Approval Date: April 13, 1995

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

ANADA 200-117

B. Sponsor

Cross Vetpharm Group Limited
Broomhill Road
Tallaght, Dublin 24, Ireland

C. Proprietary Name

Oxyshot™ LA

D. Established Name

oxytetracycline injection

E. Pharmacological Category

Antimicrobial

F. Dosage Form

sterile injectable solution

G. Amount of Active Ingredient

200 mg/mL

H. How Supplied

100 mL, 250 mL, and 500 mL vials

I. Dispensing Status

OTC marketing status

J. Dosage Regimen

CATTLE

Oxyshot™ LA is to be administered by intramuscular or intravenous injection to beef cattle and nonlactating dairy cattle.

A single dose of 9 mg of Oxyshot™ LA per pound of body weight administered intramuscularly is recommended in the treatment of the following conditions: 1) bacterial pneumonia caused by *Pasteurella* spp. (shipping fever) in calves and yearlings where retreatment is impractical. 2) infectious bovine keratoconjunctivitis (pinkeye) caused by *Moraxella bovis*.

Oxyshot™ LA can also be administered by intravenous or intramuscular injection at a level of 3 to 5 mg of oxytetracycline per pound of body weight per day. In the treatment of severe foot rot and advanced cases of other indicated diseases, a dosage level of 5 mg per pound of body weight per day is recommended. Treatment should be continued 24 to 48 hours following remission of disease signs; however, not to exceed a total of four consecutive days. Consult your veterinarian if improvement is not noted within 24 to 48 hours of the beginning of treatment.

SWINE

A single dose of 9 mg of Oxyshot™ LA per pound of body weight administered intramuscularly is recommended in the treatment of bacterial pneumonia caused by *Pasteurella multocida* in swine, where retreatment is impractical.

Oxyshot™ LA can be administered by intramuscular injection at a level of 3 to 5 mg of oxytetracycline per pound of body weight per day. Treatment should be continued 24 to 48 hours following remission of diseases signs; however, not to exceed a total of four consecutive days. Consult your veterinarian if improvement is not noted within 24 to 48 hours of the beginning of treatment.

For sows, administer once intramuscularly 3 mg of oxytetracycline per pound of body weight approximately 8 hours before farrowing or immediately after completion of farrowing.

For swine weighing 25 lb of body weight and under, Oxyshot™ LA should be administered undiluted for treatment at 9 mg/lb but should be administered diluted for treatment at 3 or 5 mg/lb.

K. Route of Administration

Intramuscular in swine, intramuscular or intravenous in cattle.

L. Species/Class

Beef Cattle, Non-lactating Dairy Cattle and Swine

M. Reference Listed New Animal Drug

LIQUAMYCIN® LA-200; oxytetracycline injection; NADA #113-232; Pfizer

N. Indication

Oxyshot™ LA is intended for use in the treatment of the following diseases in beef cattle, nonlactating dairy cattle and swine when due to oxytetracycline susceptible organisms.

CATTLE

Oxyshot™ LA is indicated in the treatment of pneumonia and shipping fever complex associated with *Pasteurella* spp. and *Hemophilus* spp.; infectious bovine keratoconjunctivitis (pinkeye) caused by *Moraxella bovis*; foot rot and diphtheria caused by *Fusobacterium necrophorum*; bacterial enteritis (scours) caused by *Escherichia coli*; wooden tongue caused by *Actinobacillus lignieresii*; leptospirosis

caused by *Leptospira pomona*; and wound infections and acute metritis caused by strains of *staphylococci* and *streptococci* organisms sensitive to oxytetracycline

SWINE

In swine, Oxyshot™ LA is indicated in the treatment of bacterial enteritis (scours, colibacillosis) caused by *Escherichia coli*; pneumonia caused by *Pasteurella multocida*; and leptospirosis caused by *Leptospira pomona*.

In sows, Oxyshot™ LA is indicated as an aid in the control of infectious enteritis (baby pig scours, colibacillosis) in suckling pigs caused by *Escherichia coli*.

II. TARGET ANIMAL SAFETY and DRUG EFFECTIVENESS

The safety and effectiveness of Oxyshot™ LA was established through the following bioequivalency and target animal safety studies.

BIOEQUIVALENCY STUDY WITH 200 MG/ML OXYTETRACYCLINE INJECTABLE IN CATTLE

A. Type of Study: Bioequivalency Study

B. Investigator(s):

Colorado Animal Research Enterprises, Inc.
6200 East County Road 56
Fort Collins, Colorado 80524

C. General Design of the Investigation:

1. **Purpose:** To assess the *in vivo* bioequivalence of Cross Vetpharm Group, Ltd. formulation of oxytetracycline injection 200 mg/mL (Test Product) in cattle compared to Pfizer's 200 mg/mL Liquamycin® LA-200® (Reference Product) at the recommended dosage of 9 milligrams per pound.

2. **Test Animals:** Crossbred, beef-type steer and heifer calves, approximately 8-10 months of age, weighing an average of 446 pounds were randomly assigned to three groups. Groups I and II contained 12 animals each and Group III contained 2 animals as negative control animals for a total of twenty-six (26) test subjects.

   The study was conducted as a two-period crossover design in which Group I received Test Product and Group II received Reference Product in Period 1. Following a 42 day washout period, Group I received the Reference Product and Group II received the Test Product as period 2.

3. **Control Group:** The two animals assigned to Group III were designated as negative control animals. These animals were subject to the same handling as the test subjects (with the exception of dosing procedures) to demonstrate a lack of background oxytetracycline contamination in the evaluation of serum levels.

4. **Diagnosis:** Healthy test subjects were used for the purpose of this study. One day prior to each period, each calf received a veterinarian conducted physical examination encompassing assessment of general appearance, respiratory and GI tract auscultation, condition of feces and rectal temperature.

5. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

6. **Route of Administration:** Intramuscular injection.

7. **Dosage(s):** 9 milligrams oxytetracycline base per pound of body weight.

8. **Test Duration:** November 5, 1991 through December 23, 1991.

9. **Pertinent Parameters Measured:**

    i. Health Examinations

    ii. Clinical Evaluations

    iii. Body Weights

    iv. Serum Oxytetracycline Levels measured at appropriate intervals after treatment to determine Maximum Concentration (C_{MAX}), Time to Maximum Concentration (T_{MAX}), Elimination Rate (Ke), Elimination Half-Life (T1/2), Area Under the Curve (AUC), Area Under the Concentration x Time Curve (AUMC),and Mean Residence Time (MRT).

D. Results:

1. **Health Examinations** - Abnormal findings in test subjects (during examination prior to drug administrations and on observation during the blood collection and washout periods) were few and of minor severity and posed no interferences with study objectives.

2. **Clinical Evaluations** - Blood hematology and total serum protein analysis performed on blood specimens collected one day prior to the start of period 2 indicated normal values for all study calves. Fecal samples were collected one day prior to period 2 in response to a suspected coccidiosis outbreak in the test subjects. Coccidia oocyst burdens were found to be generally quite light among all study animals.

3. **Body Weights** - The calves continued to gain weight during the washout period and blood collections.

4. **Serum Oxytetracycline Levels** - Blood samples were collected within ±1-3 minutes of scheduled collection times. Serum was harvested and assayed for oxytetracycline activity. The individual serum levels were tabulated and the appropriate pharmacokinetic parameters calculated for the test and reference

products.

E. Statistical Analysis:

Differences in the pharmacokinetic parameters between Test and Reference products were statistically evaluated by means of 90% confidence intervals. Each endpoint of the 90% confidence interval for the difference in product means was divided by the reference product mean. All such confidence intervals were expressed as percentages by simply multiplying the endpoints by 100. Reference and test product means for each parameter are provided below, along with the corresponding confidence intervals.

Variable	**Test Mean**	**Reference Mean**	**Lower**	**Upper**
Area Under the Curve (AUC)	158.057	158.910	-3.919%	2.845%
Area Under the Moment Curve (AUMC)	3893.12	4133.00	-9.793%	-1.819%
Maximum Concentration(C_{MAX})	5.0565	4.897	-1.616%	8.130%
Time to Maximum Concentration (T_{MAX})	6.00	4.819	6.871%	42.134%
Mean Residence Time (MRT)	24.565	25.974	-9.241%	1.604%

F. Conclusions:

Based on a criterion that the 90% confidence interval for the difference between product means be within ±20% of the Reference Product mean, the Test Product was found to be bioequivalent to the Reference Product with respect to all pharmacokinetic parameters with the exception of T_{MAX}. The Test Product took approximately 26% longer to reach Maximum concentrations; however, MRT, showed bioequivalence of the 2 products.

G. Adverse Reactions:

No adverse reactions to the test or reference product dosages were noted during this study.

BIOEQUIVALENCY STUDY WITH Oxyshot™ LA (200 MG/ML OXYTETRACYCLINE INJECTABLE) IN SWINE

A. Type of Study: Bioequivalency Study

B. Investigator(s):

Animal Research Enterprises, Inc.
6200 East County Road 56
Fort Collins, Colorado 80524

C. General Design of the Investigation:

1. **Purpose:** To assess the *in vivo* bioequivalence of Cross Vetpharm Group, Ltd. formulation of oxytetracycline injection 200 mg/mL Oxyshot™ LA in swine compared to Pfizer's 200 mg/mL Liquamycin® LA-200® (Reference Product) at the recommended dosage of 9 milligrams per pound.

2. **Test Animals:** Crossbred, production-type barrows and gilts (pigs), approximately 10-12 weeks of age, weighing an average of 52 pounds were randomly assigned to two groups. Each group contained 20 animals each.

   The study was conducted as a single-period parallel experimental design in which Group I received Test Product and Group II received Reference Product.

3. **Control Group**: No control group was used in the conduct of this bioequivalency study.

4. **Diagnosis:** Healthy test subjects were used for the purpose of this study. During acclimation, each pig received a general health examination as partial basis for selection to the study. Feed intake for each pig was also determined for a 7 day period during acclimation to assist in assessing animal normalcy for consideration as study candidates.

5. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

6. **Route of Administration:** Intramuscular injection.

7. **Dosage(s):** 9 milligrams oxytetracycline base per pound of body weight.

8. **Test Duration:** April 30, 1992 through May 4, 1992.

9. **Pertinent Parameters Measured:**

   i. Observations were made twice daily to monitor the health status of the pigs.

   ii. Serum Oxytetracycline Levels measured at appropriate intervals after treatment to determine Maximum Concentration (C_{MAX}), Time to Maximum Concentration (T_{MAX}), Elimination Rate (Ke), Elimination Half-Life (T1/2_), Area Under the Curve (AUC), Area Under the Concentration x Time Curve (AUMC), Mean Residence Time (MRT), Area Under the Curve estimated to infinity (AUC), Area Under the Concentration x Time Curve estimated to infinity (AUMC),and Mean Residence Time estimated to infinity (MRT).

D. Results:

1. Daily observations of the pigs resulted in only minor and incidental abnormalities.

2. **Serum Oxytetracycline Levels** - Blood samples were collected within ±5 minutes of scheduled collection times. Serum was harvested and assayed for oxytetracycline activity. The individual serum levels were tabulated and the appropriate pharmacokinetic parameters calculated for the test and reference products.

E. Statistical Analysis:

Differences in the pharmacokinetic parameters between Test and Reference products were statistically evaluated by means of 90% confidence intervals. Each endpoint of the 90% confidence interval for the difference in product means was divided by the reference product mean. All such confidence intervals were expressed as percentages by simply multiplying the endpoints by 100. Reference and test product means for each parameter are provided below, along with the corresponding confidence intervals.

Variable	**Test Mean**	**Reference Mean**	**Lower**	**Upper**
Area Under the Curve (AUC)	80.986	75.864	-1.606%	15.108%
Area Under the Moment Curve(AUMC)	1615.85	1523.58	-11.425%	23.538%
Maximum Concentration(C_{MAX})	4.19	3.96	-3.275%	15.00%
Time to Maximum Concentration (T_{MAX})	1.325	1.775	-71.256%	20.552%
Mean Residence Time (MRT)	19.787	19.593	-10.685%	12.66%

F. Conclusions:

Based on a criterion that the 90% confidence interval for the difference between product means be within ±20% of the Reference Product mean, the Test Product was found to be bioequivalent to the Reference Product with respect to all pivotal pharmacokinetic parameters with the exception of T_{MAX} and AUMC. The Test Product took approximately 25% less time to reach maximum concentrations; however, MRT, showed bioequivalence of the 2 products, the value for the Test Product being approximately 101% of the Reference Product. In terms of AUMC, the Test Product was found to be approximately 6% more available than the Reference Product.

G. Adverse Reactions:

No adverse reactions to the test product dosage were noted during this study.

DRUG TOLERANCE TEST WITH THE INTRAVENOUS INJECTION OF Oxyshot™ LA(200 MG/ML OXYTETRACYCLINE INJECTABLE) IN CATTLE

A. Type of Study: Drug Tolerance Study

B. Investigator(s):

HTI Bio-Services, Inc.
P.O. Box 1319
Ramona, California 92065

C. General Design of the Investigation:

1. **Purpose:** To determine the target animal response to ten times the recommended dose (5 mg/lb) of Cross Vetpharm Group Limited's 200 mg/mL oxytetracycline injectable product.

2. **Test Animals:** One group consisting of four (4) crossbred steers and heifers, weighing an average of 539 pounds was used in the conduct of this study.

3. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

4. **Dosage(s):** A single administration of 50 milligrams oxytetracycline base per pound of body weight (10 times the recommended dosage).

5. **Route of Administration:** Intravenous injection.

6. **Test Duration:** October 21, 1992 through November 4, 1992.

7. **Pertinent Parameters Measured:**

   i. Body Weights

   ii. Feed Consumption

   iii. Water Consumption

   iv. Physical Examinations

   v. Clinical Observations

   vi. Hematology

   vii. Serum Chemistries

   viii. Urinalysis

   ix. Postmortem Examination and Histopathology

D. Results:

1. **Body Weights** - The calves lost an average of 16.53% of their body weight in the fourteen days between dosing and postmortem examination. Most of the weight loss occurred in the first week after dosing.

2. **Feed Consumption** - Although there was variation between animals, all were affected with significantly decreased feed consumption after receiving the high dosage.

3. **Water Consumption** - Although there was variation between animals, all were affected with significantly decreased water consumption after receiving the high dose.

4. **Physical Examinations** - Common findings after dosing were bi-lateral nasal discharges (generally clear), slight to modest depression, weakness in gait, and reduced rumen fill. Rough hair coats and loss of condition were also noted.

5. **Clinical Observations** - The clinical observations were consistent with the physical examination and feed and water results. Generally, the calves became slightly to moderately depressed after dosing. Clear nasal discharges were seen for variable lengths of time.

6. **Hematology** - Increases in segmented neutrophil count and fibrinogen were reported. These may reflect a combination of stress and the inflammatory process.

7. **Serum Chemistries** - Increases in aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN), phosphorus, sodium and potassium were reported. The elevation in creatinine and BUN indicate a transient decrease in renal function after treatment. The changes in sodium, potassium, and phosphorus are additional indications of the reduced renal function in homeostasis. Alanine transferase (ALT) changes, although not statistically significant, indicated some level of hepatic damage after treatment. This returned to normal by day 14 and no evidence of liver damage was seen upon histological examination.

8. **Urinalysis** - Reduction in specific gravity values and the presence of red blood cells and blood in the urine were reported. The specific gravity reduction, in the face of reduced water consumption, has been described by other investigators as a direct effect of tetracycline on the ability of the kidney to concentrate urine. The red blood cells and blood in the urine suggest renal/urinary tract hemorrhage.

9. **Postmortem Examination and Histopathology** - The only organ with lesions of a consistent nature that could be related to the drug is the kidney. These kidney lesions and their association with oxytetracycline toxicity in cattle is well documented.

E. Conclusions:

The results of this study have identified the kidney as the primary organ impacted by oxytetracycline injection given intravenously to cattle at 10x the recommended dosage. The information is useful for evaluating further target animal safety studies conducted using less exaggerated overdosage.

F. Adverse Reactions:

The test article was given at 10 times higher than the recommended dosage during this study. Reactions to the test product given at this dosage are noted in the study summary above.

TARGET ANIMAL SAFETY STUDY WITH THE INTRAVENOUS INJECTION OF Oxyshot™ LA (200 MG/ML OXYTETRACYCLINE INJECTABLE) IN CATTLE

A. Type of Study: Target Animal Safety Study

B. Investigator(s):

HTI Bio-Services, Inc.
P.O. Box 1319
Ramona, California 92065

C. General Design of the Investigation:

1. **Purpose:** To determine the safety of Cross Vetpharm Group Limited's 200 mg/mL oxytetracycline injectable product in cattle by administering the product intravenously for three times the recommended Maximum duration of use at 1X, 3X, and 5X the recommended dose (5 mg/lb). The carrier (N-METHYL-2-PYRROLIDONE) alone representing the amount received at the 1X dose was also administered for 3X the recommended duration of use.

2. **Test Animals:** Healthy, crossbred steers and heifers, weighing approximately 500 pounds were randomly assigned to 5 groups containing six animals each for treatment as follows.

   Group I - Normal Saline Control
   Group II - N-METHYL-2-PYRROLIDONE Control
   Group III - Oxytetracycline Injection 5 mg/lb
   Group IV - Oxytetracycline Injection 15 mg/lb
   Group V - Oxytetracycline Injection 25 mg/lb
   Treatments were administered at 0, 72 and 144 hours

3. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

4. **Dosage(s):** 5, 15, or 25 milligrams oxytetracycline base per pound of body weight (representing 1, 3 and 5 times the recommended intravenous dosage).

5. **Route of Administration**: Intravenous injection.

6. **Test Duration:** November 7, 1992 through November 28, 1992.

7. **Pertinent Parameters Measured:**

   i. Body Weights

   ii. Feed Consumption

   iii. Water Consumption

   iv. Physical Examinations

   v. Clinical Observations

   vi. Hematology

   vii. Serum Chemistries

   viii. Urinalysis

   ix. Postmortem Examination and Histopathology

D. Results:

1. **Body Weights** - A significant transient reduction in body weight was seen only in Group V (5X) and all animals returned to normal weight gains before the end of the study.

2. **Feed Consumption** - Feed consumption showed a dose response, with Group IV (3X) and Group V (5X) showing the most marked reduction.

3. **Water Consumption** - Water consumption did not show a clear dose response. All groups showed a similar pattern of reduction and return to normal attributed to handling and facility effects on the animals.

4. **Physical Examinations** - Physical examination findings showed a dose related response with Groups IV (3X) and V (5X) showing the most significant effects. Findings of depression, incoordination and injection site swellings were more frequent in these high dose groups.

5. **Clinical Observations** - The clinical observations were consistent with the physical examination results. Findings of depression, incoordination and injection site swellings were more frequent in the high dose groups along with pale urine in increased volume.

6. **Hematology** - Increases in the segmented neutrophil count and fibrinogen were reported. In the case of segmented neutrophil counts, Group V (5X) showed a mild inflammatory response at days 3 and 21 when compared to the saline controls group but all groups were within the pathologist's reference range. Fibrinogen was elevated in a dose-dependent manner after test material administration. This is compatible with consumption and hepatic

production responses related to the renal lesions and/or consumption at the time of intravenous injections.

7. **Serum Chemistries** - Changes in creatinine, blood urea nitrogen (BUN), and potassium levels were reported. Renal damage in Group V (5X) was indicated by transient increases in both BUN and creatinine after dosing. This change was compatible with histological changes seen in this group. The only other change seen was the reduction in potassium in Group V (5X) attributed to the transient renal disease in this group.

8. **Urinalysis** - Specific gravity reduction in Groups IV (3X) and V (5X) was reported. This has been described by other investigators as a direct effect of oxytetracycline on the kidney.

9. **Postmortem Examination and Histopathology** - No gross lesions that could be attributed to administration of the test article were found. The only organ with microscopic lesions of a consistent nature that could be related to this drug is the kidney. These kidney lesions were found only in Group V (5X) animals and their association with oxytetracycline toxicity in cattle is well documented.

E. Conclusions:

The results of this study have identified the kidney as the only organ with damage that could be related to the test material. The changes were seen only in Group V (5X) animals. Additional effects, such as reduced feed and water consumption and decreased body weight, were restricted primarily to Groups IV (3X) and V (5X) and were transient in nature with all groups returning to normal by the end of the study. The data support the safety of the product administered at the recommended dosage and route of administration.

F. Adverse Reactions:

No adverse reactions to the test product administered at the recommended dosage were noted during this study.

TARGET ANIMAL SAFETY STUDY WITH THE INTRAMUSCULAR INJECTION OF Oxyshot™ LA (200 MG/ML OXYTETRACYCLINE INJECTABLE) IN CATTLE

A. Type of Study: Target Animal Safety Study

B. Investigator(s):

HTI Bio-Services, Inc.
P.O. Box 1319
Ramona, California 92065

C. General Design of the Investigation:

1. **Purpose:** To determine the safety of Cross Vetpharm Group Limited's 200 mg/mL oxytetracycline injectable product in cattle by administering the product intramuscularly for three times the recommended Maximum duration

of use at 1X, 3X, and 5X the recommended dose (9 mg/lb). The carrier (N-METHYL-2-PYRROLIDONE) alone representing the amount received at the 1X dose was also administered for 3X the recommended duration of use.

2. **Test Animals:** Healthy, crossbred steers and heifers, weighing approximately 500 pounds were randomly assigned to 5 groups containing six animals each for treatment as follows.

   Group I - Normal Saline Control
   Group II - N-METHYL-2-PYRROLIDONE Control
   Group III - Oxytetracycline Injection 9 mg/lb
   Group IV - Oxytetracycline Injection 27 mg/lb
   Group V - Oxytetracycline Injection 45 mg/lb
   Treatments were administered at 0, 72 and 144 hours.

3. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

4. **Dosage(s):** 9, 27, or 45 milligrams oxytetracycline base per pound of body weight (representing 1, 3 and 5 times the recommended intramuscular dosage).

5. **Route of Administration:** Intramuscular injection.

6. **Test Duration:** October 21, 1992 through November 11, 1992.

7. **Pertinent Parameters Measured:**

   i. Body Weights

   ii. Feed Consumption

   iii. Water Consumption

   iv. Physical Examinations

   v. Clinical Observations

   vi. Hematology

   vii. Serum Chemistries

   viii. Urinalysis

   ix. Postmortem Examination and Histopathology

D. Results:

1. **Body Weights** - A significant transient reduction in body weight was seen only in Group V (5X) and all animals returned to normal weight gains before the end of the study.

2. **Feed Consumption** - Feed consumption showed a dose response, with Group IV (3X) and Group V (5X) showing the most marked reduction. Group V (5X) also showed the most severe injection site swellings and this may have impacted the desire or ability of the calves to consume feed. All calves returned to normal feed consumption before the end of the study.

3. **Water Consumption** - Water consumption showed a dose response with Groups IV (3X) and V (5X) showing a decrease and Groups I, II and III (Controls and 1X) showing nearly unchanged consumption. Group V (5X) also showed the most severe injection site swellings and this may have impacted the desire or ability of the calves to consume water. All calves returned to normal water consumption before the end of the study.

4. **Physical Examinations** - Physical examination findings showed a dose related response with Groups IV (3X) and V (5X) showing the most significant effects. The more severe injection site swellings were in Group V (5X) animals and those animals tended to have rough haircoats and depression.

5. **Clinical Observations** - The clinical observations were consistent with the above physical examination results.

6. **Hematology** - Increases in segmented the neutrophil count and fibrinogen level were reported. In the case of segmented neutrophil counts, Group V (5X) showed a mild inflammatory response on days 3-10, with a return to normal thereafter. Fibrinogen was elevated in the treatment groups over the two control groups but returned to normal by day 15 after drug administration. This event is compatible with consumption at the injection sites and a hepatic production response.

7. **Serum Chemistries** - Changes were reported among the following: alanine transferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CK), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total protein (TP), albumen and globulin.

   Liver damage was indicated by the ALT or AST increases, however, the histological evidence showed changes compatible with past bacterial lesions.

   Muscle damage from the injection reactions was evident in the dose dependent increase in CK after injection. This muscle damage may have been responsible for the AST increase described above. ALP, an indicator of tissue necrosis, was depressed (rather than elevated) in day 3-10 post injection and was attributed to reduction in feed consumption at that time.

   Renal damage was not indicated by either BUN or creatinine (both were depressed) after dosing.

   Total protein, albumen and globulin showed a mixed response over time and across dose groups. The changes seen did not appear to reflect any drug dosage effects and were attributed to fluid balance and other bacterial lesions.

8. **Urinalysis** - No biologically significant changes were seen in the urinalysis results.

9. **Postmortem Examination and Histopathology** - The only organ with gross lesions of a consistent nature that could be related to the test substance is the muscle at the injection site. Injection site lesions were dose related in severity and there was a clear pattern of resolution over time with healing progressing at the expected rate. The injection site muscle microscopic lesion findings were consistent with the gross pathology findings of increasing number of and severity of lesions with increasing dose. These lesions are consistent with those described in literature.

E. Conclusions:

The principal effects of the drug administered in this study were local tissue inflammation and necrosis at the site of intramuscular injection that was resolving by the end of the study. The additional effects on the animals, such as reduced feed and water consumption and decreased body weight were restricted primarily to the Groups IV (3X) and V (5X) and were transient in nature with all groups returning to normal by the end of the study. The data support the safety of the product administered at the recommended dosage and route of administration.

F. Adverse Reactions:

No adverse reactions to the test product administered at the recommended dosage were noted during this study.

DRUG TOLERANCE TEST WITH THE INTRAVENOUS INJECTION OF Oxyshot™ (200 MG/ML OXYTETRACYCLINE INJECTABLE) IN SWINE

A. Type of Study: Drug Tolerance Study

B. Investigator(s):

Greenbriar Veterinary Services, Inc.
6040 Dublin Rd.
Delaware, Ohio 43015

C. General Design of the Investigation:

1. **Purpose:** To determine the target animal response to ten times a 5 mg/lb dose of Cross Vetpharm Group Limited's 200 mg/mL oxytetracycline injectable product.

2. **Test Animals:** One group consisting of four (4) healthy, uniform crossbred barrows and gilts, 11 weeks old, weighing approximately 100 pounds was used in the conduct of this study.

3. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

4. **Dosage(s):** A single administration of 50 milligrams oxytetracycline base per pound of body weight (10X dose).

5. **Route of Administration:** Slow intravenous injection by catheterization.

6. **Test Duration:** September 22, 1992 through October 6, 1992.

7. **Pertinent Parameters Measured:**

   i. Body Weights

   ii. Feed Consumption

   iii. Physical Examinations and Clinical Observations

   iv. Hematology, Serum Chemistries and Urinalysis

   v. Postmortem Examination and Histopathology

D. Results:

1. **Body Weights** - Weight gains decreased during the week immediately after treatment but the gains returned to baseline levels during the second week after treatment.

2. **Feed Consumption** - Feed consumption was not affected by the treatment.

3. **Physical Examinations and Clinical Observations** - Significant stress was placed on the animals at the time of treatment and late on day 1, one pig developed acute respiratory distress related to marked cervical swelling (at the site of intravenous catheterization and blood specimen collection). The pig died acutely on day 5 of the trial with necropsy indicating hemorrhagic ulcerative gastroenteritis. All three surviving pigs were clinically normal during the trial except for cervical swellings. By the end (day 14) of the trial there were no detectable clinical abnormalities except swelling in the cervical area in one animal.

4. **Hematology, Serum Chemistries and Urinalysis** - None of the parameters demonstrated any apparent differences across time. Serum phosphorus remained at high normal values throughout the study but were uniformly distributed across time.

5. **Postmortem Examination and Histopathology** - Two of the surviving pigs contained no gross lesions and one contained a large cavitating lesion containing a fibronecrotic exudate at the site of intravenous catheterization and blood specimen collection.

   The only significant microscopic lesion in the 3 surviving pigs was a local tissue reaction at the site of catheterization.

E. Conclusions:

Based on the parameters measured, the pigs tolerated this drug product at the dose administered. The information is useful for evaluating further target animal safety studies conducted using less exaggerated overdosage.

F. Adverse Reactions:

The test article was given at 10 times higher than the recommended dosage during this study. Reaction to the test product given at this dosage are noted in the study summary above.

TARGET ANIMAL SAFETY STUDY WITH THE INTRAMUSCULAR INJECTION OF Oxyshot™ LA (200 MG/ML OXYTETRACYCLINE INJECTABLE) IN SWINE

A. Type of Study: Target Animal Safety Study

B. Investigator(s):

Greenbriar Veterinary Services, Inc.
6040 Dublin Rd.
Delaware, Ohio 43015

C. General Design of the Investigation:

1. **Purpose:** To determine the safety of Cross Vetpharm Group Limited's 200 mg/mL oxytetracycline injectable product in swine by administering the product intramuscularly for three times the recommended Maximum duration of use at 1X, 3X, and 5X the recommended dose (9 mg/lb). The carrier (N-METHYL-2-PYRROLIDONE) alone representing the amount received at the 1X dose was also administered for 3X the recommended duration of use.

2. **Test Animals:** Healthy, uniform crossbred barrows and gilts, eleven weeks of age weighing approximately 100 pounds were randomly assigned to 5 groups containing six animals each for treatment as follows.

   Group I - Normal Saline Control
   Group II - N-METHYL-2-PYRROLIDONE Control
   Group III - Oxytetracycline Injection 9 mg/lb
   Group IV - Oxytetracycline Injection 27 mg/lb
   Group V - Oxytetracycline Injection 45 mg/lb
   Treatments were administered at 0, 72 and 144 hours.

3. **Dosage Form:** The formulation of Oxyshot™ LA to be marketed, a sterile injectable solution containing 200 mg/mL oxytetracycline base, was used as the test article for this study.

4. **Dosage(s):** 9, 27, or 45 milligrams oxytetracycline base per pound of body weight (representing 1, 3 and 5 times the recommended intramuscular dosage).

5. **Route of Administration:** Intramuscular injection.

6. **Test Duration:** September 22, 1992 through October 13, 1992.

7. **Pertinent Parameters Measured:**

   i. Body Weights

   ii. Feed Consumption

   iii. Physical Examinations and Clinical Observations

   iv. Hematology, Serum Chemistries and Urinalysis

   v. Postmortem Examination and Histopathology

D. Results:

1. **Body Weights** - Weight gains decreased for three days immediately after treatment in all groups but the gains returned to normal levels during the remainder of the trial.

2. **Feed Consumption** - Feed consumption was not affected by the treatment for any of the groups. Feed efficiency did decrease slightly in all groups during the early treatment phase of the study.

3. **Physical Examinations and Clinical Observations** - Various incidental clinical abnormalities were noted throughout the trial period, none of which held any clinical significance other than swelling observed at the injection sites. Swelling at the injection site was detected in all three treated groups. The swellings did not cause impaired function and reduced over time becoming undetectable by study end.

4. **Hematology, Serum Chemistries and Urinalysis** - Some differences in values between acclimation and day 10 of the trial were reported. In these instances there were no significant differences among treatment groups and there were no time/group interactions. There were group differences in alkaline phosphatase, hemoglobin and hematocrit. Alkaline phosphatase decreased during the study in all groups and Group V (5X) values were significantly different than the other groups. This was thought not to be indicative of any problems. Hemoglobin and hematocrit values were reduced across time in the Group V (5X) pigs possibly due to hemolysis.

5. **Postmortem Examination and Histopathology** - Injection site lesions were confirmed in Group V (5X) animals upon gross examination postmortem. No other lesions were observed. The injection site lesions observed microscopically were small, mainly fibrous scars. No significant lesions were noted in the other tissues examined.

E. Conclusions:

The principal effect of the drug administered in this study was injection site lesions in all pigs in Group IV (3X) and V (5X). None of these lesions impaired muscular function and none were detectable clinically at the end of the study. A reduction in

hematocrit and hemoglobin was also observed in the Group V (5X) animals. The data support the safety of the product administered at the recommended dosage and route of administration.

F. Adverse Reactions:

No adverse reactions to the test product given at the recommended dosage were noted during this study.

III. HUMAN FOOD SAFETY

A. TOXICITY TESTS

Oxytetracycline

Toxicology studies were not required for the oxytetracycline component of this generic product.

N-METHYL-2-PYRROLIDONE

The following studies were conducted to investigate the human food safety issues of the new vehicle used in Oxyshot™ LA, N-METHYL-2-PYRROLIDONE.

***SALMONELLA*/MAMMALIAN-MICROSOME PREINCUBATION MUTAGENICITY ASSAY (AMES TEST)**

Report Number: T8246.502036

Start Date: 08/12/88

Termination Date: 09/28/88

Test Facility:

Microbiological Associates, Inc.
9900 Blackwell Road, Rockville, MD 20850

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Methods: This mutagenicity assay evaluates the mutagenic potential of the test article (or its metabolites) for its ability to induce back mutations at selected loci of several strains of *Salmonella typhimurium* in the presence and absence of an exogenous metabolic activation system of microsomal enzymes derived from Aroclor induced rat liver. The tester strains used in this study were TA97, TA102, and TA104.

Conclusions: The results of the of the *Salmonella*/Mammalian-Microsome Preincubation Mutagenicity Assay indicate that under the conditions of the study, N-Methylpyrrolidone did not cause a positive response on any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor induced rat liver.

SALMONELLA/MAMMALIAN-MICROSOME PLATE INCORPORATION MUTAGENICITY ASSAY (AMES TEST)

Report Number: T8246.501059

Start Date: 08/12/88

Termination Date: 09/29/88

Test Facility:

Microbiological Associates, Inc.
9900 Blackwell Road, Rockville, MD 20850

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Methods: This mutagenicity assay evaluates the mutagenic potential of the test article (or its metabolites) for its ability to induce back mutations at selected loci of several strains of *Salmonella typhimurium* in the presence and absence of an exogenous metabolic activation system of microsomal enzymes derived from Aroclor induced rat liver. The tester strains used in this study were TA97, TA102, and TA104.

Conclusions: The results of the of the *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay indicate that under the conditions of the study, N-Methylpyrrolidone did not cause a positive response on any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor induced rat liver.

MUTAGENICITY TEST ON N-METHYL-2-PYRROLIDONE IN THE RAT PRIMARY HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

Report Number: HLA Study No. : 10519-0-447

Start Date: 08/22/88

Termination Date: 10/08/88

Test Facility:

Hazleton Laboratories America, Inc.
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Methods: In the *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis (UDS) Assay, the test material did not induce significant increases in UDS. Freshly prepared rat hepatocytes were exposed to N-Methylpyrrolidone at concentrations ranging from 5000 µg/ml to 0.500 µg/ml in the presence of 5 µCi/ml 3HTdr (20 Ci/mmole). Treatment at 5000 µg/ml was not analyzed for nuclear labeling due to high toxicity. Treatments from 4000 µg/ml to 250 µg/ml, which covered a good range of toxicity (75.3 to 97.6% survival), were selected for analysis. The test material was soluble in

media at all concentrations tested. None of the criteria used to indicated UDS were approached by the chemical treatments and no dose-related response was observed.

Conclusions: N-Methylpyrrolidone was evaluated as inactive in the Rat Primary Hepatocyte UDS assay.

MUTAGENICITY TEST ON N-METHYL-2-PYRROLIDONE IN THE MOUSE LYMPHOMA FORWARD MUTATION ASSAY

Report Number: HLA Study No. : 10519-0-431

Start Date: 09/15/88

Termination Date: 12/08/88

Test Facility:

Hazleton Laboratories America, Inc.
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Summary: The objective of this *in vitro* assay was to evaluate the ability of N-methyl-pyrrolidone to induce forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line. The test material was soluble in sterile deionized water at 50.0 mg/ml. In the preliminary cytotoxicity assay, cells were exposed to the test material for four hours in the presence and absence of rat liver S9 metabolic activation. The test material remained in solution and caused no adverse pH effects in the culture medium up to the maximum applied concentration of 5.0 mg/ml. The test material was nontoxic under both test conditions.

In the mouse lymphoma assay, *in vitro* treatments with the test material did not induce any significant increases in mutant frequency at the TK locus. Mutation assays were performed both with and without S9 metabolic activation conditions. Aroclor 1254 was the enzyme inducer used. The nonactivation trial included five dose levels, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. Six dose levels were used in the S9 activation portion of the study and included 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. The test material was not toxic to the mouse lymphoma cells in culture at any applied dose level. The mutant frequencies of treated cultures varied randomly with dose. Therefore, the test material was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells under the nonactivation and S9 metabolic activation conditions used in this study.

MUTAGENICITY TEST ON N-METHYL-2-PYRROLIDONE IN THE CHO/HGPRT FORWARD MUTATION ASSAY

Laboratory Number: 10194-0-435

Report Date: June 23, 1988

Test Facility:

Hazleton Laboratories America, Inc.
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Summary: The objective of this *in vitro* assay was to evaluate the ability of N-methylpyrrolidone to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary cells under conditions with and without metabolic activation.

The test material was soluble in F12 culture medium at 50.0 mg/ml. Preliminary range finding cytotoxicity testing found the test article to be nontoxic at all dose levels tested from 0.005 mg/ml to 5.0 mg/ml under both nonactivation and S9 metabolic activation test conditions. For each test condition, six dose levels that ranged from 0.5 mg/ml to 5.0 mg/ml were used in the mutation assays. The test article was not toxic at any dose level in the mutation assays.

Mutant frequencies of all cultures treated with test material varied randomly with dose within a range acceptable for negative control mutant frequencies. In the S9 metabolic activation mutation assay, one of the six treatment conditions achieved statistical significance. The culture that achieved statistical significance had a mutant frequency within the acceptable range for background mutant frequencies. The statistical significance was apparently due to normal assay variation. Without S9 metabolic activation, none of the six dose levels had mutant frequencies that achieved statistical significance. Therefore, N-methylpyrrolidone was considered negative for inducing forward mutation at the HGPRT locus in CHO cells under the S9 metabolic activation and nonactivation conditions of the assay.

EVALUATION OF THE SAFETY OF N-METHYL-2-PYRROLIDONE IN WISTAR-DERIVED RATS FOLLOWING 90-DAY ADMINISTRATION IN THE DIET

Laboratory Number: 5026

Report Date: 12/09/76

Test Facility:

Food and Drug Research Laboratories, Inc.
Route 17C
Waverly, NJ 14892-0107

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Summary: This study was conducted to evaluate the safety of the compound N-methyl pyrrolidone following a 90-day administration in the diet of weanling rats. Two hundred (200) weanling FDRL-Wistar rats, 100 of each sex, were used in the study. The compound was administered at dosage rates of 0 (control), 800, 2000, and 5000 ppm. The 800, 2000 and 5000 ppm dosages produced a significant effect ($p<0.05$) on female body weights (decrease), male thyroid weight (greater), and the chemical properties of urine (pH in males and females, albumin and specific gravity

in females, and SGPT in males at termination). It was concluded, however, that the 800 ppm dose level (which equated to a 40 mg/kg consumption level) was the no effect level (NOEL) for the compound.

90 DAY FEEDING STUDY IN BEAGLE DOGS WITH N-METHYL-2-PYRROLIDONE

Laboratory Number: 6414

Report Date: 09/03/80

Test Facility:

Food and Drug Research Laboratories, Inc.
Route 17C
averly, NJ 14892-0107

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Summary: This study was conducted to evaluate the potential systemic toxicity of dietary n-methylpyrrolidone when administered to beagle dogs for 90 days at dose levels of 0, 25, 79 and 250 mg/kg body weight. Forty-eight (24 male and 24 female) beagle dogs ranging from 5-6 months of age at the beginning of the study were included. Body weights and food consumption were measured weekly, animals were observed daily, blood samples for hematology and biochemical determination were collected prior to initiation of the study and at monthly intervals. Urine was collected and analyzed pretest, 60 days after initiation and at termination. At the termination of the study (90 days) gross and microscopic examinations were performed.

No significant differences in body weight and food consumption during the 90 day feeding period were noted among the groups nor were any signs of toxicity or behavioral abnormality observed. Intermittent significant variations in total cholesterol, serum albumin and serum total protein were noted but toxicological significance was not supported by any histopathological finding. No test article related histopathological findings were noted in any organs and analysis of organ weight failed to reveal any significant treatment differences.

TERATOLOGIC EVALUATION OF N-METHYL-2-PYRROLIDONE AFTER DERMAL APPLICATION IN SPRAGUE-DAWLEY RATS

Laboratory Number: 6161

Report Date: 10/18/79

Test Facility:

Food and Drug Research Laboratories, Inc.
Route 17C
Waverly, NJ 14892-0107

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid.

Summary: This study was conducted to evaluate the teratogenic potential of n-methylpyrrolidone administered dermally to pregnant Sprague-Dawley rats at 0 (negative, positive and aspirin control groups), 75, 237 or 750 mg/kg body weight per day on days 6 through 15 of gestation. Six (6) groups were utilized providing 25 female rats per group. On day 20 of gestation, all animals were euthanized for uterine examination and fetal examination for skeletal and soft tissue abnormalities.

Bright yellow urine and dry skin, the frequency and severity of which increased with dosage, was noted. At the 750 mg/kg dose, maternal toxicity was indicated by significantly lower dam body weights on days 15 and 20 and reduced weight gains during gestation. This dosage also resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites (although percentage of dams showing resorption was unaffected), and a significant reduction in fetal weights.

Fetal examination revealed a variety of skeletal variations and possible malformations at the 750 mg/kg dose indicating a possible retardation in fetal development and possibly the teratogenic potential of the compound at the high dose. Treatment at the 750 mg/kg/day dosage resulted in observations similar to positive controls (administered hexafluoroacetone solution) and that level could be considered maternally toxic. Treatment at levels below 750 mg/kg/day (75 or 237 mg/kg/day) had no effect on dam body weights, reproduction or fetal development.

NMP (N-METHYL-2-PYRROLIDONE) - DEVELOPMENTAL TOXICITY STUDY IN NEW ZEALAND WHITE RABBITS

Laboratory Number: 637-002

Report Date: 12/17/91

Test Facility:

International Research and Development Corporation
Mattawan, Michigan, U.S.A. 49071

Summary: Inseminated New Zealand White SPF female rabbits were used to determine the developmental toxicity including the teratogenic potential of NMP (N-METHYL-2-PYRROLIDONE). The rabbits were randomly assigned to one placebo control and three treatment groups (55, 175 and 540 mg/kg/day) consisting of twenty (20) animals each. The treatments were administered orally by gavage as a single daily dose on gestation days 6 through 18 at a volume of 3.0 ml/kg. Caesarean section examinations were performed on all females on gestation day 29, followed by teratologic examination of the fetuses.

Maternal toxicity was observed at 540 mg/kg/day. Statistically significant inhibition of maternal body weight gain and food consumption were observed at this level and an abortion at 540 mg/kg/day was confirmed. At 175 mg/kg/day, there appeared to be a dose related trend toward inhibited body weight gain; however, this inhibition was only statistically significant during first treatment subinterval (days 6-12). This, plus the fact that reduced feed consumption did not attain statistical significance when compared to the control group, and in the absence of any other overt signs of toxicity, does not clearly establish the 175 mg/kg/day dose as maternally toxic.

Developmental toxicity was observed at 540 mg/kg/day manifested by increased postimplantation loss, increased incidences of cardiovascular and skull malformations and developmental variations.

The no adverse effect level (NOAEL) based on this study was equivocally considered to be 55 mg/kg/day with respect to maternal toxicity, and 175 mg/kg/day with respect to developmental toxicity.

MULTI-GENERATION RAT REPRODUCTION STUDY WITH N-METHYL -2-PYRROLIDONE

Laboratory Number: 236535

Report Date: November 26, 1991

Test Facility:

Exxon Biomedical Sciences, Inc.
Toxicology Laboratory
Mettlers Road, CN2350
East Millstone, NJ 08875-2350

Substance/Dosage Form: N-METHYL-2-PYRROLIDONE clear liquid

Summary: A multi-generation reproduction study of N-METHYL-2-PYRROLIDONE in rats was carried out at dose levels of 50, 160 and 500 mg/kg in the diet. There were no significant differences in reproduction data for the P1 generation. However, high dose F1b (P2) male mating indices and fertility indices were lower than controls for both litters. High dose female fertility and fecundity indices were lower than controls for both litters. Additionally, there were differences in survival indices and growth rate at this 500 mg/kg dose level for all litters.

Conclusions: The 160 mg/kg/day dose was established as the parental, reproductive and developmental NOAEL in this study.

B. SAFE CONCENTRATION OF RESIDUES

The NOEL from the 90-day rat study (40 mg/kg) was used to calculate the safe concentration for total residues on N-METHYL-2-PYRROLIDONE in edible tissues. Using a safety factor of 1,000, the calculated safe concentration for the total residues of N-METHYL-2-PYRROLIDONE in muscle is 8 ppm. However, in the absence of chronic toxicity studies in the rodent and the dog, the Maximum allowable residues that can be assigned to muscle are 5 ppm. Residue levels in the cattle and swine metabolism studies described below are much less than 5 ppm (0.35 ppm and 0.069 ppm at 21 days in cattle and swine muscle, respectively).

C. METABOLISM AND TOTAL RESIDUE DEPLETION STUDIES

Since the depletion characteristics of the vehicle used in the generic product were not known, abbreviated total residue studies were conducted to determine whether the generic product would be regulated on the basis of the vehicle, N-methyl-2 pyrrolidone, or the active ingredient, oxytetracycline. The results of total residue

studies in cattle and swine demonstrated that residues of N-METHYL-2-PYRROLIDONE would deplete to their safe concentration by 21 days withdrawal. Since this is less than the 28-day withdrawal for oxytetracycline codified under 21 CFR 522.1660, the proposed generic product will be regulated based on the depletion of the active ingredient, oxytetracycline.

1. **Cattle and Swine Total Residue and Metabolism Studies**

ABBREVIATED TOTAL RESIDUE AND METABOLISM STUDY FOR 14C-N-METHYL-2-PYRROLIDONE ADMINISTERED TO BEEF CATTLE

Investigators:

Analytical Development Corporation
4405 North Chestnut Street
Colorado Springs, Colorado 80907
and
Metabolic Laboratory
Colorado State University
Fort Collins, Colorado 80523

Test Animals: Six (6) beef-type, steers and heifers 7 to 8 months of age were used.

Route, Time and Duration of Drug Administration: A single dose of ^{14}C-N-METHYL-2-PYRROLIDONE was administered intramuscularly in three portions.

Total Residues in Tissues of Cattle Treated with ^{14}C-N-METHYL-2-PYRROLIDONE:

Total Residues (ppm)

Withdrawal Time (days)	**Muscle**	**Kidney**	**Liver**	**Fat**	**Inj. Site Muscle**
1	19.93 ± 0.84	28.04 ± 4.82	23.43 ± 1.12	2.42 ± 0.66	20.82 ± 0.42
4	1.67 ± 0.34	3.42 ± 0.64	4.86 ± 1.09	0.25 ± 0.01	1.65 ± 0.30
21	0.35 ± 0.01	0.42 ± 0.01	0.78 ± 0.01	0.20 ± 0.00	0.31 ± 0.04

Summary of Metabolism Study: Extracts from tissues in this study were analyzed by TLC and HPLC to identify the major metabolite profiles.

ABBREVIATED TOTAL RESIDUE AND METABOLISM STUDY FOR ^{14}C-N-METHYL-2-PYRROLIDONE ADMINISTERED TO SWINE.

Investigators:

Analytical Development Corporation
4405 North Chestnut Street

Colorado Springs, Colorado 80907
and
Metabolic Laboratory
Colorado State University
Fort Collins, Colorado 80523

Test Animals: Seven (7) hampshire cross, barrows and gilts weighing approximately 50 pounds were used.

Route, Time and Duration of Drug Administration: A single dose of ^{14}C-N-METHYL-2-PYRROLIDONE was administered intramuscularly.

Total Residues in Tissues of Swine Treated with ^{14}C-N-METHYL-2-PYRROLIDONE:

Total Residues (ppm)

Withdrawal Time (Days)	**Muscle**	**Kidney**	**Liver**	**Fat**	**Inj.Site Muscle**
1	18.30 ± 1.27	26.55 ± 0.21	21.75 ± 3.32	5.20 ± 0.75	15.15 ± 4.17
3*	0.98	1.89	2.89	0.33	1.15
4	0.39	0.72	0.23	0.29	0.46
21	0.07 ± 0.03	0.05 ± 0.01	0.07 ± 0.02	0.14 ± 0.01	0.07 ± 0.02

*Pig died on day 3 of withdrawal; samples were collected at necropsy.

Summary of Metabolism Study: Extracts from tissues in this study were analyzed by TLC and HPLC to identify the major metabolite profiles.

2. **Total Residues of Oxytetracycline:** For a generic oxytetracycline injection, total residue studies for oxytetracycline are not required.

3. **Comparative Metabolism in the Rat:** An oral comparative metabolism study in the rat demonstrated that the metabolites of N-METHYL-2-PYRROLIDONE produced in the toxicology test species were comparable to those produced in the target species.

COMPARATIVE METABOLISM STUDY OF ^{14}C-N-METHYL-2-PYRROLIDONE ADMINISTERED ORALLY TO RATS

Investigators:

Analytical Development Corporation
4405 North Chestnut Street
Colorado Springs, Colorado 80907

Test Animals: Eleven (11) male and eleven (11) female Sprague-Dawley rats weighing approximately 250 g (males) and 225 g (females) were used.

Route, Time and Duration of Drug Administration: ^{14}C-N-METHYL-2-PYRROLIDONE was administered by oral gavage at a nominal rate of 50 mg/kg body weight (calculated to be between 48.2 and 55.7 mg/kg) for three (3) consecutive days.

Total Residue (TR) Recoveries:

Rat

Animal Type and Tissue	**Time of Sacrifice and Animal #**		**% TR in Methanol Extract**	**% TR in Post Extracted Solids**	**Total % TR Recovered**
Liver	9 hr	1 + 2	81.7	17.8	99.5
Liver	9 hr	9 + 6	68.8	25.5	94.3
Liver	15 hr	9 + 5	48.9	43.7	92.6
Liver	15 hr	1 + 3	47.4	44.6	92.0
Kidney	9 hr	1 + 2	92.8	7.57	100.4
Kidney	9 hr	9 + 6	92.1	9.07	101.2
Stomach Contents	9 hr	1 + 2	102.1	1.63	103.7
Stomach Contents	9 hr	9 + 6	101.6	1.08	102.7
Feces1	9 hr	1 + 2	91.6	9.16	100.8
Feces1	9 hr	9 + 6	96.4	3.06	99.5

Cattle

Animal Type and Tissue	**Time of Sacrifice and Animal #**		**% TR in Methanol Extract**	**% TR in Post Extracted Solids**	**Total % TR Recovered**
Liver	1 day	9177	87.9	3.95	91.9
Liver	1 day	9217A	91.6	4.48	96.1
Swine	1 day	7	94.8	8.48	103.3
Swine	1 day	1	92.4	11.4	103.8

1 48-hour samples (collected 48 hrs after initial dosing)

Summary of Metabolism Study:

Extracts from tissues and urine, feces and stomach contents in this study were analyzed by Liquid Scintillation Counting (LSC) and Thin Layer Chromatography (TLC). For metabolite characterization, the methanol extracts of male and female rat liver samples were examined, along with urine samples collected after the start of dosing from one male and one female animal. For the 9-hour animals, greater than 68% of the total ^{14}C-residue in liver was extracted with methanol. For the 15 hour animals, the ^{14}C-residues extracted with methanol decreased to an average of 48%.

Additional extractions and analysis of rat kidney and stomach content samples, as well as 48-hour feces samples, were carried out for the 9 hour rats. Greater than 90% of the total ^{14}C-residue present in these samples was extracted with methanol.

Overall, the metabolites found in rat liver samples and urine correspond to those found in liver and urine of cattle and swine. The exception is that 2-pyrrolidone has been found in cattle, but not in any rat samples examined.

D. TOLERANCE

The tolerance established for the pioneer product applies to the generic product. A tolerance of 0.1 ppm is established for the uncooked edible tissues of beef cattle, non- lactating dairy cattle and swine (21 CFR 556.500).

E. WITHDRAWAL TIME

Under the 1990 Bioequivalence Guideline, when a generic product is determined to be bioequivalent to the pioneer product in a blood level bioequivalence study, the withdrawal times for the generic product are those previously assigned to the pioneer product.

The withdrawal time for oxytetracycline injection is established in 21 CFR 522.1660 as 28 days for beef cattle, non-lactating dairy cattle, and swine.

F. REGULATORY METHOD FOR RESIDUES

The regulatory analytical method for detection of residues of the drug is a cylinder plate diffusion microbiological assay using *Bacillus cereus* var. *mycoides* (ATCC 11778). The method is published by the Food and Drug Administration, "Antibiotic Residues in Milk, Dairy Products and Animal Tissues: Methods, Reports, and Protocols", Revised October 1968, reprinted December 1974.

IV. AGENCY CONCLUSIONS

The data submitted in support of this hybrid application satisfy the requirements of Section 512(b) (1) and 512 (b)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) and 21 CFR Part 514 of the regulations. The hybrid application has been defined in the Center's Seventh Generic Animal Drug Policy Letter, dated March 20, 1991.

An applicant may seek approval of a modification of a listed animal drug that requires the review of investigations. The statute could be interpreted to require such an applicant to first obtain approval of an ANADA for the listed animal drug's approved product, and then file a 512(b)(1) supplement to the approved ANADA containing clinical data to obtain approval of the modification. If the applicant did not first obtain an ANADA for the approved product, the applicant could be required to submit a full new animal drug application (NADA) under section 512(b)(1) of the act for the modification and duplicate the basic safety and effectiveness studies conducted on the listed animal drug. The agency has concluded that such an interpretation would be inconsistent with the legislative purposes of the 1988 Amendments because it would serve as a disincentive to innovation and would require needless duplication of research.

The agency believes that a more consistent, less burdensome interpretation of the 1988 Amendments is to allow a generic applicant to submit a 512(b)(1) application for a change in an already approved animal drug that requires the submission and review of investigations conducted by or for the applicant, without first obtaining approval of an ANADA for a duplicate of the listed animal drug. Therefore, the agency accepts applications for changes requiring the review of investigations conducted by or for the applicant . These applications are known as "hybrid" applications. Like similar supplements to approved ANADA's, these applications rely on the approval of the listed animal drug, together with the data needed to support the change.

The data submitted in support of this hybrid application demonstrate that Oxyshot™ LA (oxytetracycline injection 200 mg/mL), when used under its proposed conditions of use, is safe and effective for its labeled indications. The hybrid application allows a change in the pioneer product (Liquamycin LA-200) formulation, by providing for a new vehicle (N-METHYL-2-PYRROLIDONE) for oxytetracycline injection 200 mg/mL for cattle and swine.

N-METHYL-2-PYRROLIDONE has not previously been approved as a vehicle for any new animal drug product. Therefore, data were required to test the target animal safety and human food safety of oxytetracycline with N-METHYL-2-PYRROLIDONE, under section 512(b)(1) of the act.

The hybrid application relies on the approval of a listed (pioneer) animal drug, and contains additional data needed to support the change in the generic product. The hybrid applicant is thus relying on the approval of the listed animal drug to the extent that such reliance is allowed under section 512(n) of the act, to establish the safety and effectiveness of the active ingredient. An application that relies in part on the approval of a listed animal drug, is, for this purpose, considered an application described in section 512(b)(2). Because this hybrid application is reviewed in part as an application under section 512(b)(1) of the act, the hybrid application is eligible for 3 years of exclusivity under section 512(c)(2)(F)(iii) of the act.

Under Section 512(c)(2)(F)(iii) of the Act, this approval for food-producing animals qualifies for three (3) years of marketing exclusivity beginning on the date of approval because the supplemental application contains reports of new clinical or field investigations (other than bioequivalence or residue studies) essential to the approval of the application and conducted or sponsored by the applicant.

Under the Center’s supplemental approval policy (21 CFR 514.106(b)(2)(ii)), this is a Category II change. The approval of this change is not expected to have any adverse effect on the safety or effectiveness of this new animal drug.

Patent number 4,772,460 applies to this product. The patent was issued on September 20, 1988 and expires on September 20, 2005.

The tolerance established for the pioneer product applies to the hybrid product. A tolerance of 0.1 ppm is established for oxytetracycline in the uncooked edible tissues of beef cattle, non-lactating dairy cattle, and swine (21 CFR 556.500).

The results of the total residue studies in cattle and swine demonstrated the residues of N-methyl-2-pyrrolidone would deplete to their safe concentration of the established 28 day withdrawal for oxytetracycline as codified under 21 CFR 522.1660. Therefore, the withdrawal time for the hybrid product was determined to be the same as the pioneer

product, as established in 21 CFR 522.1660, as 28 days for beef cattle, non-lactating dairy cattle, and swine.

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.