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Submitted Jan. 29, 1987
Response to Sept. 23, 1986
letter (Aug 29, 1986 memo)

Environmental Assessment

CYGRO® 1% maduramicin ammonium premix for broilers

1. Date:

2. Name of Applicant/Petitioner:

   American Cyanamid Company

3. Address:

   P. O. Box 400
   Princeton, New Jersey 08540

4. Description of the Proposed Action:

   a. Use:

      American Cyanamid Company has filed a New Animal Drug Application (NADA) for CYGRO anticoccidial, a 1% maduramicin ammonium premix for use in complete broiler feeds. CYGRO is to be mixed in complete broiler feeds to provide a range of 4.54 to 6.36 g/ton (5-7 ppm) maduramicin to be given from day one of age until 3 days before slaughter, to control coccidiosis due to Eimeria acervulina, E. tenella, E. brunetti, E. maxima, E. necatrix and E. mivati.

   b. Need:

      The cost of coccidiosis, both in terms of mortality and morbidity and decreased growth, has long been recognized as a highly significant factor in the economical production of broilers. Thus, use of an anticoccidial in the feed throughout the life of the broiler is the rule rather than the exception in modern large scale broiler production.

      Maduramicin is a highly potent member of the class of polyether ionophore anticoccidials. Because of its potency, it is effective at a low concentration in feed, 5-7 ppm, as opposed to other polyether ionophores which must be fed at substantially higher levels (monensin: 99-121 ppm; lasalocid: 75-125 ppm; salinomycin: 44-66 ppm). However, maduramicin has been shown to be safe to a number of other classes of livestock at this recommended use level (horses, swine, cattle, turkeys, layers) as opposed to the other ionophores which have demonstrated toxicity to some of these livestock species at levels well below the intended use level for broilers. (Refer to FOI Summary, p. 18 for a description of these studies).

c. Manufacturing Locations:

CYGRO premix is manufactured entirely at Cyanamid Italia S.p.A., Catania, Sicil, Italy and at Cyanamid Quimica do Brasil, Ltda., Resende, R.J., Brasil.

d. Locations and environments of use:

The marketing regions for CYGRO (sites of predominant use) are located primarily in the Southeastern area of the United States. The table below represents the number (in thousands) of one-day-old chicks placed in growout houses each week by state, for the week ending January 25, 1986. The total placements (in thousands) for the week ending January 11 and 18, 1986, were 87,719 and 88,278, respectively, indicating that the fluctuation from week to week is minimal. (Source: Feedstuff. February 10, 1986, p. 44).

State	Chicks Placed (thousands)
Alabama	11,522
Arkansas	15,519
California	3,793
Delaware	4,194
Florida	2,214
Georgia	13,501
Maryland	5,305
Mississippi	6,408
North Carolina	8,956
Pennsylvania	2,042
South Carolina	1,305
Texas	4,710
Virginia, W. Virginia	3,684
Washington, Oregon	798
Louisiana, Missouri, Tennessee	3,756

The number of broilers to be medicated with CYGRO in an average broiler facility is approximately 50,000 per flock. The average producer has five flocks or 250,000 birds per year.

5. Chemical I.D.

a. Nomenclature:

USAN (U-67)	Maduramicin
Pronunciation	ma dur' a mi sin

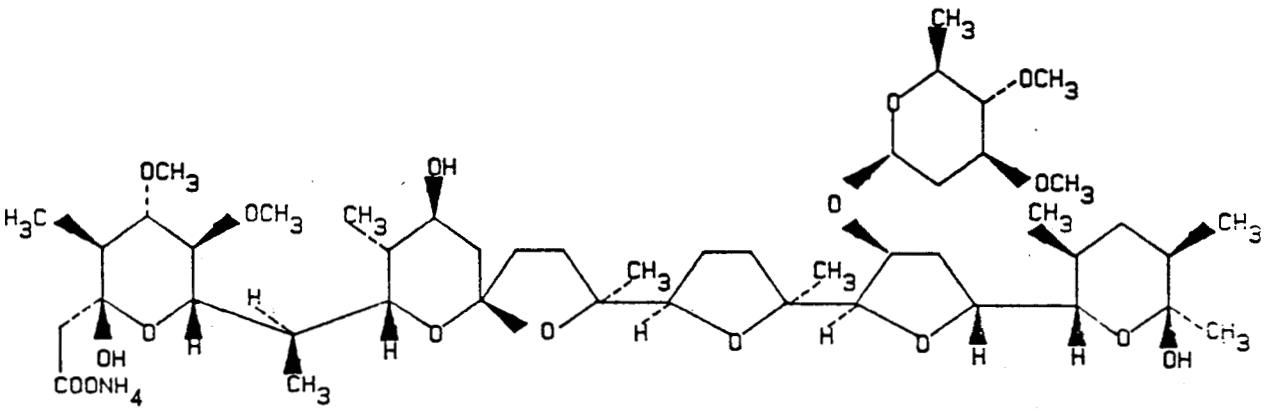
5. Chemical I.D. (Cont'd)

Chemical Names

1) (3R,4S,5S,6R,7S,22S)-23,27-didemethoxy-2,6,22-tridemethyl-11-0-demethyl-22-](2,6-dideoxy-3,4-di-0-methyl- L-arabino-hexopyranosyl) oxy[-6-methoxylonomycin A, monoammonium salt

2) Ammonium (2R,3S,4S,5R,6S)-tetrahydro-2-hydroxy-6-](R)-1-](2S,5R,7S,8R,9S)-9-hydroxy-2,8-dimethyl-2-]2S,2'R,3'S,5R,5'R)-octahydro-2-methyl-3'-]](2R,4S,5S,6S)-tetrahydro-4,5-dimethoxy-6-methyl-2H-pyran-2-yl[oxy[-5'-](2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2H-pyran-2-yl[]2,2'-bifuran[-5-yl[-1,6-dioxaspiro]4.5[dec-7-yl[ethyl[-4,5-dimethoxy-3-methyl-2H-pyran-2-acetate

Structural Formula



Molecular formula:	($C_{47}H_{83}NO_{17}$)
Molecular weight:	934.14
Trademark:	CYGRO
Manufacturer:	American Cyanamid Company
Code designation:	CL 273,703
CAS registry number:	84878-61-5
WHO number	5670

5. Chemical I.D. (Cont'd)

b. Physical-chemical description and properties.

Maduramicin is a monoglycoside polyether ionophore antibiotic. It is produced by fermentation by the organism Actinomadura yumaensis, refined by crystallization from the fermentation biomass, dissolved in benzyl alcohol and sprayed onto a corn cob fraction carrier, to produce CYGRO 1% premix.

Melting point: (Exhibit A)	165-167°C
Density: (Specific Gravity) (Exhibit B)	1.18 ± 0.01 g/cc at 23 1°C

Solubility: Slightly soluble in water, soluble in most organic solvents.

Examination of the solubility of maduramicin in distilled water and in aqueous buffers at pH 5, 7 and 9 indicates that this compound does not exhibit classic solubility characteristics. Determination of an exact value for the aqueous solubility is problematic because of it apparent tendency to form molecular aggregates in solution. This phenomenon is extremely sensitive to test conditions such as pH, ionic strength, the quantity of excess material used or the solvent from which the maduramicin was mostly recently recrystallized. Degradation of the parent compound occurs at a slow rate in aqueous solution under the experimental conditions and, therefore, does not explain the unusual solubility characteristics observed. Depending on the exact test conditions, the aqueous solubility of maduramicin at equilibrium (24°C) ranged from 100 ppm at pH 5 to 3000 ppm in distilled water. A description of the method used to conduct the solubility study is provided in Exhibit C.

Dissociation constant (Kd): Maduramicin is only slightly soluble in water. Determination of Kd would therefore have to be conducted in an organic solvent system, and would not be meaningful.

Ultraviolet/Visible Absorption Spectra: Maduramicin exhibits no significant absorbance between 290 and 750 nm. (Exhibit D).

5. Chemical I.D. (Cont'd)

c. Pharmacology:

Maduramicin is a member of the class of compounds known as polyether ionophore antibiotics. These antibiotics form electrically neutral complexes with monovalent or divalent cations and catalyze electrically silent exchanges of cations for protons or other cations across a variety of biological membranes (Ref. 1).

Other compounds in this class are monensin, lasalocid and salinomycin. These compounds as well as CYGRO are coccidiacidal. The exact mechanism of action of these compounds is not known, but is believed to be related to disturbance of electrolyte balance in the coccidial parasites during the extracellular stages of the life cycle. The affinity of CYGRO for monovalent and divalent cations has been measured by Liu et al (Ref. 2). Their findings indicate the following cation selectivity.

$K^+ > Rb^+ > Na^+ > Li^+ > Cs^+ >> Mg^{+2}, Ca^{+2}, Ba^{+2}, Sr^{+2}$

CYGRO was found to mediate efflux of intracellular potassium from chick erythrocytes in a manner typical of polyether ionophores. That is, the efflux of potassium was accompanied by an increase in the pH of the extracellular medium as protons were exchanged for intracellular potassium. The rate of K^+ efflux was observed to be linear over a wide range of ionophore concentration, from 10 to 1 mM. At concentrations above 1 mM an apparent maximum rate of potassium efflux was reached (approximately 20 µMoles/min. in this system). (Refer to Exhibit E).

6. Introduction into the environment.

a) Manufacturing:

Maduramicin ammonium, the active ingredient in CYGRO 1% Premix, is produced by deep tank aerobic fermentation of the organism Actinomadura yumaensis. The refining process results in a harvest of active material, and an aqueous waste solution which consists of fermentation biomass.

All waste streams from the fermentation and refining operations are collected and treated with caustic soda to raise the pH to 10.0, and then thermally degraded by treatment at 125-140°C for 2-4 hours. This treatment effectively detoxifies all the waste streams from the process. The detoxified material is sampled and tested by Quality Control before release for transfer to the effluent treatment facility.

6. Introduction into the environment. (Cont'd)

The composition of the waste streams is similar to fermentation mash. After alkaline treatment and heating for 4 hours at 130°C, waste characterization is as follows:

pH = 10.0
C.O.D. = 26.2 g/L
$B.O.D._5$ = 9.8 g/L
NH_3-N = 1.34 g/L

Manufacturing waste products are disposed in compliance with local environmental requirements (Catania, Sicily, Italy)-Exhibit F, (Resende, Brazil)-Exhibit G. Adequate controls over the manufacturing process, as specified by the current Good Manufacturing Practice regulations (21 CFR 200) are in place to prevent contamination of the environment with the raw compound.

b. Environmental Burden

The projected use of CYGRO involves continuous administration of complete feed containing 5 to 7 ppm maduramicin to broiler chickens throughout their lifetime, up to 3 days prior to slaughter. Generally, broiler chickens are raised in intensive confinement. Manure from confinement houses is used as fertilizer and spread on crop fields.

It is common agronomic knowledge (Ref. 5-7) that high levels of nitrogen can cause significant injury to most species of plants. This injury is known as "saltburn" and is the result of high levels of water soluble ions and/or high concentrations of ammonia such as is found in poultry manure. This factor is taken into account in University recommendations (Exhibit H) for poultry manure applications to field crops.

Application rates range from 6.73 metric tons/ha (3.0 tons/acre) for small grains to a maximum rate of 22.45 metric tons/ha (10.0 tons/acre) for corn.

Excreta from broilers fed 7 ppm of maduramicin ammonium contained 2.95 ppm maduramicin residues (Exhibit J) and contained 65% moisture. In normal use the excreta is combined with litter and applied to agricultural land at moisture levels of 15-30%. If one assumes an average moisture level of 20% and no correction for dilution by bedding (litter) materials or for degradation on storage, the actual application rate of maduramicin ammonium would be

2.95 ppm with 65% moisture = 6.74 ppm at 20% moisture

6.74 ppm x 22 mt/ha x 10^6g/mton = 148 g maduramicin/ha

6. Introduction into the environment. (Cont'd)

Assuming no degradation on storage, this would result in a soil concentration of

$$\frac{148 \text{ g maduramicin/ha}}{2.5 \text{ million kg soil/ha}} = 0.059 \text{ ppm}$$

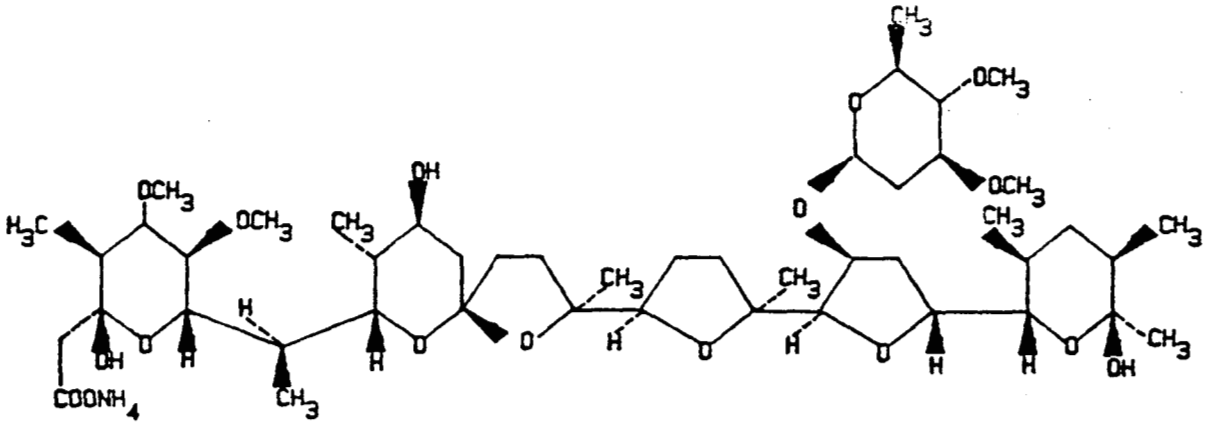
Note: 2.5 million kg soil/ha equals the dry weight of an acre furrow slice (Exhibit R).

Manure is normally added to soil and incorporated before planting in the Spring. Manure may also be applied in the Fall, after the crop has been harvested. In the case where there are two applications per year, the average storage time is 6 months, or 182 days. This is equivalent to 3.4 half-lives. A compound which degrades through 3.4 half lives will have a concentration of $(\frac{1}{2})^{3.4}$ times the initial concentration or 0.095 times the initial concentration. Thus the actual initial application is 148 g maduramicin/ha x 0.095 = 14.1 g/ha which would result in a soil concentration, after incorporation of 0.00564 ppm.

c. Metabolism of maduramicin

The only metabolic pathway of significance in the chicken is O-demethylation of a methoxy group in the A ring of maduramicin.

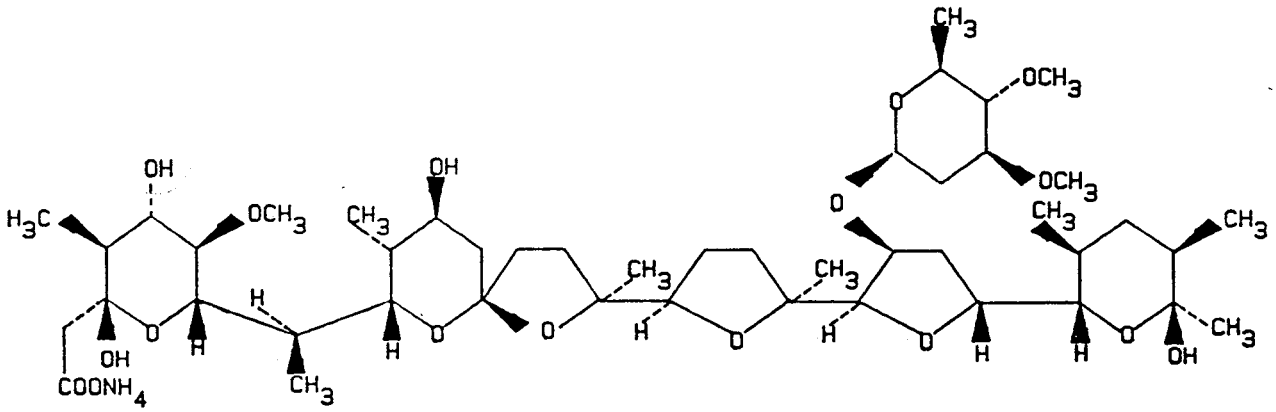
Maduramicin ammonium:



Ammonium (2R,3S,4S,5R,6S)-tetrahydro-2-hydroxy-6-](R)-1-](2S,5R,7S,8R,9S)-9-hydroxy-2,8-dimethyl-2-](2S,2'R,3'S,5'R)-octahydro-2-methyl-3'-]](2R,4S,5S,6S)-tetrahydro-4,5-dimethoxy-6-methyl-2H-pyran-2-yl[oxy[-5'-](2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2H-pyran-2-yl[ ]2,2'-bifuran[-5-yl[-1,6-dioxaspiro]4.5[dec-7-yl[ethyl[-4,5-dimethoxy-3-methyl-2H-pyran-2-acetate

6. Introduction into the environment. (Cont'd)

Metabolite CL 116,885 (β -component):



Ammonium (2R,3S,4S,5R,6S)-tetrahydro-2,4-dihydroxy-6-[(R)-1-[(2S,5R,7S,8R,9S)-9-hydroxy-2,8-dimethyl-2-[(2S,2'R,3'S,5R,5'R)-octahydro-2-methyl-3'-[[(2R,4S,5S,6S)-tetrahydro-4,5-dimethoxy-6-methyl-2H-pyran-2-yl]oxy]-5'-[(2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2H-pyran-2-yl][2,2'-bifuran]-5-yl]-1,6-dioxaspiro[4.5]dec-7-yl]ethyl]-5-methoxy-3-methyl-2H-pyran-2-acetate

Excreta from chickens fed 5 ppm of radiolabeled CYGRO for 7 consecutive days contained 21.27% AC 273,703 and 20.77% of the metabolite CL 116,885 based on total residue or 0.86 ppm and 0.85 ppm respectively. (Exhibit I).

6. Introduction into the environment. (Cont'd)

d. Stability in unextracted chicken manure.

Excreta from birds fed diets containing 5 or 7 ppm maduramicin were stored at 28 and 37°C. Maduramicin in the excreta was assayed initially and after storage at varying periods of time using an HPLC procedure with fluorometric detection.

The concentrations of maduramicin found in fresh broiler chicken excreta indicate a reasonable recovery of the compound; based on the moisture content of the feed (8%) and excreta (65%), percentage maduramicin recovered in the excreta is 92% of amount fed at 5 ppm and 110% of amount fed at 7 ppm.

During storage of excreta at 28°C maduramicin disappears quite quickly; after 12 weeks storage maduramicin concentrations are near or below the minimum detectable level of the assay method (0.5 ppm). While there is some variation in the results, this is not unexpected in view of the heterogenous nature of broiler chicken excreta.

The calculated half-lives of maduramicin in the excreta from birds fed 5 or 7 ppm maduramicin are consistent at each temperature (55 and 53 days at 28°C and 39 and 41 days at 37°C).

The initial (wet weight) concentration of CYGRO in broiler chicken feces is approximately 2 and 3 ppm for diets containing 5 and 7 ppm CYGRO, respectively.

CYGRO disappeared from chicken excreta held in laboratory incubators at 28°C and 37°C. The disappearance was temperature-related with half-lives of approximately 55 and 41 days observed for 28° and 37°C, respectively, for both concentrations of CYGRO tested. Details of the study as well as a description of methods used are contained in Exhibit J.

e. Runoff

The maximum possible concentration of maduramicin in runoff, assuming that:

(1) there is no storage of the excreta or degradation during storage
(2) all of the maduramicin in the excreta is released into the runoff,
(3) there is no adsorption onto soil, and
(4) there is no dilution of excreta with bedding (litter) normally found in poultry houses,

is calculated from the mass of maduramicin found in one-acre of

6. Introduction into the environment. (Cont'd)

soil resulting from the application of 22 mt/ha of excreta and 2 acre-inches of rainfall.

Excreta from broilers fed 7 ppm maduramicin contained 2.95 ppm maduramicin residues (Echibit J). It was shown in **6.b.**, Environmental Burden, that this would result in an application of 148 g maduramicin/ha at a 20% moisture level.

The mass of maduramicin applied to one acre in excreta is:

148 g/ha x ha/2.47 acre = 59.9 g/acre

Two acre-inches of rainfall is:

2 acre-inch x 3630 ft^3/acre-inch x 28.3 L/ft^3 = 2.06 x 10^5 L.

Thus the maximum concentration in the runoff is:

59.9 g/2.06 x 10^5 L = 59900 mg/206000 L = 0.29 ppm

This value is clearly worst case because it has been demonstrated (Exhibit J) that there is degradation during storage (half-life in excreta = 53 days at 28°C and 41 days at 37°C).

Excreta is normally added to soil and incorporated before planting in the Spring. Additionally, excreta can be applied in the Fall, after the crop has been harvested. In the case where there are two applications per year, the average storage time is 6 months or 182 days. This is equivalent to 3.4 half-lives. A compound which degrades through 3.4 half lives will have a concentration of $(\frac{1}{2})^{3.4}$ times the initial concentration or 0.095 times the initial concentration. A thus realistic initial application is 148 g maduramicin/ha x 0.095 = 14.1 g/ha. Assuming that all of the maduramicin is released into the runoff, the maximum concentration in the runoff is calculated from:

$$\frac{14.1 \text{ g maduramicin}}{\text{ha}} \times \frac{\text{ha}}{2.47 \text{ acre}} = \frac{5.71 \text{ g}}{\text{acre}}$$

Thus the maximum concentration in the runoff is:

$$\frac{5.71 \text{ g}}{2.06 \times 10^5 \text{ L}} = \frac{5720 \text{ mg}}{206000} = 0.0277 \text{ ppm}$$

Additionally it has been shown (Exhibit K) that there is adsorption by soil. Thus the actual aqueous runoff concentration would be significantly less than the calculated value of 0.0277 ppm.

7. Fate

The primary impact from the use of CYGRO in broilers on the natural environment will be the excretion of maduramicin by treated chickens in the manure. Data have been collected relevant to the environmental fate of maduramicin, and the drug sponsor has concluded that the use of maduramicin in broiler chickens does not represent an action that would have a significant impact on the quality of human environment.

In the analysis of the potential adverse impact on the environment from treating broiler chickens with maduramicin, the following areas were examined:

a. Level of excretion:

Birds fed 7 ppm maduramicin excreted manure containing a level of 2.95 ppm (Exhibit J).

b. Stability in poultry manure:

Half life of maduramicin in manure was determined to be 55 days at 28°C or 41 days at 37°C. (Exhibit J)

c. Soil sorption/desorption:

The adsorption (Freundich) coefficients were determined to be as follows:

Soil	Water	0.01 N $CaCl_2$
Princeton Sandy Loam	1.0	2.0
Nebraska Silt Loam	2.2	4.2
North Carolina Silt Loam	10.9	13.1
Wisconsin Silt Loam	3.6	4.9

There does not appear to be a significant difference in the adsorption isotherms generated using distilled water or 0.01 N calcium chloride solution.

This study demonstrates that AC 273,703 is weakly adsorbed by the four soils studied. There is also degradation of the compound during the adsorption and desorption phases. Although the adsorption is relatively weak, it is sufficient to prevent significant leaching of the parent compound under most normal use patterns, where the compound is introduced into the environment as aged residues in manure. (Exhibit K)

7. Fate (Cont'd)

d. N-octanol/water partition coefficient:

The average partition coefficient (Kow or P) of maduramicin, uncorrected for dissociation, between n-octanol and water or various pH buffers is as follows at initial concentrations of approximately 1.5 and 15 ppm in the n-octanol phase.

Solvent	Coefficient	Concentration
Distilled water	1500	1.5 ppm & 15 ppm
pH 5 buffer	4400	1.5 ppm & 15 ppm
pH 7 buffer	2900	1.5 ppm & 15 ppm
pH 9 buffer	1500	1.5 ppm & 15 ppm

By comparison, the partition coefficients of known bioaccumulators, such as PCB's are greater than 50,000. (Ref. 3)

A description of the methods used to determine the Kow of maduramicin is presented in Exhibit L.

e. Soil Biodegradation (Exhibit M)

A study was conducted to provide information concerning the extent of degradation of maduramicin during aerobic aging on soil. As part of this study carbon-14 labeled glucose, with the carbon-14 label uniformly distributed throughout the glucose, was applied to samples of the same soils to gauge micorobiological activity. Between 30% and 50% of the applied dose was trapped as $^{14}CO_2$ using sodium hydroxide over the 62 day period. The remainder of the dose was recovered in the soil. All of the ^{14}C-glucose was not converted into $^{14}CO_2$ because the C-glucose used was uniformly labeled throughout the glucose molecule and thus some of the carbon-14 would be incorporated into the cells. Total recoveries for all three soils were greater than 95%.

The aerobic biodegradation of carbon-14 labeled maduramicin on three different soils was studied. There was no detectable evolution of $^{14}CO_2$ from carbon-14 labeled maduramicin during 62 days of aging under aerobic conditions. Greater than 95% of the applied radioactivity was extractable with methanol after 60 days. Thin-layer chromatography of the extracted radioactivity, co-spotted with non-labeled maduramicin, indicated that 32-58% of the radioactivity was maduramicin. The major degradation product(s) was origin bound in two different TLC systems, and accounted between 26% and 61% of the extracted radioactivity. The behavior on the TLC plates suggest that either the degradation product(s) is more polar than maduramicin or is so strongly bound to the silica gel that it is immobile.

7. Fate (Cont'd)

This study indicates that aerobic biodegradation of maduramicin by soil microorganisms occurs with degredation to one bound and several less polar degredation products. Although complete degradation of maduramicin to CO_2 did not occur in this study, 32-58% of the original compound did degrade within a relatively short period (60 days). Therefore, biodegradation of maduramicin by soil microorganisms represents one manner by which the compound may be removed from the environment and indicates that maduramicin is not expected to accumulate in agricultural soils over time.

8. Environmental Effects of released substances.

a. Effects on microorganisms

The Minimal Inhibiting Concentration (MIC) for gram-negative bacteria was found to be greater than 125 mcg/ml, and for gram-positive bacteria was found to range from 0.25-2.0 mcg/ml (Exhibit N).

It has been calculated (6.b. Environmental Burden) that the soil concentration, after a 6" incorporation, assuming no degradation on storage or dilution by litter, would be 0.059 ppm. Under these assumptions, this soil concentration is 4.2-34 times less than the MIC's for gram-positive bacteria and 2120 times less than the MIC's for gram-negative bacteria. If decay of maduramicin during storage in excreta is considered (6.b. Environmental Burden) the initial soil concentration is 44-355 times less than the MIC for gram-positive bacteria and 22,000 times less than the MIC for gram-negative bacteria.

This determination is supported by the findings reported in two laboratory screening tests that were conducted to determine the potential for maduramicin residues in manure from treated poultry to affect two soil processes: the micorobial conversions of soil ammonia to nitrate (nitrification) and the rate of carbon utilization by bacteria (methanogenesis).

1. Nitrification

A soil column perfusion technique was used (Exhibit 0). The loss of ammonium-N from the perfusate (100 mg ammonium sulphate/L) and the accumulation of nitrite-N and Nitrate-N were followed over a 21 day period.

Nitrification, the microbiological oxidation of ammonium-N to nitrate-N via nitrite-N, was observed in all treatments. The addition of excreta (no maduramicin) to the soil columns increased nitrite concentrations in the perfusate, delayed the appearance of nitrate and slowed the rate of nitrate

8. Environmental Effects of released substances (Cont'd)

production compared to soil columns without excreta. These effects were also seen when excreta from birds fed 5 or 7 ppm were added to the soil columns; no addition effects attributable to maduramicin were observed.

Because no plateau of nitrate-N was reached in the presence of excreta a statistical model previously developed for nitrification in soil columns and applied successfully to the effect of beef faeces on the soil columns was inapplicable in the presence of broiler faeces, therefore, the following conclusions are qualitative rather than a quantitative assessment of the data.

- Broiler excreta affects the progress of nitrification in the soil column technique used.

- The use of excreta from broilers fed 5 or 7 ppm maduramicin in their diet has no greater net effect on nitrification than the use of maduramicin free excreta.

2. Methanogenesis

A model anaerobic fermentation system was used. Methane production was followed by gas head-space analysis (GLC). This procedure (unpublished) was devised by Toxicol Laboratories, Ledbury, UK to satisfy EEC requirements, and is presented here merely as auxillary information on the compound.

Methane production was observed in all cultures. Early in the incubation period (days 1 and 7), methane production in the samples obtained from the group treated with CYGRO at 7 ppm and stored was comparable with the methane production from the anaerobic sludge control and significantly higher than the other treatment groups. By day 21, all the fresh samples and the anaerobic sludge control yielded comparable methane levels. The fresh untreated and the fresh CYGRO (at 7 ppm) treated groups were also significantly higher in methane production than the stored samples. For the last 2 sampling periods (days 28 and 35), methane production from all cultures was comparable. Please refer to Exhibit P.

Storage of excreta at room temperature prior to testing depressed methane production in all three treatments (0, 5 and 7 ppm maduramicin in diet) presumably by depletion of available carbon sources by normal aerobic bacteria.

8. Environmental Effects of released substances (Cont'd)

b. Effects on Plants

1. Seedling growth and development

Using a standard protocol, maduramicin at nominal concentrations of 0, 0.01, 0.1, 1.0 and 10 ppm was tested on two monocot species, Zea mays cv. Pioneer 3541 (corn)-2 plants per replication, and Triticum aestivum cv. Fenman (wheat)-3 plants per replication and two dicot species, Glycine max cv. Jacques 99 (soybeans)-2 plants per replication, and Cucumis sativa cv. National pickling (cucumber)-2 plants per replication, for its effects on seedling growth and development (Exhibit Q).

Maduramicin in stock solution (pH = 6.7, adjusted with NH_4OH) had no effect on the shoot growth of wheat, soybeans or cucumbers at levels $\leq$ 1.0 ppm (all concentrations are nominal). In corn, there was a slight reduction in elongation of the first and second leaf, resulting in a statistically significant reduction in shoot height at the 0.1 and 1.0 ppm level on day 7 and at the 1.0 ppm level on day 10. Later corn leaf elongation appeared to be uneffected by the 0.1 ppm level and only slightly affected at the 1.0 ppm level. No other physiological or developmental differences were observed in any species between untreated plants at levels $\geq$ 1.0 ppm.

Dry weights of roots and shoots were taken at the conclusion of the test. No significant reduction in dry weight was noted with maduramicin at $\leq$1.0 ppm in any species. At 10.0 ppm, maduramicin severely inhibited root and shoot growth of all species. Shoots of treated plants were severely stunted, with smaller leaves, while roots were brown with a proliferation of short (2-3 mm) lateral roots.

Maduramicin was also tested in the Cyanamid Herbicide Screen and Evaluation on 12 plant species. At levels $\leq$ 2.0 kg/ha, maduramicin had no effect on any of the species tested. Of the 12 plant species tested, 2 were killed (rating 9), 2 were severely affected (rating 7 and 8), 5 were moderaely affected (rating 3-6) and 2 were slightly affected (rating 1 and 2) at the 4.0 kg/ha application. At the 8.0 kg/ha application, 5 were killed, 3 were severely affected, 3 were moderately affected and 1 was slighly affected.

8. Environmental Effects of released substances (Cont'd)

2. Seed germination and root growth.

Using a standard protocol, maduramicin was tested on 50 seeds per replicate on two monocot species, Zea mays cv. Pioneer 3541 (corn) and Triticum aestivum cv. Fenman (wheat), and two dicot species, Glycine max cv. Jacques 99 (soybeans) and Cucumis sativa cv. National pickling (cucumber) for its effects on seed germination and subsequent radicle growth. (Exhibit S)

At the conclusion of the test, mean radicle lenghts and shoot lengths were calculated for each individual treatment within a replicate. A grand mean was then calculated by adding together the individual treatment means for the six replicates and dividing by six. The radicle and shoot length data reported herein, is an Analysis of Variance of these grand means.

Stock solutions (pH 6.7 adjusted with NH_4OH were prepared at nominal maduramicin concentrations of 0.01, 0.1, 1.0 and 10.0 ppm. At the time of removal from the treatment solutions, all seeds were fully imbibed and radicle emergence had begun in corn, wheat, cucumber and the majority of soybeans. Care had to be taken not to break the radicle while rinsing off the test solution.

Seed germination data taken 2 and 4 days after treatment shows $< 98\%$ germination in the untreated controls of all species, except soybeans (88.6%), which suffered some cotyledon separation during the treatment period. Inbibition in the presence of maduramicin had no statistical effect on the percent of seed germination at the 5% level, but some reduction in soybean germination was observed.

Maduramicin also had no effect on the root growth of soybean and cucumber seedlings. In corn, a slight reduction in radicle length and shoot length was noted at the 10.0 ppm level, however, this effect was not statistically significant. In wheat, maduramicin did significantly reduce radicle and shoot length at the 10.0 ppm level. Wheat seedlings receiving this treatment had no physiological deformities other than this slight stunting.

The results show that maduramicin has no significant effect on seed germination at 1.0 ppm in corn, wheat and cucumbers and no significant effect at 1.0 ppm in soybeans. Little to no effect was noted on the subsequent growth of seedlings, which had their seeds germinated in the presence of maduramicin. Significant effects were noted only on wheat root and shoot growth at the 10.0 ppm level.

8. Environmental Effects of released substances (Cont'd)

3. Summary of Phytotoxicity Studies

It has been shown (6.b. Environmental Burden) that the soil concentration, assuming no degradation on storage is 0.059 ppm. The lowest level at which phytotoxic effects were observed was 1.0 ppm. This level is 17 times higher than the estimated soil concentration of maduramicin. When decay on storage is taken into account (6.b. Environmental Burden) the initial soil concentration is reduced to 0.00564 ppm which is 177 times lower than the lowest level at which phytotoxic effects were seen (1.0 ppm).

c. Toxicity to Laboratory Animals

A standard battery of acute, subchronic and chronic toxicology studies were conducted in rats, mice and dogs and rabbits. The results of these studies are presented in the Freedom of Information Summary, pages 19 to 26. Table 1 summarizes these studies.

9. Toxicity to Aquatic Organisms

Acute toxicity to 3 aquatic wildlife species was evaluated. In each study, there was no visible aggregate formation and the maduramicin was evenly distributed within the solution to the limit of sensitivity.

a. Static acute toxicity to rainbow trout (Exhibit T).

The acute toxicity of maduramicin to rainbow trout (Salmo gairdneri) was assessed. The study was conducted at the nominal maduramicin concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg/l. Samples from each test solution were collected at 0 and 96 hours of testing and shipped to American Cyanamid for analytical measurement. The results of the RIA analysis, as provided by American Cyanamid Company, are as follows and generally confirm the nominal test levels.

Fortification Level (ppm)	AC 273,703 Content (ppm) of Water at 0 Hours	96 Hours
1.0	0.86	0.83
1.8	1.41	1.54
3.2	2.64	2.83
5.6	5.90	4.54
10.0	8.37	7.66

9. Toxicity to Aquatic Organisms (Cont'd)

Ten fish with a mean weight of 0.43 (± 0.090) g and a mean standard length of 31 (± 4.1) mm, were exposed to each test concentration and control.

The results of the four-day static fish toxicity studies using maduramicin based on nominal concentrations are summarized below. The 24 and 48 hour LC_{50} values for maduramicin were also determined. The reported LC_{50} would be slightly lower (possibly as much as 20% if it had been calculated based on the measured concentration.

Compound	96-hour LC_{50} (95% C.I.)
Maduramicin	5.0 mg/l (4.1-6.6 mg/l)

The concentration at which no mortality or abnormal effects were observed after 96 hours of exposure was 1.0 mg/l. Mortality and the abnormal effects of surfacing, loss of equilibrium, dark discoloration and/or fish on the bottom of the test chamber were observed at all other test concentrations during the 96-hour exposure period.

b. Static acute toxicity to bluegill (Exhibit U)

The acute toxicity of maduramicin to bluegills (Lepomis macrochirus) was assessed.

The study was conducted at the nominal concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg/l. Samples from each test solution were collected at 0 and 96 hours of testing and shipped to American Cyanamid Company for analytical measurement. The results of the RIA analysis, as provided by American Cyanamid Company, are as follows and generally confirm the nominal test levels.

Fortification Level (ppm)	AC 273,703 Content (ppm) of Water at 0 Hours	96 Hours
1.0	0.96	1.01
1.8	1.46	1.57
3.2	3.42	3.44
5.6	4.59	4.82
10.0	8.10	8.55

9. Toxicity to Aquatic Organisms (Cont'd)

Ten fish with a mean weight of 0.19 (± 0.032) g and a mean standard length of 20 (± 1.3) mm, were exposed to each test concentration and control.

The results of the four-day static fish toxicity studies using maduramicin based on nominal concentrations are summarized below. The 24 and 48 hour LC_{50} values for maduramicin were also determined. The reported EC_{50} would be slightly lower if it has been calculated based on the measured concentration.

Compound	96-hour LC_{50} (95% C.I.)
maduramicin	1.4 mg/l (1.1-1.8 mg/l)

Mortality and the abnormal effects of surfacing, loss of equilibrium, dark discolorization and/or fish on the bottom of test chamber were observed at all test concentration during the 96-hour exposure period.

c. Static acute toxicity to Daphnia magna. (Exhibit V)

The acute toxicity of maduramicin to Daphnia magna was assessed. The study was conducted at the nominal concentrations of maduramicin of 1.0, 1.8, 3.2, 5.6 and 10 mg/l. Samples from each test solution were collected at 0 and 48 hours of testing and shipped to American Cyanamid for analytical measurement. The results of the RIA analysis, as provided by American Cyanamid Company, are as follows and generally confirm the nominal test levels.

Fortification Level (ppm)	AC 273,703 Content (ppm) of Water at 0 Hours	96 Hours
1.0	1.09	0.85
1.8	1.98	1.72
3.2	3.63	3.01
5.6	4.40	4.37
10.0	11.47	8.15

The results of the 48-hour static Daphnia magna toxicity study are summarized below. All reported values were based upon nominal concentrations. The reported EC_{50} would be slightly lower if it had been calculated based on the measured concentration.

9. Toxicity to Aquatic Organisms (Cont'd)

Compound	48-hour EC_{50} (95% C.I.)
maduramicin	7.5 mg/l (6.6-8.9) mg/l

The lowest test concentration at which the immobility or abnormal effects were observed after 48 hours of exposure was 3.2 mg/l. Immobility and the abnormal effect of lying on the bottom of the test chamber were observed at the 5.6 and 10 mg/l test concentrations.

In summary, the most sensitive species is the bluegill with a 96-hour LC_{50} of 1.4 mg/L. Assuming the runoff concentration of 0.0277 ppm, there is a minimum 150x safety factor (1.4 ppm/0.0277 ppm) for bluegill. For rainbow trout and daphnia magna the minimum safety factors are 180 and 270, respectively.

10. Effects of Potential Occupational Exposures

Numerous toxicological short and long-term tests have been conducted in several different animal species, for use in assessing the safety of maduramicin ammonium (refer to 8.c). Although some studies were conducted with technical grade maduramicin, the product to be manufactured and handled by the end user is 1% premix. The relevant toxicity data developed on the premix for assessing occupational exposure concerns are presented below:

a. Acute Studies

Oral (LD50 Rat)

Male	Female	Signs
2000 mg/kg b.w.	>2000 mg/kg b.w.	Decreased weight gain at 2000 mg/kg dose

10. Effects of Potential Occupational Exposures (Cont'd)

Oral (LD50 Mouse)

Male	Female	Signs
> 2000 mg/kg b.w.		Decreased weight gain at 2000 mg/kg dose

Dermal (LD50 Rabbit)

> 4000 mg/kg b.w.	> 2000 mg/kg b.w.	None observed

b. Hazard Evaluation Studies

Ocular Irritation in Rabbits: Moderate irritation at 100 mg instillation. With no wash, eyes cleared in 10 days; however, when eyes were rinsed 20 seconds after instillation, eye irritation was not observed.

Dermal Irritation in Rabbits: The product was moderately irritating to the rabbit skin following 24-hour skin contact of 500 mg of the 1% premix in both intact and abraded skin.

Inhalation: It is well-known that feed mill operators and poultry house workers may be constantly exposed to dust in their working environment. Although most dust originates from feed processing, a portion of dust could be attributed to the use of medicated premixes. To eliminate this potential inhalation hazard, a corn cob grit carrier was used in preparing maduramicin premixes.

In order to demonstrate the safety of the maduramicin 1% premix to feed mill operators, two studies (Exhibit W) were conducted.

In the first study workers in a feed mill wore face masks with filters throughout their normal working period. Following the normal work day, the filters were analyzed for the presence of maduramicin. The results showed that no maduramicin was detectable on any of the filters analyzed.

In the second study sample of dust were collected from around strategic areas of the feed mill and analyzed for the presence of maduramicin. No significant increase in test material was detected in any of the dust samples tested.

Based on the above results, it is concluded that the potential for exposure of the maduramicin 1% premix via inhalation is negligible.

10. Effects of Potential Occupational Exposures (Cont'd)

c. Reproduction and Teratology

Studies designed to assess the mutagenic effects of maduramicin produced negative results. Studies designed to assess reproductive effects including teratogenicity demonstrated a lack of any adverse effects on reproductive performance and a lack of teratogenicity at any dose level of maduramicin tested.

d. Conclusions

Engineering controls and good personal hygiene precautions limit exposures of maduramicin to workers in manufacturing plant operations.

Data alluded to above show that the 1% premix does not present an inhalation hazard to users (feed mill workers, farmers, etc). In addition, laboratory animal studies (refer to 8.c.) have shown that maduramicin is not a teratogen, reproductive toxin or mutagen.

The only possible significant exposure for users might occur from dermal contact. However, since the dermal LD_{50} for the 1% premix has been shown to be greater than 2000 mg/kg, it may be concluded that dermal exposure to this product would not cause any adverse effects to workers handling CYGRO 1% premix.

11. Use of Resources and Energy:

The raw materials used in fermentation of maduramicin and production of CYGRO 1% feed premix are common organic compounds, all of which are in ample supply. Energy commitment would be nominal. Though some of the raw materials are irretrievable, the proportion used in the maduramicin process compared to the total annual production of them would be minimal.

We know of no information which raises any question of an effect of the production and use of CYGRO anticoccidial for broilers on any protected or endangered species, or on any location listed on the National Register of Historic Places.

12. Mitigation measures.

As discussed previously, because of prevalent poultry waste handling practices and the degradation of maduramicin in manure, coupled with the extremely low levels of use of subsequent excretion, very little maduramicin is expected to enter the environment.

Short-term effects upon the environment, as discussed (phytotoxicity to a variety of plants, hazard to fish, aquatic animals, etc.) are therefore not expected.

12. Mitigation measures. (Cont'd)

Also, as discussed, there would be minimal short-term effect of the disposal of by-products from the manufacturing process upon the productivity of the environment.

13. Alternatives to the proposed action:

There are no alternatives to the proposed action. A variety of anticoccidial compounds, the majority which are ionophores are used in virtually all broiler production in the United States. However, maduramicin is a substantial advance over currently used products to control broiler coccidiosis, both because of its potency and its lack of toxicity to non-target species at the recommended use level. Maduramicin will not increase the total amount of anticoccidial use, but will take a share of the existing marketplace.

14. List of preparers:

	Titles
Joyce A Yates, M.S.	Product Registration Manager
Phillip L. Miller, Ph.D.	Group Leader Metabolism Research
Gary A. Mangels, Ph.D.	Senior Environmental Project Leader
Timothy Malefyt, Ph.D.	Senior Research Biologist
David L. Sharkey, Ph.D.	R & D Program Manager

15. Certification:

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the firm responsible for preparation of the environmental assessment, American Cyanamid Company.

Date: 1/23/87

Joyce A. Yates

Joyce A. Yates
Product Registration Manager

16. References:

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8. ASTM procedure D-547-41, Standard Method of test for index of Dustiness of Coal and Coke (Reapproved 1980).

9. Pulmonary Deposition and Retention of Inhaled Aerosols, Hatch, T.F. and Gross, P.

17. Exhibits: Exhibits A through W are attached.

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1/23/87

Table I

Type of Study	Species	Duration	Significant Findings	No Effect Level (mg/kg/day)
Dry Technical Material				
Acute Oral	Rat	one dose	LD_{50} 33 mg/kg	-
Acute Oral	Mouse	one dose	LD_{50} 35 mg/kg	-
Dermal	Rabbit	24 hours	LD_{50} 5.3 mg/kg	-
Eye Irritation	Rabbit	24 hours	Corrosive	-
Skin Irritation	Rabbit	24 hours	Erythema & Edema	-
Benzyl Alcohol Concentrate:				
Acute Oral	Rat	one dose	LD_{50} 05 mg/kg	-
Dermal	Rabbit	24 hours	LD_{50} 11.8 mg/kg	-
Eye Irritation	Rabbit	24 hours	Irritating	-
Acute Inhalation	Rat	4 hours	LD_{50} 0.002 mg/kg	-
1% Premix				
Acute Oral	Rat	one dose	LD_{50} 2000 mg/kg	-
Acute Oral	Mouse	one dose	LD_{50} 2000 mg/kg	-
Dermal	Rabbit	24 hours	LD_{50} 4000 mg/kg	-
Eye Irritation	Rabbit	24 hours	Irritating	
Skin Irritation	Rabbit	24 hours	Reversible Moderate erythema Mild edema	-

Table 1 (Cont'd)

Type of Study Dry Technical Material	Species	Duration	Significant Findings	No Effect Level (mg/kg b.w/day)
Subacute	Rat	28 Days	No signs of toxicity	8 ppm (87 mg/kg)
Subacute	Rat	13 Weeks	Decreased body weight. Increased relative heart weight	3 ppm (0.21 mg/kg)
Subacute	Dog	28 Days	Ataxia, hind limb paralysis	
			Atrophy, focal degeneration of skeletal muscle	12 ppm
Cardiovascular	Dog	one IV	Increased heart rate, left ventricular pressure, systolic aortic pressure	0.2 mg/kg
Cardiovascular	Dog	one oral	No signs of toxicity	6 mg/kg
Cardiovascular	Dog	one IV	No significant effect on heart rate	0-6 mg/kg
			Drug present in urine	
Teratology	Rat	Day 6-15 of Gestation	Nonteratogenic	3 ppm
Reproduction	Rat	3 Generations	No effect on reproductive function	3 ppm

Table 1 (Cont'd)

Type of Study Dry Technical Material	Species	Duration	Significant Findings	No Effect Level (mg/kg b.w.day)
Chronic	Dog	one year	Reversible multifocal water droplets on retina. Hyperreflective retina. Histopath: retinal atrophy	6 ppm (0.204 mg/kg)
Ames Bacteria	S. typhimurium	2 days	Nonmutagenic	5 mg/plate
Point Mutation CHO/HGPRT	Chinese Hamster Ovary Cells	5 hours	Nonmutagenic	0.2 mg/ml
In Vitro Metaphase	Chinese Hamster Ovary Cells	6-8 hours	Nonmutagenic	0.1 mg/ml
		14-18 Hrs	Clastogenic	0.025 mg/ml
In Vivo Metaphase Assay	Rat	6-48 Hrs	Nonmutagenic	9 mg/kg
Unscheduled DNA Synthesis	Rat	18-20 Hrs	Nonmutagenic	4 mg/ml

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