I. GENERAL INFORMATION

A. File Number

NADA 139-075

B. Sponsor

American Cyanamid Company

C. Proprietary Name

CYGRO®; 1% Type A Medicated Article

D. Established Name

maduramicin ammonium

E. Dosage Form

CYGRO® is a Type A Medicated Article (premix)

F. Amount of Active Ingredient

1% maduramicin ammonium

G. Dispensing Status

Over the Counter (OTC)

H. Dosage Regimen

Thoroughly mix CYGRO® 1% Type A Medicated Article into the feed at a rate of 1 to 1.2 lb per ton of broiler feed to obtain a level of 4.54 to 5.45 g/ton (5 ppm to 6 ppm) maduramicin ammonium in Type C medicated feed. Feed continuously as the sole ration to broiler chickens from one day of age until 5 days before slaughter.

I. Indication

For use in the manufacture of broiler chicken feeds. For the prevention of coccidiosis in broiler chickens caused by *Eimeria acervulina*, *E. tenella*, *E. brunetti*, *E. maxima*, *E. necatrix* and *E. mivati*.

II. EFFECTIVENESS

A. Establishment of Dose

1. Field Isolate Battery Trials

   Seventy-three field isolates were evaluated in 41 battery trials totaling 4,305 cockerel chickens, to titrate various levels of maduramicin against single or mixed infections of *Eimeria* spp. recently collected from commercial poultry farms at various geographical locations in North America. Each sample was analyzed to determine the major and minor *Eimeria* spp. which were present.

These trials were conducted by Dr. H. Berger at the Agricultural Research Center of American Cyanamid Company in Princeton, New Jersey.

Treatments consisted of noninfected, nonmedicated control (NNC), infected, nonmedicated control (INC) and infected, medicated treatments of 2, 4, 5, 6 or 7 ppm maduramicin. The treatments were replicated three times in each trial, with 5 birds per replicate. Ten-day-old broiler cockerels were infected with a pretitrated inoculum from the field samples which contained a single or mixed *Eimeria* spp. Weight gains for the 3 to 7 days postinfection, mortality (coccidiosis and noncoccidiosis related), and coccidial lesion scores on termination of the study were determined.

A summary of the data is given in Table 1.

The data were analyzed using Linear Plateau Model Analysis. The statistical summary of the 41 trials considered coccidial lesion scores 7 days after infection. Using these parameters, it can be concluded that maduramicin is an effective anticoccidial against the 6 pathogenic species of *Eimeria* named and that the optimum dosage range is 5-6 ppm.

		Lesion Scores [3]						
Treatment [1]	Mean Weight Gain [2]	E.A. (32)	E.M. (17)	E.T. (18)	E.B. (3)	E.N. (3)	E.Mi. (1)	Mortality [4] (%)
NNC	127.91	--	--	--	--	--	--	--
INC	54.12	2.79	2.54	2.93	3.09	2.84	2.60	25.70
maduramicin ammonium (2 ppm)	89.46	2.48	2.00	2.14	2.56	1.80	2.07	1.37
maduramicin ammonium (4 ppm)	112.94	1.88	1.54	1.27	1.44	1.64	1.73	0.00
maduramicin ammonium (5 ppm)	116.93	1.53	1.31	0.96	1.16	1.36	1.00	0.00
maduramicin ammonium (6 ppm)	119.19	1.21	1.01	0.66	0.87	1.04	1.27	0.00
maduramicin ammonium (7 ppm)	117.89	1.04	0.96	0.49	1.16	1.33	1.47	0.00

[1] NNC is noninfected, nonmedicated control. INC is infected, nonmedicated control.
[2] Average weight gain (g) 3-7 days postinoculation.
[3] Lesion scores per bird 7 days after challenge. Maximum possible score is 4. The number in parentheses below each species indicate the number of each species isolated and tested (E.A., *Eimeria acervulina*/*E. mivati*/*E. mitis* complex; E.M., *E. maxima*; E.T., *E. tenella*; E.B, *E. brunette*; E.N, *E. necatrix*; and E.Mi., *E. mitis*).
[4] Average % mortality due to coccidiosis in 19 of 41 studies that coccidiosis related mortality occurred in one or more treatments.

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2. Floor pen trials

   a. Challenge studies

      Summary:
      Five challenge floor pen studies were conducted at various geographical locations in the U.S. to evaluate the anticoccidial effect of maduramicin in broiler chickens challenged with recently collected field isolates of *Eimeria maxima*, *E. tenella*, *E. acervulina*, *E. necatrix*, *E. brunetti*, and *E. mivati*. These birds were maintained under use conditions to provide a simulated field evaluation of the effectiveness of maduramicin.

      Broiler chickens in the five studies were exposed to cocddial challenge at 14 days of age by feed and/or litter. Lesion scores were taken at 7 and 14 days postinfection (21 and 28 days of age).

      In trials 1, 2 and 3, an infected, non-medicated control group was compared with maduramicin at four dosage levels (4, 5, 6 and 7 ppm). Trial 1 added an additional group of birds treated with maduramicin at 8 ppm, and trial 4 did not have a 7 ppm treatment.

      The treated groups received anticoccidial medication in the diets from 0-44 days of age, followed by a 5-day drug withdrawal period on non-medicated feed prior to final weights. (These birds were discarded and did not enter the human food chain).

      The data from these five (5) trials are summarized in Tables 2-6

      In conclusion, maduramicin at 5 to 7 ppm in the diet effectively controlled coccidial challenge. The optimum dose range is concluded to be 5 to 6 ppm, since no significant improvement in lesion control was seen from 6 to 7 ppm.

Table 2
TRIAL 1
Larry R. McDougald
University of Georgia
Athens, GA 30002
Number of birds fed CYGRO Premix: 1000
Number of Control Birds: 200

Treatment	% Mortality 0-49 Days	Average Lesion Score Upper Intestine 21 Days	Average Lesion Score Upper Intestine 28 Days	Average Lesion Score Middle Intestine 21 Days	Average Lesion Score Middle Intestine 28 Days	Average Lesion Score Lower Intestine 21 Days	Average Lesion Score Lower Intestine 28 days	Average Lesion Score Cecum 21 Days	Average Lesion Score Cecum 28 Days	Total Average Lesion Score 21 Days	Total Average Lesion Score 28 Days	Average Weight Gain (g) 0-44 Days	Average Weight Gain (g) 0-49 Days	Average Feed/Gain 0-44 Days	Average Feed/Gain 0-49 Days
Infected non-medicated	8.5	3.19	2.38	2.88	1.88	1.06	0.13	2.31	0.63	9.44	5.00	1525	1784	2.36	2.41
maduramicin (4 ppm)	0.0	2.81	1.31	2.25	0.88	0.19	0.06	1.50	0.75	6.75	3.00	1627	1963	2.17	2.19
maduramicin (5 ppm)	0.0	2.63	1.19	2.63	0.75	0.50	0.13	1.25	0.19	7.00	2.25	1723	2017	2.15	2.21
maduramicin (6 ppm)	0.0	2.50	1.06	2.19	0.88	0.31	0.00	0.69	0.88	5.69	2.81	1675	2041	2.14	2.13
maduramicin (7 ppm)	0.0	2.00	1.19	1.75	0.69	0.13	0.00	1.00	0.88	4.88	2.75	1694	2016	2.10	2.15
maduramicin (8 ppm)	0.0	2.13	0.38	2.31	0.13	0.13	0.06	1.31	0.50	5.88	1.06	1679	1978	2.08	2.15

COMMENTS: Only coccidiosis related mortality is shown. Average lesion scores have a maximum score of 4.00. Total average lesion scores have a maximum score of 16. Challenged with Eimeria field strains of *E. tenella*, *E. necatrix*, *E. maxima*, *E. brtunetti*, *E. acervulina*, and *E. mivatiat* 14 days of age.

Table 3
TRIAL 2
Carey L. Quarles
Colorado Quality Research
Ft. Collins, CO 80524
Number of birds fed CYGRO Premix: 600
Number of Control Birds: 150

Treatment	% Mortality 0-49 Days	Average Lesion Score Upper Intestine 21 Days	Average Lesion Score Upper Intestine 28 Days	Average Lesion Score Middle Intestine 21 Days	Average Lesion Score Middle Intestine 28 Days	Average Lesion Score Lower Intestine 21 Days	Average Lesion Score Lower Intestine 28 days	Average Lesion Score Cecum 21 Days	Average Lesion Score Cecum 28 Days	Total Average Lesion Score 21 Days	Total Average Lesion Score 28 Days	Average Weight Gain (g) 0-44 Days	Average Weight Gain (g) 0-49 Days	Average Feed/Gain 0-44 Days	Average Feed/Gain 0-49 Days
Infected non-medicated	60.0	2.58	1.42	2.58	0.75	1.42	0.17	3.33	1.00	9.9	3.3	1285	1495	3.37	3.35
maduramicin (4 ppm)	0.0	1.67	0.25	0.58	0.00	0.00	0.00	1.50	0.08	3.8	0.3	1481	1728	2.05	2.09
maduramicin (5 ppm)	0.0	0.83	0.17	0.33	0.00	0.25	0.08	0.50	0.00	1.9	0.3	1515	1718	2.05	2.10
maduramicin (6 ppm)	0.0	0.17	0.08	0.08	0.00	0.00	0.00	0.42	0.00	0.7	0.8	1450	1752	2.08	2.06
maduramicin (7 ppm)	0.0	0.17	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.4	0.0	1393	1675	2.12	2.11

COMMENTS:
Only coccidiosis related mortality is shown. Average lesion scores have a maximum score of 4.00. Total average lesion scores have a maximum of 16. Challenged with Eimeria field strains of *E. tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, *E. acervulina*, and *E. mivati* at 14 days of age.

Table 4
TRIAL 3
S. A. Edgar
Auburn University
Auburn, AL 36849
Number of birds fed OYGRO Premix: 800
Number of Control Birds: 200

Treatment	% Mortality 0-49 Days	Average Lesion Score Upper Intestine 21 Days	Average Lesion Score Upper Intestine 28 Days	Average Lesion Score Middle Intestine 21 Days	Average Lesion Score Middle Intestine 28 Days	Average Lesion Score Lower Intestine 21 Days	Average Lesion Score Lower Intestine 28 days	Average Lesion Score Cecum 21 Days	Average Lesion Score Cecum 28 Days	Total Average Lesion Score 21 Days	Total Average Lesion Score 28 Days	Average Weight Gain (g) 0-44 Days	Average Weight Gain (g) 0-49 Days	Average Feed/Gain 0-44 Days	Average Feed/Gain 0-49 Days
Infected non-medicated	30.5	0.84	0.09	0.56	0.06	0.31	0.00	2.59	0.03	1.81	2.59	1403	1693	2.24	2.23
maduramicin (4 ppm)	10.0	0.72	0.28	0.16	0.06	0.06	0.16	1.25	0.06	0.94	1.25	1544	1793	2.00	2.09
maduramicin (5 ppm)	4.0	0.47	0.47	0.00	0.25	0.19	0.22	0.97	0.09	0.69	0.97	1571	1839	1.98	2.02
maduramicin (6 ppm)	4.0	0.59	0.13	0.38	0.06	0.13	0.06	0.53	0.13	1.19	0.53	1557	1834	1.94	2.00
maduramicin (7 ppm)	13.0	1.16	0.16	0.34	0.00	0.16	0.09	0.94	0.13	1.75	0.94	1589	1784	1.98	2.10

COMMENTS: Only coccidiosis related mortality is shown. AIC is acute intestinal coccidiosis and ACC is acute cecal coccidiosis lesion scores/bird at 21 days of age. Average lesion scores have a maximum score of 4.00. The maximum possible score is 4 for ACC and 12 for AIC. Challenged with Eimeria field strains of *E. tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, *E. acervulina* and *E. mivati* at 14 days of age.

Table 5
TRIAL 4
T. J. Kennedy
A.E.F. Research. Inc.
Waunakee. WI 53597
Number of birds fed CYGRO Premix: 600
Number of Control Birds: 200

Treatment	% Mortality 0-49 Days	Average Lesion Score Upper Intestine 21 Days	Average Lesion Score Upper Intestine 28 Days	Average Lesion Score Middle Intestine 21 Days	Average Lesion Score Middle Intestine 28 Days	Average Lesion Score Lower Intestine 21 Days	Average Lesion Score Lower Intestine 28 days	Average Lesion Score Cecum 21 Days	Average Lesion Score Cecum 28 Days	Total Average Lesion Score 21 Days	Total Average Lesion Score 28 Days	Average Weight Gain (g) 0-44 Days	Average Weight Gain (g) 0-49 Days	Average Feed/Gain 0-44 Days	Average Feed/Gain 0-49 Days
Infected non-medicated	31.5	3.18	1.68	2.73	1.90	2.10	0.95	4.00	0.70	12.0	5.2	1750	1960	2.76	2.86
maduramicin (4 ppm)	1.0	2.10	0.78	1.95	1.03	0.70	0.70	2.70	0.35	7.4	2.9	1810	2000	2.32	2.50
maduramicin (5 ppm)	0.0	1.95	0.33	1.63	0.70	0.45	0.28	1.90	0.00	5.9	1.3	1830	2020	2.24	2.44
maduramicin (6 ppm)	0.0	1.65	0.28	1.40	0.60	0.28	0.33	1.33	0.08	4.6	1.3	1800	2000	2.23	2.41

COMMENTS: Only coccidiosis related mortality is shown. Average lesion scores have a maximum score of 4.00. Total average lesion scores have a maximum score of 16. Challenged with Eimeria field strains of *E. tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, *E. acervulina*, and *E. mivati* at 14 days of age.

Table 6
TRIAL 5
J. D. Kobland
Agricultural Research Center
American Cyanamid Company
Princeton, NJ 08540
Number of birds fed CYGRO Premix: 1408
Number of control birds: 352

Treatment	% Mortality 0-49 Days	Average Lesion Score Upper Intestine 21 Days	Average Lesion Score Upper Intestine 28 Days	Average Lesion Score Middle Intestine 21 Days	Average Lesion Score Middle Intestine 28 Days	Average Lesion Score Lower Intestine 21 Days	Average Lesion Score Lower Intestine 28 days	Average Lesion Score Cecum 21 Days	Average Lesion Score Cecum 28 Days	Total Average Lesion Score 21 Days	Total Average Lesion Score 28 Days	Average Weight Gain (g) 0-44 Days	Average Weight Gain (g) 0-49 Days	Average Feed/Gain 0-44 Days	Average Feed/Gain 0-49 Days
Infected non-medicated	53.7	1.81	0.00	2.75	0.00	2.19	0.00	3.00	0.19	9.75	0.19	1473.1	1784.1	2.51	2.44
maduramicin (4 ppm)	1.4	2.06	0.00	1.94	0.00	0.88	0.00	2.50	0.06	7.38	0.06	1802.9	2071.0	1.84	1.92
maduramicin (5 ppm)	0.9	0.94	0.00	2.13	0.00	0.56	0.00	1.69	0.00	5.32	0.00	1786.2	2104.2	1.85	1.92
maduramicin (6 ppm)	0.3	0.38	0.06	0.69	0.00	0.00	0.00	0.69	0.00	1.42	0.06	1784.3	2107.7	1.84	1.89
maduramicin (7 ppm)	0.9	1.00	0.13	1.69	0.00	0.19	0.00	0.31	0.00	3.19	0.13	1700.8	2039.7	1.87	1.88

COMMENTS: Only coccidiosis related mortality is shown. Average lesion scores have a maximum score of 3.00. Total average lesion scores have a marlmum score of 12. Challenged with Eimeria field strains of *E. tenella*, *E. necatrix*, *E. brunetti*, *E. acervulina*, and *E. mivati* at 14 days of age.

III. TARGET ANIMAL SAFETY

Pivotal Studies

A. Target Animal (broiler chicken) Safety

1. Floor Pen Safety Study in Broiler Chickens

   Investigator:

   P.E. Gingher
   Agricultural Research Center
   American Cyanamid Company
   Princeton, New Jersey 08540

   In this pivotal study broiler chickens were grown to market age (49 days) under simulated commercial conditions and fed dietary levels of 0, 5, 6, 7.5, 9 and 15 ppm of maduramicin ammonium. Medication was fed from 0-44 days and followed with a 5-day medication withdrawal period. There were 8 replicates of 100 chickens of equal sex in each group.

   Parameters for determining the safety of maduramicin ammonium in broiler diets were growth, feed efficiency, mortality, hematology (hemoglobin, hematocrit, RBC and clotting times) and histopathology of various body organs and tissues as well as effects on pigmentation and feathering.

   The Linear Plateau Model analysis of 49-day weight gains indicated that in the absence of coccidiosis there was a significant dose related decrease in weight gains ($P<0.05$).

   Feed efficiency, as determined by feed gain ratios was comparable for all groups at the end of 49 days, except that the 15 ppm level of maduramicin ammonium significantly impaired feed efficiency during the medication period.

   Percent mortality for the 9 and 15 ppm groups was higher than for all other groups by 49 days.

   There were no effects on hemoglobin, hematocrit or red blood cell counts in males or females fed maduramicin ammonium for 44-45 days at levels up to 7.5 ppm. Reduced blood values were seen in males at the 9 and 15 ppm levels andfemales at 15 ppm at the 44-45 day period.

   Blood clotting times ranged from a low of 145 to a high of 1355 seconds, about the normal variation associated with chicken blood. There were no apparent trends with regard to treatment at the 44-45 day sampling.

   Histopathology of various tissues indicated that changes ascribable to maduramicin ammonium were apparent in the bursa of Fabricius and thymus. Partial atrophy of the bursa of Fabricius and of the thymus was an observable effect at 9 ppm (earliest) and 15.0 ppm (highest frequency). No other treatment related microscopic changes were observed. There was no adverse drug related effect on pigmentation. At levels above 5 ppm there were reduced

floor feathers at the end of the experiment which is indicative of feather eating by broilers.

The production data from the trial can be summarized as follows:

Table 7

Treatment	Weight Gain Average (g/bird) 0-44 Days	Weight Gain Average (g/bird) 0-49 Days	Feed Gain 0-44 Days	Feed Gain 0-49 Days	Avg. % Mortality 0-44 Days	Avg. % Mortality 0-49 Days
Non-medicated	1867.1	2168.6	1.96	2.04	2.3	2.4
maduramicin ammonium (5 ppm)	1807.3	2092.4	1.97	2.05	1.9	1.9
maduramicin ammonium (6 ppm)	1738.8	2018.5	1.99	2.05	2.6	3.4
maduramicin ammonium (7.5 ppm)	1686.4	1967.2	2.01	2.06	2.1	2.4
maduramicin ammonium (9.0 ppm)	1629.0	1962.8	1.98	2.01	2.4	3.0
maduramicin ammonium (15.0 ppm)	980.4	1317.2	2.12	2.00	4.5	6.6

2. Immunocompetency Study

In order to confirm that there was no biological significance to the histological changes observed in the bursa of Fabricus and thymus in the Floor Pen Safety study an additional study was conducted to determine immunocompetency of broiler chickens fed dietary concentration of maduramicin ammonium at 5, 7, 10 or 15 ppm in the starter diets during the first 3 weeks followed by 5 - 7 ppm of maduramicin ammonium during the remaining study period of 22 - 41 days. Since the Newcastle Disease (ND) involves both the humoral and cell-mediated immunity of chickens[1] the ND vaccine was selected to test the immunocompetency of maduramicin- medicated chickens. Newcastle Disease vaccine was given in drinking water at 7 and 21 days of age. A group of chickens served as the nonmedicated, nonvaccinated control and another group served as the nonmedicated, vaccinated control. At 31 days of age, all birds were challenged with a pretitrated dose of ND virus. Blood samples were taken from each bird at 7, 21 and 31 days of age for hemaglutination inhibition (HI) test. Mortality, morbidity, weight gain and feed intake were measured during the ND challenge period.

The HI titers at 21 and 31 days show that maduramicin ammonium medicated birds developed good primary and secondary antibody response to ND vaccine and their titers were comparable to those of the nonmedicated, vaccinated chickens. The mortality due to Newcastle disease virus challenge in the nonvaccinated controls was 12/15 (80%). One bird died prior to challenge.

[1] Y. E. Perey, G.B. Cleland and P.B. Dent, Am. J.. Vet. Res., 36, (1975): 513-517.

Neither mortality nor morbitity was observed in any of the vaccinated chickens.

It is concluded that the immunocompetency of chickens treated with maduramicin ammonium as high as 15 ppm for 3 weeks followed by 7 ppm for 3 more weeks was not compromised.

Data from the study are summarized in tables 8-9 on the following page. There were 16 chickens per group with 4 replicates of 2 males and 2 females per replicate.

Table 8 Summary of Body Weight, Weight Gain and Feed Intake of Birds Fed Maduramicin Ammonium for 41 days

Group	Maduramicin (ppm) Days 1-21	Maduramicin (ppm) Days 22-41	ND Vac.	Avg. Wt. (g/) Bird 31	Avg. Wt. (g/) Bird 41	Avg. Wt. (g/) Bird Gain	Feed Intake (g)/Bird/Day 0-31	Feed Intake (g)/Bird/Day 0-41	Feed Intake (g)/Bird/Day 31-41
A	0	0	No	1031	1212	181	54	47	22
B	0	0	Yes	1090	1630	541	65	69	80
C	5	5	Yes	1033	1463	430	60	67	88
D	7	7	Yes	1084	1511	427	52	59	81
E	10	7	Yes	1029	1583	554	59	60	63
F	15	7	Yes	912	1458	559	47	54	76

Table 9 Hemaglutination Inhibition (HI) Titers at 7, 21 and 31 Days of Age and Mortality (%) After Vital Challenge

Group	Maduramicin (ppm) Days 1-21	Maduramicin (ppm) Days 22-41	% Mortality	ND Vac. Day 7[1]	ND Vac. Day 21[2]	ND Vac. Day 31[2]	Mean HI Titers at Days 31-41
A	0	0	No	--	0	0	80
B	0	0	Yes	0.88	5.13	21.9	0
C	5	5	Yes	0.67	3.63	20.0	0
D	7	7	Yes	0.17	3.80	17.4	0
E	10	7	Yes	0.38	4.79	17.4	0
F	15	7	Yes	1.38	4.17	29.5	0

[1]arithmetic mean
[2]geometric mean

3. Non-Challenge Studies Summary:

Five pivotal non-challenge floor pen studies were conducted in various geographical locations in the U.S. to demonstrate the safety and dose range of maduramicin in broiler chickens under simulated commercial conditions, in the absence of coccidiosis. Maduramicin was examined at four dosage levels (5, 6, 7 and 8 ppm).

The primary parameters for measuring safety and efficacy of maduramicin were weight gain, feed efficiency and mortality.

The various treatment groups, except the non-medicated control group, received anticoccidial medication in the diets from 0-44 days of age, followed by a 5-day withdrawal period on non-medicated feed.

The body weight, feed efficiency and mortality of broiler chickens receiving maduramicin at various levels, was compared to the non-medicated control group. There were no significant differences in feed efficiency or mortality at 49 days of age between broilers receiving either of the four levels of maduramicin (5, 6, 7 and 8 ppm) and the non-medicated control group. A dose related adverse affect on weight gain was seen. Doses greater than 6 ppm showed a statistically significant decrease in weight gain as compared to the non-medicated control group.

Other observations included mortality, feed consumption, pigmentation and feathering. Feed consumption, pigmentation and feathering were normal in all trials. In trials 1 and 4 which were conducted in the same house, increased litter moisture was noted in the CYGRO pens.

Each trial is summarized as follows:

Trial 1

Investigator:

P.E. Gingher
Agricultural Research Center
American Cyanamid Company
Princeton, New Elersey 08540

Number of birds fed CYGRO® Premix: 2400
Number of control birds: 600

The data from the trial is summarized as follows:

Table 10

Treatment	% Mortality[1] 0-44 Days	% Mortality[1] 0-49 Days	Avg. Weight Gain (g) 0-44 Days	Avg. Weight Gain (g) 0-49 Days	Avg. Feed/ Gain 0-44 Days	Avg. Feed/ Gain 0-49 Days
Non-medicated	3.7	4.0	1841	2141	1.87	1.95
CYGRO (5 ppm)	3.7	3.8	1805	2117	1.86	1.93
CYGRO (6 ppm)	3.7	3.0	1767	2090	1.88	1.93
CYGRO (7 ppm)	2.5	2.5	1712	2047	1.89	1.93
CYGRO (8 ppm)	1.2	1.2	1645	1990	1.92	1.94

[1]Total mortality shown

Trial 2

Investigator:

P. M. Waldroup
University of Arkansas
Fayetteville, AR 72701

Number of birds fed CYGRO Premix: 1680
Number of control birds: 420

The data from trial 2 is summarized as follows:

Table 11

Treatment	% Mortality[1] 0-49 Days	Avg. Weight Gain (g) 0-44 Days	Avg. Weight Gain (g) 0-49 Days	Avg. Feed/ Gain 0-44 Days	Avg. Feed/ Gain 0-49 Days
Non-medicated	8.51	1486	1759	1.84	1.92
CYGRO (5 ppm)	6.37	1514	1797	1.88	1.95
CYGRO (6 ppm)	6.37	1472	1748	1.83	1.92
CYGRO (7 ppm)	1.88	1497	1777	1.86	1.93
CYGRO (8 ppm)	6.15	1406	1707	1.85	1.86

[1]Total mortality shown

Trial 3

Investigator:

Carey Quarles
Colorado Quality Research
Ft. Collins, CO 80524

Number of birds fed CYGRO® Premix: 1200
Number of control birds: 300

The data from trial 3 is summarized as follows:

Table 12

Treatment	% Mortality[1] 0-49 Days	Avg. Weight Gain (g) 0-44 Days	Avg. Weight Gain (g) 0-49 Days	Avg. Feed/ Gain 0-44 Days	Avg. Feed/ Gain 0-49 Days
Non-medicated	3.0	1644	1904	1.81	1.89
CYGRO (5 ppm)	2.3	1619	1929	1.80	1.87
CYGRO (6 ppm)	4.7	1610	1924	1.77	1.86
CYGRO (7 ppm)	3.4	1565	1918	1.79	1.86
CYGRO (8 ppm)	2.7	1428	1829	1.86	1.87

[1]Total mortality shown

Trial 4

Investigator:

P.E. Gingher
Agricultural Research Center
American Cyanamid Company
Princeton, New Jersey 08540

Number of birds fed CYGRO® Premix: 2400
Number of control birds: 600

The data from trial 4 is summarized as follows:

Table 13

Treatment	% Mortality[1] 0-44 Days	% Mortality[1] 0-49 Days	Avg. Weight Gain (g) 0-44 Days	Avg. Weight Gain (g) 0-49 Days	Avg. Feed/ Gain 0-44 Days	Avg. Feed/ Gain 0-49 Days
Non-medicated	2.7	2.8	1862	2189	1.88	1.94
CYGRO (5 ppm)	2.2	2.5	1849	2158	1.85	1.91
CYGRO (6 ppm)	2.0	2.0	1828	2139	1.86	1.92
CYGRO (7 ppm)	3.5	3.7	1790	2119	1.86	1.91
CYGRO (8 ppm)	3.3	3.3	1748	2086	1.83	1.87

[1]Total mortality shown

Trial 5

Investigator:

J. F. Ort
North Carolina State University
Raleigh, N.C. 27695

Number of birds fed CYGRO Premix: 1920
Number of control birds: 480

The data from the trial 5 is summarized as follows:

Table 14

Treatment	% Mortality[1] 0-44 Days	% Mortality[1] 0-49 Days	Avg. Weight Gain (g) 0-44 Days	Avg. Weight Gain (g) 0-49 Days	Avg. Feed/ Gain 0-44 Days	Avg. Feed/ Gain 0-49 Days
Non-medicated	6.88	8.75	1807	1981	1.74	2.01
CYGRO (5 ppm)	11.25	11.67	1756	1983	1.77	1.98
CYGRO (6 ppm)	6.46	8.33	1723	2018	1.76	1.90
CYGRO (7 ppm)	3.75	5.63	1668	1969	1.82	1.97
CYGRO (8 ppm)	4.58	6.25	1614	1919	1.86	1.99

[1]Total mortality shown

In summary, it can be concluded from the data in these nonchallenge floor pen studies that in the absence of coccidiosis, a significant dose related adverse effect on growth with no improvement in feed efficiency was seen starting at 6 ppm maduramicin ammonium. No significant adverse effect on growth was seen at 5 ppm maduramicin ammonium.

4. Clinical Studies - Commercial Scale Field Trials - Corroborative Studies

Summary:

Four field trials were conducted in the U.S. to evaluate the safety of maduramicin ammonium at 5 ppm in large scale broiler production.

A total of 67,941 broiler chickens were reared under commercial conditions with maduramicin ammoniurn included in the ration at the 5 ppm level from 1-day-old to seven days before slaughter. Parameters measured were: observations for clinical coccidiosis, mortality, live bird weight and feed conversion. Any possible adverse drug effects on feathering or litter condition were also monitored.

The performance of broilers in the four trials was as follows:

Table 15

Location	Processing Age (Days)	Average Live Weight (lb)	Feed Conversion	Mortality (%)
NC	46	4.09	1.86	3.44
MD	52	4.58	1.95	13.79*
GA	49	4.58	2.01	6.48
AR	42	2.97	1.95	2.16

* Mortality primarily due to vaccine reaction.

No sign of drug related toxicity or clinical coccidiosis was observed throughout the four trials. Litter condition and feathering were normal.

These reports further support the safety and effectiveness of maduramicin at 5 ppm in the complete feed of broiler chickens when fed under commercial conditions.

Name and Address of Investigator	No. of Birds Fed CYGRO
1. Tom Hester B&L Feeds Morganton, NC	16,320
2. Tom Holder Perdue Farms, Inc. Salisbury, MD	10,700
3. Birch McMurrray Seaboard Farms Athens, GA	20,921
4. John D. Story Campbells Insititute of Research & Technology Farmington, AR	20,000

Four field trials were conducted in the U.S. to evaluate the safety of maduramicin at 7 ppm in large scale broiler production.

A total of 85,572 broiler chickens were reared under commercial conditions with maduramicin included ln the ration of the 7 ppm level from 1-day-old to nine to twelve days before slaughter. Parameters measured were: observations for clinical coccidiosis, mortality, live bird weight and feed conversion. Any possible adverse drug effects on feathering or wet litter were also monitored.

The performance of broilers in the four trials were as follows:

Table 16

Location	Processing Age (Days)	Average Live Weight (lb)	Feed Conversion	Mortality (%)
NC	47	3.88	1.91	4.67
GA	52	3.97	2.03	2.04
AL	50	3.88	1.87	1.67
AR	42	3.367	1.94	3.24

No sign of drug related toxicity or clinical coccidiosis was observed throughout the four trials. Litter condition and feathering were normal.

These reports support the safety and effectiveness of maduramicin at 7 ppm in the complete feed of broiler chickens when fed under commercial conditions.

Name and Address of Investigator	No. of Birds Fed CYGRO
1. Tom Hester B&L Feeds Morganton, NC	16,320
2. Birch McMurrray Seaboard Farms Athens, GA	21,300
3. Gerald Bailey Goldkist Poultry Cullman, AL	21,352
4. Phil Dorr Con Agra Poultry El Dorado, AR	26,600

5. Additional Corroborative Information Concerning Animal Safety

CYGRO maduramicin ammonium has been approved and marketed internationally since 1984. It is currently marketed in 28 countries throughout the world, including most of the major broiler producing countries. Approximately 1.5-2.0 billion broilers per year are currently receiving CYGRO maduramicin ammonium. No adverse commercial reports attributable to CYGRO maduramicin ammonium including unacceptable litter condition, disease outbreaks or poor feathering have been received.

In addition, to determine the effect, if any, of maduramicin ammonium on fecal moisture, carefully controlled battery experiments were conducted by Dr. Carey Quarles of Colorado Quality Research, Fort Collins, CO. These studies of 4 weeks duration with 5 different U.S. broiler diets showed that CYGRO maduramicin ammonium caused no increase in water consumption or fecal moisture. These data are shown in the following Table 17.

Table 17 Fecal Moisture and Total Water Consumption at 28 days of Age Study A-84-30

Diet	Level of maduramicin (ppm)	Fecal Moisture (%)	Total 28 day Water Consumption (ml)
1	0	65	2302
2	0	71	2293
3	0	68	2269
4	0	71	2285
5	0	71	2282
1	5	70	2318
2	5	68	2291
3	5	70	2345
4	5	67	2286
5	5	69	2299

Additionally, a series of both floor pen and field trials with either 5 or 7 ppm CYGRO maduramicin ammonium in combination with various antibiotics and/or roxarsone have been conducted in support of combination NADAs. This

information has been submitted under the following NADAs: 140-821, 140-822, 140-823, 140-899, 140-902 and 140888. Nearly 1 million birds were involved in these studies. No adverse commercial effects attributable to CYGRO maduramicin ammonium on litter conditions, disease outbreaks or feathering were observed in any of these studies.

B. Effects on microbial populations

1. Antimicrobial Resistance

   Investigator:

   D. Fagerberg
   CARE, Inc.
   Fort Collins, CO

   A study was conducted to evaluate the effect of maduramicin ammonium fed *ad libitumto* chickens at 7 ppm on antimicrobial resistance of indigenous coliforms. Fecal samples of medicated and non-medicated birds were collected prior to treatment initiation for base-lines and weekly thereafter during an eight week treatment period. Ten coliform isolates per bird per sampling were tested for susceptibility to amikacin, ampicillin, carbenicillin, cefoxitin chloramphenicol, gentamicin kanamycin, nalidixic acid, streptomycin, sulfadiazine, tetracycline and trimethoprim/sulfamethoxazole.

   Essentially no antimicrobial resistance was found among indigenous fecal coliforms of birds in either the non-medicated or the maduramicin treated group. Therefore, maduramicin ammonium had no effect on coliform antimicrobial resistance in poultry when administered at 7 ppm in the diet.

2. Salmonella Shedding

   Investigator:

   D. Fagerberg
   CARE, Inc.
   Fort Collins, CO

   A study was conducted to evaluate the effect of maduramicin ammonium fed to chickens at 7 ppm on *Salmonella typhimurium* shedding (quantity, prevalence and duration), antimicrobial resistance and tissue levels. Fecal samples of medicated and nonmedicated birds were collected prior to treatment initiation to assure freedom from indigenous salmonellae. Chicks were orally inoculated with *Salmonella typhimurium* at 11 and 12 days of age. Medicated diet was initiated to the appropriate treatment group five days after the final challenge and continued throughout the remainder of the eight week study. Fecal samples were collected 14 times during the 56 day treatment period and were directcounted for quantities of test salmonellae and enriched for detection of non-countable levels of test salmonellae. From ten of the post-challenge sampling periods, five *S. typhumurium* isolates per bird were tested by a microdilution, MIC method for susceptibility to amikacin, ampicillin, carbenicillin, cefoxitin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfadiazine, tetracycline and trimethoprim/sulfamethoxazole. All

Salmonella-challenged birds were necropsied at time of death or at study end, and samples of liver, spleen, colon contents and cecal contents were analyzed for presence of test salmonella.

Maduramicin ammonium had no adverse effect on the quantity, prevalence or duration of salmonella shedding, nor on antimicrobial resistance of the salmonella, nor did it have any effect on the presence of test salmonella in tissues.

IV. HUMAN FOOD SAFETY

A. Toxicity tests

1. Acute toxicity tests

Acute toxicology:

Rabbit eye irritation-maduramicin technical

Six male rabbits received 0.1 g of the test material in the conjunctival sac of one eye. Within 24 hours signs of severe conjunctival and corneal irritation were apparent in all animals. These animals all died by day 7. Three other rabbits received the test material followed by a 20 second rinse immediately after instillation. Only mild signs of conjunctival irritation which reversed by 3 days were observed.

Rabbit skin irritation-maduramicin technical

Six male rabbits were patched with 0.2 g of the test material as an aqueous paste. The occluded patches were held in place for 24 hours, then removed and the skin was examined for signs of irritation. The primary irritation score which resulted was 2.9. Two rabbits died 72 hours after application. The material produces both moderate erythema and edema upon skin application.

Rabbit eye irritation-maduramicin in benzyl alcohol

Six rabbits were dosed with 0.1 ml of this mixture instilled into the conjunctival sac. All eyes were rinsed after 24 hours. Results showed that the product was irritating to the cornea, iris and conjunctivae of most animals. All animals had died by day 4.

American Cyanamid Report A-83-10:

The following studies were conducted with the 1% premix formulation which is the subject of this NADA with the exception that the final premix contained approximately 5% corn oil.

Rabbit eye irritation-premix

A standard dose (0.1 g) of formulation was instilled into the conjuctival sac of 9 rabbits. After 20 seconds the eyes of three of the rabbits were rinsed thoroughly, white the other 6 rabbits' eyes were washed after 24 hours. The

animals whose eyes had been washed after 20 seconds showed no signs of irritation. The animals receiving the 24 hour rinse showed corneal and conjunctival irritation as well as iritis. All eyes returned to normal before l4 days.

Rabbit skin irritation-premix

A standard dose (0.5 g) of the product as an aqueous paste was held under an impervious patch for 24 hours on 6 rabbits and the skin was examined and scored at 24 and 72 hours. The primary irritation score was 2.0. Moderate erythema and mild edema were observed which was more pronounced at the abraded sites.

2. Subchronic Toxicology:

American Cyanamid Report L-1966B: Rat 28-day feeding study-maduramicin technical

Five rats/sex/group were fed diets containing test material at concentrations of 4, 6 and 8 ppm for 28 consecutive days. A control group of 10 rats/sex received basal diet at the same time. No signs of toxicity were observed at any treatment level over the course of the study. The no observable effect level, therefore, was considered to be >8 ppm, equivalent to 0.87 mg/kg maduramicin body weight.

American Cyanamid Report L-1983: Rat 13-week feeding study-maduramicin technical

Thirty rats/sex/group were fed diets containing maduramicin at levels of 0, 1, 3, 5, and 7 ppm for 13 weeks. A significant decrease in body weight over the course of the study was observed for males receiving 7 ppm. Males receiving 5 and 7 ppm also showed significantly increased relative heart weights as compared to the controls. For these reasons the no effect level was considered to be 3 ppm (0.21 mg/kg).

American Cyanamid Report L-l984: Dog 28-day feeding study-maduramicin technical

Two dogs/sex were fed diets containing 0, 6, 12, 24 and 48 ppm maduramicin for 28 days. No effects were observed in any parameters measured in any animals receiving 12 ppm or less. The only effect observed in animals receiving 24 ppm was an intermittent hindlimb weakness/stiffness observed in 2/4 dogs. The effect was transient and was not seen after 16 days of test material administration. At 48 ppm ataxia which progressed to hindlimb paralysis was seen in all dogs. Atrophy, focal degeneration and diffuse sarcolemmal proliferation of skeletal muscle were observed microscopically in these dogs. These changes were not observed in the animals receiving 24 ppm. The no effect level was considered to be 12 ppm.

BioResearch Laboratories Report 50716-maduamicin technical

A range finding study to determine doses to be used in the definitive cardiovascular screening of maduramicin in the beagle dog following single oral or intravenous administration.

Ten dogs (5 male, 5 females) received incremental doses of maduramicin in ethyl alcohol at about 1 hour intervals in order to find a dose which would produce an effect and one which would produce no effect. Following each dose all dogs were monitored for heart rate, respiration rate, blood pressure, left ventricular pressure, dp/dt, aortic pressure and cardiac output. Coronary blood flow was determined at termination. The time course of the response was determined also. In addition, eight (4 male and 4 female) dogs were fed the test material in a ball of ground meat and the time course of the response was again determined measuring the same parameters. The results showed that after intravenous administration the apparent response was over in about 1 hour, and after oral administration the first signs of response could be observed after about 2 hours. Therefore, the dose levels chosen for intravenous dosing in the main study were 0.05, 0.20 and 0.60 mg/kg, and for oral dosing 0.5, 2.0 and 6.0 mg/kg. The optimum time to begin data collection in orally dosed dogs appeared to be 1 ½ hours post-dose. The most sensitive parameters measured appeared to be left ventricular and aortic pressure.

BioResearch Laboratories Report 50724-maduramicin technical

Cardiovascular profile of maduramicin in the beagle dog following single oral or intravenous administration.

Three dogs/sex/dose group were administered test material at dose levels of 0.05, 0.20, 0.60 mg/kg (intravenous), and 0.5, 2.0 and 6.0 mg/l (oral) in a single dose and various cardiovascular parameters were measured. Intravenous injection of the test material caused an increase in heart rate, left ventricular pressure, systolic aortic pressure and dp/dt in dogs given a single dose of 0.60 mg/kg. No significant cardiovascular effect was observed following injection of 0.20 mg/kg. None of these changes was detected consistently in dogs given the test material orally at dose levels up to ten times higher than the IV dose.

Lederle Laboratories Report 36624-maduramicin technical

Cardiovascular screening profile

The effects of the test material on parameters such as blood pressure, urine output and renin production were studied in rats.

Isolated heart muscle was examined to determine the effect of perfusion of test material on contractile force, coronary vasodilatation, etc. Many other cardiovascular parameters were examined in this study. Results showed that there was nothing remarkable in the cardiovascular screening profile of maduramicin.

American Cyanamid Report C-84-1-maduramicin technical

Changes in heart rate, ECG measurements and amount of drug in urine measured at different intervals from dogs injected intravenously with maduramicin.

The purpose of this experiment was to measure heart rate changes, electrocardiographic recordings and maduramicin residues in urine of conscious trained beagle dogs intravenously injected with maduramicin in order to find one or several parameters which can be used for monitoring the degree of accidental human exposure to maduramicin.

The results of this experiment indicate that the-analysis for determination of maduramicin residues in the urine is the best parameter which can be used for monitoring the degree of accidental human exposure to maduramicin since electrocardiographic changes are very slight or not existent.

3. Teratology and Reproductive Toxicology

Toxigenics Study 450-1252:
Teratology pilot study in albino rats with with maduramicin-maduramicin technical

Five females/group were administered the test material in corn oil on gestation days 6 through 15 at dosage levels of 0 (corn oil only), 1, 5, 10 or 15 mg/kg maduramicin. All females receiving 5, 10 or 15 mg/kg died during the dosing period. Animals receiving 1 mg/kg showed no signs of toxicity. From the study dose levels of 0.1, 1.0 and 3.0 mg/kg were chosen for the definitive study.

Toxigenics Study 450-1253:
Teratology study in albino rats with maduramicin-maduramicin technical

Twenty-five virgin female rats/group were mated and treated with test material via gavage on gestation days 6 through 15 at dose levels of 0, 0.3, 1.0 or 3.0 mg/kg/day. All females treated with 3.0 mg/kg died prior to gestation day 20. Animals treated with lower dosages showed no signs of maternal toxicity, and offspring of these two groups of females showed no signs of teratogenicity.

Hazelton Laboratories study 6123-110:
Three generation reproduction study with maduramicin in rats-maduramicin technical

Groups of 12 male and 24 female immature rats were fed diets containing 0, 1, 3 or 5 ppm maduramicin for 71 days. They were then mated twice to produce the F1a and F1b litters. After a minimum of 100 days of exposure to the same diets as the parents, the breeding program was repeated with F1b animals to produce the F2a and F2b pups and with F2b animals to produce the F3a and F3b pups. The only finding was slight inconsistent body weight changes in adults and pups which did not interfere with development and had

no adverse effects on the reproductive function of either sex in any of the 3 generations.

4. Chronic Toxicology

BioResearch Laboratories Project 81603:
A one-year dietary toxicity study in dogs with maduramicin-technical

No observable effects were seen in dogs administered 6 ppm or less for one year. Target organs seen in higher dose groups were the heart and the eye.

Five beagles/sex/group were fed diets containing maduramicin at levels of 0, 3, 6, 12 and 24 ppm for one year. Two dogs receiving 24 ppm died during the course of the study. In both cases the cause of death was considered to be congestive heart failure. Electrocardiograms showed that a significant increase in heart rate was observed in surviving dogs receiving 24 ppm after 1 year of study. At necropsy, a slight increase was seen in the mean (absolute and relative) heart weights of high dose group dogs, probably related to the ventricular dilation seen on gross examination. Subsequent microscopic examination of the heart tissue showed no histological changes that could definitely be attributed to test material administration. Eye lesions were observed in two females receiving 12 ppm and three males and five females receiving 24 ppm. In-life examinations showed the presence of what appeared to be reversible lesions described as multifocal lesions appearing as water droplets and hyperreflectivity. Histopathological examination of the eye confirmed retinal.atrophy in dogs showing hyperreflectivity. The no observable effect level was 6 ppm (0.204 mg/kg).

5. Genetic Toxicology Summary

A battery of *in vitro* and *in vivo* tests directed at assessing mutagenic/carcinogenic potential (*in vitro*) and to confirm presence/absence of mutagenic activity (*in vivo*) of maduramicin have been undertaken. Such systems are thought to possess high predictive accuracy for carcinogenicity and hence constitute a useful tool to obtain data which can be used together with our toxicological data for the evaluation of the safety of the product.

The *in vitro* and *in vivo* tests completed with maduramicin technical are:

a. A bacterial/microsome reverse mutation test (Ames).

b. A Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase test (CHO/HGPRT).

c. In vitro chromosome aberration analysis in Chinese hamster ovary cells (CHO).

d. In vivo bone marrow cytogenetic rat metaphase analysis.

e. Unscheduled DNA Synthesis in rat primary hepatocytes.

Ames Test

In the Ames test, maduramicln was tested using six bacterial strains (*S. Typhimurium* TA-98, TA-100, TA-1535, TA-1537 and TA-1538 and *E. coli* WP-2 uvrA-) in both the presence and absence of arochlor 1254 - induced rat liver S-9 homogenate. The dose levels in the plate assay ranged from 15.8 to 5000 mcg/plate; the maximum dose level tested for each strain was limited by solubility or cytotoxicity. In addition, a disc test was performed. Non-mutagenic responses were obtained for all strains at all dose levels.

CHO/HGPRT Assay

In the CHO/HGPRT assay, maduramicin again was tested in both the presence and absence of S-9. The highest dose level was chosen based on cytotoxicity. The dose levels tested ranged from 1 to 100 mcg/ml in the presence of S-9 and from 10 to 150 mcg/ml in the absence of 5-9. Non-mutagenic responses were again obtained at all dose levels.

Unscheduled DNA Synthesis in Rat Primary Hepatocytes

In this in vitro unscheduled DNA Synthesis Test maduramicin was tested using rat primary hepatocytes. Based on the results of an initial toxicity test, the test article was tested at live dose levels ranging from 0.10 to 4.0 mg/ml.

The results of the UDS assay indicate that under the test conditions, the test article did not cause a significant increase in the number of labelled nuclear grain counts and therefore is considered negative in this study.

CHO/Chromosome Aberration Assay

In the *in vitro* CHO/Chromosome aberration assay, the highest dose levels were again limited by cytotoxicity. Maduramicin was tested in the presence and absence of S-9 with cultures harvested at two time points, 6-8 hours and 14-18 hours after treatment. Uniformly non-mutagenic results were obtained for the 6-8 hour sampling time. For the 14-18 hours sampling time, chromosomal breakage was observed at the high dose level (100 mcg/ml) without S-9, and at the middle (50 mcg/ml) and high (100 mcg/ml) dose levels in the presence of S-9. The response at the middle dose level was greater than the response at the high dose level.

Since the induction of chromosomal aberrations was the only positive response in an in vitromutagenicity test battery, and since *in vivo* systems provide closeness to the physiological and biological complexities of the intact mammalian organism, it was decided to test CYGRO for its clastogenic potential using rat bone marrow metaphase analysis system. In this *in vivo* study, CYGRO was tested at intraperitoneally (ip) administered doses of 0.9, 4.5 and 9 mg/kg; 9 mg/kg was the maximum tolerated dose permitting adequate metaphase cells for analysis. Bone marrow cells were harvested at 6, 24 and 48 hours after chemical injection for chromosomal aberration analysis. Negative results were obtained at all dose levels.

Thus positive results obtained under *in vitro* conditions are not corroborated by *in vivo* evaluation. Also, the *in vivo* test was performed at considerably higher doses than could result from potential human exposure. Greater reliance is

placed on results obtained in living animals and it is concluded that CYGRO does not have genotoxic potential using chromosome breakage as a genetic endpoint.

B. Safe Concentration of Residues

The dog was the most sensitive species with a no-effect level of 0.204 mg/kg/day. Applying a 100-fold safety factor, the safe concentration of residues is as follows:

Calculation of Maduramicin Ammonium Acceptable Daily, Intake (ADI)

ADI = 0.204 mg/kg/day (lowest NOEL) / 100 fold safety factor = 0.00204 mg/kg/day

ppm in muscle
= ADI x 60 kg (weight of average human) / 0.5 kg/day (daily consumption of meat)
= .00204 mg/kg/day x 60 kg / 0.5 kg/day = 0.240 ppm or 240 ppb

Safe concentrations of residues in edible tissues other than muscle:

Tissue	Consumption Factor	Safe Concentration
Chicken Liver	3	720 ppb
Chicken Skin	2	480 ppb
Chicken Fat	2	480 ppb

C. Metabolism and Total Residue Depletion Studies, Demonstration of Withdrawal Time

In study PD-M Volume 21-1, six Hubbard x Hubbard broilers were offered feed medicated with 5.5 ppm carbon-14 labeled maduramicin ammonium *ad libitum* for 7 consecutive days. The animals were sacrificed on the 0 day of withdrawal and the appropriate tissues were excised. The total carbon-14 residue levels of the tissues are given below:

Tissue	Total Carbon-14 Content (ppm)
Fat	1.19
Liver	0.67
Skin	0.49
Kidney	0.22
Muscle	0.08

Extracts of each tissue were subjected to two-dimensional TLC. Maduramicin ammonium was the most abundant residue found in each tissue. In fat, parent maduramicin ammonium represented 91.9% of the total residue. 6.4% of the total residue was observed to co-migrate in the TLC system with the beta-isomer of maduramicin ammonium. The remaining 1.7% consisted of unidentified minor components. The beta-derivative is produced by O-demethylation of a methoxy group in the A ring of maduramicin ammonium.

This metabolite would not be expected to produce toxicity different from that observed with the parent drug. Therefore, separate toxicity testing of the metabolite was not required.

In Study A-87-44, male and female broiler chickens were fed diets containing 6 ppm maduramicin ammonium for 44 days. The birds were sacrificed at 0, 1, 2, 3, 4 and 5 days withdrawal and the abdominal fat analyzed using the HPLC/F method for maduramicin ammonium residues. The results were as follows:

Withdrawal Period in Days	Range of Assay Values (ppm)
0	1.01-1.18
1	0.57-0.74
2	0.31-0.36
3	0.11-0.23
4	<0.10-0.26
5	<0.10-0.11

Limit of measurement = 0.10 ppm

In Study L-2295, carbon-14 labeled maduramicin ammonium was administered to broilers at a concentration of 6 ppm for seven consecutive days. At the conclusion of the medication period, chickens were sacrificed on 0, 1, 3 and 5 days withdrawal. Tissues were collected at time of sacrifice. Abdominal fat was analyzed via combustion analysis for total radioactivity with the following results:

Withdrawal Period in Days	Total Radioactivity (mean ppm maduramicin ammonium equivalents)
0	1.409
1	0.771
3	0.206
5	0.102

The results of the HPLC analyses obtained from Study A-87-44 were compared with those of combustion analyses from Study L-2295. At 0, 1, 3 and 5 days of withdrawal, parent maduramicin ammonium was 78%, 85%, 80% and 81% respectively, of the total residue.

Based on parent maduramicin ammonium being 80% of the total residue in fat (safe concentration, 480 ppb) as measured by the HPLC/F method, an R(m) of 380 ppb has been established for the marker residue maduramicin ammonium, in fat, the target tissue.

The HPLC data from Study A-87-44 was subjected to a statistical analysis (99% tolerance limit with 95% confidence) to determine the minimum withdrawal period. Based on this, a minimum withdrawal period of 4 days was calculated. It should be pointed out, however, that target animal safety and efficacy considerations will limit the withdrawal period to 5 days.

D. Regulatory Methods

The determinative method for quantitating residues of maduramicin in chicken fat is based on high pressure liquid chromatography. The confirmatory assay is a Liquid chromatography/mass spectrometry/mass spectrometry procedure.

The determinative and confirmatory methods were validated satisfactorily by FDA and USDA laboratories. The validated regulatory methods are filed in the Food Additives Manual on display in FDA's Freedom of Information Public Room (Room 12A30) 5600 Fishers Lane, Rockville, Maryland 20857.

V. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Act and demonstrate that maduramicin ammonium (CYGRO) at a concentration ranging from 5 ppm to 6 ppm in finished feed is safe and effective when fed to broiler chickens for the indications stated in the product labeling.

Maduramicin ammonium has been subjected to a threshold assessment in accordance with the procedures contained in the agency's "Guideline for Threshold Assessment" dated September, 1986. The submitted data are adequate to satisfy the Agency's food safety requirements. The safe concentration for total residues in noncooked edible chicken tissues are: 720 ppb in liver; 480 ppb in skin and 480 ppb in fat.

Based on parent maduramicin ammonium being 80% of total residue in fat, the target tissue, an R(m) of 380 ppb has been established for the marker residue. The data supports a minimum withdrawal time of 4 days. All studies conducted to document efficacy of the drug product included a 5-day withdrawal period. This provides the basis for the established withdrawal time.

Proper use by non-veterinarians can be expected because poultry producers routinely use medicated feed containing an animal drug for the prevention of coccidiosis in broiler chickens. 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. The Agency has concluded that this product can be approved for over-the-counter use.

VI. ATTACHMENTS

Package Label, Type C Broiler Feed, Medicated
Package Label, Type A Medicated Article

Copies of these labels may be obtained by writing to the:

Food and Drug Administration
Freedom of Information Staff (HFI-35)
5600 Fishers Lane
Rockville, MD 20857

Or requests may be sent via fax to: (301) 443-1726. If there are problems sending a fax, call (301) 443-2414.

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