

U.S. DEPARTMENT OF AGRICULTURE

SPEED MEMO

PART NUMBER

DATE

TO

Biometrics, Poultry, Aerobes

1

8-29-00

SUBJECT

Ft. Hodge (112)

code 1901.00

FROM

M. Cox

S. typhimurium efficacy proposal

MESSAGE (WRITE CONCISE MESSAGE SIGN AND FORWARD PARTS 1 AND 3 TO ADDRESSEE RETAIN PART 2.)

OK, open for comments. Typical efficacy proposal from 112 - this for S. typhimurium avo-A. I would like to see testing of the

(b)(4)

(b)(4)

Otherwise the

isolation procedures, etc seems OK to me. They only intend to look for

(b)(4)

of short to me - what do you all think? by other comments?

Thanks - MJC

SIGNATURE

REPLY (USE THIS SPACE FOR REPLY SIGN AND DATE. RETURN PART 3 TO SENDER. RETAIN PART 1.)

SIGNATURE

DATE

Center for Veterinary Biologics

USDA, APHIS, VS, CVB-LPD, Biometrics

Memorandum

To Michel Carr
From David Siev
Date 8/29/00
Subject Ft. Dodge Protocol
Efficacy of *Salmonella typhimurium* aroA live vaccine
against *Salmonella enteritidis* challenge

According to the protocol. 

(b)(4) 

Reports of at least two similar studies have been submitted for this product. The protocol should refer to all earlier work and describe the need for the current proposal in the context of the earlier work. Subsequent submissions should reference the earlier data, so that they may be analyzed and evaluated together, if appropriate.



FORT DODGE ANIMAL HEALTH
DIVISION OF AMERICAN HOME PRODUCTS CORPORATION

MAILING ADDRESS:
P.O. BOX 518
FORT DODGE, IOWA 50501

2000 AUG 29 AM 9 43

DELIVERY ADDRESS:
800 5TH STREET N.W.
FORT DODGE, IOWA 50501

TELEPHONE: 515-955-4600
FAX: 515-955-9189

August 28, 2000

Dr. Michel Carr
Staff Microbiologist
USDA APHIS VS
CVB-LPD
510 South 17th Street
Suite 104
Ames, IA 50010

RE: VS Code 19C1.00, Salmonella Typhimurium Vaccine, Live Bacteria
(UNLICENSED)

Dear Dr. Carr:

Enclosed, please find the protocol entitled "Efficacy of a Salmonella typhimurium aroA⁻ Live Vaccine in Layers Against Salmonella enteritidis Challenge".

Approval of the enclosed protocol is respectfully requested.



Global Bio. R&D Support

bj/0811 effy of styph against SE.doc
9505

CL
16351

PROTOCOL TITLE:

Efficacy of a *Salmonella typhimurium aroA*-Live Vaccine in Layers Against
Salmonella enteritidis Challenge

VS Code 19C1.00

August 28, 2000

U.S. Veterinary License No. 112
Fort Dodge Animal Health
Fort Dodge, Iowa
USA

16350^{el}

CONTENTS:

- 1.0 STATEMENTS OF APPROVAL AND COMPLIANCE
- 2.0 GENERAL INFORMATION
 - 2.1 Title
 - 2.2 Protocol No.
 - 2.3 Study Support
- 3.0 INTRODUCTION
 - 3.1 Background
 - 3.2 Objective
- 4.0 MATERIALS AND METHODS
 - 4.1 Event Log
 - 4.2 Animal Selection
 - 4.3 Test Vaccine
 - 4.4 Experimental Design
 - 4.5 Vaccination
 - 4.6 Challenge and Observation Procedure
 - 4.7 Sample Collection and Testing
 - 4.8 Data Analysis
- APPENDIX 1 Environmental Monitoring: Methods
- APPENDIX 2 Vaccine Preparation Form
- APPENDIX 3 Vaccination Record Form
- APPENDIX 4 Post-Vaccination Observation Form
- APPENDIX 5 Post-Challenge Observation Form
- APPENDIX 6 Sample Collection and Disposition Form

1.0 STATEMENTS OF APPROVAL AND COMPLIANCE

We have reviewed this protocol and agree that it accurately reflects the proposed study, Efficacy of a *Salmonella typhimurium aroA*- Live Vaccine in Layers Against *Salmonella enteritidis* Challenge. Amendments or deviations to this protocol will be approved in writing by undersigned and documented with explanation(s) in the final report as protocol amendments or deviations.

(b)(6)



2.0 GENERAL INFORMATION

2.1 **Title:** Efficacy of a *Salmonella typhimurium aroA*- Live Vaccine in Layers Against *Salmonella enteritidis* Challenge.

2.2 **Protocol No:** B-393-00-A3

2.3 **Study Support:**

2.3.1 Trial Site

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.2 Study Sponsor

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.3 (b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.4 (b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.5 Veterinary Support

Veterinary Services
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.6 Laboratory Support

Biological R&D Department
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.0 INTRODUCTION

3.1 Background

Only a few strains of *Salmonella* cause severe disease in poultry. Those strains that are known to cause disease, such as *Salmonella pullorum* and *Salmonella gallinarum*, are serologically screened for by poultry producers. If flocks are found to be positive, the practice in the United States is eradication. Most *Salmonella* species isolated from poultry have little or no effect on the overall health of the bird. Rather, the biggest concern is one of public health. *Salmonella* food poisoning is a major health issue throughout the world. By effectively reducing the *Salmonella* bioburden found in poultry, a significant reduction in potential food poisoning is achieved, both from eggs and meat.

Due to the public health significance of *Salmonella enteritidis* (SE), protection against SE by a live vaccine is essential. An apathogenic strain of *Salmonella typhimurium* containing an aromatic mutation (*aroA*-) has been developed for the purpose of protecting chickens against disease caused by pathogenic strains of SE.

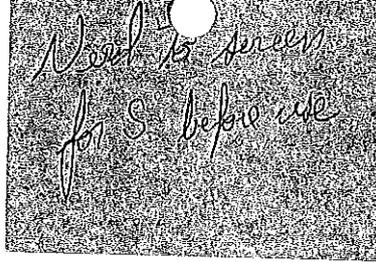
3.2 Objective

To evaluate the efficacy of an apathogenic *aroA*- strain of *Salmonella typhimurium* vaccine in the reduction of SE colonization of the internal organs, including the reproductive tract, and intestines.

4.0 MATERIAL AND METHODS

4.1 Event Log

| Proposed Date | Bird Age | Procedure |
|---------------|-----------------|--------------------|
| 19 Sep 00 | 12 Weeks of Age | First Vaccination |
| 17 Oct 00 | 16 Weeks of Age | Second Vaccination |
| 14 Nov 00 | 20 Weeks of Age | Challenge |
| 21 Nov 00 | 21 Weeks of Age | Necropsy |



4.2 Animal Selection

4.2.1 Test Animals

Type: SPF Pullets
 Number: 90 (Chickens about 8 to 12 weeks of age will be purchased.)
 Sex: Female
 Identification: Controls and vaccinates will be housed in separate isolation units by groups for the duration of the study. Cage cards on the units will denote groups. All the birds will be wing-banded prior to the start of the study.
 Source: SPAFAS or equivalent

4.2.2 Housing and Care of Animals

All the chickens will be reared in isolation units at Fort Dodge Animal Health, Fort Dodge, Iowa until the completion of the study. Any ill-thriven chickens will be excluded.

All the chickens will be under veterinary care and will be fed a standard antibiotic-free commercial feed, with feed and water available *ad libitum*. Each batch of feed and the source of drinking water will be screened for the presence of *Salmonella* contamination as described in Appendix 1.

4.3 Test Vaccine

4.3.1 Composition of Vaccine

The lyophilized experimental vaccine consisted of *Salmonella typhimurium* strain STM-1 (*aroA*-)^{(b)(4)}

4.3.2 Source of Vaccine

Vaccine for use in this study was prepared by the Biological R&D Department, Fort Dodge Animal Health, Fort Dodge, Iowa.

4.3.3 Shipment of Samples

No shipping of vaccine samples will be performed.

4.3.4 Lot Number

Salmonella typhimurium AWC 591 [REDACTED]
Lot# 1108-54-082997

4.3.5 Vaccine Testing

Vaccine testing was conducted by the QC Department, Fort Dodge Animal Health, Fort Dodge, Iowa in accordance with the Outline of Production, VS Code 19C1.00.

4.3.6 Storage

Vaccine will be stored between 2°C and 7°C before their use in the present study.

4.4 Experimental Design

| Test Group | Vaccination Route | Targeted Vaccination Dose (CFU/mL) (1mL/bird dose) | Challenge Route | No. of Birds |
|------------|----------------------|--|-----------------|--------------|
| 1 | CS ¹ /CS* | [REDACTED] | [REDACTED] | 30 |
| 2 | NA | None | [REDACTED] | 30 |
| 3 | NA | None | [REDACTED] | 30 |

¹ CS=Coarse Spray

*Second vaccination administered by coarse spray at four weeks post first vaccination.

Chickens will be randomized into groups by their wing-band numbers using a random number generator. Each treatment group will have 6 isolator units and each isolator unit will house 5 chickens. Cage cards on the isolator units will denote group.

4.5 Vaccination

Vaccination by coarse spray will be accomplished using a hand-held sprayer device. At 12 weeks of age the birds will be grouped together in one corner of the isolator, or in an appropriate smaller container, and spray applied to the heads of the birds until the calibrated total dosage had been given.

Salmonella typhimurium aroA- vaccine (Lot# 1108-54-082997) will be diluted to [REDACTED] with sterile phosphate buffered saline (PBS). Vaccine preparation will be documented using the Vaccine Preparation Form (Appendix 2).

Each bird will be vaccinated with a [REDACTED] of the *Salmonella typhimurium aroA-* vaccine. The vaccine administered will be titrated at the time of vaccination. The coarse spray procedure will be repeated 4 weeks later (16 weeks of age) to administer the vaccine two times.

Vaccination of the *Salmonella typhimurium aroA-* vaccine will be documented using the Vaccination Record Form (Appendix 3).

Birds will be observed daily and any clinical signs of disease will be recorded on the Post-Vaccination Observation Form (Appendix 4).

4.6 Challenge and Observation Procedure

At 20 weeks of age, birds in groups 1 and 2 will be challenged by [REDACTED] with a [REDACTED] dose of a [REDACTED] strain of SE (FDAH 1105-03). The SE challenge culture will be titrated at the time of challenge.

The birds in group 3 will remain unvaccinated, and unchallenged as negative controls.

Birds will be observed for 7 days and any clinical signs of disease will be recorded on the Post-Challenge Observation Form (Appendix 5). Any birds that die during the observation period will be necropsied as described in Section 4.7.

4.7 Sample Collection and Testing

At the end of the 7-day observation period all surviving chickens will be necropsied and examined for the presence of grossly visible lesions. Any organ displaying grossly visible lesions will be aseptically obtained from each bird and cultured separately. Necropsy findings will be documented using the Sample Collection and Disposition Form (Appendix 6).

If no gross lesions are seen, approximately one gram each of the ovary, oviduct (infundibulum, magnum, isthmus and uterus), spleen, kidney and liver will be aseptically obtained from each bird. Tissues obtained from each bird will be pooled (ovary and oviduct in pool 1; spleen, kidney and liver in pool 2). Pools will be placed in sterile whirl pak bags and then transferred to the lab. Ten

milliliters (10 mL) of BGTB (Brilliant Green Tetrathionate Broth) will be added to each bag and the contents will be macerated in a Stomacher blender for a period of 30 seconds. The bags will be incubated for 24 hours at $37^{\circ}\text{C}\pm 2$.

Also, a 10 mm sample of the duodenum (bottom of the duodenal loop below the pancreas), jejunum (region of yolk sac diverticulum), and the ileum (anterior to the ileocecal junction) will be aseptically collected from each bird, flushed internally and externally with sterile PBS (pH 7.2), pooled (pool 3), and processed similarly. The bags will be incubated for 24 hours at $42^{\circ}\text{C}\pm 2$.

After 24 hours incubation, a loopful from each whirl pak bag culture will be streaked onto brilliant green agar containing 100 $\mu\text{g}/\text{mL}$ nalidixic (BGAN) and onto xylose-lysine tergitol 4 agar (XLT4). Plates will be examined after 24 hours of incubation at $37^{\circ}\text{C}\pm 2$. If SE grows on either of the plate media the pool will be considered positive.

After incubating for 48 hours, 1 mL of the BGTB from each bag culture containing a pool that plated negative for SE on both BGAN and XLT4 will be transferred to a tube of fresh BGTB. The tubes will be incubated for 24 hours at $37^{\circ}\text{C}\pm 2$ and then streaked onto BGAN and XLT4. Plates will be examined after 24 hours of incubation at 37°C for the presence of SE. If SE grows on either of the plated media the pool will be considered SE positive.

Salmonella colonization of the ceca will be assessed as follows. Cecal contents will be collected from each bird (pool 4) and placed in a whirl pak bag. The net weight of the contents of each bag will be determined by weighing on a tare-adjusted balance. Sterile PBS (pH 7.2) will be added to each bag in the ratio of 100 mL PBS per gram of cecal contents. The contents will be mixed thoroughly and 0.1 mL of the suspension of cecal contents will be plated on duplicate plates of BGAN. The plates will be incubated for 24 hours at $37^{\circ}\text{C}\pm 2$. If SE grows on either of the plate media the pool will be considered positive.

An agglutination test with group D *Salmonella* antiserum will be performed on at least one colony per plate from all positive plates to confirm the presence of a group D *Salmonella* species.

Sample collection and lab culture results will be documented using the Sample Collection and Disposition Form (Appendix 6).

4.8 Data Analysis

The direct plate recovery rates will be compared between vaccinates and controls by Fisher's exact test. The level of significance will be set at $P < 0.05$.

4.8.1 Safety

The lack of post-vaccination pre-challenge clinical signs of salmonellosis (anorexia, emaciation, closed eyes, drooping wings, ruffled feathers, profuse watery diarrhea) and mortality attributed to *S. typhimurium*, will be an indication that the vaccine is safe.

4.8.2 Vaccine Efficacy

A statistically significant difference in the recovery of SE from the internal organ pools (liver, spleen, and kidney), reproductive pools (ovary and oviduct), intestinal pools or cecal contents between the vaccinated challenged chickens and non-vaccinated challenged control chickens will be considered satisfactory to demonstrate the vaccine efficacy.

4.8.3 Trial Validity

The trial will be considered valid if non-vaccinated non-challenged chickens remained negative for *Salmonella sp.* throughout the study.

APPENDIX 1

Environmental Monitoring: Methods

Culture Method for Feed and Drinking Water

Approximately 5 grams of feed or 5 mL of source water for drinking will be mixed with 45 mL of BGTB and incubated for 5 days at 42°C. After incubation 0.1 mL subcultures will be streaked onto BG (Brilliant Green) agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies will be subcultured onto blood agar and identified using either API strips or the Vitek system.

Environmental Monitoring

2x skim milk will be used to dampen sterile gauze prior to sampling of the following areas:

- Feed bins
- Water dishes
- Unit grate/flooring
- Unit -top and front
- Floor drain basket
- Hallway floor outside of isolation room

Each individual gauze/sample will be placed in a container with approximately 50 mL of 2x skim milk (transport media). One mL of milk/sample will be added to 9 mL of BGTB and incubated at 42°C for approximately 24 hours. After incubation 0.1 mL subcultures will be streaked onto XLT4 agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies will be subcultured onto blood agar and identified using either API strips or the Vitek system.

APPENDIX 2

Vaccine Preparation Form

Vaccine group: _____

Vaccine Lot No.: _____

Targeted titer: _____

Diluent used: _____

Dilution scheme:

Performed by/Date: _____

Observed by/Date: _____

APPENDIX 6

Sample Collection and Disposition Form

Vaccine group: _____

Bird ID: _____

Date of necropsy and sampling: _____

Necropsy performed by: _____

Samples taken by: _____

Samples taken: (Circle samples collected)

Pool 1 Pool 2 Pool 3 Pool 4 Others (describe)_____

Grossly visible lesions? Yes (describe below) No

Lab culture results: (Circle appropriate test result for each sample pool)

| | Pool 1 | Pool 2 | Pool 3 | Pool 4 | Others |
|---------------------------------|---------------|---------------|---------------|---------------|---------------|
| 1st isolation | + NG NT - | |
| 2nd isolation | + NG NT - | |
| Agglutination test | + NT - | + NT - | + NT - | + NT - | |

+ = Positive
 NG = No growth
 NT = Not tested
 - = Negative

Lab results recorded by: _____

Lab results verified by: _____

PENDING

November 29, 2000

(b)(6)

Fort Dodge Laboratories
800 5th Street, NW
P.O. Box 518
Fort Dodge, IA 50501

Dear (b)(6)

This is in response to your November 22, 1999, submission regarding your Salmonella Typhimurium Vaccine, Live Bacteria, APHIS Product Code 19C1.00 (unlicensed), consisting of the report entitled "Efficacy of a Salmonella aroA Live Vaccine in Day Old Chicks Against Salmonella Heidelberg Challenge."

The above report has been reviewed, and supports a [redacted]

Please note, however, that while this lowered MPD does demonstrate a distinguishable effect in reducing colonization by the challenge organism, this reduction is not as great as that provided by the previously used, higher dose.

The above report is therefore filed as acceptable for the purpose intended, supporting a [redacted]

If you have any questions regarding this letter, please contact me at your convenience.

Sincerely,

/s/Michel Y. Carr

Michel Y. Carr
Staff Microbiologist
Licensing and Policy Development

cc:
CVB-IC
CVB-L

APHIS:VS:CVB-LPD:MYCarr:cl:232-5785:11-29-00
G:\Prog\cvb-lpd\data\112\Correspondence:112_19C100_001122_LTR.lwp

FPA 11-30-00

DMG

TO

Biometrics

2

11/29/99

FROM

M. Carr

SUBJECT

Ft Hodge (112)

6de - 19C1.00

S. typhimurium - reduced titer efficiency vs.
S. Heidelberg

MESSAGE (WRITE CONCISE MESSAGE SIGN AND FORWARD PARTS 1 AND 3 TO ADDRESSEE RETAIN PART 2.)

Attached the original study (in green folder). No need to worry about the stuff about ^{(b)(4)} [REDACTED] I'll handle that with Harry.

Study itself looks fairly routine - did I miss anything odd?

Thanks -
MJC

SIGNATURE

REPLY (USE THIS SPACE FOR REPLY SIGN AND DATE. RETURN PART 3 TO SENDER. RETAIN PART 1.)

SIGNATURE

DATE

(DESTROY THIS PART 2 UPON RECEIPT OF REPLY)

FORM AD-311 (REV. 3/81)

SENDER'S COPY

Center for Veterinary Biologics

USDA, APHIS, VS, CVB-LPD, Biometrics

Memorandum

To Michel Carr
From David Siev
Date 3/23/00
Subject Ft. Dodge Report B-393-99-A7.R
Salmonella typhimurium aroA efficacy against *S. heidelberg* challenge

I have reviewed this as well as the two earlier efficacy studies. The data support a reduction in colonization by the three species in internal organs including intestines and ceca in birds vaccinated by the two dose regimen.

The current study was done to support



FORT DODGE ANIMAL HEALTH

DIVISION OF AMERICAN HOME PRODUCTS CORPORATION

MAILING ADDRESS:
P.O. BOX 518
FORT DODGE, IOWA 50501

'99 NOV 24 AM 10:09

DELIVERY ADDRESS:
800 5TH STREET N.W.
FORT DODGE, IOWA 50501

TELEPHONE: 515-955-4600
FAX: 515-955-9189
November 22, 1999

Dr. Michel Carr
Staff Microbiologist
Bacteriology and Biotechnology Section
USDA APHIS VS
CVB-LPD
510 South 17th Street
Suite 104
Ames, IA 50010

RE: VS Code 19C1.00, Salmonella Typhimurium Vaccine, Live Bacteria

Dear Dr. Carr:

Enclosed, please find the following report entitled "Efficacy of a Salmonella *aroA* Live Vaccine in Day Old Chicks Against Salmonella Heidelberg Challenge" (b)(4)

(b)(4)

A disk of data tables and statistical analysis is enclosed. Approval of the enclosed report is respectfully requested.

Consistent with our telephone calls on November 10, 1999 with you and Dr. Larry Elsken, we understand the true name of this product and its VS Code assignment are being reassessed at this time. Should you find any corrective action necessary, your earliest possible action on this would be appreciated. For your information, the (b)(4) On November 10th, we printed information about the (b)(4) from the internet. The printout is enclosed.

Sincerely,

(b)(6)

bj/1112 sthy eff rept shaidelberg.doc
8068

Enclosure

NOV 25 1999

11519

REPORT TITLE:

Efficacy Of A Salmonella Typhimurium aroA-Live Vaccine In Day Old Chicks Against
Salmonella Heidelberg Challenge

VS Code 19C1.00

November 22, 1999

U.S. Veterinary License No. 112
Fort Dodge Animal Health
Fort Dodge, Iowa
USA

CONTENTS:

1.0 STATEMENTS OF APPROVAL AND COMPLIANCE

2.0 STUDY SUMMARY

3.0 GENERAL INFORMATION

3.1 Title

3.2 Report No.

3.3 Study Support

4.0 INTRODUCTION

4.1 Background

4.2 Objective

4.3 Proposed Label Claim

5.0 MATERIALS AND METHODS

5.1 Event Log

5.2 Animal Selection

5.3 Test Vaccine

5.4 Experimental Design

5.5 Vaccination

5.6 Challenge and Observation Procedure

5.7 Sample Collection and Testing

5.8 Data Analysis

6.0 RESULTS

7.0 DISCUSSION

8.0 CONCLUSION

TABLE 1

APPENDIX 1 - Environmental Monitoring: Methods

ATTACHMENT 1 - QC Vaccine Testing Results

ATTACHMENT 2 - *Salmonella heidelberg* Organ Pool Reisolation Data

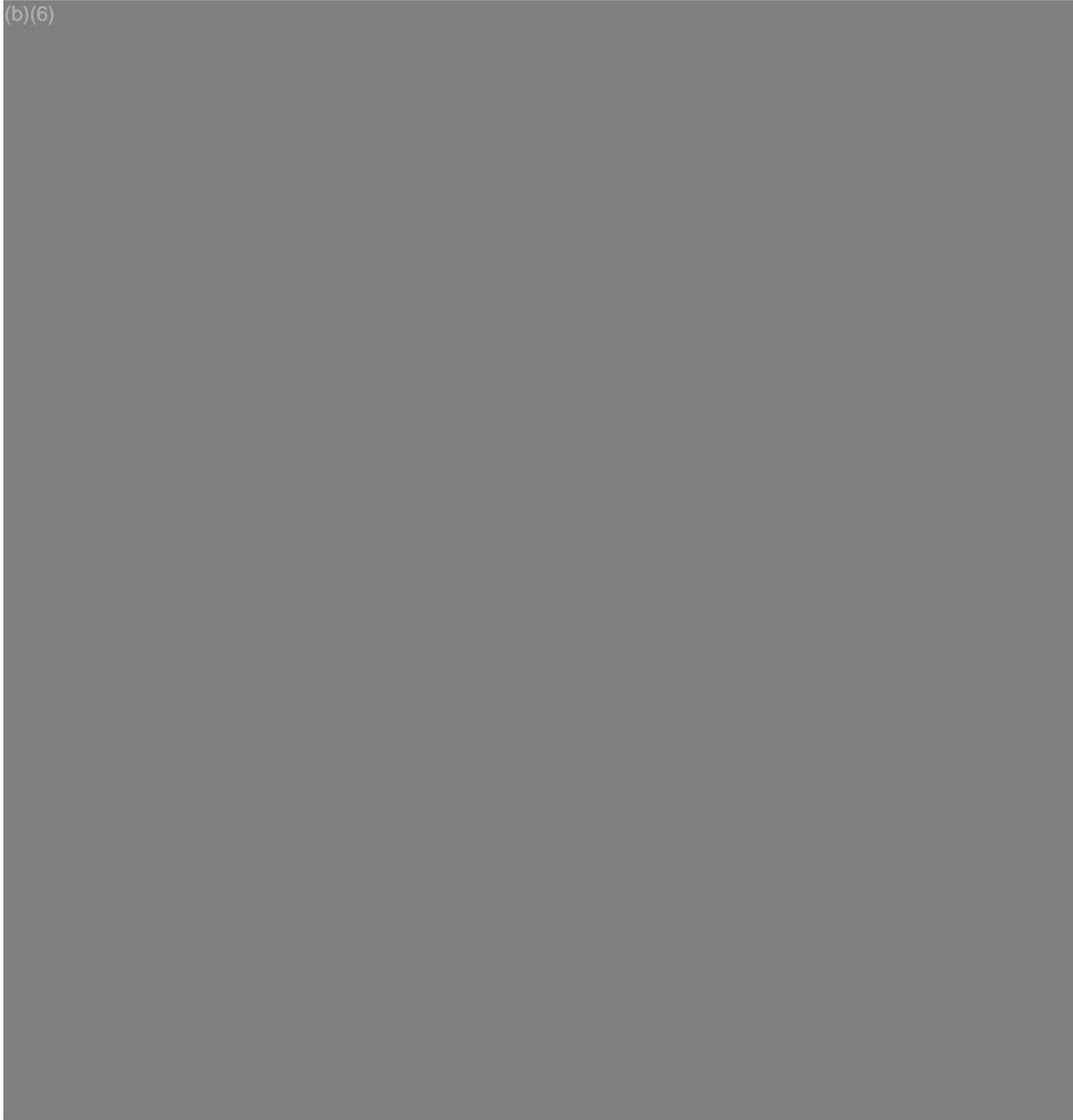
ATTACHMENT 3 - Agglutination Data

ATTACHMENT 4 - Post-Vaccination Observation

1.0 STATEMENTS OF APPROVAL AND COMPLIANCE

We have reviewed this report and agree that it accurately reflects the study, Efficacy of a *Salmonella typhimurium* aroA- live vaccine in day old chicks against *Salmonella heidelberg* challenge.

(b)(6)



2.0 STUDY SUMMARY

Vaccination of chicks with a live *Salmonella typhimurium* aroA- vaccine against a pathogenic *Salmonella heidelberg* challenge was investigated. One group of 37 chicks was vaccinated one time at one day of age by the coarse spray route with [REDACTED]. Another group of 40 chicks was vaccinated at one day of age by the coarse spray route with [REDACTED] and again at 2 weeks of age by the oral route [REDACTED]. All the birds, including the non-vaccinated challenged controls, were challenged [REDACTED] of a [REDACTED] of *Salmonella heidelberg* then necropsied at 7 days post challenge. The liver, spleen, kidney, duodenum, ileum, jejunum and cecal contents were collected and cultured from each bird.

The recovery of *Salmonella heidelberg* from internal organ pools (liver, spleen and kidney), intestinal pools (duodenum, ileum and jejunum) and cecal contents was statistically analyzed between vaccinated, challenged birds and non-vaccinated, challenged control birds. The data showed that there was a significant difference ($P < 0.05$) between the vaccinated and non-vaccinated challenged birds in recovery of *Salmonella heidelberg* from the internal organ pool. In addition, the results showed that birds vaccinated at one day of age and again at two weeks of age had a greater reduction of *Salmonella heidelberg* recovery from intestinal pool and ceca than birds vaccinated only once at one day of age.

The data also showed that the birds vaccinated at one day of age and again at two weeks of age had a significant reduction of *Salmonella heidelberg* recovery from intestinal pool and ceca compared to non-vaccinated challenged controls. However, the birds vaccinated only once at one day of age had no significant difference in recovery from intestinal pool and ceca compared to non-vaccinated challenged controls.

There was no mortality attributable to the administration of the vaccine during this study. Therefore, it was concluded that the FDAH *Salmonella typhimurium* aroA- live vaccine, [REDACTED] is safe and efficacious for the reduction of internal organ, intestinal and cecal colonization of *Salmonella heidelberg* in chickens when vaccine is administered at one day of age and again at two weeks of age.

Based on the results from all efficacy studies (including the present study) submitted to USDA, the [REDACTED]. At this level the vaccine has been shown to be an aid in the reduction of *Salmonella enteritidis*, *Salmonella heidelberg* and *Salmonella typhimurium* colonization of the internal organs, including the intestines and ceca.

3.0 GENERAL INFORMATION

3.1 **Title:** Efficacy of a *Salmonella typhimurium* aroA- live vaccine in day old chicks against *Salmonella heidelberg* challenge.

3.2 **Report No:** B-393-99-A7.R

3.3 **Study Support:**

3.3.1 **Trial Site**

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.2 **Study Sponsor**

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.3 (b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.4 (b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.5 **Veterinary Support**

Veterinary Services
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

4.0 INTRODUCTION

4.1 Background

Based on the efficacy reports previously submitted to USDA, the titer of FDAH *Salmonella typhimurium* aroA- live vaccine used in each of the efficacy studies varied. [REDACTED]

Based on the above results, USDA required the MPD for all 3 species (*Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella heidelberg*) be set at the [REDACTED]

4.2 Objective

To attempt to reduce the MPD for the FDAH *Salmonella typhimurium* aroA- live vaccine.

4.3 Proposed Label Claim

A modified live *Salmonella typhimurium* vaccine for day of age chicks as an aid in the reduction of *Salmonella enteritidis*, *Salmonella heidelberg* or *Salmonella typhimurium* colonization of the internal organs, including the intestines and ceca.

5.0 MATERIALS AND METHODS

5.1 Event Log

| Date | Bird Age | Procedure |
|-----------|----------------|--------------------|
| 24 Aug 99 | One Day of Age | First Vaccination |
| 07 Sep 99 | 2 Weeks of Age | Second Vaccination |
| 05 Oct 99 | 6 Weeks of Age | Challenge |
| 12 Oct 99 | 7 Weeks of Age | Necropsy |

Each bird was vaccinated with target [REDACTED] mL/bird) of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was titrated before vaccination.

Birds were observed daily and any clinical signs of disease were recorded.

5.5.3 Oral Route Vaccination

Chickens in group 2 were boosted at two weeks post first vaccination. The vaccine was administered orally in the drinking water as follows.

On the day of vaccination, the birds were deprived of drinking water for three hours. The vaccine was rehydrated, diluted as required, and mixed into a measured quantity of cool distilled water that was previously determined to be consumed in one hour by the birds so as to provide [REDACTED]. The vaccine-containing water was the sole source of drinking water. Once the vaccine-containing water was consumed, the drinker was removed from the isolator and the regular drinking water source was turned back on.

Each bird was vaccinated with [REDACTED] of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was also titrated before vaccination.

Birds were also observed daily and any clinical signs of disease were recorded.

5.6 Challenge and Observation Procedure

At 6 weeks of age, birds in groups 1, 2 and 3 were challenged by oral gavage with the [REDACTED] resistant strain of *Salmonella heidelberg* (FDAH 1220-42-061898). The *Salmonella heidelberg* culture was titrated before challenge.

The birds in group 4 remained unvaccinated, and unchallenged as negative controls.

Birds were observed daily and any clinical signs of disease were recorded.

5.7 Sample Collection and Testing

At necropsy, 1 gram each of the spleen, kidney, liver, and any organ displaying grossly visible lesions was aseptically obtained from each bird. Tissues from each individual bird were pooled and placed in individual sterile whirl pak bags. Ten milliliters (10 mL) of Brilliant Green Tetrathionate Broth (BGTB) were added to each bag and the contents were macerated in a Stomacher blender for a period of 30 seconds. The bags were incubated at 37°C. Any organs displaying grossly visible lesions were collected and cultured individually.

Also, a 10 mm sample of the duodenum (bottom of the duodenal loop below the pancreas), jejunum (region of yolk sac diverticulum), and the ileum (anterior to the ileocecal junction) were aseptically collected from each bird, flushed internally and externally with phosphate buffered saline, pooled, and processed similarly. The bags were incubated for 24 hours at 42°C.

After 24 hours incubation, a loopful from each whirl pak culture was streaked onto brilliant green agar containing 100 µg/mL nalidixic acid plus 15 µg/mL of novobiocin (BGAN) and onto xylose-lysine tergitol 4 agar (XLT4) containing 100 µg/mL nalidixic acid. Plates were examined after 24 hour of incubation at 37°C (42°C for intestinal pools). If *Salmonella heidelberg* grew on either of the plate media the pool was considered positive.

After incubating 48 hours, 1 mL of the BGTB from each bag culture containing a pool that plated negative for *Salmonella heidelberg* on both BGAN and XLT4 was transferred to a tube of fresh BGTB (9 mL). The tubes were incubated for 24 hours at 37°C (42°C for intestinal pools) and then streaked onto BGAN and XLT4. Plates were examined after 24 hours of incubation at 37°C for the presence of *Salmonella heidelberg*. If *Salmonella heidelberg* grew on either of the plated mediums the pool was considered *Salmonella heidelberg* positive.

An agglutination test with group B *Salmonella* antiserum was performed on at least one colony per plate from all positive plates to confirm the presence of a group B *Salmonella* species.

The amount of *Salmonella* colonization of the ceca was assessed as follows. Approximately one gram of cecal content was aseptically weighed and placed in 100 mL of sterile PBS (pH 7.2). The contents were mixed thoroughly and 0.1 mL of the suspension was plated on duplicate plates of XLT4 containing nalidixic acid. The plates were incubated for 24 hours at 37°C and the resulting colonies counted.

5.8 Data Analysis

A Fisher's exact test was used to analyze the direct plate recovery rate between vaccinates and the controls at a significance level of $P < 0.05$.

6.4 Titration Results

The titer of *Salmonella typhimurium* aroA- vaccine administered by coarse spray route was [REDACTED]

The titer of *Salmonella heidelberg* challenge isolate (FDAH 1220-42-061898) [REDACTED]

6.5 Culture Results

The data in Table 1 (raw data is in Attachment 2) indicate that the *Salmonella typhimurium* aroA- vaccine administered once (coarse spray route at one day of age) or twice (coarse spray route at one day of age and then oral route at 2 weeks of age) both offered significant protection ($P < 0.05$) against *Salmonella heidelberg* colonization of the internal organs (liver, spleen and kidney) as compared to the non-vaccinated challenged controls

The data in Table 1 (raw data is in Attachment 2) also indicate that the *Salmonella typhimurium* aroA- vaccine offered significant protection ($P < 0.05$) from *Salmonella heidelberg* challenge strain colonization of the intestines and the ceca when birds were vaccinated twice. However, the *Salmonella typhimurium* aroA- vaccine offered no significant protection from *Salmonella heidelberg* challenge strain colonization of the intestines and the ceca when birds were vaccinated only once.

6.6 Agglutination Test

An agglutination test with group B *Salmonella* antiserum was performed to confirmed the presence of *Salmonella heidelberg*. The results in Attachment 3 confirmed the results obtained from the cultures. Every colony tested was positive for group B *Salmonella*.

7.0 DISCUSSION

Reisolation results indicate that the live *Salmonella typhimurium* vaccine was capable of reducing internal organ, intestinal and cecal colonization following challenge with a pathogenic *Salmonella heidelberg* isolate when administered twice (Table 1).

There was no mortality in this trial attributed to the FDAH *Salmonella typhimurium* aroA- live vaccine, indicating that it was safe for day of age birds. The result indicated the FDAH *Salmonella typhimurium* aroA- live vaccine to be safe for coarse spray and oral administration.

Table 1

Efficacy of Live Salmonella typhimurium aroA- Vaccine in Preventing Colonization by Salmonella heidelberg: Individual Organ Pool Data

| Group No. | Vaccination Route | Challenge Route | No. <i>S. heidelberg</i> Positive / No. Cultured | | |
|-----------|-------------------|-----------------|--|--------------------|-----------------------------|
| | | | Organ Pool ³ | Intestine Pool | Cecal Contents ¹ |
| 1 | CS ² | (b)(4) | 15/37 ^a | 34/37 ^b | 34/37 ^b |
| 2 | CS/Oral | (b)(4) | 8/40 ^a | 27/40 ^a | 27/40 ^a |
| 3 | None ⁴ | (b)(4) | 29/30 ^b | 28/30 ^b | 30/30 ^b |
| 4 | None | (b)(4) | 0/20 | 0/20 | 0/20 |

¹ No enrichment = Direct plate culture

² CS = Coarse Spray

³ Values within the same column followed by different letters are significantly different (Fisher's exact test: $P < 0.05$).

⁴ Groups 3 & 4 are the positive and negative control groups.

APPENDIX 1

Environmental Monitoring: Methods

Culture Method for Feed and Drinking Water

Approximately 5 grams of feed or 5 mL of source water for drinking were mixed with 45 mL of BGTB and incubated for 5 days at 42°C. After incubation 0.1 mL subcultures were streaked onto BG (Brilliant Green) agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

Environmental Monitoring

2x skim milk was used to dampen sterile gauze prior to sampling of the following areas:

- Feed bins
- Water dishes
- Isolator grate/flooring
- Isolator - top and front
- Floor drain basket
- Hallway floor outside of isolation room

Each individual gauze/sample was placed in a container with approximately 50 mL of 2x skim milk (transport media). One mL of milk/sample was added to 9 mL of BGTB and incubated at 42°C for approximately 24 hours. After incubation 0.1 mL subcultures were streaked onto XLT4 agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

ORDER#: 34791

MATERIAL :

S TYPHIMURUM AWC 591

MATERIAL#:

B393 R2415

ASPECT: ---

SECTION: BRD REQUESTED BY: (b)(6)

DATE LOGGED: 08/29/97 DATE SCHEDULED: 09/02/97

LOT/SERIAL#:

LOT/SERIAL#: 1108-54-082997

SPECIAL INSTRUCTIONS: REHYDRATE TO 5ML W/PBS-LIVE MUTANT-AUTOClave

BLEND DATE: VOLUME:

2008 SUBMITTED

| REQUESTED TEST | RESULT | SPECS-COMMENTS |
|---|---|------------------------------|
| BT18.2 10/80 THIO 30-35C STERILITY | | |
| START DATE: 9/2/97 END DATE: 9/16/97 ANALYST: SLB BOOK#: STATUS: <u>S</u> | | |
| BT18.2 | 10/10 NO CONTAMINATION SEEN THIO 30-35C | 10/10 NO GROWTH THIO 30-35C |
| FB14.2 10/40 TSB 20-25C STERILITY | | |
| START DATE: 9/2/97 END DATE: 9/16/97 ANALYST: SLB BOOK#: STATUS: <u>S</u> | | |
| FB14.2 STER | 10/10 NO CONTAMINATION SEEN TSB 20-25C | 10/10 NO GROWTH TSB @ 20-25C |
| MO MOISTURE 029-CH0 | | |
| START DATE: 9/10/97 END DATE: 9/10/97 ANALYST: LD BOOK#: STATUS: <u>S</u> | | |
| MO MOISTURE | 0.795 | AVG: 0.8 |
| ID32 BACTERIAL IDENTITY-BIO (VIT | | |
| START DATE: 9/4/97 END DATE: 9/5/97 ANALYST: WD BOOK#: STATUS: <u>R</u> | | |
| ID32 VITEK | SALMONELLA SPECIES | |

ORDER STATUS:

APPROVED BY: (b)(6)

APPROVAL DATE: 10/08/97

10/8/97

THIS IS A TRUE COPY OF ORIGINAL DATA

NAME: (b)(6)

DATE: 10/5/97

ATTACHMENT 2

Salmonella heidelberg Organ Pool Reisolation Data

Isolation Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Tissue Isolation Data
 Group 1 Vaccinated (CS)/Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------|-----------------|---------------|-----------------|---------|----------------|------------------|----------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 2 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 3 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 4 | + | + | NT | NT | + | + | + | + | + | + | + |
| 5 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 6 | + | + | NT | NT | + | + | + | + | + | + | + |
| 7 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 8 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 9 | + | + | NT | NT | + | + | + | + | + | + | + |
| 10 | + | + | NT | NT | + | + | + | + | + | + | - |
| 11 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 12 | + | + | NT | NT | + | + | NG | NT | + | + | + |
| 13 | + | + | NT | NT | + | + | + | + | + | + | + |
| 14 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 15 | NG | NG | NG | NG | - | + | + | + | + | + | - |
| 16 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 17 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 18 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 19 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 20 | + | + | NT | NT | + | + | + | + | + | + | + |
| 21 | + | + | NT | NT | + | + | + | + | + | + | + |
| 22 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 23 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 24 | + | + | NT | NT | + | + | + | + | + | + | + |
| 25 | + | + | NT | NT | + | + | + | + | + | + | + |
| 26 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 28 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 29 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 30 | NG | NG | NG | NG | - | NG | NG | + | + | + | + |
| 31 | + | + | NT | NT | + | + | + | + | + | + | + |
| 32 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 33 | + | + | NT | NT | + | + | + | + | + | + | + |
| 34 | + | + | NT | NT | + | NG | NG | + | + | + | + |
| 35 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 36 | NG | NG | NG | NG | - | + | + | + | + | + | + |
| 37 | + | + | NT | NT | + | + | + | + | + | + | + |
| 38 | + | + | NT | NT | + | + | + | + | + | + | + |

NG = No Growth + = Positive
 NT = Not Tested - = Negative

pos
 1
 -
 2
 3
 4
 5
 6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26
 27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37

Isolation Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Tissue Isolation Data
 Group 2 Vaccinated (CS & Oral)/Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------|-----------------|---------------|-----------------|---------|----------------|------------------|----------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | NG | NG | NG | NG | - | NG | NG | + | + | + | - |
| 2 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 3 | NG | NG | NG | NG | - | NG | NG | + | + | + | + |
| 4 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 5 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 6 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 7 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 8 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 9 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + | - |
| 11 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 12 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 13 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 14 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 15 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 16 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 17 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 18 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 19 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 20 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 21 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 22 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 23 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 24 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 25 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 26 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 27 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 28 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 29 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 30 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 31 | + | NG | NT | NG | + | NG | NG | NG | NG | - | + |
| 32 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 33 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 34 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 35 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 36 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 37 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 38 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 39 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 40 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |

Pos.
 1
 2
 3
 4
 5
 6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26
 27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37
 38
 39
 40

Isolation Data

NG = No Growth + = Positive
 NT = Not Tested - = Negative

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Tissue Isolation Data
 Group 3 Non-Vaccinated/Non-Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------|-----------------|---------------|-----------------|---------|----------------|------------------|----------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 2 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 3 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 4 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 5 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 6 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 7 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 8 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 9 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 11 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 12 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 13 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 14 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 15 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 16 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 17 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 18 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 19 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 20 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 21 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 22 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 23 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 24 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 25 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 26 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 27 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 28 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 29 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 30 | + | + | NT | NT | + | + | + | NT | NT | + | + |

NG = No Growth + = Positive
 NT = Not Tested - = Negative

Isolation Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Tissue Isolation Data
 Group 4 Non-Vaccinated/Non-Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------|------------|---------------|------------|---------|---------------|-------------|---------------|-------------|---------|------------|
| | Organ Pool | Organ Pool | Organ Pool | Organ Pool | | Intest.Pool | Intest.Pool | Intest.Pool | Intest.Pool | | |
| | BG | XLT4 | BG | XLT4 | | BG | XLT4 | BG | XLT4 | | |
| 1 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 2 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 3 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 4 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 5 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 6 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 7 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 8 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 9 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 10 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 11 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 12 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 13 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 14 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 15 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 16 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 17 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 18 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 19 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 20 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |

NG = No Growth
 NT = Not Tested
 + = Positive
 - = Negative

0
 20 (A)

Agglutination Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Agglutination Data
 Group 1 Vaccinated (CS)/Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary |
|--------|---------------|-----------------|---------------|-----------------|---------|----------------|------------------|----------------|------------------|---------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | |
| 1 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 2 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 3 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 4 | + | + | NT | NT | + | + | + | NT | NT | + |
| 5 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 6 | + | + | NT | NT | + | + | + | NT | NT | + |
| 7 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 8 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 9 | + | + | NT | NT | + | + | + | NT | NT | + |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + |
| 11 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 12 | + | + | NT | NT | + | + | NT | NT | + | + |
| 13 | + | + | NT | NT | + | + | + | NT | NT | + |
| 14 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 15 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 16 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 17 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 18 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 19 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 20 | + | + | NT | NT | + | + | + | NT | NT | + |
| 21 | + | + | NT | NT | + | + | + | NT | NT | + |
| 22 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 23 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 24 | + | + | NT | NT | + | + | + | + | + | + |
| 25 | + | + | NT | NT | + | + | + | + | + | + |
| 26 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 28 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 29 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 30 | NT | NT | NT | NT | NT | NT | NT | + | + | + |
| 31 | + | + | NT | NT | + | + | + | + | + | + |
| 32 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 33 | + | + | NT | NT | + | + | + | + | + | + |
| 34 | + | + | NT | NT | + | NT | NT | + | + | + |
| 35 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 36 | NT | NT | NT | NT | NT | + | + | + | + | + |
| 37 | + | + | NT | NT | + | + | + | + | + | + |
| 38 | + | + | NT | NT | + | + | + | + | + | + |

positive

16-18 were secure

27

NT=No agglutination test was performed because there were no colonies recovered.
 "+" =Agglutination test was positive for a group B Salmonella.
 "-" =Agglutination test was negative for a group B Salmonella.

Agglutination Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
 Agglutination Data
 Group 2 Vaccinated (CS & Oral)/Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary |
|--------|---------------|-----------------|---------------|-----------------|---------|----------------|------------------|----------------|------------------|---------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | |
| 1 | NT | NT | NT | NT | NT | NT | NT | + | + | + |
| 2 | + | + | NT | NT | + | + | + | NT | NT | + |
| 3 | NT | NT | NT | NT | NT | NT | NT | + | + | + |
| 4 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 5 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 6 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 7 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 8 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 9 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + |
| 11 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 12 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 13 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 14 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 15 | + | + | NT | NT | + | + | + | NT | NT | + |
| 16 | + | + | NT | NT | + | NT | NT | NT | NT | NT |
| 17 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 18 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 19 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 20 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 21 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 22 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 23 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 24 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 25 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 26 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 27 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 28 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 29 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 30 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 31 | + | NT | NT | NT | + | NT | NT | NT | NT | NT |
| 32 | + | + | NT | NT | + | NT | NT | NT | NT | NT |
| 33 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 34 | + | + | NT | NT | + | + | + | NT | NT | + |
| 35 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 36 | + | + | NT | NT | + | + | + | NT | NT | + |
| 37 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 38 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 39 | NT | NT | NT | NT | NT | + | + | NT | NT | + |
| 40 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |

NT=No agglutination test was performed because there were no colonies recovered.

"+" =Agglutination test was positive for a group B Salmonella.

"-" =Agglutination test was negative for a group B Salmonella.

Agglutination Data

Efficacy of Salmonella Typhimurium Aro A- Live Vaccine
 In Day Old Chicks Against Salmonella heidelberg Challenge
 Protocol # B393-99-A7
Agglutination Data
Group 4 Non-Vaccinated/Non-Challenged

| Bird # | 1st Isolation | | 2nd Isolation | | Summary | 1st Isolation | | 2nd Isolation | | Summary |
|--------|------------------|--------------------|------------------|--------------------|---------|-------------------|---------------------|-------------------|---------------------|---------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | |
| 1 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 2 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 3 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 4 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 5 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 6 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 7 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 8 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 9 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 10 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 11 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 12 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 13 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 14 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 15 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 16 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 17 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 18 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 19 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 20 | NT | NT | NT | NT | NT | NT | NT | NT | NT | NT |

NT=No agglutination test was performed because there were no colonies recovered.

"+" =Agglutination test was positive for a group B Salmonella.

"-" =Agglutination test was negative for a group B Salmonella.

ATTACHMENT 4*Post-Vaccination Observation*

Chick #39 (Group 1), #27 (Group 1) and #40 (Group 1) died on 25 August 99, 30 August 99 and 01 September 99, respectively due to cage accident.

Post-vaccination Observation

Study No. _____ Vac. Date ^{1st} 8/24/99 _____ Isolator No. _____

| Group No. | Date | Observations (See Key below) | Initial |
|-----------|------------|---|---------|
| 1-4 | 8/25/99 | one dead due to cage accident | (b)(6) |
| | 8/26/99 | all Normal | |
| | 8/27/99 | ALL look good | |
| 1 | 8/30/99 | Iso #5 -1 ALL OTHERS GOOD | |
| | 8/31/99 | all look normal | |
| 1 | 9-1-99 | Iso #5 -1 ALL OTHERS GOOD | |
| | 9-2-99 | Normal | |
| | 9/3/99 | Normal except bird Iso #9 got foot stuck - block cause of circulation | |
| | 05 Sept 99 | normal - all groups | |
| | 06 Sept 99 | all OK | |
| | 07 Sept 99 | Normal | |
| | 09 Sept 99 | normal | |
| | 10 Sept 99 | normal | |
| | 13 Sept 99 | Normal | |
| | 14 Sept 99 | Normal | |
| | 15 Sept 99 | Normal | |
| | 16 Sept 99 | Normal | |
| | 17 Sept 99 | Normal | |
| | 19 Sept 99 | Normal | |
| | 20 Sept 99 | Normal | |
| | 21 Sept 99 | Normal | |
| | 22 Sept 99 | Normal | |
| | 23 Sept 99 | Normal | |
| | 24 Sept 99 | Normal | |
| | 26 Sept 99 | Normal | |
| | 27 Sept 99 | Normal | |
| | 28 Sept 99 | NORMAL | |

29 Sept 99 Normal

Score Key: R = Respiratory Signs W = Weakness
 L = Lameness P = Depression
 N = Normal D = Death

30 Sept 99 Normal
 01 Oct 99 Normal
 02 Oct 99 Normal
 04 Oct 99 Normal

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (Include Zip Code)
AMERICAN HOME PRODUCTS CORPORATION
Subsidiary: **Fort Dodge Laboratories, Inc.**
800 5th Street NW
PO Box 588
Fort Dodge, IA 50501

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

VEB (4)

3. ESTABLISHMENT LICENSE NO.

112 #1

4. DATE SUBMITTED

May 3, 2000

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Bacteria

6. PRODUCT CODE
19C1.00

7. 'X' IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES

4

13. TYPE OF SUBMISSION

New Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED

N/A

15. DATE OF PREVIOUS OUTLINE

May 14, 1999

16. COMMENTS

(b)(6)

EXCEPTIONS

See Attachment.

20. REVIEWED BY (Signature, Veterinary Biologics)

Michael J. Carr

DMG
MYCarr:jw

21. DATE RETURNED

December 19, 2000

11-29-00 mjc

JW

14290

MAY - 5 2000

8-8-00 L

This revised Outline of Production is filed with the following pen-and-ink changes:

(b)(4)



Case
112

Code
19C1.00

OUTLINE OF PRODUCTION ✓
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA ✓

VS Code No. 19C1.00 ✓

Complete Revision ✓

May 14, 1999 ✓

Supersedes All Previous Revisions ✓

American Home Products Corporation
through its producing subsidiaries: ✓

U.S. Veterinary License No. 112 ✓

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA ✓

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

I. COMPOSITION OF THE PRODUCT ✓

A. Microorganism Used ✓

1. Identification of the Microorganism, Including Strain or Isolate

[Redacted]

2. Isolation and Known Passage History, Including the Number of Passages Since Isolation in Each Type of Medium, Cell Culture and/or Animal

[Redacted]

3. Identification of the Master Seed

[Redacted]

4. Date of Notification from the CVB that the Master Seed is Eligible for Use in Production & Name and License Number of the Firm at the Time Notification is Received from CVB that the Master Seed is Eligible for Use in Production

[Redacted]

B. Source and Date of Accession

[Redacted]

C. Strain

Refer to Section 1. A. 1. - *referenced another section*

[Redacted]

D. Proportions of Each Strain

N/A

II. CULTURES

A. Identification

[Redacted]

Completely Revised.

for

JUL 15 1999

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 14, 1999

II.

A. *(handwritten)*



B. Virulence and Purity of Cultures



C. Composition and Reaction of Media Used for Seed and Production Cultures



D. Character, Size, and Shape of Containers Used for Growing Cultures ✓

1.



2.

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENFORCEMENT EXPENSES

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 14, 1999

I. [Redacted]

E. Storage Conditions of Seed Cultures ✓

[Redacted]

F. Method of Preparing Suspensions for Seeding or Inoculation ✓

[Redacted]

G. Technique of Inoculating

1.

[Redacted]

2.

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 14, 1999

II. H. Period of Time and Conditions for Incubation and Degree of Temperature Used

1.

2.

I. Character and Amount of Growth; Observation as to Contamination of Growth

J. Method of Attenuation

III. HARVEST

A. Handling and Preparation of Cultures and Media for Harvest

B. Minimum and Maximum Harvest Period from Inoculation

C. Technique of Harvest

added

D. Specifications for Acceptable Harvest Material

CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA ✓

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

III. E. Handling of Discarded Material Not Used in Production ✓

[Redacted]

F. Additional Pertinent Information

N/A ✓

IV. PREPARATION OF THE PRODUCT

A. Method of Inactivation, Attenuation, or Detoxification ✓

[Redacted]

B. Composition of Preservative, Adjuvant or Stabilizer, and Proportions Used

[Redacted]

C. Method and Degree of Concentration ✓

[Redacted]

D. Standardization of the Product

[Redacted]

E. Assembly of Units to Make a Serial - *continued*

[Redacted]

USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
U.S. DEPARTMENT OF AGRICULTURE

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA ✓

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

IV. E. 2.



3.

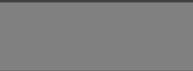
4.

F. Volume of Fill for Each Size Vial

| <u>Dose</u> | <u>Fill Volume (mL)</u> | | <u>Container</u> | <u>Stopper</u> |
|-------------|-------------------------|----------------|------------------|----------------|
| | <u>Minimum</u> | <u>Maximum</u> | | |



G. Method and Technique of Filling and Sealing of Final Containers



H. Desiccation and Moisture Control



1. Procedures ✓



2. Testing



JUL 15 1999

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA ✓

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

IV. I. Amount of Antigenic Material Per Dose(s) in Final Container

[Redacted]

V. TESTING

A. Purity

[Redacted]

B. Safety

[Redacted]

C. Potency

[Redacted]

[Redacted]

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

OUTLINE OF PRODUCTION ✓
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

V. D. Moisture, if Desiccated

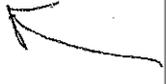
[Redacted]

E. Any Other Tests

Identity Test ✓

[Redacted]

What?



VI. POST PREPARATORY STEPS

A. Form and Size of Final Containers in Which the Product is to Be Distributed

[Redacted]

B. Collection, Storage and Submission of Representative Samples

[Redacted]

C. Expiration Date

[Redacted]

190



D. Use, Dosage and Route of Administration

[Redacted]

1.

[Redacted]

Pending approval



2/2/99

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

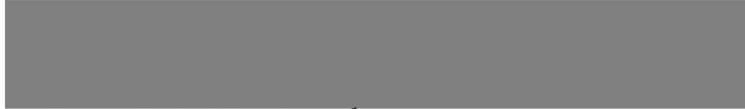
OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00 ✓

May 14, 1999 ✓

VI. D. 2. Dosage



3. Route of Administration ✓



E. Confidential Information

I.

II.

III.

IV.

V.



American Home Products Corporation
through its producing subsidiaries

(b)(6)



FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

APPENDIX I

SITE OF MANUFACTURE

(b)(4)



FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

(b)(6)



OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE BACTERIA

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 14, 1999

PAGE CHANGES

| Page and Section | Revision | Reason for Change |
|---------------------------|----------|--|
| Page 1 I.A. ✓ | (b)(4) | Per USDA requirements. |
| Page 1 II.A. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 1 I.C. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 2 II.C. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 2 II.D. a. 2, a ✓ | | Per USDA comments dated June 26, 1997. |
| Page 3 II.E. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 3 II.G.1.a. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 4 II.H.1. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 4 II.H.2. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 4 III.B. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 4 III.C. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 4 III.D. ✓ | | Typo. |
| Page 5 IV.B. ✓ | | FDL Mfg. recommendation. |
| Page 5 IV.C. ✓ | | FDL Mfg. recommendation. |
| Page 5 IV.E.1. ✓ | | Per USDA comments dated June 26, 1997. |
| Page 6 IV.E.2.&3 ✓ | | Per USDA comments dated June 26, 1997. |
| Page 6 IV.G. ✓ | | FDL Mfg. recommendation. |

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUN 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

(b)(6)

| | | |
|-------------------|----------|--|
| Page 6 IV.H.1. | ✓ (b)(4) | FDL Mfg. recommendation. |
| Page 7 IV.I. | ✓ | Based on the Immuno titer. |
| Page 7 V.B.1. | ✓ | To use the same method that is required for live NDV vaccines. |
| Page 7 V.C.1. | ✓ | Per USDA comments dated June 26, 1997. |
| Page 7 V.C.2. | ✓ | Per USDA requirements. |
| Page 8 V.E. | ✓ | Per USDA comments dated June 26, 1997. |
| Page 8 VI.A. | ✓ | Per Marketing recommendation. |
| Page 8 VI.C. | ✓ | Based on the real time stability data. |
| Page 8 VI.D. | ✓ | Per USDA comments dated June 26, 1997. |

6-2-99
L

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 15 1999

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT EXPRESSED

(b)(6)

VEB (4) pend
117

July 27, 1999

(b)(6)

800 5th Street, NW
P.O. Box 518
Fort Dodge, IA 50501

Dear (b)(6)

This is in response to your March 25 and May 14, 1999, submissions for your Salmonella Typhimurium Vaccine, Live Bacteria, APHIS Product Code 19C1.00 (unlicensed). These submissions consisted of reports describing efficacy trials of this product in day old chicks when challenged with *Salmonella typhimurium*, *S. heidelberg*, and *S. enteritidis* (2 reports).

These reports have been reviewed. The initial (March 25) study of chickens challenged with *S. enteritidis* demonstrated efficacy of the product only in internal organs for both one- and two-dose regimes. In the other 3 studies, statistical estimates demonstrate acceptable efficacy of this product in internal organs, intestines, and ceca when the two-dose regimen is used, but not with one dose alone.

It is noted that the titer of the vaccine used in each of these 4 studies varied. For a label claim against all 3 species (*S. typhimurium*, *S. heidelberg*, and *S. enteritidis*), these reports are acceptable in demonstrating efficacy of the product in internal organs, intestines, and ceca of chickens at (b)(4) when vaccinated once at day-of-age and again at 2 weeks of age.

If you have any questions regarding this letter, please contact me at your convenience.

Sincerely,

/s/ Michel Y. Carr

Michel Y. Carr
Staff Microbiologist
Licensing and Policy Development

cc:
CVB-IC
CVB-L

APHS:CVB-LPD:MYCarr:kb:232-5785:7-27-99
P:\mycarr\Ft. Dodge 112:19C1.00_0399_Efficacy - 3 challenges.lwp

JAE for LH

VEB(4) pend
117

July 27, 1999

(b)(6)

800 5th Street, NW
P.O. Box 518
Fort Dodge, IA 50501

Dear (b)(6)

This is in response to your March 25 and May 14, 1999, submissions for your Salmonella Typhimurium Vaccine, Live Bacteria, APHIS Product Code 19C1.00 (unlicensed). These submissions consisted of reports describing efficacy trials of this product in day old chicks when challenged with *Salmonella typhimurium*, *S. heidelberg*, and *S. enteritidis* (2 reports).

These reports have been reviewed. The initial (March 25) study of chickens challenged with *S. enteritidis* demonstrated efficacy of the product only in internal organs for both one- and two-dose regimes. In the other 3 studies, statistical estimates demonstrate acceptable efficacy of this product in internal organs, intestines, and ceca when the two-dose regimen is used, but not with one dose alone.

It is noted that the titer of the vaccine used in each of these 4 studies varied. For a label claim against all 3 species (*S. typhimurium*, *S. heidelberg*, and *S. enteritidis*), these reports are acceptable in demonstrating efficacy of the product in internal organs, intestines, and ceca of chickens at (b)(4) when vaccinated once at day-of-age and again at 2 weeks of age.

If you have any questions regarding this letter, please contact me at your convenience.

Sincerely,

/s/ Michel Y. Carr

Michel Y. Carr
Staff Microbiologist
Licensing and Policy Development

cc:
CVB-IC
CVB-L

ke
JAE for LH
APHIS:CVB-LPD:MYCarr:kb:232-5785:7-27-99
P:\mycarr\Ft. Dodge 112:19C1.00_0399_Efficacy - 3 challenges.lwp

Center for Veterinary Biologics

USDA, APHIS, VS, CVB-LPD, Biometrics

Memorandum

To Michel Carr
From David Siev
Date 6/22/99
Subject Ft. Dodge Report B-393-A2.R
Efficacy of *Salmonella typhimurium* vaccine against *Salmonella enteritidis* challenge
Code 19C1.00

This report makes no mention of the previous study. The background section should refer to the previous study and justify repeating it. I gather from your comments that the intent was to increase the *Salmonella enteritidis* potency to achieve acceptable efficacy. This objective should also have been clearly stated in the report.

Estimates of vaccine efficacy in preventing *Salmonella enteritidis* colonization are shown in the following tables (estimates are given only if a 95% confidence interval does not include zero).

One Dose Regimen

| | Study | | |
|-----------|-------|-----|------|
| | March | May | Both |
| organ | 44% | 54% | 49% |
| intestine | -- | -- | -- |
| ceca | -- | -- | -- |

Two Dose Regimen

| | Study | | |
|-----------|-------|-----|------|
| | March | May | Both |
| organ | 38% | 67% | 51% |
| intestine | -- | 25% | -- |
| ceca | -- | 55% | 41% |

No change is evident for the single dose regimen. For the two dose regimen, the results of the second study appear to indicate somewhat better efficacy. Should the studies be analyzed separately or combined in a stratified analysis? The answer is clear from neither a clinical nor a statistical perspective.

The statistical approach indicates possible heterogeneity in vaccine efficacy between the two studies (p values ranging from 0.01 to 0.05) for the two dose regimen. Results that are not homogeneous should not be indiscriminately combined.

A clinical perspective would consider the possibility of a dose-response relationship between the two studies. It is difficult to verify the presence of a dose-response relationship with only two dose levels, particularly since dose is completely confounded with trial. It is possible that the results of the second trial are due to increased vaccine potency, and, if so, vaccine efficacy may be estimated from this trial without considering the initial results (assuming that release potency will be based on the second trial). It is also possible that the discordant results are random or due to trial-specific factors, in which case the data of both trials would have to be considered in some fashion.

In any case, the intestine is the only one of the three sites for which it matters whether a separate or combined analysis is done. The combined data are insufficient to detect a difference between vaccinates and controls. The data of the second trial support a difference, although not a very large one (vaccine efficacy = 25%). Perhaps the proposed label claim could be appropriately modified.



FORT DODGE ANIMAL HEALTH

DIVISION OF AMERICAN HOME PRODUCTS CORPORATION

MAILING ADDRESS:
P.O. BOX 518
FORT DODGE, IOWA 50501

DELIVERY ADDRESS:
99 MAR 31 1999
800 5TH STREET, N.W.
FORT DODGE, IOWA 50501

TELEPHONE: 515-955-4600
FAX: 515-955-9183

March 25, 1999

Dr. Michel Carr
Staff Microbiologist
Bacteriology and Biotechnology Section
USDA APHIS VS
CVB-LPD
510 South 17th Street
Suite 104
Ames, IA 50010

RE: VS Code 19C1.00 - Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Typhimurium Challenge; Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Heidelberg Challenge and Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Enteritidis Challenge

Dear Dr. Carr:

Enclosed please find three reports entitled "Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Typhimurium Challenge; Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Heidelberg Challenge and Efficacy of a Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Enteritidis Challenge".

Approval of the enclosed report is respectfully requested.

(b)(6)



lg
Enclosures

REPORT TITLE:

Efficacy Of A Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against
Salmonella Enteritidis Challenge

VS Code 19C1.00

March 25, 1999

U.S. Veterinary License No. 112
Fort Dodge Animal Health
Fort Dodge, Iowa
USA

CONTENTS:

- 1.0 STATEMENTS OF APPROVAL AND COMPLIANCE
 - 2.0 STUDY SUMMARY
 - 3.0 GENERAL INFORMATION
 - 3.1 Title
 - 3.2 Report No.
 - 3.3 Study Support
 - 4.0 INTRODUCTION
 - 4.1 Background
 - 4.2 Objective
 - 4.3 Proposed Label Claim
 - 5.0 MATERIALS AND METHODS
 - 5.1 Event Log
 - 5.2 Animal Selection
 - 5.3 Test Vaccine
 - 5.4 Experimental Design
 - 5.5 Vaccination
 - 5.6 Challenge and Observation Procedure
 - 5.7 Sample Collection and Testing
 - 5.8 Data Analysis
 - 6.0 RESULTS
 - 7.0 DISCUSSION
 - 8.0 CONCLUSION
- TABLE 1 Efficacy of Live *Salmonella typhimurium* aroA- Vaccine in Preventing Colonization of *Salmonella enteritidis*: Individual Organ Pool Data
- APPENDIX 1 Environmental Monitoring: Methods

ATTACHMENT 1

QC Vaccine Testing Results

ATTACHMENT 2

Salmonella enteritidis Organ Pool Reisolation Data

ATTACHMENT 3

Agglutination Data

2.0 STUDY SUMMARY

Vaccination of chicks with a live *Salmonella typhimurium* aroA- vaccine against a pathogenic *Salmonella enteritidis* challenge was investigated. One group of 30 chicks was vaccinated one time at one day of age by the coarse spray route [REDACTED]

[REDACTED] Another group of 30 chicks was vaccinated at one day of age by the coarse spray route with [REDACTED] and again at 2 weeks of age by the oral route with [REDACTED]

[REDACTED] All the vaccinates, including the non-vaccinated challenged controls, were challenged [REDACTED] at 6 weeks of age [REDACTED] of a [REDACTED]

[REDACTED] strain of *Salmonella enteritidis* then necropsied at 7 days post challenge. The liver, spleen, kidney, duodenum, ileum, jejunum and cecal contents were collected and cultured from each bird.

The recovery of *Salmonella enteritidis* from internal organ pools (liver, spleen and kidney), intestinal pools (duodenum, ileum and jejunum) and cecal contents was statistically analyzed between vaccinated, challenged birds and non-vaccinated, challenged control birds. The data showed that there was a significant difference ($P < 0.05$) between the vaccinated and non-vaccinated challenged birds in recovery of *Salmonella enteritidis* from the internal organ pool. The results showed that birds vaccinated once (at one day of age) or twice (at one day of age and again at two weeks of age) did not have significant reduction of *Salmonella enteritidis* recovery from the intestinal pool or cecal contents compared to the non-vaccinated challenged controls. However, the titer of vaccine administered was below the planned titer on both occasions. Also, the challenge was more severe than intended. That combination of factors may have contributed to the failure of significant reduction of SE colonization of intestine and cecum.

There was no mortality attributable to the administration of the vaccine during this study. Therefore, it was concluded that the FDAH *Salmonella typhimurium* aroA- live vaccine, consisting of [REDACTED] is safe and efficacious for the reduction of internal organ colonization of chickens by *Salmonella enteritidis* when it is administered at one day of age and again at two weeks of age.

3.0 GENERAL INFORMATION

3.1 **Title:** Efficacy of a *Salmonella typhimurium* aroA- live vaccine in day old chicks against *Salmonella enteritidis* challenge.

3.2 **Report No:** B-393-98-A4.R

3.3 **Study Support:**

3.3.1 **Trial Site**

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.2 **Study Sponsor**

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.3

(b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.4

(b)(6)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.5 **Veterinary Support**

Veterinary Services
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.6 **Laboratory Support**

Biological R&D Department
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

5.0 MATERIAL AND METHODS

5.1 Event Log

Start Date: May 12, 1998
 Completion Date: July 17, 1998

| Date | Bird Age | Procedure |
|---------|----------------|------------------------------|
| May 12 | One Day of Age | First Vaccination |
| May 26 | 2 Weeks of Age | Second Vaccination (Group 2) |
| June 23 | 6 Weeks of Age | Challenge |
| June 30 | 7 Weeks of Age | Necropsy |

5.2 Animal Selection

5.2.1 Test Animals

Type: SPF White Leghorns
 Number: 90 birds at one day of age
 Identification: Controls and vaccinates were housed in separate isolators. Identified with cage cards to denote group.
 Source: Hy-Vac (Flock # B31)

5.2.2 Housing and Care of Animals

All the chickens were reared in isolators until the completion of the study. Standard infrared heating lamps were used as needed. Only healthy chickens were used for this study. Any ill-thriven chickens were excluded.

All the chickens were under veterinary care and were fed a standard antibiotic-free commercial diet, with food and water available *ad libitum*. Each batch of feed and the source of drinking water were screened for the presence of *Salmonella* contamination as described in Appendix 1.

5.3 Test Vaccine

5.3.1 Composition of Vaccine

The experimental vaccine was produced according to the VS Code 19C1.00 Outline of Production. The lyophilized experimental vaccine consisted of *Salmonella typhimurium* strain STM-1 (aroA-) a (b)(4)

(b)(4)

5.3.2 Source of Vaccine

Vaccine for use in this study was prepared by Biological R&D Department, Fort Dodge Animal Health, Fort Dodge, Iowa.

5.3.3 Shipment of Samples

No shipping of vaccine samples was performed.

5.3.4 Lot Number

Salmonella typhimurium AWC 591 [REDACTED]
Lot# 1108-54-082997

5.3.5 Vaccine Testing

Testing has been conducted on the prototype vaccine at Fort Dodge Animal Health, Fort Dodge, Iowa in accordance with the Outline of Production, VS Code 19C1.00. See Attachment 1 for QC vaccine testing results.

5.3.6 Storage

All the vaccines were stored between 2°C and 7°C before their use in the present study.

5.4 Experimental Design

| Test Group | Vaccination Route | Targeted Vaccination Dose (CFU/mL) | Challenge Route | No. of Birds |
|------------|-------------------|------------------------------------|-----------------|--------------|
| 1 | CS ¹ | [REDACTED] | [REDACTED] | 30 |
| 2 | CS/Oral* | [REDACTED] | [REDACTED] | 30 |
| 3 | Control | None | [REDACTED] | 20 |
| 4 | Control | None | [REDACTED] | 10 |

¹ CS=Coarse Spray

* Second vaccination administered by oral route at two weeks post first vaccination.

5.5 Vaccination

5.5.1 Calculation of Vaccine Dosage Levels

Experimental vaccine stock was titrated 5 times before vaccination. Dosages of the experimental vaccine were calculated based on the titers and appropriate dilutions made in sterile deionized water.

5.5.2 Coarse Spray Vaccination

Vaccination by coarse spray was accomplished using a hand-held sprayer device, previously calibrated as to average volume of liquid delivered as spray per unit of time at the desired standard settings. The birds were grouped together in one corner of the isolator, or in an appropriate smaller container, and spray applied to the heads of the birds until the calibrated total dosage had been given.

Each bird was vaccinated with target titer [REDACTED] (1 mL/bird) of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was titrated before vaccination.

Birds were observed daily and any clinical signs of disease were recorded.

5.5.3 Oral Route Vaccination

Chickens in group 2 were boosted at two weeks post-first vaccination. The vaccine was administered orally in the drinking water as described below. The vaccine administered was titrated 5 times.

On the day of vaccination, the birds were deprived of drinking water for three hours. The vaccine was rehydrated, diluted as required, and mixed into a measured quantity of cool distilled water that was previously determined to be consumed in one hour by the birds so as to provide 1.0 x [REDACTED]. The vaccine-containing water was the sole source of drinking water. Once the vaccine-containing water was consumed, the drinker was removed from the isolator and the regular drinking water source was turned back on.

Each bird was vaccinated with target titer [REDACTED] (1 mL/bird) of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was also titrated before vaccination.

Birds were also observed daily and any clinical signs of disease were recorded.

6.3 Post-challenge Observation

All the birds challenged were also observed daily. There was no mortality attributable to the administration of the SE challenge culture.

6.4 Titration Results

The titer of *Salmonella typhimurium* aroA- vaccine administered by coarse spray route was [REDACTED] and the titer of the same vaccine administered by oral route was [REDACTED]

The titer of SE challenge culture (FDAH 1105-03) was [REDACTED]

6.5 Culture Results

The data in Table 1 (raw data in Attachment 2) indicate that the FDAH *Salmonella typhimurium* aroA- vaccine administered once (coarse spray route at one day of age) or twice (coarse spray at one day of age and then oral route at two weeks of age) both offered significant protection ($P < 0.05$) from SE colonization of the internal organs (liver, spleen and kidney) compared to the non-vaccinated challenged controls. There was no significant difference in SE colonization between birds vaccinated once or twice with FDAH live *Salmonella typhimurium* aroA- vaccine.

The data in Table 1 (raw data in Attachment 2) also indicate that FDAH *Salmonella typhimurium* aroA- vaccine administered once or twice offered no significant protection against SE colonization of the intestines and the ceca.

6.6 Agglutination Test

An agglutination test with group D *Salmonella* antiserum was used to confirm the presence of a group D *Salmonella* species. The results in Attachment 3 confirmed the results obtained from the cultures. Every colony tested was positive for group D *Salmonella*.

7.0 DISCUSSION

Reisolation results (Table 1) indicate that FDAH *Salmonella typhimurium* aroA-vaccine was capable of reducing internal organ colonization following challenge with a pathogenic SE isolate.

Following challenge, SE colonization of the intestines and cecal contents was not significantly different between vaccinates and non-vaccinated, challenged controls (Table 1). The doses of vaccine actually given both at day of age and at two weeks of age were lower than targeted. In addition, the challenge was given at nearly twice the intended

titer. That combination of factors may have contributed to the failure of the vaccine to significantly reduce SE colonization of the intestines and cecum. Because the planned vaccine and challenge titer levels were not achieved in this study, the study will be repeated in order to demonstrate significant reduction in SE colonization of intestines and cecum.

No mortality was observed that was attributable to FDAH *Salmonella typhimurium* aroA-vaccine, indicating that it was safe for coarse spray and oral administration.

8.0 CONCLUSION

The FDAH live *Salmonella typhimurium* vaccine, formulated according to the Outline of Production for VS Code 19C1.00, with a titer of [REDACTED] is safe and efficacious for the reduction of SE colonization of the internal organs of chickens when it is administered one time at one day of age or at one day of age and again at two weeks of age.

Table 1

Efficacy of Salmonella typhimurium Vaccine in Preventing Colonization by Salmonella enteritidis: Individual Organ Pool Data

| Group No. | Vaccine | Challenge Route | No. SE Positive / No. Cultured | | |
|-----------|-----------------------|-----------------|--------------------------------|--------------------|-----------------------------|
| | | | Organ Pool ³ | Intestine Pool | Cecal Contents ¹ |
| 1 | CS | (b)(4) | 15/27 ^a | 22/27 ^a | 24/27 ^a |
| 2 | CS ² /Oral | (b)(4) | 18/29 ^a | 27/29 ^a | 29/29 ^a |
| 3 | None ⁴ | (b)(4) | 20/20 ^b | 18/20 ^a | 19/20 ^a |
| 4 | None | (b)(4) | 0/8 | 0/8 | 0/8 |

¹ No enrichment = Direct plate culture

² CS=Coarse Spray

³ Values within the same column followed by different letters are significantly different (Fisher's exact test: $P < 0.05$).

⁴ Please note that groups 3 & 4 are the positive and negative control groups.

APPENDIX 1

Environmental Monitoring: Methods

Culture Method for Feed and Drinking Water

Approximately 5 grams of feed or 5 mL of source water for drinking were mixed with 45 mL of BGTB and incubated for 5 days at 42°C. After incubation 0.1 mL subcultures were streaked onto BG (Brilliant Green) agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

Environmental Monitoring

2x skim milk was used to dampen sterile gauze prior to sampling of the following areas:

- Feed bins
- Water dishes
- Isolator grate/flooring
- Isolator - top and front
- Floor drain basket
- Hallway floor outside of isolation room

Each individual gauze/sample was placed in a container with approximately 50 mL of 2x skim milk (transport media). One mL of milk/sample was added to 9 mL of BGTB and incubated at 42°C for approximately 24 hours. After incubation 0.1 mL subcultures were be streaked onto XLT4 agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

ATTACHMENT 1

QC Vaccine Testing Results

FINAL REPORT

ORDER#: 34791
 MATERIAL : S TYPHIMURTIUM AWC 591 MATERIAL#: B393 R2415
 ASPECT: ---
 SECTION: BRD REQUESTED BY: MALLINGER
 DATE LOGGED: 08/29/97 DATE SCHEDULED: 09/02/97
 LOT/SERIAL#: 1108-54-082997
 SPECIAL INSTRUCTIONS: REHYDRATE TO 5ML W/PBS-LIVE MUTANT-AUTOClave
 BLEND DATE: VOLUME:

2008 SUBMITTED

| REQUESTED TEST | RESULT | SPECS-COMMENTS |
|---|--|------------------------------|
| BT18.2 10/80 THIO 30-35C STERILITY START DATE: 9/2/97 END DATE: 9/16/97 ANALYST: SLB BOOK#: STATUS: <u>S</u> | BT18.2 10/10 NO CONTAMINATION SEEN THIO 30-35C | 10/10 NO GROWTH THIO 30-35C |
| FB14.2 10/40 TSB 20-25C STERILITY START DATE: 9/2/97 END DATE: 9/16/97 ANALYST: SLB BOOK#: STATUS: <u>S</u> | FB14.2 STER 10/10 NO CONTAMINATION SEEN TSB 20-25C | 10/10 NO GROWTH TSB @ 20-25C |
| MO MOISTURE 029-CHO START DATE: 9/10/97 END DATE: 9/10/97 ANALYST: LD BOOK#: STATUS: <u>S</u> | MO MOISTURE 0.795 | AVG: 0.8 |
| ID32 BACTERIAL IDENTITY-BIO (VIT) START DATE: 9/4/97 END DATE: 9/5/97 ANALYST: WD BOOK#: STATUS: <u>R</u> | ID32 VITEK SALMONELLA SPECIES | |

ORDER STATUS: P
 APPROVED BY: (b)(6)
 APPROVAL DATE: 10/08/97

10/8/97

THIS IS A TRUE COPY OF ORIGINAL DATA

NAME: (b)(6)
 DATE: 10/5/97

ATTACHMENT 2

Salmonella enteritidis Organ Pool Reisolation Data

Isolation Data

Efficacy of Salmonella Typhimurium Aro A⁺ Live Vaccine
 In Day Old Chicks Against Salmonella enteritidis Challenge
 Protocol # B393-98-A4
 Tissue Isolation Data
 Group 1 Vaccinated (CS) / Challenged

| Bird # | 1 st Isolation | | 2 nd Isolation | | Summary | 1 st Isolation | | 2 nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------------------|-----------------|---------------------------|-----------------|---------|---------------------------|------------------|---------------------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 2 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 3 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 4 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 5 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 6 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 7 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 8 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 9 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 11 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 12 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 13 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 14 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 15 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 16 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 17 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 18 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 19 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 20 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | + |
| 21 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 22 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 23 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 24 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 25 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 26 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 27 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |

NG=No Growth "+"=Positive
 NT=Not Tested "-"=Negative

total 24 (+)
 27

Isolation Data

Efficacy of Salmonella Typhimurium Aro A⁻ Live Vaccine
 In Day Old Chicks Against Salmonella enteritidis Challenge
 Protocol # B393-98-A4
 Tissue Isolation Data
 Group 2 Vaccinated (CS&Oral) / Challenged

| Bird # | 1 st Isolation | | 2 nd Isolation | | Summary | 1 st Isolation | | 2 nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------------------|-----------------|---------------------------|-----------------|---------|---------------------------|------------------|---------------------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 2 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 3 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 4 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 5 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 6 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 7 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 8 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 9 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 10 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 11 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 12 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 13 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 14 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 15 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 16 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 17 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 18 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 19 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 20 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 21 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 22 | NG | NG | + | + | + | NG | NG | NG | NG | - | + |
| 23 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 24 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 25 | NG | NG | + | + | + | NG | NG | + | + | + | + |
| 26 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 27 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 28 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 29 | + | + | NT | NT | + | + | + | NT | NT | + | + |

NG=No Growth "+"=Positive
 NT=Not Tested "-"=Negative

Isolation Data

Efficacy of Salmonella Typhimurium Aro A Live Vaccine
 In Day Old Chicks Against Salmonella enteritidis Challenge
 Protocol # B393-98-A4
 Tissue Isolation Data
 Group 3 Non-Vaccinated / Challenged

| Bird # | 1 st Isolation | | 2 nd Isolation | | Summary | 1 st Isolation | | 2 nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------------------|-----------------|---------------------------|-----------------|---------|---------------------------|------------------|---------------------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 2 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 3 | NG | NG | + | + | + | NG | NG | NG | NG | - | + |
| 4 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 5 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 6 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 7 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 8 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 9 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 10 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 11 | NG | NG | + | NG | + | + | + | NT | NT | + | - |
| 12 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 13 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 14 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 15 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 16 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 17 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 18 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 19 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 20 | + | + | NT | NT | + | + | + | NT | NT | + | + |

NG=No Growth "+"=Positive
 NT=Not Tested "-"=Negative

total 20/20

Isolation Data

Efficacy of Salmonella Typhimurium Aro A⁻ Live Vaccine
 In Day Old Chicks Against Salmonella enteritidis Challenge
 Protocol # B393-98-A4
 Tissue Isolation Data
 Group 4 Non-Vaccinated / Non-Challenged

| Bird # | 1 st Isolation | | 2 nd Isolation | | Summary | 1 st Isolation | | 2 nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------------------|-----------------|---------------------------|-----------------|---------|---------------------------|------------------|---------------------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 2 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 3 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 4 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 5 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 6 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 7 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |
| 8 | NG | NG | NG | NG | 0 | NG | NG | NG | NG | 0 | 0 |

NG=No Growth "+"=Positive
 NT=Not Tested "-"=Negative

TO

Biometrics / D. Clough

1

6/9/99

FROM

M. Ken

SUBJECT

Ft Hodge (112) Prod. Code 19C1.00
(unlicensed)
S. Typhimurium - avo-A / Efficacy in chick

MESSAGE (WRITE CONCISE MESSAGE SIGN AND FORWARD PARTS 1 AND 3 TO ADDRESSEE RETAIN PART 2.)

This is their 2nd attempt at this with a

(b)(4)

Comments ?

Dave; the previous version is attached

MPK

SIGNATURE

REPLY (USE THIS SPACE FOR REPLY SIGN AND DATE. RETURN PART 3 TO SENDER. RETAIN PART 1.)

SIGNATURE

DATE

TO Biometrics / N. Clough

2

6/9/99

FROM M. Len

SUBJECT Ft Hodge (112) Prod. Code 19C1.00 (unlicensed) S. Typhimurium - avirulent / Efficiency in chick

MESSAGE (WRITE CONCISE MESSAGE SIGN AND FORWARD PARTS 1 AND 3 TO ADDRESSEE RETAIN PART 2.)

This is their 2nd attempt at this with a [redacted]

(b)(4) [Large redacted area]

SIGNATURE [Signature]

REPLY (USE THIS SPACE FOR REPLY SIGN AND DATE. RETURN PART 3 TO SENDER. RETAIN PART 1.)

The study appears to have been properly conducted, and the data support the conclusions.

Needs comments -

Biometrics

SIGNATURE N Clough (DESTROY THIS PART 2 UPON RECEIPT OF REPLY)

-18-99

PART NUMBER

DATE

To Tanner/Siew → Hyde/Taylor

1

4/5/99

SUBJECT

St Dodge (112) Code 19C1.00

FROM

M. Cox

S. typhimurium Pro-A live (unhoused)
= 3 efficacy studies

MESSAGE: WRITE CONCISE MESSAGE SIGN AND FORWARD PARTS 1 AND 2 TO ADDRESSEE RETAIN PART 3

(b)(4)

SIGNATURE

MJC

REPLY (USE THIS SPACE FOR REPLY SIGN AND DATE. RETURN PART 3 TO SENDER. RETAIN PART 1.)

SIGNATURE

DATE

Center for Veterinary Biologics

USDA, APHIS, VS, CVB-LPD, Biometrics

Memorandum

To Michel Carr
 From David Siev
 Date 4/19/99
 Subject Ft. Dodge Reports B-393-98-A4.R, B-393-98-A6.R, B-393-98-A8.R
 Efficacy of *Salmonella typhimurium* vaccine against three challenges
 Code 19C1.00

Groups of chicks either vaccinated by one of two regimens (coarse spray at one day, or coarse spray at one day followed by oral vaccination at two weeks) or held as unvaccinated controls were challenged at six weeks with one of three organisms (*S. enteritidis*, *S. typhimurium*, *S. heidelberg*).

The proposed label claim is "an aid in the reduction of ... colonization of the internal organs, including the intestines and ceca."

The table summarizes vaccine efficacy estimated from the data. The proposed claim is well supported only for *S. typhimurium* and *S. heidelberg*, and only with the two-dose regimen. There is only weak support for any claim regarding *S. enteritidis*.

| | | Vaccination Regimen | | | | |
|-----------------------|------------|---------------------|------------|----------|-----|-----|
| | | One dose | | Two dose | | |
| <i>S. enteritidis</i> | organ | 44% | organ | 38% | 67% | |
| | intestines | - | intestines | - | | 25% |
| | ceca | - | ceca | - | | 55% |
| <i>S. typhimurium</i> | organ | 88% | organ | 90% | | |
| | intestines | - | intestines | 65% | | |
| | ceca | - | ceca | 60% | | |
| <i>S. heidelberg</i> | organ | 39% | organ | 86% | | |
| | intestines | - | intestines | 49% | | |
| | ceca | - | ceca | 54% | | |

New study, 2 dose

Table. Vaccine Efficacy (estimates shown only where significantly different than zero at 95% confidence level).



FORT DODGE ANIMAL HEALTH
DIVISION OF AMERICAN HOME PRODUCTS CORPORATION

MAILING ADDRESS:
P.O. BOX 518
FORT DODGE, IOWA 50501

99 MAY 12 DELIVERY ADDRESS:
800 5TH STREET N.W.
FORT DODGE, IOWA 50501

TELEPHONE: 515-955-4600
FAX: 515-955-9183

May 14, 1999

Dr. Michel Carr
Staff Microbiologist
Bacteriology and Biotechnology Section
USDA APHIS VS
CVB-LPD
510 South 17th Street
Suite 104
Ames, IA 50010

RE: VS Code 19C1.00, Salmonella Typhimurium Vaccine, Live Bacteria

Dear Dr. Carr:

Enclosed please find the report entitled "Efficacy Of A Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against Salmonella Enteritidis Challenge".

Approval of the enclosed report is respectfully requested.

(b)(6)

lg/0512carr.doc
Enclosures

REPORT TITLE:

Efficacy Of A Salmonella Typhimurium Aro A-Live Vaccine In Day Old Chicks Against
Salmonella Enteritidis Challenge

VS Code 19C1.00

May 14, 1999

U.S. Veterinary License No. 112
Fort Dodge Animal Health
Fort Dodge, Iowa
USA

CONTENTS:

- 1.0 STATEMENTS OF APPROVAL AND COMPLIANCE
 - 2.0 STUDY SUMMARY
 - 3.0 GENERAL INFORMATION
 - 3.1 Title
 - 3.2 Report No.
 - 3.3 Study Support
 - 4.0 INTRODUCTION
 - 4.1 Background
 - 4.2 Objective
 - 4.3 Proposed Label Claim
 - 5.0 MATERIALS AND METHODS
 - 5.1 Event Log
 - 5.2 Animal Selection
 - 5.3 Test Vaccine
 - 5.4 Experimental Design
 - 5.5 Vaccination
 - 5.6 Challenge and Observation Procedure
 - 5.7 Sample Collection and Testing
 - 5.8 Data Analysis
 - 6.0 RESULTS
 - 7.0 DISCUSSION
 - 8.0 CONCLUSION
- TABLE 1 Efficacy of Live *Salmonella typhimurium* aroA- Vaccine in Preventing Colonization of *Salmonella enteritidis*: Individual Organ Pool Data
- APPENDIX 1 Environmental Monitoring: Methods

1.0 STATEMENTS OF APPROVAL AND COMPLIANCE

We have reviewed this report and agree that it accurately reflects the study, Efficacy of a *Salmonella typhimurium* aroA- live vaccine in day old chicks against *Salmonella enteritidis* challenge.

(b)(6)



| | |
|--------------|---|
| ATTACHMENT 1 | QC Vaccine Testing Results |
| ATTACHMENT 2 | Titration Data |
| ATTACHMENT 3 | <i>Salmonella enteritidis</i> Organ Pool Reisolation Data |
| ATTACHMENT 4 | Agglutination Data |

2.0 STUDY SUMMARY

Vaccination of chicks with a live *Salmonella typhimurium* aroA- vaccine against a pathogenic *Salmonella enteritidis* challenge was investigated. One group of 39 chicks was vaccinated one time at one day of age by the coarse spray route with [REDACTED]. Another group of 40 chicks was vaccinated at one day of age by the coarse spray route with [REDACTED] and again at 2 weeks of age by the oral route with [REDACTED]. All the vaccinates, including the non-vaccinated challenged controls, were challenged orally at 6 weeks of age with [REDACTED] of *Salmonella enteritidis* (FDAH 1105-03) then necropsied at 7 days post challenge. The liver, spleen, kidney, duodenum, ileum, jejunum and cecal contents were collected and cultured from each bird.

The recovery of *Salmonella enteritidis* from internal organ pools (liver, spleen and kidney), intestinal pools (duodenum, ileum and jejunum) and cecal contents was statistically analyzed comparing vaccinated, challenged birds and non-vaccinated, challenged control birds. The data showed that there was a significant difference ($P < 0.05$) between the vaccinated and non-vaccinated challenged birds in recovery of *Salmonella enteritidis* from the internal organ pool. In addition, the results showed that birds vaccinated at one day of age and again at two weeks of age had a significant reduction ($P < 0.05$) of *Salmonella enteritidis* recovery from intestinal pool and ceca compared to birds vaccinated only once at one day of age.

The data also showed that the birds vaccinated at one day of age and again at two weeks of age had a significant reduction ($P < 0.05$) of *Salmonella enteritidis* recovery from intestinal pool and ceca compared to non-vaccinated challenged controls. However, the birds vaccinated only once at one day of age had no significant difference in recovery from intestinal pool and ceca compared to non-vaccinated challenged controls.

There was no mortality attributable to the administration of the vaccine during this study. Therefore, it was concluded that the FDAH *Salmonella typhimurium* aroA- live vaccine, consisting of [REDACTED] is safe and efficacious for the reduction of internal organ, intestinal and cecal colonization of *Salmonella enteritidis* in chickens when it is administered at one day of age and again at two weeks of age.

3.0 GENERAL INFORMATION

3.1 Title: Efficacy of a *Salmonella typhimurium* aroA- live vaccine in day old chicks against *Salmonella enteritidis* challenge.

3.2 Report No: B-393-99-A2.R

3.3 Study Support:

3.3.1 Trial Site

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.2 Study Sponsor

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.3

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.4

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.5 Veterinary Support

Veterinary Services
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

3.3.6 Laboratory Support

Biological R&D Department
Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

4.0 INTRODUCTION

4.1 Background

Only a few strains of *Salmonella* cause severe disease in poultry. Those strains that are known to cause disease, such as *Salmonella pullorum* and *Salmonella gallinarum*, are serologically screened for by poultry producers. If flocks are found to be positive, the practice in the United States is eradication. Most *Salmonella* species isolated from poultry have little or no effect on the overall health of the bird. Rather, the biggest concern is one of public health. *Salmonella* food poisoning is a major health issue throughout the world. By effectively reducing the *Salmonella* bioburden found in poultry, a significant reduction in potential food poisoning is achieved, both from eggs and meat.

Traditional formalin-killed *Salmonella* bacterins are available commercially. These bacterins often provide only limited protection against *Salmonella* as they do not confer significant immunity in the intestinal tract, the normal site of infection. Attenuated live strains may be proven to be more promising as they may be able to temporarily colonize the intestine and stimulate both local and systemic immunity.

Due to the public health significance of *Salmonella enteritidis* (SE), cross protection against SE, *Salmonella typhimurium* and *Salmonella heidelberg* by a live vaccine is essential. An apathogenic strain of *Salmonella typhimurium* containing an aromatic mutation (aroA-) has been developed for the purpose of protecting chickens against disease caused by pathogenic strains of SE, *Salmonella typhimurium* and *Salmonella heidelberg*.

4.2 Objective

To evaluate the efficacy of an apathogenic aromatic mutation (aroA-) strain of *Salmonella typhimurium* vaccine in preventing infection following oral challenge with a pathogenic SE isolate.

4.3 Proposed Label Claim

A modified live *Salmonella typhimurium* vaccine for day of age chicks as an aid in the reduction of *Salmonella enteritidis*, *Salmonella heidelberg* or *Salmonella typhimurium* colonization of the internal organs, including the intestines and ceca.

5.0 MATERIAL AND METHODS

5.1 Event Log

Start Date: March 9, 1999
 Completion Date: May 4, 1999

| Date | Bird Age | Procedure |
|----------|----------------|------------------------------|
| March 9 | One Day of Age | First Vaccination |
| March 23 | 2 Weeks of Age | Second Vaccination (Group 2) |
| April 20 | 6 Weeks of Age | Challenge |
| April 27 | 7 Weeks of Age | Necropsy |

5.2 Animal Selection

5.2.1 Test Animals

Type: SPF White Leghorns
 Number: 119 birds at one day of age
 Identification: Controls and vaccinates were housed in separate isolators. Identified with cage cards to denote group.
 Source: Hy-Vac (Flock # RF1-7)

5.2.2 Housing and Care of Animals

All the chickens were reared in isolators until the completion of the study. Standard infrared heating lamps were used as needed. Only healthy chickens were used for this study. Any ill-thriven chickens were excluded.

All the chickens were under veterinary care and were fed a standard antibiotic-free commercial diet, with food and water available *ad libitum*. Each batch of feed and the source of drinking water were screened for the presence of *Salmonella* contamination as described in Appendix 1.

5.3 Test Vaccine

5.3.1 Composition of Vaccine

The experimental vaccine was produced according to the VS Code 19C1.00 Outline of Production. The lyophilized experimental vaccine consisted of *Salmonella typhimurium* strain STM-1 (aroA-) at passage

(b)(4)

5.3.2 Source of Vaccine

Vaccine for use in this study was prepared by the Biological R&D Department, Fort Dodge Animal Health, Fort Dodge, Iowa.

5.3.3 Shipment of Samples

No shipping of vaccine samples was performed.

5.3.4 Lot Number

Salmonella typhimurium AWC 591 (b)(4)
Lot# 1108-54-082997

5.3.5 Vaccine Testing

Testing was conducted on the prototype vaccine at Fort Dodge Animal Health, Fort Dodge, Iowa in accordance with the Outline of Production, VS Code 19C1.00. See Attachment 1 for QC vaccine testing results.

5.3.6 Storage

All the vaccines were stored between 2°C and 7°C before their use in the present study.

5.4 Experimental Design

| Test Group | Vaccination Route | Targeted Vaccination Dose (CFU/mL) | Challenge Route | No. of Birds |
|------------|-------------------|------------------------------------|-----------------|--------------|
| 1 | CS ¹ | | | 39 |
| 2 | CS/Oral* | | | 40 |
| 3 | Control | None | | 25 |
| 4 | Control | None | | 15 |

¹CS=Coarse Spray

* Second vaccination administered by the oral route at two weeks post first vaccination.

5.5 Vaccination

5.5.1 Calculation of Vaccine Dosage Levels

Experimental vaccine stock was titrated 5 times before vaccination. Dosages of the experimental vaccine were calculated based on the titers and appropriate dilutions made in sterile deionized water.

5.5.2 Coarse Spray (First) Vaccination

Vaccination by coarse spray was accomplished using a hand-held sprayer device, previously calibrated as to average volume of liquid delivered as spray per unit of time at the desired standard settings. The birds were grouped together in one corner of the isolator, or in an appropriate smaller container, and spray applied to the heads of the birds until the calibrated total dosage had been given.

Each bird was vaccinated with target titer [REDACTED] of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was titrated before vaccination.

Birds were observed daily and any clinical signs of disease were recorded.

5.5.3 Oral Route (Second) Vaccination

Chickens in group 2 were boosted at two weeks post first vaccination. The vaccine was administered orally in the drinking water as described below. The vaccine administered was titrated 5 times.

On the day of vaccination, the birds were deprived of drinking water for three hours. The vaccine was rehydrated, diluted as required, and mixed into a measured quantity of cool distilled water that was previously determined to be consumed in one hour by the birds so as to provide [REDACTED]. The vaccine-containing water was the sole source of drinking water. Once the vaccine-containing water was consumed, the drinker was removed from the isolator and the regular drinking water source was turned back on.

Each bird was vaccinated with target titer [REDACTED] (1 mL/bird) of the *Salmonella typhimurium* aroA- vaccine. The vaccine administered was also titrated before vaccination.

Birds were also observed daily and any clinical signs of disease were recorded.

5.6 Challenge and Observation Procedure

At 6 weeks of age, birds in groups 1, 2, and 3 were [REDACTED] with the target titer of [REDACTED] of SE (FDAH 1105-03). The SE culture was titrated before challenge.

The birds in group 4 remained unvaccinated, and unchallenged as negative controls.

Birds were observed daily and any clinical signs of disease were recorded.

5.7 Sample Collection and Testing

At necropsy, 1 gram each of the spleen, kidney, liver, and any organ displaying grossly visible lesions was aseptically obtained from each bird. Tissues from each individual bird were pooled and placed in individual sterile whirl pak bags. Ten milliliters (10 mL) of BGTB (Brilliant Green Tetrathionate Broth) were added to each bag and the contents were macerated in a Stomacher blender for a period of 30 seconds. The bags were incubated for 24 hours at 37°C. Any organs displaying grossly visible lesions were collected and cultured individually.

Also, a 10 mm sample of the duodenum (bottom of the duodenal loop below the pancreas), jejunum (region of yolk sac diverticulum), and the ileum (anterior to the ileocecal junction) were aseptically collected from each bird, flushed internally and externally with phosphate buffered saline (PBS), pooled, and processed similarly. The bags were incubated for 24 hours at 42°C.

After 24 hours incubation, a loopful from each whirl pak bag culture was streaked onto brilliant green agar containing 100 µg/mL nalidixic (BGAN) and onto xylose-lysine tergitol 4 agar (XLT4). Plates were examined after 24 hour of incubation at 37°C. If SE grew on either of the plate media the pool was considered positive.

After incubating for 48 hours, 1 mL of the BGTB from each bag culture containing a pool that plated negative for SE on both BGAN and XLT4 was transferred to a tube of fresh BGTB. The tubes were incubated for 24 hours at 37°C and then streaked onto BGAN and XLT4. Plates were examined after 24 hours of incubation at 37°C for the presence of SE. If SE grew on either of the plated media the pool was considered SE positive.

An agglutination test with group D *Salmonella* antiserum was performed on at least one colony per plate from all positive plates to confirm the presence of a group D *Salmonella* species. Ten percent of all group D positive colonies were typed for species by NVSL.

The amount of *Salmonella* colonization of the ceca was assessed as follows. Approximately one gram of cecal content was aseptically weighed and placed in 100 mL of sterile PBS (pH 7.2). The contents were mixed thoroughly and 0.1 mL of the suspension was plated on duplicate plates of BGAN. The plates were incubated for 24 hours at 37°C and the resulting colonies counted.

5.8 Data Analysis

A Fisher's exact test was used to analyze the direct plate recovery rate between vaccinates and the controls at a significance level of $P < 0.05$.

5.8.1 Safety

Mortality, if any, due to the vaccine was also recorded.

5.8.2 Trial Efficacy

A statistically significant difference in the recovery of SE from the internal organ pools (liver, spleen, and kidney), intestinal pools or cecal contents between the vaccinated challenged chickens and non-vaccinated challenged control chickens was considered to demonstrate efficacy.

5.8.3 Trial Validity

The trial was considered valid if non-vaccinated non-challenged chickens remained negative for *Salmonella sp.* throughout the study.

6.0 RESULTS

6.1 Environmental Monitoring

There was no *Salmonella* detected in the chickens' environment, when tested twice, once before putting chicks into isolators and again before challenge. The feed and water were tested free of *Salmonella sp.* (see Appendix 1).

6.2 Post-vaccination Observation

It was observed that there was no mortality attributable to the administration of FDAH *Salmonella typhimurium* aroA- vaccine during this study. In addition, there were no clinical signs or lesions attributable to *Salmonella typhimurium*.

6.3 Post-challenge Observation

All the birds challenged were also observed daily. There was no mortality attributable to the administration of the SE challenge culture.

6.4 Titration Results

The mean titer of *Salmonella typhimurium* aroA- vaccine administered by coarse spray route was [REDACTED] and the mean titer of the same vaccine administered by oral route was [REDACTED] (Attachment 2).

The mean titer of SE challenge culture (FDAH 1105-03) was [REDACTED] [REDACTED] Attachment 2).

6.5 Culture Results

The data in Table 1 (raw data in Attachment 3) indicate that the FDAH *Salmonella typhimurium* aroA- vaccine administered once (coarse spray route at one day of age) or twice (coarse spray at one day of age and then oral route at two weeks of age) both offered significant protection ($P < 0.05$) from SE colonization of the internal organs (liver, spleen and kidney) compared to the non-vaccinated challenged controls. There was no significant difference in SE colonization between birds vaccinated once or twice with FDAH live *Salmonella typhimurium* aroA- vaccine.

The data in Table 1 (raw data in Attachment 3) also indicate that FDAH *Salmonella typhimurium* aroA- vaccine administered twice offered significant protection ($P < 0.05$) against SE colonization of the intestines and the ceca compared to the non-vaccinated challenged controls.

6.6 Agglutination Test

An agglutination test with group D *Salmonella* antiserum was used to confirm the presence of a group D *Salmonella* species. The results in Attachment 4 confirmed the results obtained from the cultures. Every colony tested was positive for group D *Salmonella*.

7.0 DISCUSSION

Reisolation results indicate that FDAH *Salmonella typhimurium* aroA- vaccine was capable of reducing internal organ, intestinal and cecal colonization following challenge with a pathogenic SE isolate when administered twice.

No mortality was observed that was attributable to FDAH *Salmonella typhimurium* aroA- vaccine, indicating that it was safe for coarse spray and oral administration.

8.0 CONCLUSION

The FDAH live *Salmonella typhimurium* vaccine, formulated according to the Outline of Production for VS Code 19C1.00, with a [REDACTED] is safe and efficacious for the reduction of SE colonization of the internal organs, intestines and ceca of chickens when it is administered at one day of age and again at two weeks of age.

Table 1***Efficacy of Salmonella typhimurium Vaccine in Preventing Colonization by Salmonella enteritidis: Individual Organ Pool Data***

| Group No. | Vaccine | Challenge Route | No. SE Positive / No. Cultured | | |
|-----------|-------------------|-----------------|--------------------------------|--------------------|-----------------------------|
| | | | Organ Pool ¹ | Intestine Pool | Cecal Contents ² |
| 1 | CS ³ | | 16/39 ^a | 36/39 ^b | 34/39 ^b |
| 2 | CS/Oral | | 12/40 ^a | 30/40 ^a | 17/40 ^a |
| 3 | None ⁴ | | 18/20 ^b | 20/20 ^b | 19/20 ^b |
| 4 | None | | 0/15 | 0/15 | 0/15 |

¹ Values within the same column followed by different letters are significantly different (Fisher's exact test: $P < 0.05$).

² No enrichment = Direct plate culture

³ CS=Coarse Spray

⁴ Please note that groups 3 & 4 are the positive and negative control groups respectively.

APPENDIX 1

Environmental Monitoring: Methods

Culture Method for Feed and Drinking Water

Approximately 5 grams of feed or 5 mL of source water for drinking were mixed with 45 mL of BGTB and incubated for 5 days at 42°C. After incubation 0.1 mL subcultures were streaked onto BG (Brilliant Green) agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

Environmental Monitoring

2x skim milk was used to dampen sterile gauze prior to sampling of the following areas:

- Feed bins
- Water dishes
- Isolator grate/flooring
- Isolator - top and front
- Floor drain basket
- Hallway floor outside of isolation room

Each individual gauze/sample was placed in a container with approximately 50 mL of 2x skim milk (transport media). One mL of milk/sample was added to 9 mL of BGTB and incubated at 42°C for approximately 24 hours. After incubation 0.1 mL subcultures were streaked onto XLT4 agar plates and incubated at 42°C for 24 to 48 hours. Suspect colonies were subcultured onto blood agar and identified using either API strips or the Vitek system.

ATTACHMENT 1

QC Vaccine Testing Results

ATTACHMENT 2***Titration Data***

| Replicate | 1st Vaccination (cfu/dose) | 2nd Vaccination (cfu/dose) | Challenge (cfu/dose) |
|-----------|-------------------------------|-------------------------------|-------------------------|
| 1 | (b)(4) | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| Mean | 1.0×10^8 | 5.2×10^8 | 5.0×10^8 |

ATTACHMENT 3

Salmonella enteritidis Organ Pool Reisolation Data

Isolation Data

Efficacy of Salmonella Typhimurium AroA⁺ Live Vaccine
 In Day Old Chicks Against Salmonella enteritidis Challenge
 Protocol # B393-99-A2
 Tissue Isolation Data
 Group 1 Vaccinated (CS) / Challenged

| Bird # | 1 st Isolation | | 2 nd Isolation | | Summary | 1 st Isolation | | 2 nd Isolation | | Summary | Cecal XLT4 |
|--------|---------------------------|-----------------|---------------------------|-----------------|---------|---------------------------|------------------|---------------------------|------------------|---------|------------|
| | Organ Pool BG | Organ Pool XLT4 | Organ Pool BG | Organ Pool XLT4 | | Intest.Pool BG | Intest.Pool XLT4 | Intest.Pool BG | Intest.Pool XLT4 | | |
| 1 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 2 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 3 | NG | NG | + | + | + | + | + | NT | NT | + | + |
| 4 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 5 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 6 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 7 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 8 | + | + | NT | NT | + | + | + | NT | NT | + | - |
| 9 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 10 | NG | NG | NG | NG | - | NG | NG | NG | NG | - | - |
| 11 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 12 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 13 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 14 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 15 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 16 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 17 | + | + | NT | NT | + | NG | NG | NG | NG | - | + |
| 18 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 19 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 20 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 21 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 22 | NG | NG | NG | NG | - | + | + | NT | NT | + | - |
| 23 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 24 | NG | NG | NG | NG | - | NG | NG | + | NG | + | + |
| 25 | + | + | NT | NT | + | 0 | + | NT | NT | + | + |
| 26 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 27 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 28 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 29 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 30 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 31 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 32 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 33 | + | + | NT | NT | + | NG | NG | + | + | + | - |
| 34 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 35 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 36 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 37 | + | + | NT | NT | + | + | + | NT | NT | + | + |
| 38 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |
| 39 | NG | NG | NG | NG | - | + | + | NT | NT | + | + |

NG=No Growth "+"=Positive
 NT=Not Tested "-"=Negative

ATTACHMENT 4

Agglutination Data

PROTOCOL TITLE:

**EGG REISOLATION EVALUATION FOLLOWING VACCINATION WITH LIVE STM-1
AND CHALLENGE WITH VIRULENT SALMONELLA ENTERITIDIS**

VS Code

Date

AUGUST 19, 1997

U.S. Veterinary License No. 112

CONTENTS:

1.0 STATEMENTS OF APPROVAL AND COMPLIANCE

We have reviewed this protocol and agree that it accurately reflect the proposed study, Egg Reisolation Evaluation Vaccination with Live STM-1 and Challenged with virulent Salmonella Enteritidis.

(b)(6)



2.0 GENERAL INFORMATION

2.1 Title:

Egg Reisolation Evaluation Vaccination with Live STM-1 and Challenge with Virulent Salmonella Enteritidis.

2.2 Protocol No: B-393-97-004.01

2.3 Study Support:

2.3.1 Trial Site

South Atlantic Area
Southeast Poultry Research Laboratory
Athens, Georgia 30604

2.3.2 Study Sponsor

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.3 (b)(4)

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.4 Study Investigator

Dr. Jean Petter-Guard
USDA ARS
Southeast Poultry Research Laboratory
934 College Station Road
P.O. Box 5657
Athens, Georgia 30604

2.3.5 Veterinary Support

Fort Dodge Animal Health
800 5th Street NW
Fort Dodge, Iowa 50501

2.3.6 Laboratory Support

Fort Dodge Animal Health
800 5 th Street NW
Fort Dodge, Iowa 50501

3.0 BACKGROUND AND OBJECTIVE

3.1 Background

Only a few strains of *Salmonella* cause severe disease in poultry. Those that are known to cause disease, such as *Salmonella pullorum* and *Salmonella gallinarum*, are serologically screened for by poultry producers and if found, the practice in the United States is eradication. Most of the other *Salmonella* species isolated from poultry have little or no effect on the overall health of the bird. Rather, the biggest concern is one of public health. *Salmonella* food poisoning is a major health issue throughout the world. By effectively reducing the *Salmonella* bioburden found in poultry, a significant reduction in potential is achieved, both from eggs and meat.

Traditional formalin-killed *Salmonella* bacterins are available commercially. These bacterins often provide limited protection against *Salmonella* as they do confer significant immunity in the intestinal tract, the normal site of infection. Attenuated live strains may prove to be more promising as they may be able to stimulate both local and systemic immunity.

An avirulent strain of *Salmonella typhimurium* (STM-1) containing an aromatic mutation (aro-A) has been developed and demonstrated to protect chickens against disease caused by virulent *Salmonella* challenge. This strain has a limited ability to proliferate *in vivo* or in the environment. Fort Dodge Animal Health is in the process of developing an avian *Salmonella* vaccine utilizing this mutant strain.

3.2 Objectives

The objective of this study is to determine if the potential vaccine strain, STM-1, VS Code 19C1.00 (True Name: *Salmonella Typhimurium Vaccine, Live Culture*) will provide a significant reduction in transmission of *Salmonella* to the egg after a virulent challenge with *Salmonella enteritidis*. SPF leghorns (layer birds) will be vaccinated ^{(b)(4)} at 14 weeks of age, and challenge with a virulent *Salmonella enteritidis* at 24 weeks of age. Eggs will be collected three weeks prior to challenged, and for at least ten weeks post challenge for cultures to determine if egg colonization following vaccination is significantly reduced following challenge.

4.0 STUDY DESIGN

Proposed Start Date:

Proposed Completion Date:

Event Log:

4.1 Animal Selection

4.1.1 Test Animals

Type : Layers
Number: 60 birds at 14 weeks age
Sex: Female
Identification : all birds are to be wing banded
Source: SPF facility (Hyvac or equiv.)

4.1.2 Housing and Care of Animals

All birds will be under veterinary care and will be fed a standard commercial diet, with food and water available ad libitum. Each batch of food and the source of drinking water will be prescreened for the presence of *Salmonella* contamination (see Appendix 1). Vaccinates and controls will housed separately through the study. Housing will comply with applicable welfare regulations.

Birds will treated as deemed necessary by the investigator after consultation with the study director. Any treatment before and during will be documented. Any birds that die during this study will be necropsied and tissues collected for culture for *Salmonella*.

4.2 Test Vaccine

4.2.1 Composition of vaccine

Vaccine will consist of *Salmonella typhimurium* strain STM-1 (aro-A). Specific vaccine composition will be listed in the final report.

4.2.2 Source of Vaccine

Vaccine will be prepared at the South Atlantic Area Southeast Poultry Research Laboratory, Athens, Georgia 30604.

4.2.3 Batch Number

To be recorded and identified on final report.

4.3 Trial Design

Table 1. Test Groups and Vaccine Schedule

| Test Group | # of Birds | Route of Administration | Dose (CFU/ML) |
|------------|------------|-------------------------|---------------|
| 1 | 20 | Intramuscular | [REDACTED] |
| 2 | 20 | Subcutaneous | [REDACTED] |
| 3 | 20 | No vaccine - control | none |

Vaccinates will be housed separately from controls.

Groups 1 and 2 will be vaccinated as per **Table 1** at fourteen weeks of age. Group 3 (controls) will not be vaccinated. All groups will be challenged at 24 weeks of age. Eggs will be collected at 3 weeks prior to a challenge to establish a baseline for the egg cultures. Eggs will be collected from one day to thirty five (but not limited to) days post challenge for the detection of Salmonella.

4.4 Methods

4.4.1 Route of Vaccination

Vaccine will contain approximately [REDACTED]. Dose size will be between 0.5 to 1.0 ml/bird. IM group will receive the inoculation in the thigh muscle while the SQ group will be inoculated midway down the back of the neck.

4.4.2 Egg Culture Procedure Pre and Post Challenge

Eggs will be cultured individually. Eggs will be broken out into sterile bags and stomached (mixed by being pulverized). Egg will be transferred to 160 ml trypticose soy broth with supplemental iron and incubated for 36 hours. After preincubation, 1 ml will be transferred to brain-heart-infusion (BHI) broth and incubated at 37°C for 16 hours. Culture will be streaked on Brilliant Green (BG) agar and incubated at 37°C. Colonies that grow will be identified by genus and species using Enterotube biochemical strips. Any putative Salmonella or Hafnia strains will be serotyped using Difco O- and H-antigen immune serum.

4.4.3 Reisolation Egg Culture Procedure Pre and Post Challenge

The procedures are as above (section 4.4.2), except that ampicillin will be included in the media for post-challenge recovery of challenge strain SE-HCD17.

4.4.4 Sick birds will be euthanized and necropsied. Spleen, liver, mature ovum (3 largest), Immature ovum (between 10-15 mm), and oviduct will be cultured for SE.

4.4.5 At the end of egg collection, all birds will be euthanized, and the spleens and ovaries will be collected to see if the vaccine or challenge strain can be recovered from these tissues. Briefly, spleens and ovaries will be macerated in 4 parts broth (w/v), incubated at 37°C for 16 hours, and then 100ul will be plated on BG agar. Any bacterial colonies will be identified as described above.

4.5 Assessment of Results

4.5.1 Pre and Post Challenge Egg Cultures

Culture results from each group will be recorded.

5.0 REFERENCES

APPENDIX 1

Culture Method for Feed and Drinking Water

One kilogram of feed will be incubated for 2 days in 1 liter of TSB-iron broth at 37°C. Ten ml of broth will be transferred to 100 mls of RV broth, which will be incubated for 16 hours at 37°C. 100 ul will be spread on BG plates and incubated 16 hours at 37°C.

One liter of water will be incubated for 2 days at 37°C after addition of 100 ml of 10X concentrate TSB-iron broth. Ten ml of broth will be transferred to 100 mls of RV broth, which will be incubated for 16 hours at 37°C. 100 ul will be plated on BG plates and incubated 16 hours at 37°C.

Est. 112
Code 19C1.00
#1 File

June 20, 2007

(b)(6)

800 5th Street, NW
P.O. Box 518
Fort Dodge, IA 50501

Dear (b)(6)

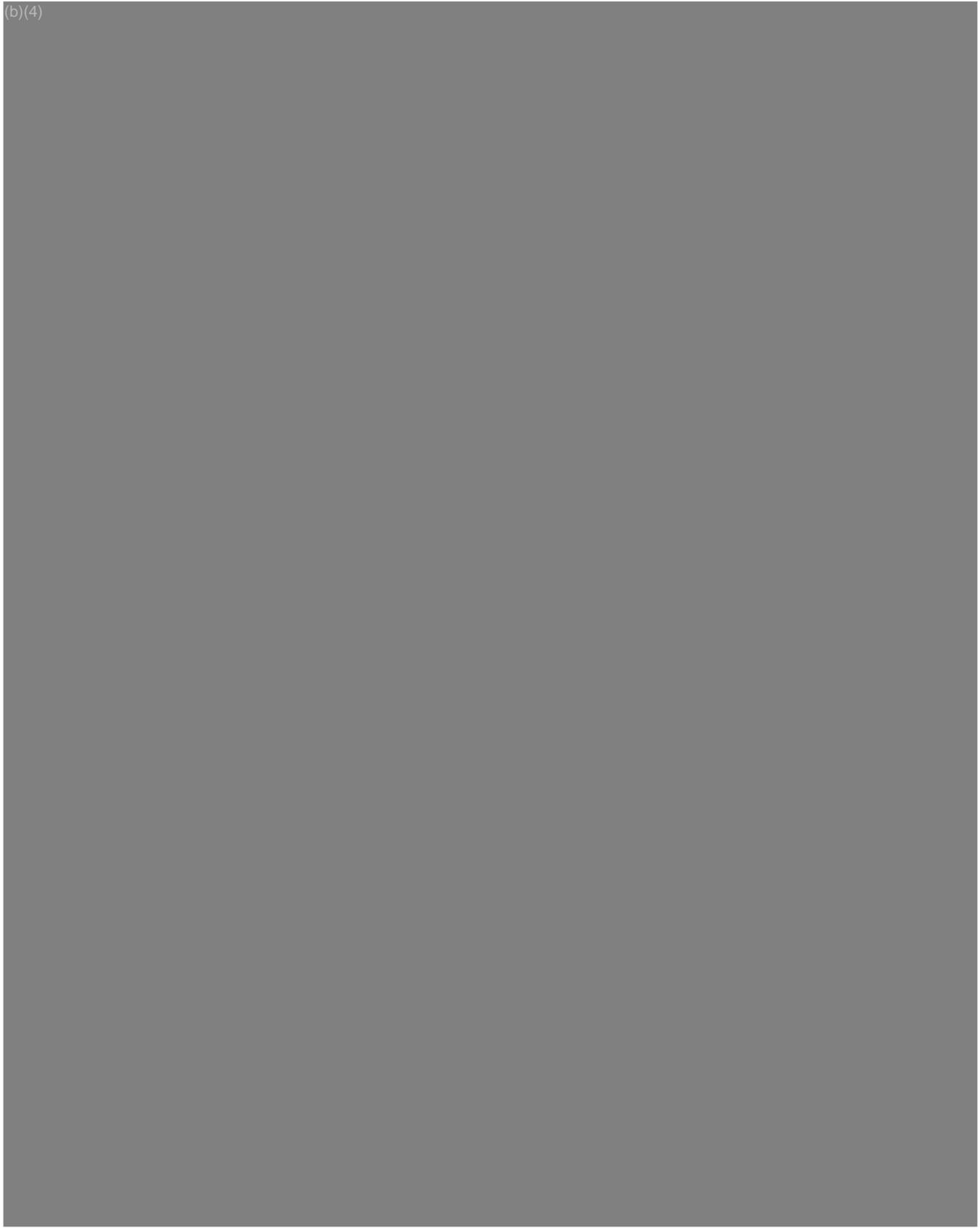
This letter is in response to (b)(6) June 4, 2007, submission of a confirmation of dating report for Salmonella Typhimurium Vaccine, Live Culture, Code 19C1.00 (licensed).

(b)(4)


RTS 06-20-07
FPD 06-21-07

(b)(6)

(b)(4)



(b)(6)

3

(b)(4)

If you have any questions regarding this letter, please contact me at your convenience.

Sincerely,

/s/ Connie S. Schmellik-Sandage

Connie S. Schmellik-Sandage
Senior Staff Microbiologist
Policy, Evaluation, and Licensing

✓ em:
R. D. Owen, CVB-IC
L. R. Ennis, BCA, CVB-IC

B4 (Actg. SL)
APHIS:CVB-PEL:CSSchmellik-Sandage:rp:232-5785:6-20-07
W:\Firms Correspondence\112\Correspondence\112_19C100_070620_ltr.doc

| | |
|---|---|
| U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES CENTER FOR VETERINARY BIOLOGICS AMES, IOWA 50010 TRANSMITTAL OF LABELS AND CIRCULARS OR OUTLINES | 1. NAME AND ADDRESS OF LICENSEE (include Zip Code) Wyeth Subsidiary: Fort Dodge Laboratories, Inc. PO Box 518 Fort Dodge, IA 50501-0518 |
|---|---|

| | | | | |
|--|--|---|--|---|
| NOTE: Submit original and 6 copies. Retain last copy. | | 2. DATE OF PRIOR RELATED CORRESPONDENCE | 3. ESTABLISHMENT LICENSE NO. 112 | 4. DATE SUBMITTED August 10, 2007 |
| 5. NAME OF PRODUCT (Use separate form for each product) Salmonella Typhimurium Vaccine, Live Culture | | | 6. PRODUCT CODE 19C1.00 | 7. "X" IF NEW PRODUCT <input type="checkbox"/> |

| LABELS AND CIRCULARS SUBMITTED | | | | | | |
|--------------------------------|----------------|--------------------------|---|----------------|--------------------------|---|
| TYPE | FINISHED | | | SKETCHES | | |
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

| OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars) | | | |
|--|--|--|---|
| 12. NO. COPIES 4 | 13. TYPE OF SUBMISSION <input type="checkbox"/> New Outline <input type="checkbox"/> Complete Revision <input checked="" type="checkbox"/> Pages Amended <input type="checkbox"/> Pages Added | 14. PAGE NUMBERS AMENDED OR ADDED 9 and 10 | 15. DATE OF PREVIOUS OUTLINE May 16, 2001 |

16. COMMENTS

17. SIGNATURE (b)(6)

EXCEPTIONS

NC

| | |
|--|---|
| 20. REVIEWED BY (Signature, Veterinary Biologics) <i>Cennie S. Schmellik-Sandaga</i> CSSchmellik-Sandaga:brm | 21. DATE RETURNED August 30, 2007 |
|--|---|

APHIS FORM 2015 (JAN 98) Replaces APHIS Form 2015 (APR 89) which may be used.

YM *RTS - Aug 29, 2007 - CSS* 68603 **AUG 19 2007** *8-29-07 L*

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 25, 2005
Supersedes May 16, 2001 ✓

V. C. Potency

1.



2. Master Seed Immunogenicity Information

| Immunogenicity | <i>Salmonella typhimurium</i> Challenge | <i>Salmonella enteritidis</i> Challenge | <i>Salmonella heidelberg</i> Challenge |
|----------------|--|--|---|
|----------------|--|--|---|



D. Moisture, if Desiccated

Refer to Section IV. H. of this Production Outline.

E. Any Other Tests

Identity Test



FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUN 21 2005

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

06-02-05
#6088
[Signature]

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE *chg from "Bacteria"*

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

VI. POST PREPARATORY STEPS

A. Form and Size of Final Containers in Which the Product is to Be Distributed



B. Collection, Storage and Submission of Representative Samples



C. Expiration Date



D. Use, Dosage and Route of Administration

1.

2.



USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

PEL copy could
not be located
as of 6-26-08.

AB

**OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE**

VS Code No. 19C1.00

COMPLETE REVISION

May 16, 2001

Replaces the Outline dated January 23, 2001, inclusive of subsequent revisions.

American Home Products Corporation
through its producing subsidiaries.

U.S. Veterinary License No. 112

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

CG COPY

All redactions on this page are pursuant to (b)(4).

Page 1

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

I. COMPOSITION OF THE PRODUCT

A. Microorganism Used

1. Identification of the Microorganism, Including Strain or Isolate

[Redacted]

2. Isolation and Known Passage History, Including the Number of Passages Since Isolation in Each Type of Medium, Cell Culture and/or Animal

[Redacted]

3. Identification of the Master Seed

[Redacted]

4. Date of Notification from the CVB that the Master Seed is Eligible for Use in Production & Name and License Number of the Firm at the Time Notification is Received from CVB that the Master Seed is Eligible for Use in Production

[Redacted]

B. Source and Date of Accession

[Redacted]

C. Strain

Refer to Section 1. A. 1.

D. Proportions of Each Strain

N/A

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

 COPY

All redactions on this page are pursuant to (b)(4).

Page 2
OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

October 15, 2003
Supersedes December 13, 2002

II. CULTURES

A. Identification

[Redacted]

B. Virulence and Purity of Cultures

1. Virulence
N/A

2. Purity

[Redacted]

3. Range of Passage Used in Production

[Redacted]

C. Composition and Reaction of Media Used for Seed and Production Cultures

1.

[Redacted]

2.

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

OCT 30 2003

COPY POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

(b)(6)

All redactions on this page are pursuant to (b)(4).

Page 3
OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

January 11, 2006
Supersedes May 16, 2001

II. D. Character, Size, and Shape of Containers Used for Growing Cultures

1.



2. Production cultures are grown in:

Container

Volume of Media



E. Storage Conditions of Seed Cultures



USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JAN 17 2006

(b)(6)



COPY POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

All redactions on this page are pursuant to (b)(4).

Page 4

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

II. F. Method of Preparing Suspensions for Seeding or Inoculation

[Redacted]

G. Technique of Inoculating

1. Seed Media

a.

[Redacted]

b.

2. Production Media

[Redacted]

H. Period of Time and Conditions for Incubation and Degree of Temperature Used

1.

[Redacted]

2.

I. Character and Amount of Growth; Observation as to Contamination of Growth

[Redacted]

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

 COPY

All redactions on this page are pursuant to (b)(4).

Page 5

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURJUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

II. I. Method of Attenuation

[Redacted]

III. HARVEST

A. Handling and Preparation of Cultures and Media for Harvest

[Redacted]

B. Minimum and Maximum Harvest Period from Inoculation

[Redacted]

C. Technique of Harvest

[Redacted]

D. Specifications for Acceptable Harvest Material

[Redacted]

E. Handling of Discarded Material Not Used in Production

[Redacted]

F. Additional Pertinent Information

N/A

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

 COPY

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

All redactions on this page are pursuant to (b)(4).

Page 6

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

IV. PREPARATION OF THE PRODUCT

A. Method of Inactivation, Attenuation, or Detoxification

[Redacted]

B. Composition of Preservative, Adjuvant or Stabilizer, and Proportions Used

[Redacted]

C. Method and Degree of Concentration

[Redacted]

D. Standardization of the Product

[Redacted]

B. Assembly of Units to Make a Serial

1.

[Redacted]

FILED WITH
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

COPY

All redactions on this page are pursuant to (b)(4).

Page 7

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

- IV. E. 1.
- 2.
- 3.
- 4.



N/A

F. Volume of Fill for Each Size Vial

| <u>Dose</u> | <u>Fill Volume (mL)</u> | | <u>Container</u> | <u>Stopper</u> |
|-------------|-------------------------|----------------|------------------|----------------|
| | <u>Minimum</u> | <u>Maximum</u> | | |



G. Method and Technique of Filling and Sealing of Final Containers



FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

COPY

Page 8
OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

October 28, 2009
Supersedes March 5, 2008 ✓

IV. H. Desiccation and Moisture Control

1. Procedures

[Redacted]

2. Testing

[Redacted]

I. Amount of Antigenic Material Per Dose(s) in Final Container

[Redacted]

V. TESTING

A. Purity

[Redacted]

B. Safety

[Redacted]

(b)(6)
[Redacted]

USDA/APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

NOV 12 2009

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

82314 RTG - NOV 10, 2009 - C88

All redactions on this page are pursuant to (b)(4).

Page 9

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

August 8, 2007
Supersedes May 25, 2005

V. C. Potency

1.

2. Master Seed Immunogenicity Information

| Immunogenicity | <i>Salmonella typhimurium</i> Challenge | <i>Salmonella enteritidis</i> Challenge | <i>Salmonella heidelberg</i> Challenge |
|----------------|--|--|---|
|----------------|--|--|---|



D. Moisture, if Desiccated

Refer to Section IV. H. of this Production Outline.

E. Any Other Tests

Identity Test



(b)(6)



CC COPY

FILED with
USDA-AFIS-VS
CENTER FOR VETERINARY
MICROBIOLOGICS

AUG 30 2007

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

All redactions on this page are pursuant to (b)(4).

Page 10

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

August 8, 2007
Supersedes May 16, 2001

VI. POST PREPARATORY STEPS

A. Form and Size of Final Containers in Which the Product is to Be Distributed

[Redacted]

B. Collection, Storage and Submission of Representative Samples

[Redacted]

C. Expiration Date

[Redacted]

D. Use, Dosage and Route of Administration

1. Use

[Redacted]

2. Dosage and Route of Administration

[Redacted]

USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

AUG 8 0 2007

COPY

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

(b)(6)

[Redacted]

All redactions on this page are pursuant to (b)(4).

Page 11

OUTLINE OF PRODUCTION
SALMONELLA TYPHMURIUM VACCINE, LIVE CULTURE

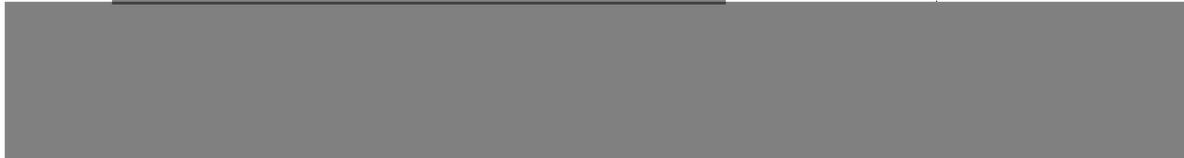
U.S. Veterinary License No. 112

VS Code No. 19C1.00

August 14, 2002
Supersedes May 16, 2001

VI. E. Confidential Information

- I.
- II.
- III.
- IV.
- V.



Wyeth
through its producing subsidiaries



bo

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

COPY

NOV 20 2002

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

APPENDIX 1

SITE OF MANUFACTURE

(b)(4)



(b)(6)



 **COPY**

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 16, 2001

SUMMARY OF CHANGES

| Page and Section | Revision | Reason for Change |
|--------------------|----------|--|
| Throughout Outline | (b)(4) | Correction per USDA phone contact dated 5/8/01 with (b)(6) |
| 4 II. F. | | In response to the 2015 comments dated 5/3/2001. |
| 4 II. G. 1. b. | | Clarification & in response to the 2015 comments dated 5/3/2001. |
| 7 IV. E. | | (b)(4) |

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 02 2001

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

COPY

(b)(6)

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

August 14, 2002

SUMMARY OF CHANGES

| | | |
|-------------|--------|------------------------|
| Cover & 11 | (b)(4) | Corporate name change. |
| 2 II. C. | | |

COPIY

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

NOV 20 2002

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

(b)(6)

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

December 13, 2002

SUMMARY OF CHANGES

| | | |
|-------------|-------------------|---|
| 2 II. C. | Revised verbiage. | Per USDA recommendations and comply with CFIA VBS Memo 2002-01. |
|-------------|-------------------|---|



CC COPY

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

DEC 18 2002

LICENSING & POLICY
DEVELOPMENT
NO ENDORSEMENT
EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

October 15, 2003

SUMMARY OF CHANGES

| | | |
|---------|--------|-----------------------|
| 2 | (b)(4) | |
| II.A. | | Unnecessary verbiage. |
| 2 | | New title. |
| II.C.1. | | |
| 8 | | |
| V.B. | | |

(b)(6)

COPY

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

OCT 30 2003

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

June 25, 2004

SUMMARY OF CHANGES

| | | |
|--------------|--------|---------------------------------|
| 8 IV.H.2. | (b)(4) | Updated to new standard method. |
|--------------|--------|---------------------------------|

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUL 14 2004

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

COPI

(b)(6)

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

May 25, 2005

SUMMARY OF CHANGES

| | |
|---------------------|---------------|
| <p>9 V.C.2.</p> | <p>(b)(4)</p> |
|---------------------|---------------|

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUN 21 2005

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

 COPY

(b)(6)

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

January 11, 2006

SUMMARY OF CHANGES

| | |
|---------------------|---------------|
| <p>3 I.D.2.</p> | <p>(b)(4)</p> |
|---------------------|---------------|

(b)(6)

COPI

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JAN 17 2006

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

August 8, 2206

SUMMARY OF CHANGES

| File No. | Revision | Description |
|--------------|----------|--|
| 9 V.C.I., | (b)(4) | In response to (b)(6) July 25, 2007 letter. |
| 10 V.I.C. | | |

QC COPY

FILED with
USDA-APHIS-WS
CENTER FOR VETERINARY
BIOLOGICS

AUG 8 2007

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

(b)(6)

BMPs

05/26/2008 11:14 5156637359

Product labels and circulars and outline of production have been reviewed (9 CFR 102.112, and 114).
FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (include Zip Code)

Wyeth
Subsidiary: Fort Dodge Laboratories, Inc.
PO Box 518
Fort Dodge, IA 50501

(b)(6)

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES

4

13. TYPE OF SUBMISSION

New Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED

9 and 10

15. DATE OF PREVIOUS OUTLINE

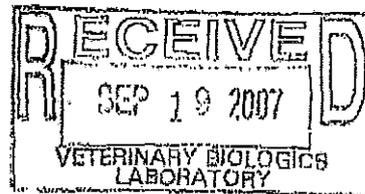
May 16, 2001

16. COMMENTS

(b)(6)

EXCEPTIONS

COPY



20. REVIEWED BY (Signature, Veterinary Biologics)

21. DATE RETURNED

CSEhmellik-Sandgren:brs August 30, 2007

no. U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (S'GFR 102, 112, and 114). FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (Include Zip Code)

Wyath
Subsidiary: Fort Dodge Laboratories, Inc.
P.O. Box 518
Fort Dodge, IA 50501

(b)(6)

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.

112

4. DATE SUBMITTED

January 12, 2006

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE

1901.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES

4

13. TYPE OF SUBMISSION

New Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED

3

15. DATE OF PREVIOUS OUTLINE

May 16, 2001

16. COMMENTS

(b)(6)

EXCEPTIONS

RECEIVED
JAN 25 2006
VETERINARY BIOLOGICS
LABORATORY

COPY

20. REVIEWED BY (Signature, Veterinary Biologist)

21. DATE RETURNED

HYCaribma

January 17, 2006

[Handwritten signature]

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a
 valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete
 this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching
 existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
 ANIMAL AND PLANT HEALTH INSPECTION SERVICE
 VETERINARY SERVICES
 CENTER FOR VETERINARY BIOLOGICS
 AMES, IOWA 50010
**TRANSMITTAL OF LABELS
 AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (Include Zip Code)
Wyeth
Subsidiary: Fort Dodge Laboratories, Inc.
808 5th Street NW, PO Box 518
Fort Dodge, IA 50501 (b)(6)

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED
May 27, 2005

5. NAME OF PRODUCT (Use separate form for each product)
Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|---|-------------|-----------------------|---|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No. (s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No. (s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES
4

13. TYPE OF SUBMISSION
 Now Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED
9

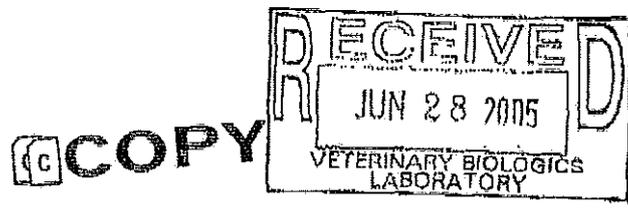
15. DATE OF PREVIOUS OUTLINE
May 16, 2001

16. COMMENTS
 (b)(6)
 REVIEW BY VETERINARY BIOLOGICS

EXCEPTIONS

This amended Outline of Production page is filed with the following pen-and-ink change:

1. Page 9, Section V.C.2: A blank was provided for the date that this product was exempted from the three year immunogenicity requirement (CVB Notice 04-17). The date of June 21, 2005, was added in the space provided. Please consider this product as exempted with the stamping of this Outline of Production page.



20. REVIEWED BY (Signature, Veterinary Biologics)
 [Signature]

21. DATE RETURNED
June 21, 2005

CSSchnellik-Sandagataip

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (9 CFR 102.112, and 114). FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average 12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (include Zip Code)
Wyeth
Subsidiary: **Fort Dodge Laboratories, Inc.**
800 5th Street NW
PO Box 516
Fort Dodge, IA 50501

NOTE: Submit original and 6 copies. Retain last copy.
2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED
June 25, 2004

5. NAME OF PRODUCT (Use separate form for each product)
Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|---|-------------|-----------------------|---|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No. (s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No. (s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES
4

13. TYPE OF SUBMISSION
 New Outline Complete Revision Pages Amended Pages Added

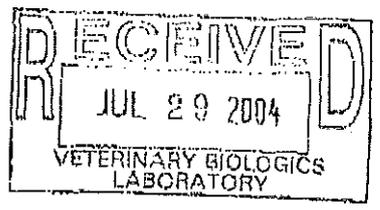
14. PAGE NUMBERS AMENDED OR ADDED
8

15. DATE OF PREVIOUS OUTLINE
May 16, 2001

16. COMMENTS

(b)(6)

EXCEPTIONS



COPY

20. REVIEWED BY (Signature, Veterinary Biologics)
[Signature] **CS Schmallik-Sandegard**

21. DATE RETURNED
July 14, 2004

lets

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (9 CFR 102, 112, and 114). FORM APPROVED OMB NO. 0678-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0679-0013. The time required to complete this information collection is estimated to average 12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. (b)(6)

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (include Zip Code)

WYETH
Subsidiary: Fort Dodge Laboratories, Inc.
PO Box 518
Fort Dodge, IA 50501

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED
October 27, 2003

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 6. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES
4

13. TYPE OF SUBMISSION
 New Outline
 Complete Revision
 Pages Amended
 Pages Added

14. PAGE NUMBERS AMENDED OR ADDED
2 & 8

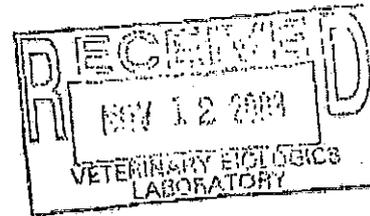
15. DATE OF PREVIOUS OUTLINE
5/16/01

16. COMMENTS

(b)(6)

REVIEW BY VETERINARY BIOLOGICS

EXCEPTIONS



6 COPY

20. REVIEWED BY (Signature, Veterinary Biologics)

Caroline S. ...

CSSandage/jw

21. DATE RETURNED

October 30, 2003

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (9 CFR 102, 112, and 114). FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (include Zip Code)

~~XXXXX~~
Subsidiary: Fort Dodge Laboratories,
PO Box 318
Fort Dodge, IA 50501

(b)(6)

(b)(6)

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.

112

4. DATE SUBMITTED

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE

19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|---|-------------|-----------------------|---|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No. (s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No. (s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES

4

13. TYPE OF SUBMISSION

New Outline
 Complete Revision
 Pages Amended
 Pages Added

14. PAGE NUMBERS AMENDED OR ADDED

2

15. DATE OF PREVIOUS OUTLINE

5/16/01

16. COMMENTS

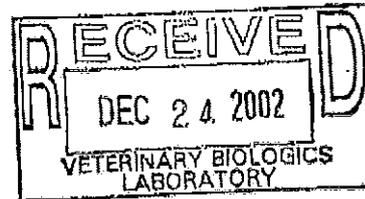
17. SIGNATURE OF LICENSEE REPRESENTATIVE

18. TITLE

(b)(6)

EXCEPTIONS

COPY



20. REVIEWED BY (Signature, Veterinary Biologist)

21. DATE RETURNED

FLFoley:sw

December 18, 2002

L

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (4 CFR 102, 112, and 114). FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (Include Zip Code)
**WVSPM
Subsidiary: Fort Dodge Laboratories, Inc.
1800 24th Street NW
PO Box 518
Fort Dodge, IA 50501**

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. "X" IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES
4

13. TYPE OF SUBMISSION

New Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED
Cover, 2 & 11

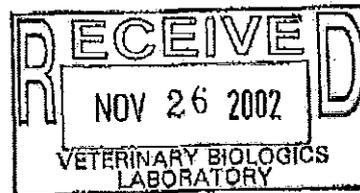
15. DATE OF PREVIOUS OUTLINE
5/16/02

16. COMMENTS

17. SIGNATURE OF LICENSEE REPRESENTATIVE

REVIEW BY VETERINARY BIOLOGICS

EXCEPTIONS



COPY

20. REVIEWED BY (Signature, Veterinary Biologics)

21. DATE RETURNED

PLFoley:aw

November 20, 2002

L

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed, 9 CFR 102, 112, and 114). FORM APPROVED OMB NO. 0578-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0578-0013. The time required to complete this information collection is estimated to average 12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

| | | | |
|---|--|---|---|
| U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES CENTER FOR VETERINARY BIOLOGICS AMES, IOWA 50010 TRANSMITTAL OF LABELS AND CIRCULARS OR OUTLINES | | 1. NAME AND ADDRESS OF LICENSEE (Include Zip Code) AMERICAN BUREAU PRODUCTS CORPORATION Subsidiary: Fort Dodge Laboratories, Inc. 602 5th Street NE PO Box 518 Fort Dodge, IA 50501 | |
| NOTE: Submit original and 6 copies. Retain last copy. 2. DATE OF PRIOR RELATED CORRESPONDENCE | | 3. ESTABLISHMENT LICENSE NO. 112 | 4. DATE SUBMITTED 1/23/01 |
| 6. NAME OF PRODUCT (Use separate form for each product) Salmonella Typhimurium Vaccinae, Live Culture | | 8. PRODUCT CODE 19CL-00 | 7. "X" IF NEW PRODUCT <input type="checkbox"/> |

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|---|-------------|-----------------------|---|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No. (s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No. (s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

| | | | |
|----------------------------|---|---|--|
| 12. NO. COPIES 4 | 13. TYPE OF SUBMISSION <input type="checkbox"/> New Outline <input checked="" type="checkbox"/> Complete Revision <input type="checkbox"/> Pages Amended <input type="checkbox"/> Pages Added | 14. PAGE NUMBERS AMENDED OR ADDED N/A | 15. DATE OF PREVIOUS OUTLINE 1/23/01 |
|----------------------------|---|---|--|

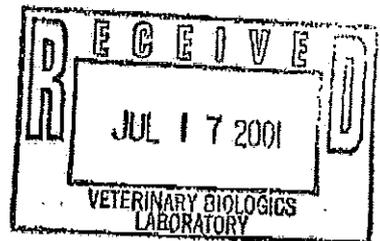
16. COMMENTS

(b)(6)

EXCEPTIONS

REVIEW BY VETERINARY BIOLOGICS

(c) COPY



| | |
|---|--|
| 20. REVIEWED BY (Signature, Veterinary Biologist) <i>[Signature]</i> | 21. DATE RETURNED July 2, 2001 |
|---|--|

L

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

March 5, 2008

SUMMARY OF CHANGES

| Page and Section | Revision | Reason for Change |
|------------------|----------|--|
| 8 IV.I. | (b)(4) | In response to (b)(6) July 25, 2007 letter. |

(b)(6)

06-26-08

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

JUN 30 2008

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

(b)(6)

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (9 CFR 102.112, and 114).
FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average 12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
CENTER FOR VETERINARY BIOLOGICS
AMES, IOWA 50010
**TRANSMITTAL OF LABELS
AND CIRCULARS OR OUTLINES**

1. NAME AND ADDRESS OF LICENSEE (Include Zip Code)

Wyeth
Subsidiary: Fort Dodge Laboratories, Inc.
PO Box 518
Fort Dodge, IA 50501

RCUD MAR 10 2008

NOTE: Submit original and 6 copies. Retain last copy.

2. DATE OF PRIOR RELATED CORRESPONDENCE

3. ESTABLISHMENT LICENSE NO.
112

4. DATE SUBMITTED
March 7, 2008

5. NAME OF PRODUCT (Use separate form for each product)

Salmonella Typhimurium Vaccine, Live Culture

6. PRODUCT CODE
19C1.00

7. 'X' IF NEW PRODUCT

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

12. NO. COPIES

4

13. TYPE OF SUBMISSION

New Outline Complete Revision Pages Amended Pages Added

14. PAGE NUMBERS AMENDED OR ADDED

8

15. DATE OF PREVIOUS OUTLINE

May 16, 2001

16. COMMENTS

17. SIGNATURE OF LICENSEE REPRESENTATIVE

18. TITLE

(b)(6)

REVIEW BY VETERINARY BIOLOGICS

EXCEPTIONS

20. REVIEWED BY (Signature, Veterinary Biologics)

Connie S. Schmellik-Sandage

CSSchmellik-Sandage:brm

21. DATE RETURNED

June 30, 2008

OUTLINE OF PRODUCTION
SALMONELLA TYPHIMURIUM VACCINE, LIVE CULTURE

U.S. Veterinary License No. 112

VS Code No. 19C1.00

October 28, 2009

SUMMARY OF CHANGES

| Page and Section | Revision | Reason for Change |
|------------------|---|--|
| 8 V.A. | Added "APHIS approval granted July 30, 2009." | Per (b)(6) July 30, 2009 letter the data are accepted as supporting (b)(4) |

(b)(4)

They are working on
confirmation (b)(6)

(b)(6)

11-09-09

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

NOV 12 2009

POLICY, EVALUATION, AND LICENSING
NO ENDORSEMENT
EXPRESSED

(b)(6)



RECEIVED

AUG - 3 2009

July 30, 2009

United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

1920 Dayton Avenue
P.O. Box 844
Ames, IA 50010

(515) 337-6100

(b)(6)

800 5th Street NW
Fort Dodge, IA 50501

**BIOLOGICAL
REGULATORY AFFAIRS**

Dear (b)(6)

This is in response to (b)(6) submission of June 12, 2009, and July 2, 2009, concerning the dilution of preservative testing for the following (all licensed):

- Escherichia Coli Vaccine, Live Culture, Code 1551.R0
- Salmonella Typhimurium Vaccine, Live Culture, Code 19C1.00
- Salmonella Dublin Vaccine, Live Culture, Code 19D1.R0

The purpose of the submission was to comply with 9 CFR 113.25(d). (b)(6)

(b)(4)

Sincerely,

Eleanor V. Eagly
Senior Staff Veterinarian
Policy, Evaluation, and Licensing

FILED with
USDA-APHIS-VS
CENTER FOR VETERINARY
BIOLOGICS

NOV 12 2009



Safeguarding American Agriculture

APHIS is an agency of USDA's Marketing and Regulatory Programs
An Equal Opportunity Provider and Employer

FEDERAL RELAY SERVICE
(Voice/TTY/ASL/Sign/Spoken)
1-800-877-8339 EXPRESSED

No U.S. Veterinary Biological Product license may be issued until product labels and circulars and outline of production have been reviewed (9 CFR 102, 112, and 114).
FORM APPROVED OMB NO. 0579-0013

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0013. The time required to complete this information collection is estimated to average .12 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

| | |
|---|---|
| U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES CENTER FOR VETERINARY BIOLOGICS AMES, IOWA 50010 TRANSMITTAL OF LABELS AND CIRCULARS OR OUTLINES | 1. NAME AND ADDRESS OF LICENSEE (Include Zip Code) WYETH Division: Fort Dodge Animal Health 800 5th Street N.W. Fort Dodge, IA 50501 |
|---|---|

NOV 13 2009

| | | | |
|--|--|---------------------------------------|--|
| NOTE: Submit original and 6 copies. Retain last copy. | | | |
| 2. DATE OF PRIOR RELATED CORRESPONDENCE | 3. ESTABLISHMENT LICENSE NO. 112 | 4. DATE SUBMITTED 29 Oct 09 | |
| 5. NAME OF PRODUCT (Use separate form for each product) Salmonella Typhimurium Vaccine, Live Culture | | 6. PRODUCT CODE 19C1.00 | 7. 'X' IF NEW PRODUCT <input type="checkbox"/> |

LABELS AND CIRCULARS SUBMITTED

| TYPE | FINISHED | | | SKETCHES | | |
|---------------|-------------|-----------------------|--|-------------|-----------------------|--|
| | A. No. Sets | B. No. Copies of Each | C. Item on File Being Replaced (Give No.(s)) | D. No. Sets | E. No. Copies of Each | F. Item on File Being Replaced (Give No.(s)) |
| 8. CONTAINER | | | | | | |
| 9. BOX | | | | | | |
| 10. CIRCULARS | | | | | | |
| 11. OTHER | | | | | | |

OUTLINE SUBMITTED (Do not submit with same form covering Labels and Circulars)

| | | | |
|----------------------------|---|---|---|
| 12. NO. COPIES 2 | 13. TYPE OF SUBMISSION <input type="checkbox"/> New Outline <input type="checkbox"/> Complete Revision <input checked="" type="checkbox"/> Pages Amended <input type="checkbox"/> Pages Added | 14. PAGE NUMBERS AMENDED OR ADDED 8 | 15. DATE OF PREVIOUS OUTLINE May 16, 2001 |
|----------------------------|---|---|---|

16. COMMENTS

17. SIGNATURE OF LICENSEE REPRESENTATIVE: (b)(6)
 18. TITLE: _____

EXCEPTIONS

| | |
|--|---|
| 20. REVIEWED BY (Signature, Veterinary Biologics) Connie S. Schmellik-Sandage CSSchmellik-Sandage:brm | 21. DATE RETURNED November 12, 2009 |
|--|---|

**SUMMARY INFORMATION FORMAT FOR
CATEGORY II VETERINARY BIOLOGICS**

(b)(4)



January 31, 1997

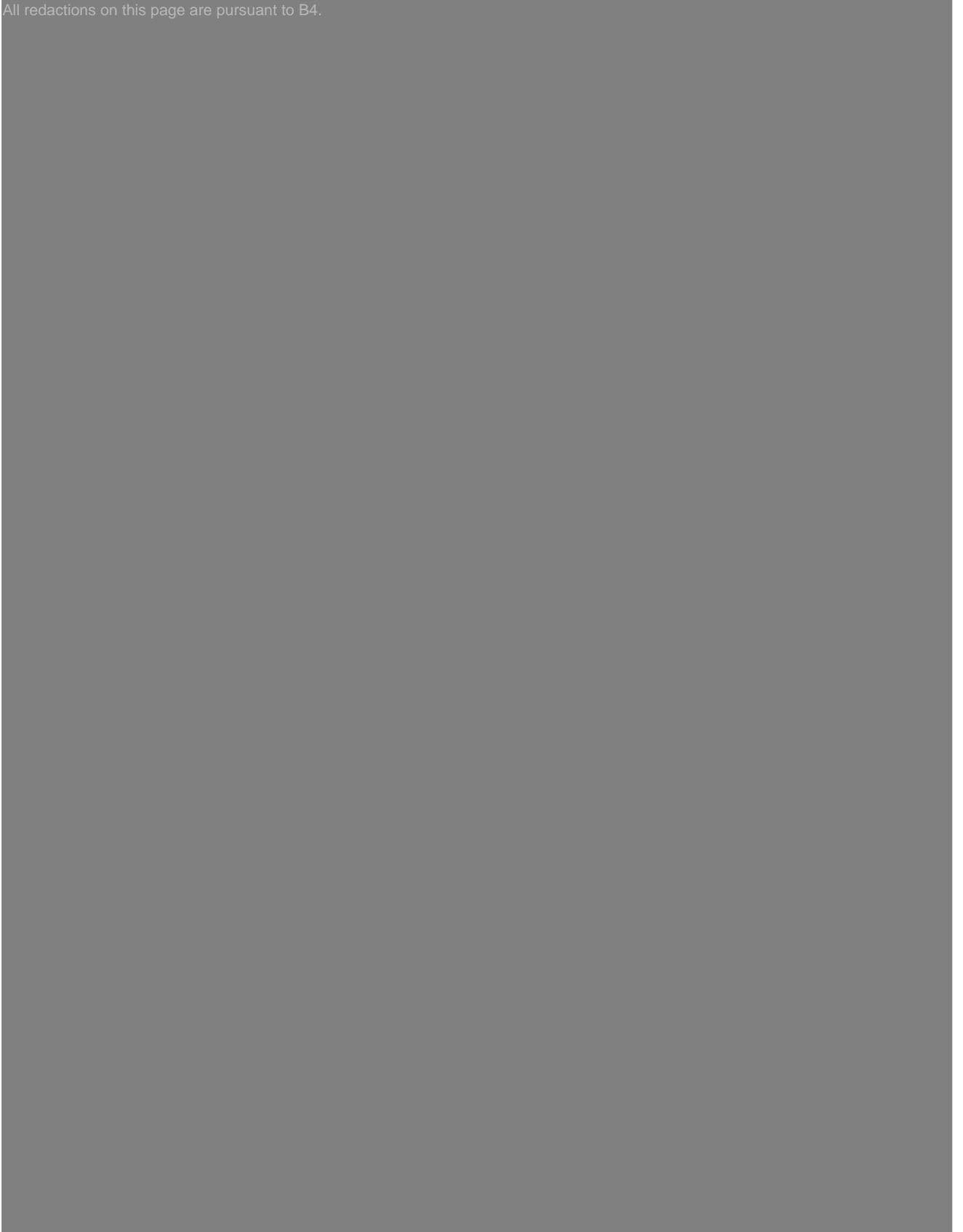
**Prepared by Fort Dodge Laboratories
800 5th Street NW
Fort Dodge, IA 50501
U.S. Establishment License 112**

I. INTRODUCTION

All redactions on this page are pursuant to (b)(4).



All redactions on this page are pursuant to B4.



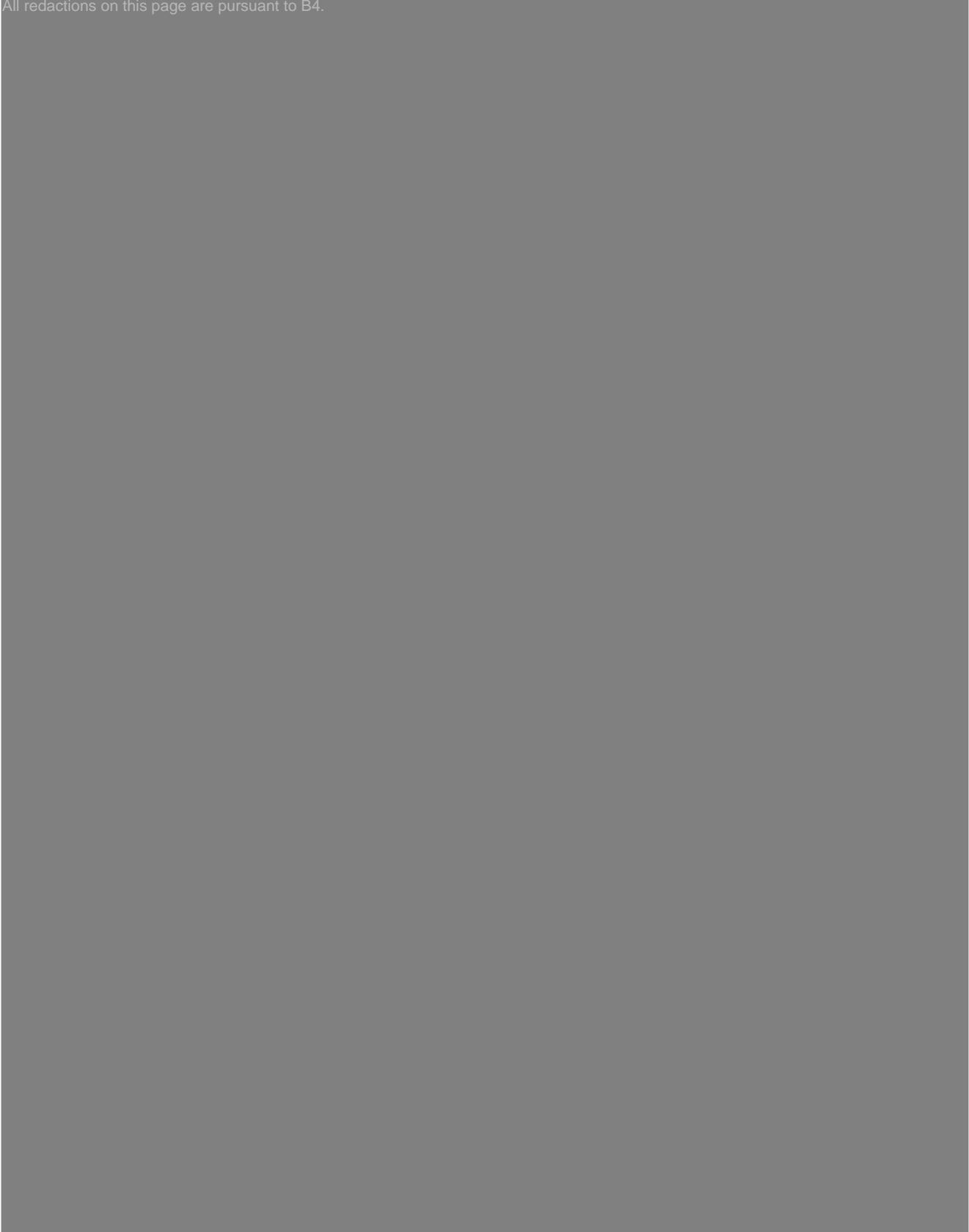
All redactions on this page are pursuant to B4.



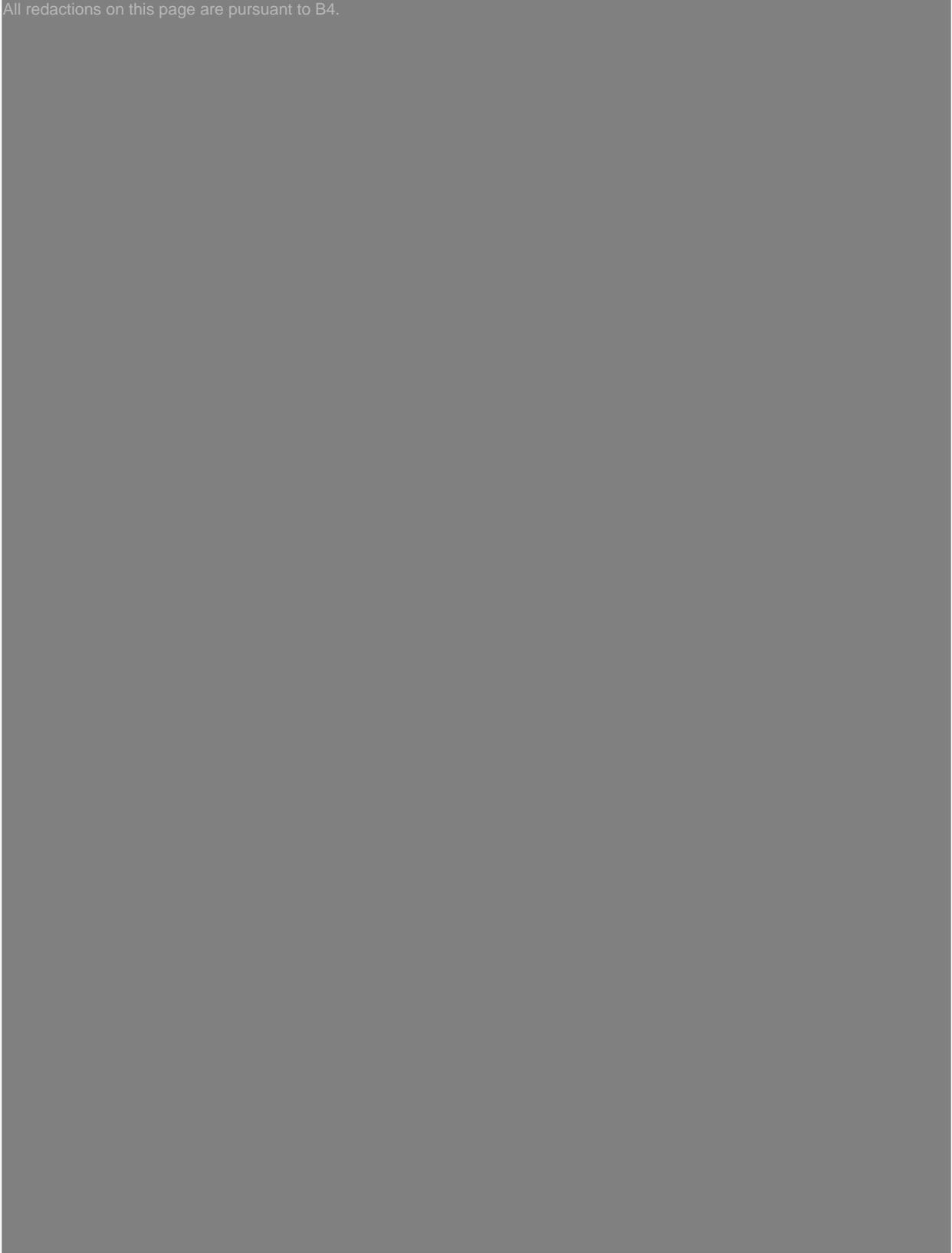
All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



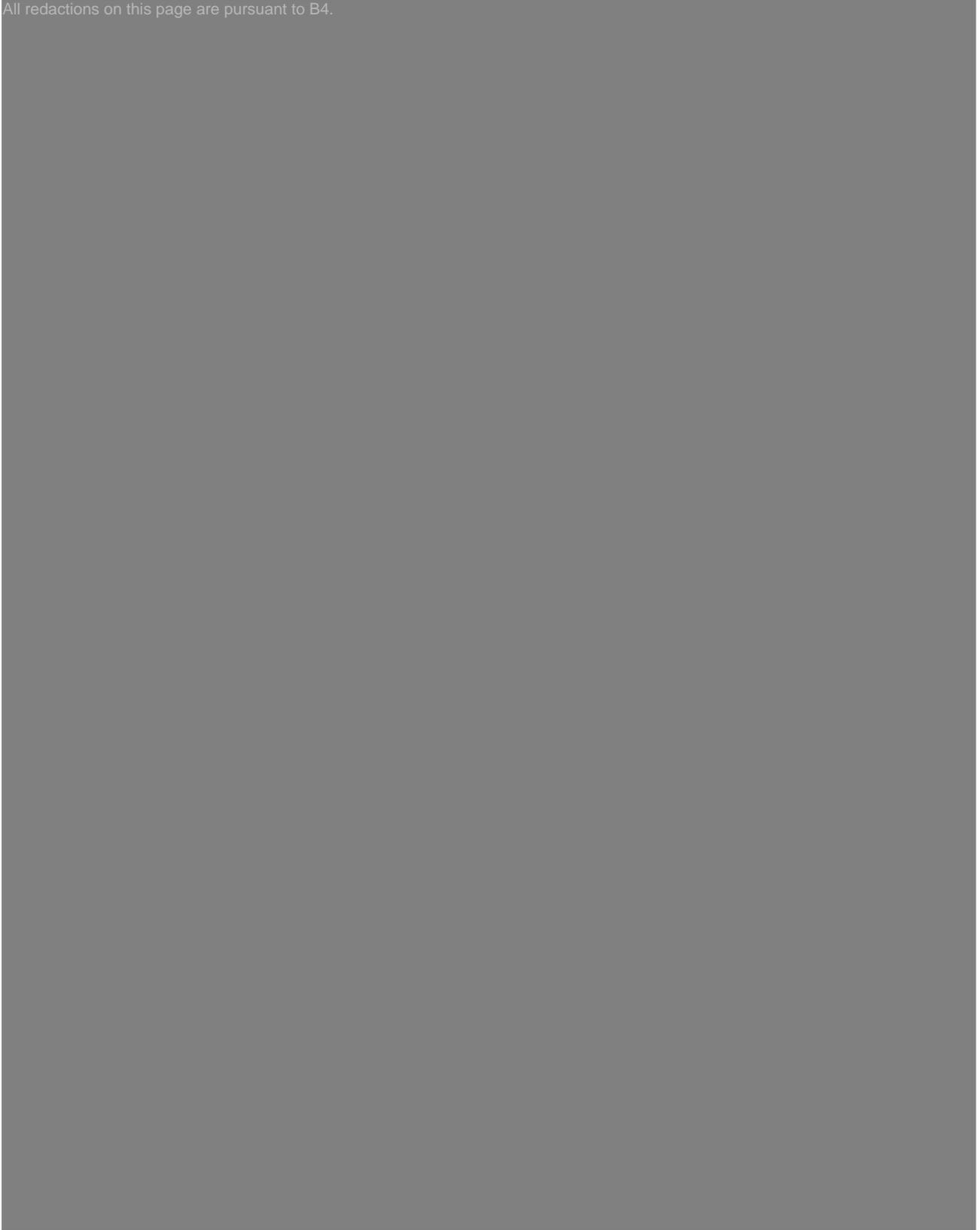
All redactions on this page are pursuant to B4.



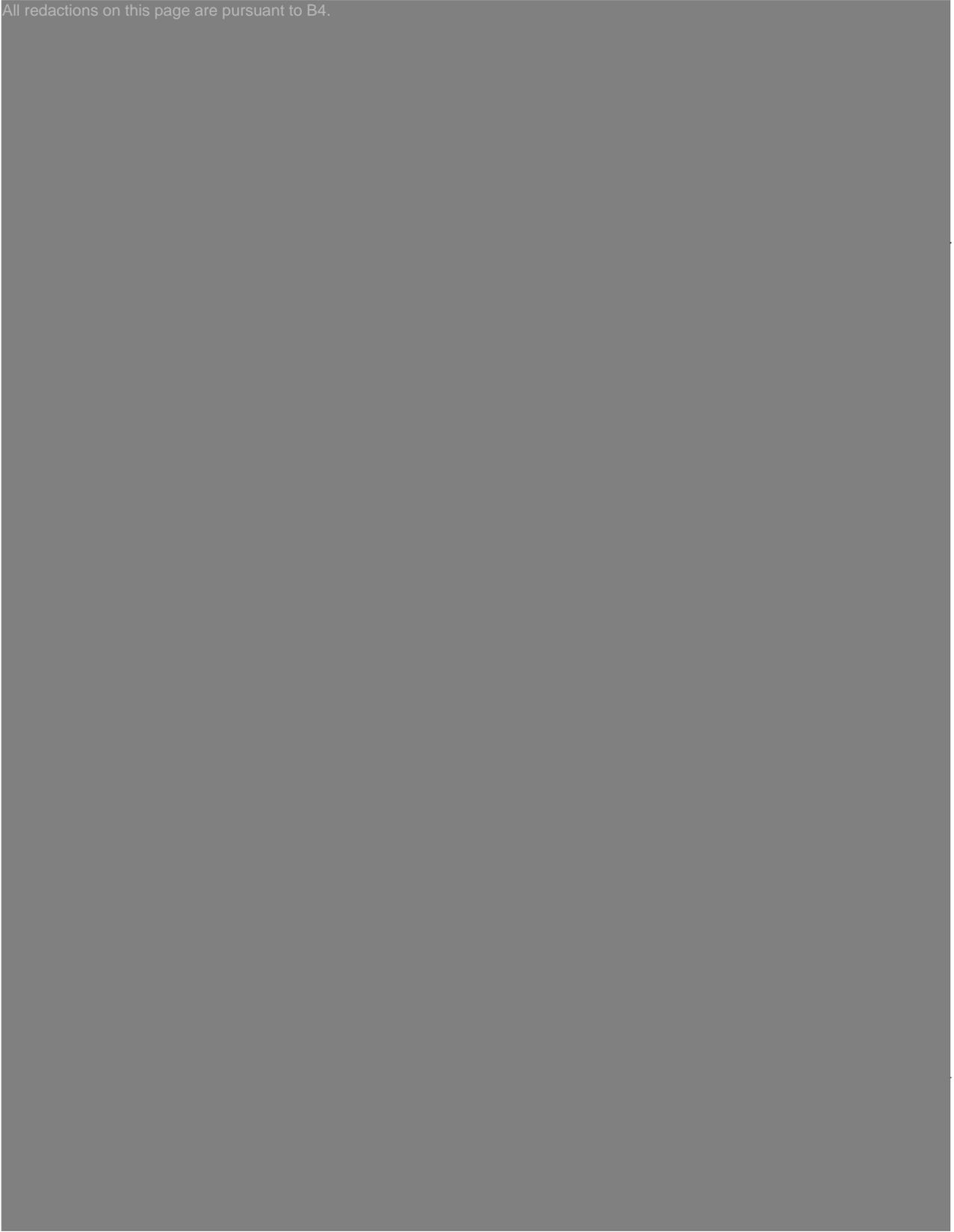
All redactions on this page are pursuant to B4.



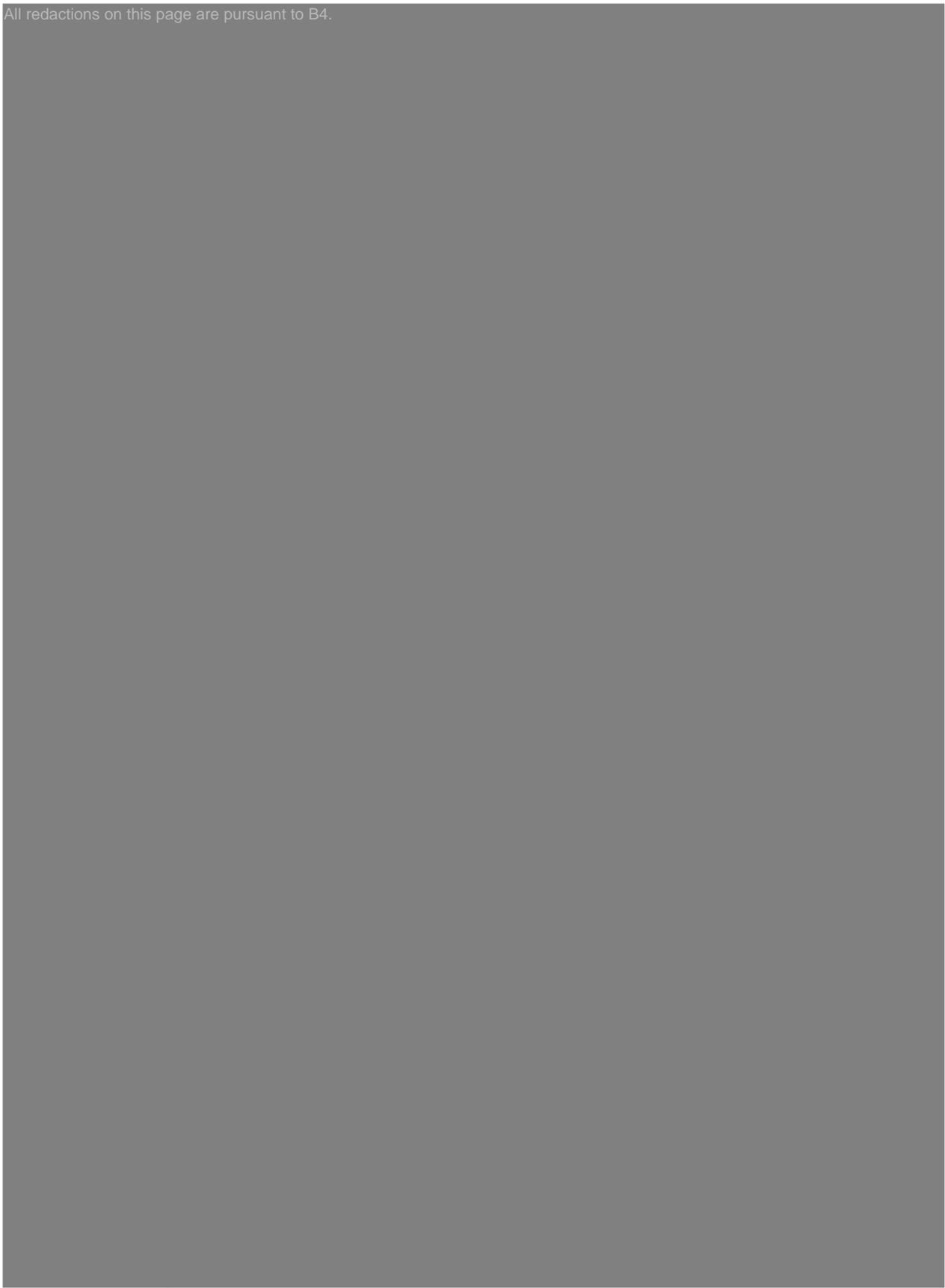
All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



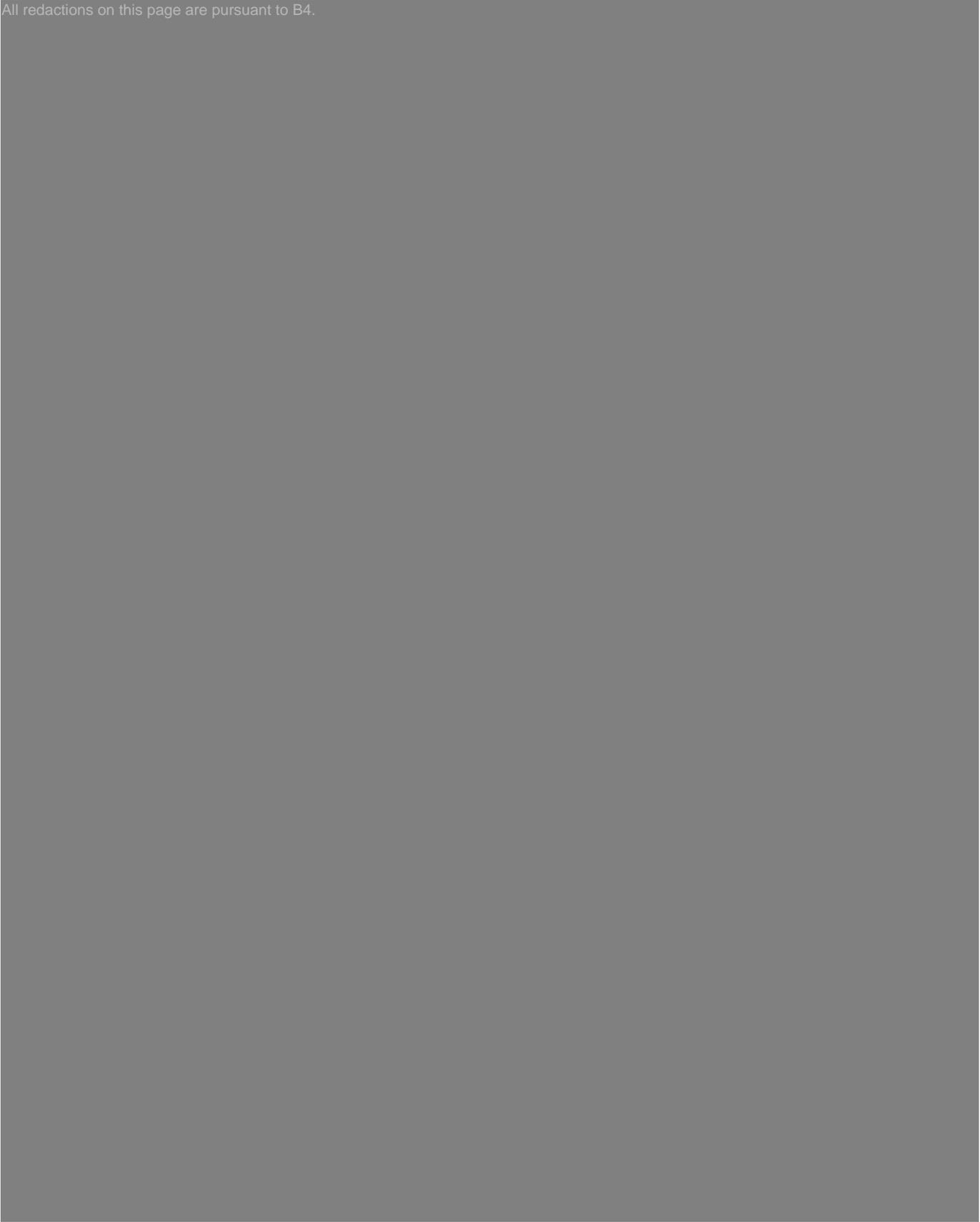
All redactions on this page are pursuant to B4.



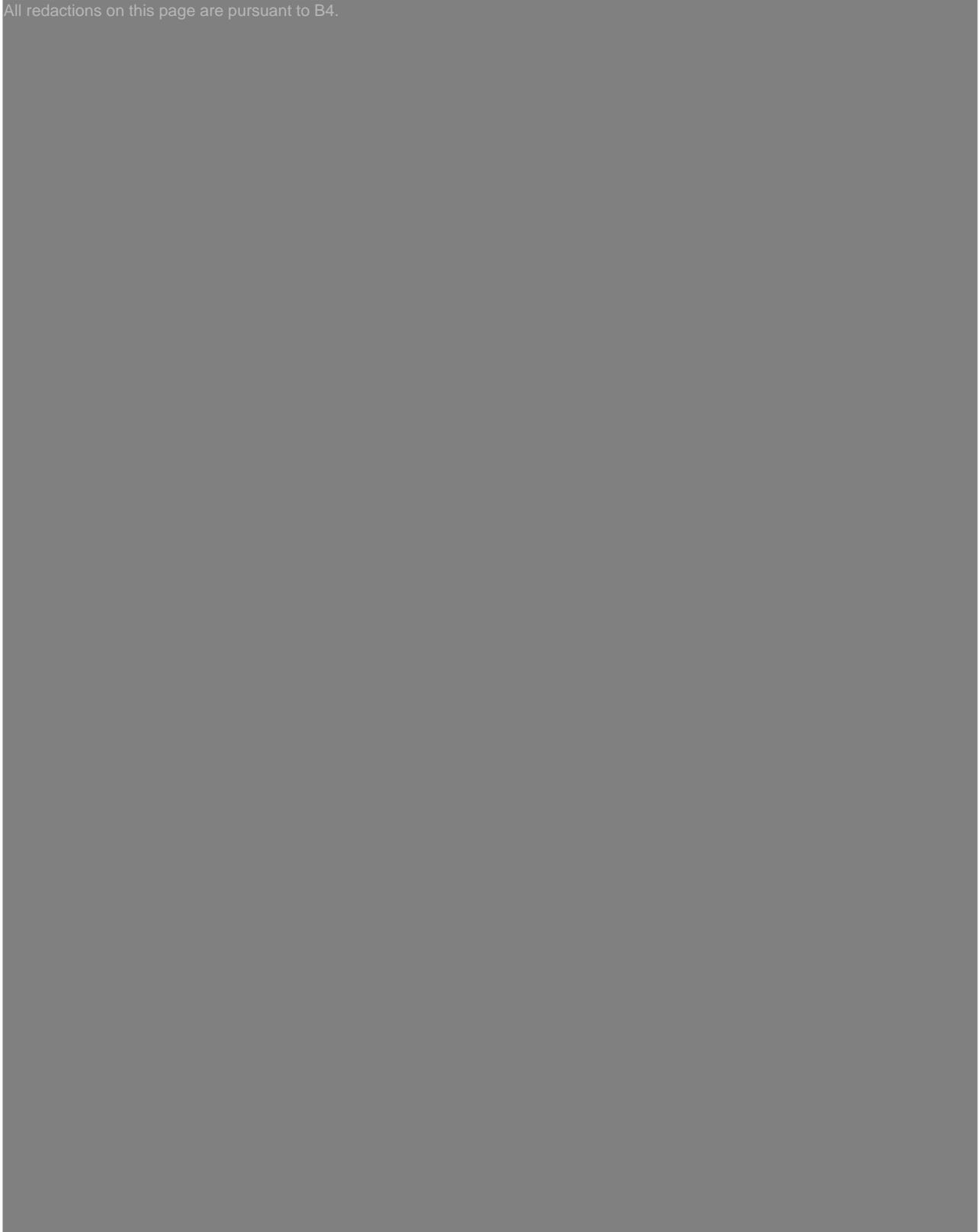
All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



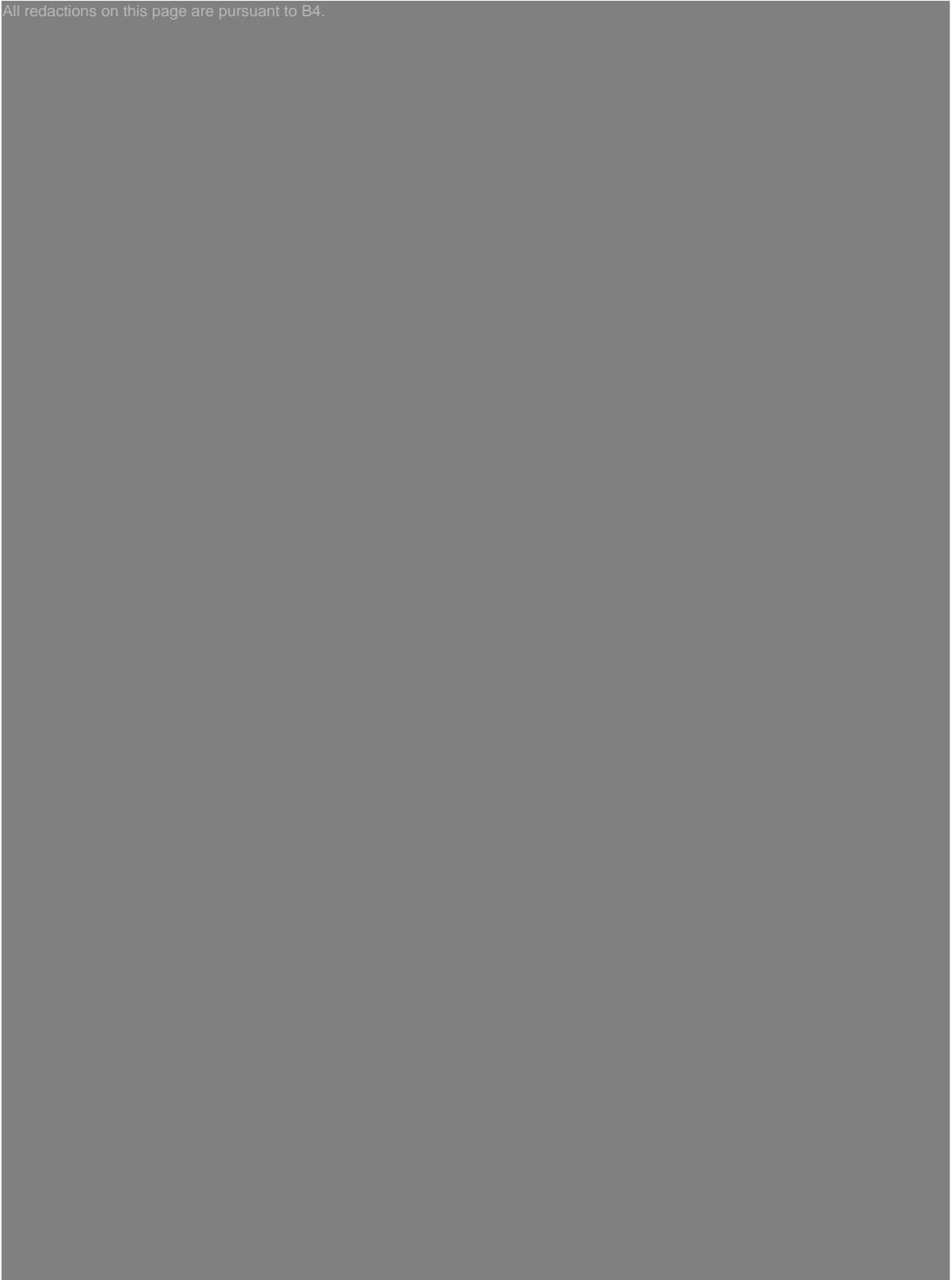
All redactions on this page are pursuant to B4.



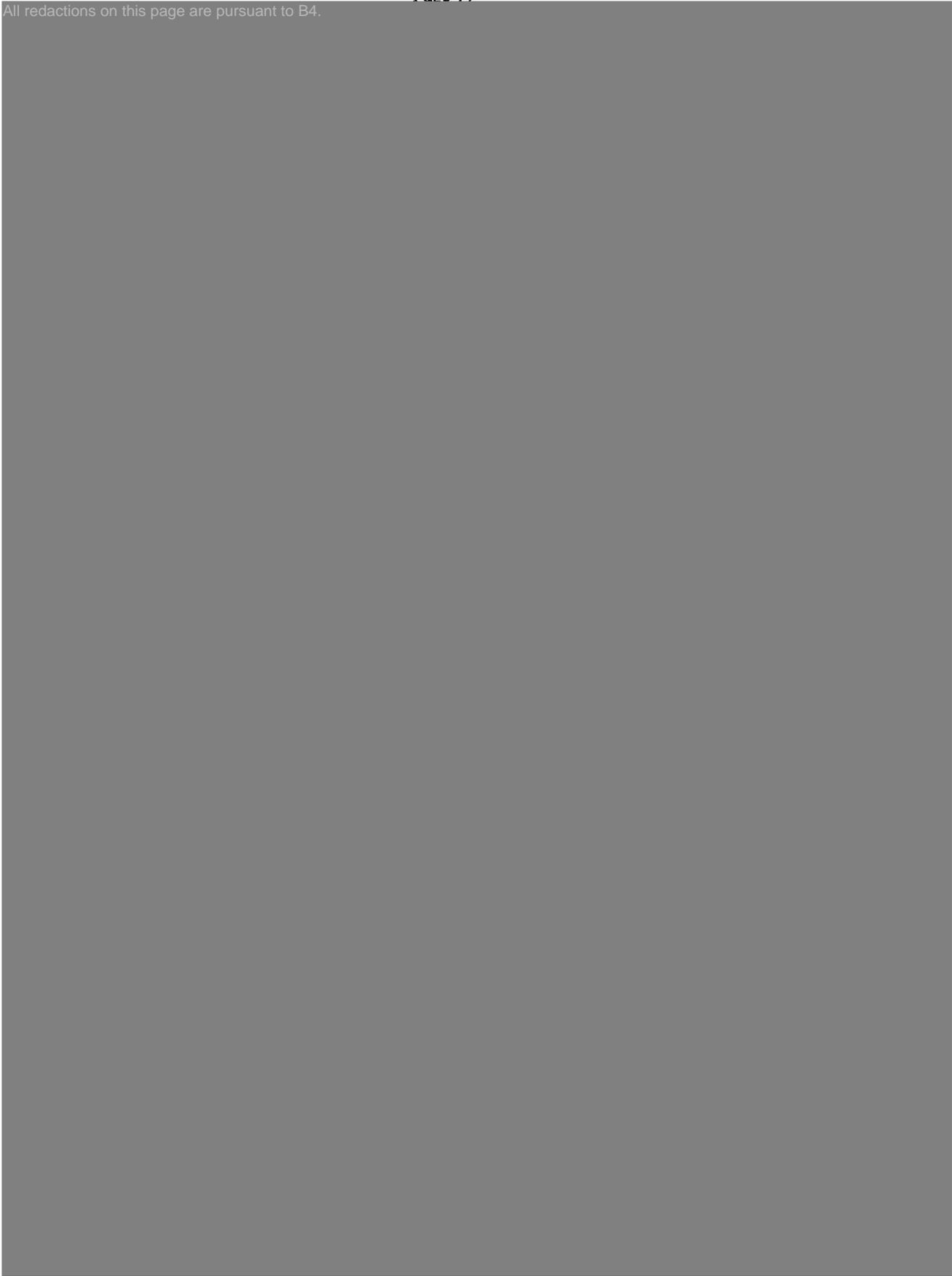
All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



All redactions on this page are pursuant to B4.



B4.



(b)(6)



The French text is a true translation of the English text. FOR EXPORT ONLY.

WYETH *from American Home Products*

Subsidiary:

FORT DODGE LABORATORIES, INC.

P. O. Box 518

Fort Dodge, Iowa 50501

U. S. Vet. License No. 112

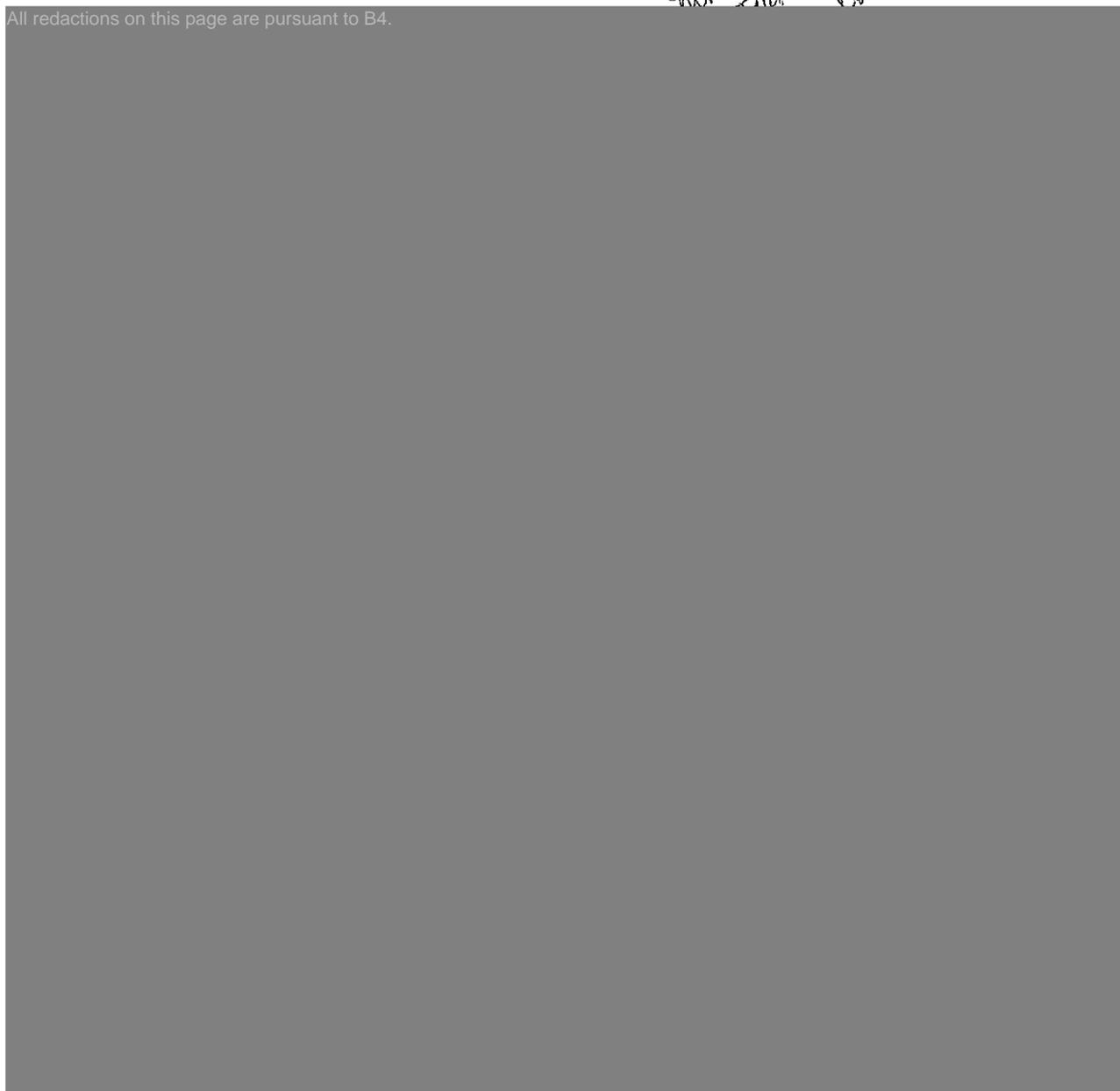
Salmonella Typhimurium Vaccine, Live Culture

(Poulvac® ST)

VS Code: 19C1.00

more full than on label 5170

All redactions on this page are pursuant to B4.



(b)(6)

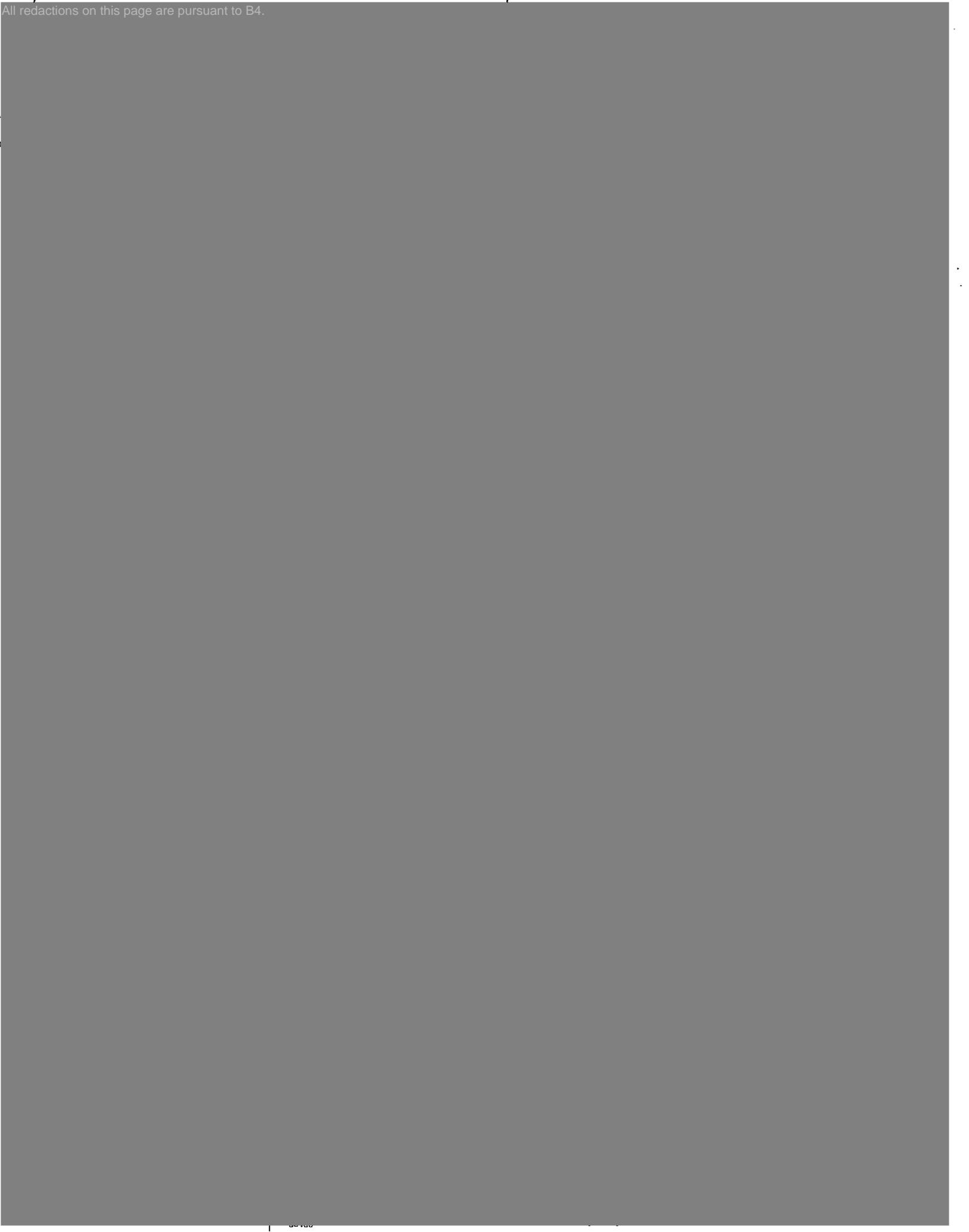
POLICY, EVALUATION, AND LICENSING
USE PERMITTED UNTIL FURTHER NOTICE

Compared to like kind label 5170 - Different from [unclear]

New

(b)(6)

Company to
New



| Est | Firm | Code | ML | Sub Date | Appr Date | type | study | V age | route | cfu/dose | C age | challenge |
|-----|------------|---------|-------|-----------|-----------|----------|-----------------|----------|--------------------------|----------|-------|--------------|
| 112 | Fort Dodge | 19C1.00 | 11519 | 22-Nov-99 | 29-Nov-99 | efficacy | accine in day c | 1d + 14d | coarse spray | | 6w | S heidelberg |
| 112 | Fort Dodge | 19C1.00 | 11519 | 22-Nov-99 | 29-Nov-99 | efficacy | accine in day c | 1d + 14d | coarse spray + drink H2O | | 6w | S heidelberg |
| 112 | Fort Dodge | 19C1.00 | 11519 | 22-Nov-99 | 29-Nov-99 | efficacy | accine in day c | n/a | n/a | non-vac | 6w | S heidelberg |
| 112 | Fort Dodge | 19C1.00 | 11519 | 22-Nov-99 | 29-Nov-99 | efficacy | accine in day c | n/a | n/a | non-vac | n/a | non-chal |
| 112 | Fort Dodge | 19C1.00 | 7332 | 27-Jul-99 | 25-Mar-99 | efficacy | vaccine in day | 1d + 14d | coarse spray | | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 7332 | 27-Jul-99 | 25-Mar-99 | efficacy | vaccine in day | 1d + 14d | coarse spray + drink H2O | | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 7332 | 27-Jul-99 | 25-Mar-99 | efficacy | vaccine in day | n/a | n/a | non-vac | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 7332 | 27-Jul-99 | 25-Mar-99 | efficacy | vaccine in day | n/a | n/a | non-vac | n/a | non-chal |
| 112 | Fort Dodge | 19C1.00 | 8143 | 27-Jul-99 | 14-May-99 | efficacy | vaccine in day | 1d + 14d | coarse spray | | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 8143 | 27-Jul-99 | 14-May-99 | efficacy | vaccine in day | 1d + 14d | coarse spray + drink H2O | | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 8143 | 27-Jul-99 | 14-May-99 | efficacy | vaccine in day | n/a | n/a | non-vac | 6w | SE |
| 112 | Fort Dodge | 19C1.00 | 8143 | 27-Jul-99 | 14-May-99 | efficacy | vaccine in day | n/a | n/a | non-vac | n/a | non-chal |

| Est | organs | # pos | organs | # pos | organs | # pos | TOTAL | # pos | notes |
|-----|---------------------|-------|-----------|-------|--------|-------|-------|--------------|--|
| 112 | liver spleen kidney | 15/37 | intestine | 34/37 | ceca | 34/37 | TOTAL | not reported | spray + spray not approved for intestine or ceca claim |
| 112 | liver spleen kidney | 8/40 | intestine | 27/40 | ceca | 27/40 | TOTAL | not reported | |
| 112 | liver spleen kidney | 29/30 | intestine | 28/30 | ceca | 30/30 | TOTAL | not reported | |
| 112 | liver spleen kidney | 0/20 | intestine | 0/20 | ceca | 0/20 | TOTAL | not reported | |
| 112 | liver spleen kidney | 15/27 | intestine | 22/27 | ceca | 24/27 | TOTAL | not reported | spray + spray not approved for intestine or ceca claim |
| 112 | liver spleen kidney | 18/29 | intestine | 27/29 | ceca | 29/29 | TOTAL | not reported | |
| 112 | liver spleen kidney | 20/20 | intestine | 18/20 | ceca | 19/20 | TOTAL | not reported | |
| 112 | liver spleen kidney | 0/8 | intestine | 0/8 | ceca | 0/8 | TOTAL | not reported | |
| 112 | liver spleen kidney | 16/39 | intestine | 36/39 | ceca | 34/39 | TOTAL | not reported | spray + spray not approved for intestine or ceca claim |
| 112 | liver spleen kidney | 12/40 | intestine | 30/40 | ceca | 17/40 | TOTAL | not reported | |
| 112 | liver spleen kidney | 18/20 | intestine | 20/20 | ceca | 19/20 | TOTAL | not reported | |
| 112 | liver spleen kidney | 0/15 | intestine | 0/15 | ceca | 0/15 | TOTAL | not reported | |