Approval Date: January 23, 1990

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 111-636

B. Sponsor

The Upjohn Company
Agricultural Division
Kalamazoo, MI 49001

C. Proprietary Name

Lincomix Soluble Powder

D. Established Name

lincomycin hydrochloride

E. Dosage Form

Water soluble powder

F. Dispensing Status

OTC

G. Dosage Regimen

64 mg of lincomycin per gallon of drinking water continuously for seven days.

H. Route of Administration

Oral, via medicated drinking water

I. Indication

For the control of necrotic enteritis in broiler chickens caused by *Clostridium perfringens* susceptible to lincomycin.

J. Effect of Supplement

This supplement provides for use of this product in broiler chickens. It also provides for the deletion of the tolerance for lincomycin residues in chickens.

II. EFFECTIVENESS

The efficacy of lincomycin for the control of necrotic enteritis in broiler chickens is well documented in NADA 34-085 and approved on July 22, 1977; 42 FR 37545 (21 CFR558.325). For the purposes of this application, the efficacy of Lincomix Soluble Powder for the control of necrotic enteritis in broilers was evaluated in several well controlled studies including a five trial evaluation in experimentally induced infections.

A. Pivotal Studies Study Number 1

1. Type of Study: Dose determination

2. Investigator:

Richard B. Davis, PhD
953 College Station Road.
Athens, GA 30605

3. General design of investigation:

a. Purpose of Study: To determine the therapeutic effect of various concentrations of lincomycin in drinking water for the selection of the optimal dose for the control of necrotic enteritis in broiler chickens under simulated natural conditions. Necrotic enteritis was produced by *C. perfringens* in the litter.

b. Test Animals: Species: Chicken, Number/Group: 578 to 580 per treatment Subgroup identity: Day-old, mixed sex, broiler type chickens.

c. Control Group: A nonmedicated group served as a negative control.

d. Diagnosis: Necrotic enteritis was diagnosed by mortality, gross pathology and recovery of *C. perfringens* from liver or intestines.

e. Dosage Form: Water soluble powder

f. Route of Administration: Oral via medicated drinking water

g. Doses: The doses used were 0, 2, 8, 32 and 128 mg of lincomycin activity per gallon of drinking water continuously for seven days.

h. Test Duration: The total test duration time was for three weeks after the first medication day. Medication was delayed as indicated above until necrotic enteritis was diagnosed and continued for seven days. (Birds were observed for three weeks after the first medication day).

i. Pertinent parameters: Mortality due to necrotic enteritis as determined by gross pathology and recovery of *C. perfringens* from livers or intestines.

4. Results:

Lincomycin water medication was effective for the control of necrotic enteritis in chickens at concentrations greater than or equal to 8 mg per gallon of water. By interpolation, it was estimated that 64 mg of lincomycin per gallon of drinking water was an acceptable dose for this claim. Tables 1, 2, and 3 present the efficacy of various dose levels of lincomycin on necrotic enteritis in broilers.

Table 1. Efficacy of Various Dose Levels (Concentrations) of Lincomycin on Necrotic Enteritis in Broilers - Replicate I

Lincomycin mg/gal	No. Used	Mortality No.	Mortality %
0.0	295	31	10.50
2.0	295	27	9.15

Lincomycin mg/gal	No. Used	Mortality No.	Mortality %
8.0	295	5	1.69
32.0	295	0	0.00
128.0	295	0	0.00

Table 2. Efficacy of Various Dose Levels (Concentrations) of Lincomycin on Necrotic Enteritis in Broilers - Replicate II

Lincomycin mg/gal	No. Used	Mortality No.	Mortality %
0.0	284	16	5.63
2.0	284	11	3.87
8.0	285	5	1.75
32.0	283	1	0.36
128.0	284	0	0.00

Table 3. Efficacy of Various Dose Levels (Concentrations) of Lincomycin on Necrotic Enteritis in Broilers - Both Replicates

Lincomycin mg/gal	No. Used	Mortality No.	Mortality %
0.0	579	47	8.01
2.0	579	38	6.51
8.0	580	10	1.72
32.0	579	1	0.18
128.0	579	0	0.00

5. Conclusion:

   The data generated by this study demonstrated that lincomycin water medication was effective for the control of necrotic enteritis in broiler chickens at an estimated 64 mg/gallon.

6. Adverse Reactions:

   No adverse reactions due to treatment with lincomycin were reported.

7. Special Issues: None

B. Pivotal Studies Study Number 2

1. Type of Study: Dose confirmation

2. Investigator:

   Richard B. Davis, PhD
   953 College Station Rd.
   Athens, GA 30605

3. General Design of Investigation:

   a. Purpose of Study: Under simulated natural conditions, confirm the efficacy of 64 mg lincomycin/gallon of drinking water for the control of necrotic enteritis. Necrotic enteritis was produced by *C. perfringens* in the litter.

   b. Test Animals:
   Species: Chicken, Number/Group: 372 per treatment
   Subgroup Identity: Day old, mixed sex broiler chickens.

   c. Control Group: A non-medicated group served as a negative control.

   d. Diagnosis: Necrotic enteritis was diagnosed by mortality, gross pathology and recovery of *C. perfringens* from liver or intestines.

   e. Dosage Form: Water soluble powder. Medication was deleted until necrotic enteritis was diagnosed.

   f. Route of Administration: Oral via medicated drinking water.

   g. Doses: The dose evaluated was 64 mg lincomycin/gallon of drinking water.

   h. Test Duration: Three weeks after initiation of therapy.

4. Pertinent Parameters: Mortality due to necrotic enteritis was determined by gross pathology.

5. Results:

   Lincomycin, when mixed in the drinking water at 64 mg/gallon was effective for the control of necrotic enteritis in broilers. Mortality data are presented in Table 4.

Table 4. Study Number 2 -- Effects of Lincomycin Water Medication on Mortality Due to Necrotic Enteritis in Broilers

Group No.	Lincomycin mg/gallon of water	No. Birds Use	Replicate No.						Total No.	Total %
			1	2	3	4	5	6		
1	64	372	1	0	0	0	0	0	1	0.27a
3	0	372	14	10	15	1	9	3	52	13.98b

a,b = Different superscript letters are significantly different at $P<0.01$.

6. Conclusions:

   The data generated by this study demonstrated that 64 mg lincomycin/gallon of drinking water was effective for the control of necrotic enteritis in broiler chickens.

7. Adverse Reactions: No adverse reactions due to lincomycin were reported.

8. Special Issues: None.

C. Pivotal Studies Study Number 3

1. Type of Study: Multi-location five trial induced infection evaluation.

2. Investigators:

Robert K. Page, DVM, M.S.
Route 2
Winder, GA 30680
Trial Nos. 783-9690-0-AHH-83-003; 783-9690-0-AHH-83-009

Aziz H. Hamdy, DVM, PhD
The Upjohn Co.
Kalamazoo, MI 49001
Trial No. 783-9690-0-AHH-83-008

Diane J. Fagerberg, PhD
Colorado Animal Research Enterprises
6200 E. County Rd. 56
Fort Collins, CO 80524
Trial Nos. 783-7922-0-AHH-84-001; 783-9690-TRS-84-003

3. General Design of Investigation:

a. Purpose of Study: To decide if lincomycin soluble powder, administered in drinking water at a level of 64 mg of lincomycin activity per gallon of water, controls necrotic enteritis in broiler chickens. Necrotic enteritis was produced by *C. perfringens* in the feed.

b. Test Animals:
Species: Chicken; Number/Group: 100 to 120 per treatment/location
Subgroup identity: Day old, sexed, broiler type chickens, 50% male and 50% female per treatment.

c. Control Groups: A non-medicated group served as a negative control.

d. Diagnosis: Necrotic enteritis was diagnosed by mortality, gross pathology and recovery of *C. perfringens* from liver or intestines.

e. Dosage Form: Water soluble powder.

f. Route of Administration: Oral via medicated drinking water.

g. Doses: The dose evaluated was 64 mg lincomycin/gallon of drinking water. Medication was delayed until one day after challenge with *C. perfringens*.

h. Test Duration: Three weeks after initiation of therapy.

i. Pertinent Parameters: Mortality due to necrotic enteritis as determined by gross pathology.

4. Results:

Lincomycin water medication at a concentration of 64 mg/gallon of drinking water is effective for the control of necrotic enteritis in broiler chickens. Mortality data are presented in Table 5.

Table 5. Study Number 3 -- Mortality Due to Necrotic Enteritis

Trial Number	Total Mortality - Lincomycin	Total Mortality - Control	Percent Mortality - Lincomycin	Percent Mortality - Control
733-9690-0-AHH- 83-003	0/100	6/100	0	6
733-9690-0-AHH- 83-008	0/120	5/120	0	4.2
783-9690-0-AHH- 83-009	0/100	8/100	0	8
783-9690-0-AHH- 84-001	4/118	21/118	3.4	17.8
783-9690-0-TRS- 84-003	0/120	1/120	0	0.8
Total	4/558	42/558		
Mean			0.68a	7.36b

a,b = Different superscript letters are significant at P<.01

5. Conclusion:

The pooled data generated by this five trial study demonstrated that 64 mg lincomycin/gallon of drinking water was effective for the control of necrotic enteritis in broiler chickens.

6. Adverse Reactions: No adverse reactions due to lincomycin were reported.

7. Special Issues: None.

III. TARGET ANIMAL SAFETY

Information regarding animal safety studies in poultry may be referred to in the Freedom of Information Summaries of NADAs 34-085 (Lincomix Premix) and, 97-505 (Lincomix-20 Premix). Additionally, the following 21 day oral safety study in poultry was conducted with Lincomycin Soluble Powder to establish target animal safety.

1. Type of Study: Subacute toxicity in target species.

2. Investigator:

A.D. Hall, DVM, PhD
The Upjohn Company
Kalamazoo, MI 49001

3. General Study Design:

a. Purpose of Study: To determine the relative safety/toxicity of lincomycin soluble powder when administered to broiler chickens at 1X, 3X and 5X the recommended therapeutic dose of 64 mg lincomycin/gallon of drinking water.

b. Test Animals:
Species: Chicken; Number/Group: 120 birds/treatment
Subgroup identity: Three week old broiler type chickens.

c. Dosage Form: Water soluble powder.

d. Doses: 0, 64, 192 and 320 mg lincomycin/gallon of drinking water.

e. Route of Administration: Oral via medicated drinking water.

f. Test Duration: 21 days.

g. Parameters Measured:

1) Clinical Observations:

a) Observations for pharmacologic and toxicologic effects were performed twice daily (AM and PM) during the week and once daily on weekends and holidays during the animal phase of the study. All birds were monitored daily for signs of loose stools (pasted vents).

b) Body Weights: Body weights were recorded weekly on a per pen basis except at study initiation and study termination when the males and females within a pen were weighed separately.

c) Water Consumption: Water consumption (medicated or unmedicated) was recorded daily for each pen.

d) Food Consumption: Food consumption was done on a per pen basis. For the first week of study, determinations were done every two or three days, then daily for the remaining 14 days.

2) Clinical Pathology

a) Hematology: Red blood cell count (Coulter ZBI) and hematocrit (manual method).

b) Serum Chemistry: Blood samples were also taken for determination of the following serum chemistry parameters: Calcium, inorganic phosphorus, glucose, blood urea nitrogen, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase (AST).

3) Necropsy

a) A total of 477 birds were necropsied in a predetermined order over a six day period at study termination. Five birds (25%) of the 20 birds in each pen were randomly selected prior to study initiation for tissue preservation for histopathologic examination.

b) Organ weights: The following organs were weighed at necropsy: liver, spleen, paired ceca (the intestine was ligated immediately above and below the ceca before they were removed for weighing).

4) Histopathology

The following tissues were collected for histopathologic evaluation: liver, lung, kidney, spleen, heart, thymus, skeletal muscle (pectoral), proventriculus, small intestine, cecum, colon, Bursa of Fabricius, bone marrow (femur), any gross lesion.

4. Results:

a. Clinical observations: During the course of this study, no observations of drug related problems were noted.

b. Body weight: The body weight data were used to calculate average daily gain (ADG) on a per bird basis. A significant linear trend was detected with the average daily gain increasing as the dosage levels increased.

c. Food consumption: Food consumption on a per bird basis increased relatively uniformly across treatment groups as the birds increased in age.

d. Water consumption: Daily water consumption on a per bird basis also increased relatively uniformly across treatment groups as the birds increased in age.

e. Hematologic observations: The statistical analysis of the hematologic parameters (RBC and HCT) revealed no drug related changes in any of the dose groups.

f. Serum chemistries: No significant drug related changes were produced among the serum chemistries after 21 days of oral dosing.

g. Gross lesions: The most frequent lesion noted at necropsy was the presence of scattered petechial and ecchymotic hemorrhages along the mucosal surface in the middle of the small intestine. Curled toes, slipped tendons, and pasted vents were occasionally observed and were uniformly distributed among control and each of the treatment groups. Enlarged ceca was an infrequent observation in treated birds.

h. Histologic lesions: A variety of incidental findings was observed histologically in the birds from which tissues were saved. Liver sections contained the presence of small multifocal accumulations of lymphocytes scattered throughout the parenchyma. Occasionally, granulocytic cells, possibly eosinophils, were present in small numbers within the portal was seen in approximately 25% of the birds in all four groups. A focal acute exudative myocarditis was observed with relative frequency and probably was a sequela of the intracardial bleeding that each bird underwent several hours prior to euthanasia. The gross lesion of scattered petechiae and ecchymoses along the mucosa of the jejunum was observed histologically as a pooling of red blood cells within the lamina propria at the tips of several adjacent villi. No immediate reason for this pooling was detected. No treatment related effects of this drug were observed.

5. Statistical Analysis:

The analyses of hematology, serum chemistry and average daily gain data were done using weighted least squares analysis of variance (weighted by number of observation in mean) on the sex pen means according to the following table:

Table 6. Statistical Analysis

Source	**DF**	**Testing Term**
Block	5	-
Treatment	3	E1 (3)
Linear	1	E1 (1)
Lack of Fit	2	E1 (2)
Comparisons	-	-
0X vs 1X	1	E1 (4)
0X vs 3X	1	E1 (4)
0X vs 5X	1	E1 (4)
Error 1 (E1)	15	-
Sex	1	E2 (5)
Sex by Treatment	3	E2 (6)
Error 2 (E2)	20	-

a. This was the first test conducted. Significance (two sided 0.05) in this test provided evidence of a linear change due to increasing level of lincomycin. The test for lack of fit was then conducted (see [2]). If a significant linear trend was not obtained, then the overall test for a treatment effect was computed (see [3]).

b. Significance in this test (two sided 0.05) provided evidence that there are significant deviations from linearity. Inferences would not be made to a linear trend in this case and the overall test for a treatment effect was computed (see [3]). If significant lack of fit is not obtained and there is a significant linear trend, then inferences will be made to linear trend.

c. Significance in this test (alpha = 0.05) was required before proceeding with the contrasts described in [4].

d. Significance in this test (two-sided 0.05) indicated a significant change for that dose group when compared to control.

e. Significance in this test (two-sided 0.05) provides evidence that there are significant differences between males and females.

f. Significance in this test provided evidence that the response of a bird to a dose of lincomycin depends on the sex of the bird. In this case, all inferences were made to sex treatment means and comparisons of each dose group to control within each sex were made. If this interaction test was significant, then inferences were made to the sex and treatment means.

6. Conclusions:

No adverse clinical signs were observed relative to the presence of lincomycin in the drinking water. There were no statistically significant differences noted between

treated and control birds for hematologic or serum chemistry parameters nor average daily gain. The incidence of the observed gross and histologic lesions could not be correlated with treatment, since these lesions were evenly distributed across all dose groups. Likewise, the occurrence of soft stools or pasted vents was observed equally in all groups and never exceeded four birds per pen.

Based upon the results of this study, the recommended therapeutic dose of 64 mg of lincomycin soluble powder per gallon of drinking water for the control of necrotic enteritis in growing broiler chickens appears to be non-toxic when administered for up to 21 consecutive days.

IV. HUMAN FOOD SAFETY

A. Drugs for Use in Food Animals Toxicity Tests

1. Teratology Study in Rats

a. Title: U-10,149A (Lincomycin HCl Premix): A segment II Teratology Study in Rats

b. Report number: 768-9610-80-001

c. Starting date: March 6, 1979

d. Termination date: May 9, 1979

e. Name(s) and address(es) of investigator(s) who did the study:

D.F. Morris, S.B. Harris, S.M. Poppe, J.L. Stuckhardt, J.H. Harris
The Upjohn Co.
Kalamazoo, MI. 49001

f. Name and address of laboratory where study was done:

Pathology and Toxicology
Research Unit
The Upjohn Co.
Kalamazoo, MI. 49001

g. Identity of substance and dosage form tested: Lincomycin HCl premix grade, U-10,149A, Lot # 025EU. Dosage form: oral gavage.

h. Species and strain of animal used: Rat, Upj:TUC (SD) spf.

i. Number of animals of each sex in each group: 96 females, 24 per treatment group.

j. Levels and duration of dosing: Dosage levels (mg/kg) 0.0, 10.0, 30.0 and 100.0. Duration of dosing: Ten days (days 6 through 15 of gestation).

k. Route of drug administration: Oral by gastric gavage.

l. Parameters studies: Dam conception rates and body weights were evaluated. Fetuses were weighted, sexed and evaluated for gross, visceral and skeletal anomalies.

m. Significant toxicities observed: The litter proportion of resorption increased significantly (P<.05) and the litter proportions of implants resulting in live fetuses decreased significantly (P<.05) at the 100.0 mg/kg dose level. However, the incidence (8.0%) of resorption in the 100.0 mg/kg group is similar to the overall incidence (5.3%) of resorption found in historical controls.

n. No-observed-effect-level: 30 mg/kg body weight.

o. Statistical analysis: The modified Cochran and Armitage Chi Square Statistic (Thomas, Breslow and Gart, 1977) was used to test whether or not there was a significant dose response with respect to the proportion of dams that conceived. Dam body weight gains were analyzed using a one-way analysis of variance (Snedecor and Cochran, 1971).

p. Fetal body weights were analyzed using a weighted analysis of variance with the litter as the experimental unit (Healy, 1972). Proportions per litter of live fetuses, resorption and alterations in development were analyzed using Alternatives Test (Lin and Haseman, 1976) to test for a significant dose response.

q. Conclusions drawn from study: It is concluded from the results that Lincomycin HCl premix was not teratogenic at 100 mg/kg, and was not embryotoxic at the 30 mg/kg dose level.

2. Tolerance Study in the Dog

a. Title: Lincomycin One Year Oral Tolerance Study in the Dog.

b. Report number: 768-9610-79-002

c. Starting date: May 27, 1977

d. Termination date: June 5, 1978

e. Name(s) and address(es) of investigator(s) who did the study: L.S. Goyings, R. Thomas, C.N. VanHuysen, R.G. Bastianse.

f. Name and address of laboratory where study was done: The Upjohn Co., Kalamazoo, MI.

g. Identity of substance and dosage form tested: Lincomycin premix grade, U-10,149A and lincomycin USP grade, U-10,149A. Dosage form: Oral.

h. Species and strain of test animal: Purebred Beagle dogs.

i. Number of animals of each sex in each group: Five males and five females per treatment group.

j. Levels and duration of dosing: Dosage levels (mg/kg/day): 0.0, lincomycin premix grade at 0.375, 0.75 and 1.5; lincomycin USP grade at 1.5. Duration of Dosing: One year.

k. Route of administration: Oral, gelatin capsules.

l. Parameters studied: Measurements to evaluate potential drug effects included clinical observations, ophthalmic examinations, food consumption, body weights, hematologic and blood chemistry determinations, urinalysis, organ weights and gross and microscopic observations.

m. Significant toxicities observed: None.

n. No-observed-effect-level: > or = 1.5 mg/kg/day.

o. Statistical analysis: The data were analyzed using least squares analysis of variance techniques.

p. Conclusion(s) drawn from the study: Analysis of the data from the study indicated no adverse toxicologic or pharmacologic effects due to either grade of lincomycin.

3. Two Feeding Studies in Rats

a. Titles:

1) Twenty-six Month Oral Feeding Study in Rats With Lincomycin.
2) Twenty-six Month Oral Feeding Study in Rats With Lincomycin: Complete Histopathological Observations of Thyroids, Supplemental Report.

b. Report numbers: 1) 768-9610-80-003; 2) 768-9610-81-002

c. Starting date: August 3, 1977

d. Termination date: October 11, 1979

e. Name(s) and address(es) of investigator(s) who did the study: L.S. Goyings, J.E. Lund, W.J. Seaman, The Upjohn Company, Kalamazoo, MI.

f. Name and address of laboratory where study was done: The Upjohn Co., Kalamazoo, MI.

g. Identity of substance and dosage form tested: Lincomycin premix grade, Lot # 60109, and lincomycin USP grade, Lot # 157EW. Dosage Form: Oral in the diet.

h. Species and strain of test animal: Rat, Upjohn Sprague-Dawley.

i. Number of animals of each sex in each group: 60 males and 60 females were assigned to each treatment group.

j. Levels and duration of dosing:

Table 7. Levels and Duration of Dosing

Group #	Grade of lincomycin	Dose mg/kg/day	Duration (days)
1	Control	0	784
2	Premix	0.375	784
3	Premix	0.750	784
4	Premix	1.500	784
5	USP	1.500	784

Group #	Grade of lincomycin	Dose mg/kg/day	Duration (days)
6	USP	100.000	784

k. Route of administration: lincomycin was administered orally in the daily diet.

l. Parameters studied: Percent survival, clinical observations, ophthalmology, food consumption, body weight changes, necropsy observations, organ weights, hematology, serum chemistry, urinalysis, histology (including complete thyroid examination).

m. Significant toxicities observed: The incidence of acute prostatitis and seminal vesiculitis was increased (P<.05) in males that received 1.50 mg/kg (premix grade) and 100 mg/kg (USP grade).

n. No-observed-effect-level: 0.375 mg/kg/day

o. Statistical analysis, where appropriate: The data were analyzed using least squares analysis of variance. The model consisted of treatment effect, sex effect, and the treatment by sex interaction.

p. Conclusion(s) drawn from the study: No real differences attributable to lincomycin treatment were found for survival percentage, clinical and ophthalmological examinations, food consumption, organ weights, hematology, serum chemistry determinations other than cholesterol and urinalysis. Histopathologic examination revealed no real differences in tumor incidence attributable to treatment. Complete histopathological observation of the thyroids (report number 768-9610-81-002) indicated no treatment related effects of lincomycin on the thyroid. Incidence of acute prostatitis and seminal vesiculitis was increased (P<.05) in males treated with 1.50 mg/kg (premix grade) and 100 mg/kg (USP grade). These lesions may be associated with the antibiotic properties of lincomycin. Lincomycin premix grade appeared to be growth promoting in that the .750 mg/kg/day dose group increased body weight faster from initiation of the study through 574 days.

4. Two-Generation Reproduction Study (Oral) in Rats

a. Title: A Two-Generation Reproduction Study (Oral) in Rats Given U-10,149A.

b. Report number: 7259-87-024

c. Starting date: February 2, 1987

d. Termination date: November 16, 1987

e. Name(s) and address(es) of investigators who did the study: D.L. Black, T. A. Marks, D. F. Morris, C.I. Shaw, S.M. Poppe, R.D. Terry, A.D. Hall

f. Name and address of laboratory where study was done: The Upjohn Co., Kalamazoo, MI.

g. Identity of substance and dosage form tested: Lincomycin HCl premix,U-10,149A in sterile water given orally.

h. Species and strain of test animal used: Rat, Upj:TUC (SD) spf.

i. Number of animals of each sex in each group: 120 males and 120 females, 30 males and 30 females per treatment group.

j. Levels and duration of dosing: Dosage Levels (mg/kg) 0.0, 100.0, 300.0 and 1,000.0. Duration of Dosing: 60 days prior to mating until delivery of the F1 generation (males) or 14 days prior to mating until 21 days postpartum (female).

k. Route of drug administration: Oral by gastric gavage.

l. Parameters studied: Spermatogenesis, fertility, length of gestation, parturition, lactation and offspring survival and growth in both the F0 and F1 generations.

m. Significant toxicities observed: None.

n. No-observed-effect-level: < or = 1,000 mg/kg

o. Statistical analysis, where appropriate: Not done.

p. Conclusion(s) drawn from the study: In spite of the extremely high doses employed in this study, U-10,149A was not embryotoxic, fetotoxic, teratogenic (external exams performed on all pups; skeletal exams performed on pups that died on postpartum (days zero through four), and did not adversely affect lactation, survival or growth of F0 or F1 offspring until weaning. In addition, the reproductive performance of F0 or F1 males and females, and the estrous cycles of F0 and F1 females, were not affected at the dosages and route of employed in this study.

5. Evaluation of Two Lots of Lincomycin HCl in the *In Vitro* Unscheduled DNA Synthesis (UDS)

a. Title: Evaluation of Two Lots of Lincomycin HCl in the In Vitro Unscheduled DNA synthesis (UDS) Assay in Rat Primary Hepatocytes.

b. Report number: 7268-87-038

c. Starting date: August 8, 1987

d. Termination date: September 14, 1987

e. Name(s) and address(es) of investigators who did the study: P.R. Harbach, S.K. Wiser, C.S. Aaron.

f. Name and address of laboratory where study was done: The Upjohn Co., Kalamazoo, MI.

g. Identity of substance and dosage form used: Lincomycin HCl, bulk drug, Lot # 647AF and reference standard issue D.

h. Species and strain of test animal used: Not applicable.

i. Number of animals of each sex in each group: Not applicable.

j. Levels and duration of dosing: 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1,000 and 3,000 µg U-10,149A/ml and solvent controls (DMSO in the negative control and 2-AAF in the positive control).

k. Route of drug administration: Treated culture.

l. Parameters studied: This DNA repair assay measures unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes treated with the test compound in vitro. This assay is useful as a genotoxicity screen because it measures the repair of DNA damage induced by many classes of mutagens and carcinogens. Genotoxic compounds or their metabolites react with DNA to form adducts which are repaired by an enzymatic process in which the adduct is excised, and the DNA strand is polymerized and ligated. This process, referred to as unscheduled DNA synthesis, can be quantitated by measuring the incorporation of labeled thymidine into the nuclear DNA of cells not in S phase. Thus, compounds that induce repairable DNA damage without inhibiting that repair can be detected by measuring UDS.

m. Primary hepatocytes are particularly useful for this assay because of their high capacity to metabolize procarcinogens to a genotoxic form. In addition, the cultures are essentially nondividing, so that normal replicative DNA synthesis is too low to interfere with UDS detection.

n. Significant toxicities observed: For Lot # 647AF, the grains/nucleus (NG) counts were slightly increased (0-5 NG) at 1000 and 3000 µg/ml in the second experiment. For the Control Reference Standard U-10,149A, NG counts were also slightly increased at 1000 ug/ml. NG increases at 300, 1000 and 3000 µg/ml in the first experiment, and at 1000 µg/ml in the second experiment were statistically significant, as were the dose responses for the NG and the percent of cells in repair. These high doses showed a dose related cytotoxicity as indicated by a granular appearance of the cells, which probably accounts for the NG increases. All of the NG values met our criteria for a non-positive or inconclusive UDS response.

o. No-observed-effect-level: Not applicable.

p. Statistical analysis, where appropriate: Regression analysis of the NG means was used to test for a significant dose response.

q. Conclusion(s) drawn from this study: The results with both lots of U-10,149A were considered non-positive or inconclusive under the conditions of this in vitro rat hepatocyte DNA repair assay. The results of this study are evaluated in the context of all genetic toxicology data available on lincomycin.

6. Evaluation of Lincomycin Lot 647AF in the *In Vitro* Unscheduled DNA Synthesis (UDS)

a. Title: Evaluation of Lincomycin Lot 647AF in the In Vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Primary Hepatocytes.

b. Report number: 7268-87-035

c. Starting date: April 22, 1987

d. Termination date: July 1, 1987

e. Name(s) and address(es) of investigator(s) who did the study: T.R. Barfknecht, Study Director

f. Name and address of laboratory where study was done: Pharmakon Research International, Inc., Waverly, PA.

g. Identity of substance and dosage form used: Lincomycin HCl, U-10,149A, bulk drug, Lot #647AF.

h. Species and strain of test animal used: Not applicable.

i. Number of animals of each sex in each group: Not applicable.

j. Levels and duration of dosing: 0.167, 0.50, 5.0, 16.7, 50, 167, 500, 1670 and 5000 µg U-10,149A/ml and deionized H20 and 2-AAF serving as negative and positive controls, respectively.

k. Route of drug administration: Treated culture.

l. Parameters studied: This DNA repair assay measures unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes treated with the test compound in vitro. This assay is useful as a genotoxicity screen because it measures the repair of DNA damage induced by many classes of mutagens and carcinogens. Genotoxic compounds or their metabolites react with DNA to form adducts which are repaired by an enzymatic process in which the adduct is excised, and the DNA strand is polymerized and ligated. This process, referred to as unscheduled DNA synthesis, can be quantitated by measuring the incorporation of labeled thymidine into the nuclear DNA of cells not in S phase. Thus, compounds that induce repairable DNA damage without inhibiting that repair can be detected by measuring UDS.

m. Primary hepatocytes are particularly useful for this assay because of their high capacity to metabolize procarcinogens to a genotoxic form. In addition, the cultures are essentially non-dividing, so that normal replicative DNA synthesis is too low to interfere with UDS detection.

n. Significant toxicities observed: Due to unexplained toxicity, doses above 16.7 µg/ml could not be scored. This toxic phenomenon was also observed in a repeat assay. The doses scored were 0.167, 0.50, 1.67, 5.0 and 16.7 µg/ml.

o. In both the initial and repeat assays on this lot (Lot # 647AF) of lincomycin, the compound produced a positive response. In the initial experiment, a clear dose related increase in net nuclear grains was in evidence from 1.67 to 16.7 µg/ml, whereas only 5.0 and 16.7 mg/ml produced a positive response in the replicated assay. Nevertheless, the number of cells in repair increased significantly in both experiments (maximum of about 80% of cells in repair at 16.7 µg/ml). The positive (2-Acetylaminofluorene, 1x10 7 M) and negative (H20) controls performed as expected.

p. No-observed-effect-level: Not applicable.

q. Statistical analysis, where appropriate: None conducted.

r. Conclusions drawn from this study: The result of this assay of lincomycin (U-10,140=9A, Lot #647AF) is positive in the rat primary hepatocyte UDS assay under the conditions of the test. However, an in-depth analysis of all UDS data determined these results to be artifactitious, and not indicative of lincomycin-related genotoxic activity. (See "Summary of UDS Assays" p.18)

7. Evaluation of Lincomycin Control Reference Standard D in the *In Vitro* Unscheduled DNA

a. Title: Evaluation of Lincomycin Control Reference Standard D in the In Vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Primary Hepatocytes.

b. Report number: 7268-87-039

c. Starting date: July 17, 1987

d. Termination date: August 8, 1987

e. Name(s) and address(es) of investigator(s) who did the study: R.R. Barfknecht, Study Director

f. Name and address of laboratory where study was done: Pharmakon Research International, Inc., Waverly, PA.

g. Identity of substance and dosage form used: Lincomycin HCl, U-10,149A, reference standard issue D.

h. Species and strain of test animal used: Not applicable.

i. Number of animals of each sex in each group: Not applicable.

j. Levels and duration of dosing: 0.167, 0.50, 1.67, 5.0, 16.7, 50, 167, 500, 1670 and 5000 µg U-10,149A/ml and deionized H2O and 2-AAF serving as negative and positive controls, respectively.

k. Route of drug administration: Treated culture.

l. Parameters studied: This DNA repair assay measures unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes treated with the test compound in vitro. This assay is useful as a genotoxicity screen because it measures the repair of DNA damage induced by many classes of mutagens and carcinogens. Genotoxic compounds or their metabolites react with DNA to form adducts which are repaired by an enzymatic process in which the adduct is excised, and the DNA strand is polymerized and ligated. This process, referred to as unscheduled DNA synthesis, can be quantitated by measuring the incorporation of labeled thymidine into the nuclear DNA of cells not in S phase. Thus, compounds that induce repairable DNA damage without inhibiting that repair can be detected by measuring UDS.

m. Primary hepatocytes are particularly useful for this assay because of their high capacity to metabolize procarcinogens to a genotoxic form. In addition, the cultures are essentially non-dividing, so that normal replicative DNA synthesis is too low to interfere with UDS detection.

n. Significant toxicities observed: None observed at the scored doses.

o. No-observed-effect-level: Not applicable.

p. Statistical analysis, where appropriate: None conducted.

q. Conclusion(s) drawn from this study: In the assay on this lot (control reference standard issue D) of lincomycin, the compound produced a negative response under the conditions of the test. The positive and negative controls performed as expected. Thus, the result of this assay of lincomycin (U-10,149A, control reference standard D) is negative in the rat primary hepatocyte UDS assay under the conditions of the test.

Summary of UDS Assays:

Supplemental UDS data and documentation, including photomicrographs, presented to the Agency indicated that the suspect UDS activity of lincomycin was: a) artifactitious in the case of the studies conducted by Pharmakon (Report No. 7268-87-035), and; b) biologically meaningless in the case of studies conducted by the Upjohn Co. (Report No. 7268-87-038). Therefore, any UDS activity observed was not considered indicative of lincomycin related genotoxic activity. The Agency has determined that the mutagenicity prerequisites of the Threshold Assessment of lincomycin have been satisfied.

8. Evaluation of Lincomycin in the Salmonella/Microsome Test (Ames Assay)

a. Title: Evaluation of Lincomycin in the Salmonella/Microsome Test (Ames Assay).

b. Report Number: 7268-87-016

c. Starting Date: April 17, 1987

d. Termination Date: May 3, 1987

e. Name(s) and address(es) of investigator(s) who did the study: C.S. Aaron, J.H. Mazurek

f. Name and address of laboratory where study was done: Pathology and Toxicology Research Unit, The Upjohn Co. Kalamazoo, MI 49001

g. Identity of substance and dosage form tested: Lincomycin HCl, U-10,149A, Lot #647AF.

h. Species and strain of test animal used: In vitro study.

i. Number of animals of each sex in each group: Not applicable

j. Levels and duration of dosing: 625, 1250, 2500 and 5000 µg/plate plug negative and strain specific positive controls.

k. Route of drug administration: Not applicable

l. Parameters studied: The procedures for the Ames Assay are the standard ones developed in the laboratory of Dr. B.N. Ames. Briefly, histidine auxotrophes of Salmonella typhimurium are mixed with the test compound in 0.1 ml dimethylsulfoxide, the 9,000xg supernatant of liver homogenates (or saline) in

molten (45° C) agar. The molten agar mix is poured onto a Petri plate containing a histidine prototrophy are scored as colonies, after incubation at 37° C for 22 days. A single plate is used for each dose level and the experiment is repeated. Vehicle controls are run in triplicate for each strain in each experiment and reported as an average of the three values.

m. Significant toxicities observed: The data show no evidence of bacterial mutagenicity at any dose, whether or not exogenous in vitro liver homogenate (5-9) was used to supply metabolic activation.

n. No-observed-effect-level: Not applicable.

o. Statistical analysis, where appropriate: None conducted.

p. Conclusion(s) drawn from this study: Based on the results of this study, lincomycin was not mutagenic in the Salmonella/microsome test (Ames Assay).

9. Evaluation of Lincomycin in the *Drosophila* Sex Linked Recessive Lethal Assay

a. Title: Evaluation of Lincomycin in the *Drosophila* Sex Linked Recessive Lethal Assay.

b. Report number: 7268-87-045

c. Starting date: March 24, 1987

d. Termination date: October 20, 1987

e. Name(s) and address(es) of investigator(s) who did the study: R. Valencia, Study Director

f. Name and address of laboratory where study was done: Zoology Department University of Wisconsin Madison, WI.

g. Identity of substance and dosage form tested: Lincomycin HCl, U-10,149A, Lot# 647AF.

h. Species and strain of test animal used: *Drosophila melanogaster*; Canton-S (males) and Basic strains (females).

i. Number of animals of each sex in each group: 15 males per treatment group.

j. Levels and duration of dosing: 25,000 ppm and 50,000 ppm in feeding solution administered for three days.

k. Route of drug administration: Oral in sucrose feeding solution.

l. Parameters studied: The sex-linked recessive lethal test detects the occurrence of mutations in the germ line of an insect. The assay is capable of screening for forward mutations at about 800 loci on the X chromosome. This represents about 80% of the X chromosome or approximately 1/5 of the entire genome. The mutations detected are recessive and are lethal to the carrier when expressed.

m. Significant toxicities observed: The mutation frequency in the offspring of males treated with lincomycin was not enhanced.

n. No-observed-effect-level: Not applicable.

o. Statistical analysis, where appropriate: None conducted.

p. Conclusion(s) drawn from the study: Lincomycin is not a germ line mutagen under the conditions of this assay.

Summary of Toxicity Tests:

The data presented in this section support the conclusion that lincomycin is not genotoxic or mutagenic. Additionally, a no-observed-effect-level has been established as 30 mg/kg on the basis of embryotoxicity in the rat.

B. Resideue Information

1. Safe Concentration of Total Residue

a. On the basis of the toxicology studies, the no-observed-effect-level from the most sensitive study in the most sensitive species was 30 mg/kg for embryotoxicity in segment II teratology study in rats. A 1000X safety factor was used in calculating the safe concentration of total residue in edible tissue since the NOEL was established on the basis of subchronic data.

b. The safe concentration of total residue is derived as follows:

No Observable Effect Level:

Lowest NOEL is 30 mg/kg based on embryotoxicity in the rat teratology study.

Safe Concentration: Allowable Daily Intake X Human Weight / Daily Consumption of Meat

Where Human Weight = 60 kg
Daily Consumption of Meat = 500 gm

ADI = NOEL/Safety Factor

SF = 1000X

Therefore,
ADI = 30 mg/kg divided by 1000 or 0.03 mg/kg and
SC = .03 mg/kg X 60 kg / 500 g = 3.6 mg/kg or 3.6 ppm

Present FDA policy limits the SC in muscle to no more than 3.0 ppm unless chronic toxicity data exist. Therefore, the SC of total residues is 3.0 ppm.

The factors used to adjust for consumption along with the safe concentration in each tissues are as follows:

Table 8. Factors used to adjust for consumption and the safe concentration in each tissues

Consumption Factor	Safe Concentration, ppm

Consumption Factor	Safe Concentration, ppm
Muscle = 1X	3
Skin/Fat = 2X	6
Liver = 3X	9

2. Total Residue Depletion and Metabolism Studies

a. Names and address of Investigators:

R.E. Hornish, R.E. Gosline, J.M. Nappier, T.J. Butine and C.J. Subacz.
The Upjohn Company
Kalamazoo, MI 49001

b. Description of study animals: 42 Hubbard-cross-Hubbard broiler chickens 25 days old. Twenty one (21) males and 21 females housed individually in stainless steel cages.

c. Route of administration: Oral via drinking water.

d. Time and duration of dosing: Ad libitum water treatment for seven days.

e. Particular radioisotope used: C14

f. Tissue residue levels: Mean Values for 6 Birds, in parts per million of C14 residue.

Table 9. Tissue Residue Levels: Mean Values in ppm of C14 Residue

Withdrawal	Liver	Kidney	Muscle	Skin/Fat
0 Day	1.580	1.26	0.052	0.132
1/2 Day	0.503	0.56	0.027	0.051
1 Day	0.224	0.23	0.027	0.065
2 Day	0.107	0.10	<0.005	0.028
4 Day	0.028	0.03	<0.005	0.017
7 Day	0.020	0.01	<0.005	<0.005

g. Summary of metabolism studies leading to identification of major metabolites in target and test species: A study completed to determine the total residue levels and metabolites in tissues of chickens treated with C-14 lincomycin is described in parts a. through f. above. In this study, liver had the highest level of total residues, 1.58 ppm at 0 hours, and is therefore the limiting tissue relative to the calculated safe concentration. The major metabolites found in the liver at 0 hours in this study were lincomycin (20%), lincomycin sulfoxide (40%), N-demethyllincomycin (5%), and N-demethyllincomycin sulfoxide (10%).

Rats were orally dosed at 300 mg/kg body weight with C-14 labeled lincomycin for seven consecutive days, then sacrificed while on treatment. A comparison of the lincomycin metabolites in the chicken to the lincomycin metabolites in the rat reveals that the rat can serve as the suitable test animal for determination of the toxicological profile of the antibiotic since each metabolite present in the chicken is also present in the rat. These data support the

contention that the rat was autoexposed to metabolites which humans would be exposed to from consuming edible tissues of chickens treated with lincomycin.

Table 10. HPLC Retention Times of the Various metabolites in the Livers of Chickens and Rats, A-BE/AR Fraction
Retention Time in Minutes

Peak ID	Chicken (0 Hr)	Chicken (12 Hr)	Rat (Male)	Rat (Female)
Unknown	3.0-3.2 min		.0-3.5	2.8-3.2
Unknown				3.4-3.6
Unknown	3.6-4.0		4.0-4.5	4.0-4.8
Unknown	4.4-5.0	4.4-4.8	4.8-5.3	
Unknown			9.4-9.8	9.4-10.0
Linco Sulfoxide	10.3-11.4	10.5-11.4		10.2-10.7
Linco Sulfoxide				13.0-13.3
Linco Sulfoxide				16.5-16.8
Lincomycin	17.2-19.0	17.8-19.2	18.0-18.5	17.7-18.6
Lincomycin	19.0-19.6*	19.6-20.0		

* May be tailing part of the lincomycin peak.

3. Tolerance for the Marker Residue

Based on the toxicology data, and using the lowest No-Observed-Effect-Level of 30 mg/kg, a permitted Safe Concentration (SC) of 3 ppm in muscle, 6 ppm in skin/fat and 9 ppm in liver was established (Part 2 of this section).

Total residues at zero day withdrawal are substantially lower than corresponding calculated safe concentrations for edible tissues, therefore, neither a marker compound nor a tolerance for a marker compound is required. The permitted safe concentration for total residues in edible tissues and the actual residues found are as follows:

Table 11. Permitted Safe Concentration for Total Residues in Edible Tissues and the Actual Residues Found

Tissue	SC, ppm	Mean Lincomycin Residue, ppm
muscle	3.0	0.052
skin/fat	6.0	0.132
liver	9.0	1.580

4. Study Establishing Withdrawal Period

Data presented in Parts 3 and 4 of this section showed that the mean concentrations of total lincomycin residues at zero day withdrawal following the administration of 128 mg/gallon (2X label dosage) were well below the permitted Safe Concentration. Therefore, a zero day pre-slaughter drug withdrawal has been established and no residue decline studies were required.

5. Regulatory Method

A regulatory method is not required since the residue and toxicology data support a zero withdrawal period (see Part 5 of this section).

V. AGENCY CONCLUSIONS

The data submitted in support of the supplemental new animal drug application satisfy the requirements of Section 512 of the Act and demonstrate that the lincomycin hydrochloride when used under its proposed conditions of use, is safe and effective for the control of necrotic enteritis caused by *Clostridium perfringens* in broiler chickens. Because the supplement provided for a new indication for use of the drug product, this is a Category II change under the Center's supplemental approval policy (42 FR 64367).

Human food safety data submitted in the application to establish a safe concentration for lincomycin in the edible tissues of chickens show that the total residue at zero day withdrawal are substantially lower than corresponding calculated safe concentrations for edible tissues, i.e., 3 ppm in muscle, 6 ppm in skin/fat, and 9 ppm in liver. Under the Center's supplemental approval policy (42 FR 64367), this is a Category III change. The existing tolerance of 0.1 ppm (21 CFR 556.360) for negligible residues in the edible tissues of chickens is deleted.

Proper use by non-veterinarians can be expected because the conditions of necrotic enteritis can be easily recognized by non-veterinarians. Directions are clearly written and there is reasonable certainty that the conditions of use, including mixing directions, on the label can and will be followed by the producer. For these reasons, the agency has concluded that the directions are adequate for lay use, and the product is therefore approved for over-the-counter use.

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