NAME OF SITE & ADDRESS: _____ (b) 4
 PRODUCT: Meril HVT / Meril SB-1 SERIAL NO: _____ (b)(4)
 VACCINATION DATE: 9/18/06 VACCINATION ROUTE: In ovo
 SPECIES: CHICKEN AGE OF EMBRYOS: 18 to 19 days
 FLOCK ID: _____ (b) 4 NUMBER OF EMBRYOS VACCINATED: 24,548

Placed 22,024

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds through 21 days of age, day 0 is day of hatch.
3. Daily – record mortality
4. Daily – if birds are normal, record "N" under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks (") or a line with an arrow.

Day of Age	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	9/21	0	Date of Hatch	
1	9/22	24	N	
2	9/23	17	N	
3	9/24	15	N	
4	9/25	14	N	
5	9/26	12	N	
6	9/27	15	N	
7	9/28	11	N	
8	9/29	8	N	
9	9/30	7	N	
10	10/1	10	N	
11	10/2	8	N	
12	10/3	6	N.	
13	10/4	24	N	
14	10/5	15	N	
15	10/6	14	N	
16	10/7	11	N	
17	10/8	21	N	
18	10/9	17	N	
19	10/10	15	N.	
20	10/11	23	N.	
21	10/12	14	N.	

(b) 6

Biomune® Field Safety Study (Subcutaneous Route)

NAME OF SITE & ADDRESS: _____ (b) 4 _____
 PRODUCT: HVT-NDV+SB1 vaccine SERIAL NO: _____ (b)(4) _____
 VACCINATION DATE: 10/3/06 VACCINATION ROUTE: Subcutaneous
 SPECIES: CHICKEN AGE OF BIRDS: 1 day of age
 FLOCK ID: _____ (b) 4 _____ NUMBER OF BIRDS VACCINATED: 19,000

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds for a total of 21 days post vaccination, day 0 is day of vaccination.
3. Daily - record mortality
4. Daily - if birds are normal, record "N" under observations & comments
5. Daily - record your initials
6. Please complete each line. The USDA will not accept the use of "ditto" marks (") or a line with an arrow.

Day post vaccination	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	10-3	0	Date of Vaccination	
1	10-4	8	N	
2	10-5	29	N	
3	10-6	40	N	
4	10-7	52	N	
5	10-8	60	N	
6	10-9	37	N	
7	10-10	20	N	
8	10-11	20	N	
9	10-12	12	N	
10	10-13	13	N	
11	10-14	8	N	
12	10-15	5	N	
13	10-16	3	N	
14	10-17	3	N	
15	10-18	4	N	
16	10-19	6	N	
17	10-20	7	N	
18	10-21	5	N	
19	10-22	8	N	
20	10-23	6	N	
21	10-24	7	N	

(b) 6

CONTROL GROUP

Biomune® Field Safety Study (Subcutaneous Route)

NAME OF SITE & ADDRESS: _____ (b) 4
PRODUCT: Merical / AVT / Merical 5b1 / Intervet 8903 SERIAL NO: _____ (b)(4)
VACCINATION DATE: _____ VACCINATION ROUTE: Subcutaneous
SPECIES: CHICKEN AGE OF BIRDS: 1 day of age
FLOCK ID: _____ (b) 4 NUMBER OF BIRDS VACCINATED: 19,000

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds for a total of 21 days post vaccination, day 0 is day of vaccination.
3. Daily - record mortality
4. Daily - if birds are normal, record "N" under observations & comments
5. Daily - record your initials
6. Please complete each line. The USDA will not accept the use of "ditto" marks ("") or a line with an arrow.

Day post vaccination	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	10-3	0	Date of Vaccination	
1	10-4	23	N	
2	10-5	41	N	
3	10-6	30	N	
4	10-7	12	N	
5	10-8	9	N	
6	10-9	4	N	
7	10-10	4	N	
8	10-11	6	N	
9	10-12	5	N	
10	10-13	10	N	
11	10-14	9	N	
12	10-15	7	N	
13	10-16	10	N	
14	10-17	3	N	
15	10-18	9	N	
16	10-19	6	N	
17	10-20	11	N	
18	10-21	3	N	
19	10-22	8	N	
20	10-23	10	N	
21	10-24	6	N	

(b) 6

Biomune® Field Safety Study (Subcutaneous Route)

NAME OF SITE & ADDRESS: _____ (b) 4

PRODUCT: HVT-NDV+SB1 vaccine SERIAL NO: _____ (b)(4)

VACCINATION DATE: 10/10/06 VACCINATION ROUTE: Subcutaneous

SPECIES: CHICKEN AGE OF BIRDS: 1 day of age

FLOCK ID: _____ (b) 4 NUMBER OF BIRDS VACCINATED: 18,680

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds for a total of 21 days post vaccination, day 0 is day of vaccination.
3. Daily – record mortality
4. Daily – if birds are normal, record "N" under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks (") or a line with an arrow.

Day post vaccination	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	10/10/06	0	Date of Vaccination	
1	10/11/06	9	N	
2	10/12/06	8	N	
3	10/13/06	26	N	
4	10/14/06	33	N	
5	10/15/06	47	N	
6	10/16/06	43	N	
7	10/17/06	63	N	
8	10/18/06	55	N	
9	10/19/06	38	N	
10	10/20/06	29	N	
11	10/21/06	27	(b) 4 N	(b) 6
12	10/22/06	12	N	
13	10/23/06	11	N	
14	10/24/06	15	N	
15	10/25/06	14	N	
16	10/26/06	12	N	
17	10/27/06	10	N	
18	10/28/06	10	N	
19	10/29/06	6	N	
20	10/30/06	4	N	
21	10/31/06	12	N	

CONTROL GROUP

Biomune® Field Safety Study (Subcutaneous Route)

NAME OF SITE & ADDRESS: _____ (B) (4)

PRODUCT: VAC Check Vac / Business 2 / Merial HVT / Merial SBT SERIAL NC (b)(4)

VACCINATION DATE: 10/10/06 VACCINATION ROUTE: Subcutaneous

SPECIES: CHICKEN AGE OF BIRDS: 1 day of age

FLOCK ID (B) (4) NUMBER OF BIRDS VACCINATED: 18600

DIRECTIONS

1. Fill form out in ink (pen)
2. Observe birds for a total of 21 days post vaccination, day 0 is day of vaccination.
3. Daily – record mortality
4. Daily – if birds are normal, record "N" under observations & comments
5. Daily – record your initials
6. **Please complete each line.** The USDA will not accept the use of "ditto" marks ("") or a line with an arrow.

Day post vaccination	DATE (m/d/y)	MORTALITY (No. Dead)	OBSERVATIONS & COMMENTS	INITIALS
0	10/10/06	0	Date of Vaccination	
1	10/11/06	8	N	
2	10/12/06	21	N	
3	10/13/06	20	N	
4	10/14/06	30	N	
5	10/15/06	35	N	
6	10/16/06	46	N	
7	10/17/06	47	N	
8	10/18/06	54	N	
9	10/19/06	38	N	
10	10/20/06	30	N	
11	10/21/06	27	N	
12	10/22/06	27	N	
13	10/23/06	19	N	
14	10/24/06	26	N	
15	10/25/06	13	N	
16	10/26/06	15	N	
17	10/27/06	21	N	
18	10/28/06	20	N	
19	10/29/06	13	N	
20	10/30/06	11	N	
21	10/31/06	12	N	

(B) 6

October 10, 2006

(B) 6

Biomune Company
8906 Rosehill Road
Lenexa, KS 66215

Dear (B) 6

The purpose of this letter is to inform you that I attended the in-ovo vaccination process for the recombinant HVT- NCD and Sbl vaccine trial at (b)(4) on September 26, 2006. At the time of my visit, I observed the vaccine mixing and administration, and I personally labeled the eggs after vaccination. There were no problems associated with the vaccination process.

Please contact me if you have any questions.

Sincerely,

(B) 6

October 10, 2006

(B) 6

Biomune Vaccines
8906 Rosehill Road
Lenexa, KS 66215

Dear (B) 6

The purpose of this letter is to inform you that on September 28, 2006, I was present for the hatching and placement of chicks vaccinated with the recombinant HVT-NCD and Sb1 at (b)(4) Chicks looked normal and healthy on the day of hatch and placement on the grow farm was uneventful.

Please contact me if you have any questions.

Sincerely,

(B) 6

October 18, 2006

(B) 6

Biomune Company

Dear (B) 6

On October 18, 2006; (b)(4) was visited with the purpose to evaluate chicks vaccinated at the hatchery subcutaneously with Newcastle Disease-Marek's Disease Vaccine, Serotypes 2 & 3, Live Virus, Live Marek's Disease Vector (rHVT/NDV) vaccine. During the observation period, 13 days post vaccination, none of the groups, vaccinated and controls, showed evidence of adverse reactions and no clinical signs of Marek's or Newcastle Disease were observed.

If you have any question, please contact me.

Sincerely,

(B) 6

October 30, 2006

(B) 6

**Biomune Vaccines
8906 Rosehill Road
Lenexa., KS 66215**

Dear] (B) 6

The purpose of this letter is to inform you that on October 12, 2006, I inspected the birds vaccinated with the recombinant HVT-NCD and Sb1 vaccine a (b)(4)
(b)(4) On the day of my visit, the birds were exactly 2 weeks old and looked normal and healthy. No signs of any problems were noted on that day or in the flock records.

Please contact me if you have any questions.

Sincerely,

(B) 6
