ENVIRONMENTAL ASSESSMENT FOR THE USE OF RUMENSIN® PREMIXES IN THE FEED OF BEEF CATTLE FOR THE PREVENTION AND CONTROL OF COCCIDIOSIS

Elanco Products Company
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

August 1989

2

TABLE OF CONTENTS

3

4

Page

APPENDICES (Continued)

5

ENVIRONMENTAL ASSESSMENT FOR THE USE OF RUMENSIN® PREMIXES IN THE FEED OF BEEF CATTLE FOR THE PREVENTION AND CONTROL OF COCCIDIOSIS

1. DATE — July 1989

2. APPLICANT — Elanco Products Company
A Division of Eli Lilly and Company

3. ADDRESS — Lilly Corporate Center
Indianapolis, Indiana 46285

4. DESCRIPTION OF THE PROPOSED ACTION

A Supplemental New Animal Drug Approval has been requested for use of RUMENSIN® Premixes in the feed of beef cattle for the prevention and control of coccidiosis. When incorporated into feed, RUMENSIN Premixes can prevent and control coccidiosis in feedlot cattle due to Eimeria bovis and Eimeria zuernii. Monensin sodium is the active ingredient in the RUMENSIN Premixes. Premixes would be incorporated into feeds to provide monensin levels of 10 to 30 g/ton, or 100 to 360 mg monensin/head/day. RUMENSIN Premixes are already approved at these levels for the rations of feedlot cattle to increase efficiency of feed utilization (21 CFR 558.355; December 16, 1975). When incorporated into cattle rations, monensin alters the production of volatile fatty acids in the rumen. As a result of increased production of propionic acid, the usable energy derived from the ration is increased. An Environmental Impact Analysis Report was provided for this use of RUMENSIN Premixes in the feed of cattle.

RUMENSIN® (monensin sodium, Elanco)

6

RUMENSIN Premixes are also approved (21 CFR 558.355; Federal Register, July 28, 1978) for use in the rations of growing cattle in pastures (up to 200 mg monensin/head/day). In 1983 approval for use of RUMENSIN Premixes in pastured cattle was expanded to include beef and dairy replacement heifers. In 1987 approval was granted for the use of RUMENSIN Premixes in reproducing beef cattle (21 CFR 558.355; Federal Register, December 15, 1988) and an Environmental Assessment was submitted for this use.

The current Environmental Assessment provides updated information and specifically addresses the use of RUMENSIN Premixes for the prevention and control of coccidiosis in feedlot cattle. Approval of the proposed action would authorize the fermentation and processing plants of Eli Lilly and Company at Clinton and Lafayette, Indiana to manufacture and package the RUMENSIN Premixes to be sold in the United States for prevention and control of coccidiosis in feedlot cattle.

Based on the proposed action, monensin could potentially be introduced into the following environments:

a) The environment adjacent to the manufacturing plants.

b) The environment adjacent to facilities which mix RUMENSIN with feed.

c) Feedlots where residues may be found in cattle excreta.

d) Agricultural lands where waste products from cattle are used as fertilizer.

e) Aquatic systems where runoff may flow from sites receiving waste products of cattle.

7

5. IDENTIFICATION OF CHEMICAL SUBSTANCE

A. RUMENSIN PREMIXES

RUMENSIN Premixes will be incorporated into rations of feedlot cattle. Monensin sodium is the active ingredient in the RUMENSIN Premixes and is produced in dried mycelial biomass and crystalline forms. The raw material is added to the premixes to achieve monensin concentrations of 20, 30, 45, and 60 g/lb. RUMENSIN Premixes may contain diluents such as rice hulls.

B. MYCELIAL MONENSIN

Monensin is produced by the fermentation of a strain of Streptomyces cinnamonensis, an organism isolated from soil (Haney and Hoehn, 1968). The most economical procedure to prepare a usable form of monensin is to harvest the fermentation culture in such a way as to combine monensin with the mycelial cells of the producing organisms and the unused components of the feed-stock used in the fermentation to achieve growth of the organism. Thus, the dried mycelial or biomass form of monensin contains nutrients which can commonly be found in cattle feedstuff.

C. MONENSIN

Monensin consists primarily of monensin factor A, but small amounts of monensin factor B and very small amounts of factors C and D do occur. Monensin factor A accounts for at least 90 percent of the microbiologically active material of mycelial monensin. The characteristics of

8

monensin factor A are discussed in this section. Monensin is a monocarboxylic polyether compound which complexes with monovalent alkali cations and shows ionophorous activity with a selectivity of $Na^+ > K^+ > Rb^+ > Li > ^+Cs^+$ (Haney and Hoehn, 1968; Pressman, 1976).

Monensin Sodium:

During the manufacturing process, monensin is exposed to sodium ions during a pH adjustment giving rise to monensin sodium which is the chemical form in the product.

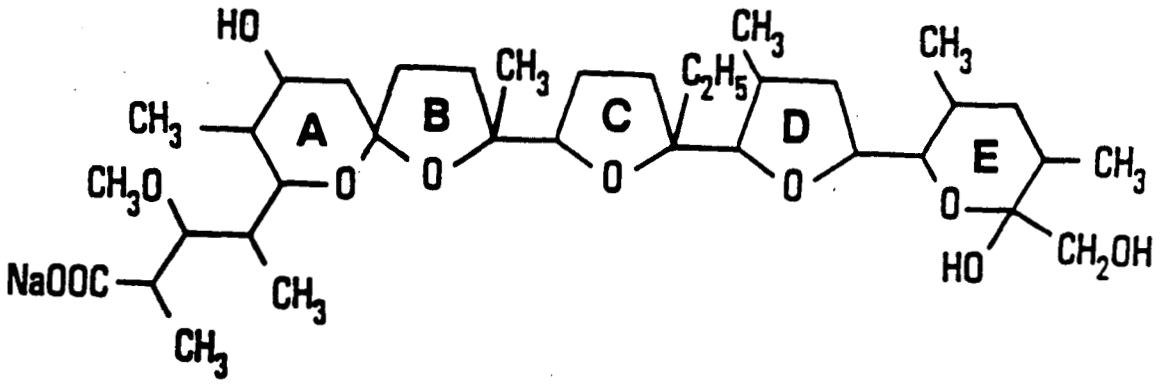
Chemical Name (acid form):

Stereoisomer of 2-[2-ethyloctahydro-3'-methyl-5'-tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl][2,2'-bifuran]-5-yl]-9-hydroxy-β-methoxy-α,γ'2,8-tetramethyl-1,6-dioxaspiro[4,5]decane-7-butanoic acid.

CAS Registry Number:	17090-79-8
Molecular Formula:	$C_{36}H_{62}O_{11}$ (acid), $C_{36}H_{61}O_{11}Na$ (salt)
Molecular Weight:	670 (acid), 692 (sodium salt)

9

Structural Formula:



Solubility (Appendix A):

water	pH 7	63 mg/L
	pH 9	0.85 mg/L
ethyl acetate	very soluble	
chloroform	very soluble	
acetone	very soluble	
benzene	very soluble	
methanol	very soluble	
hexane	slightly soluble	

Melting Point: 103-105°C (acid)
267-269°C (sodium salt)

UV absorption: None

pKa value: 6.65 (66% DMF)

Specific Rotation: + 47.7° (acid), + 57.3° (sodium salt)

Vapor pressure: Non-volatile solid based on molecular weight, melting point, and thermogravimetric analysis.

N-octanol/Water Partition Coefficient at 25°C (Appendix B):

17329 at pH 5
567 at pH 7
6135 at pH 9

10

6. INTRODUCTION OF SUBSTANCE INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCES FROM THE MANUFACTURING SITE

The manufacturing process for monensin, in conjunction with the corresponding pollution control practices at each of the plant sites is designed to have minimal environmental impact. These plant sites are located near Clinton and Lafayette, Indiana. Monensin is produced by a fermentation process and is recovered by processes utilizing unit operations such as evaporation, centrifugation or filtration, drying, pelletizing, granulation by crushing, screening and blending.

Essentially no monensin will be released from the manufacturing process. The only releases of monensin from manufacturing operations will be in dilute washwaters used to rinse the empty fermentation and processing facilities. At these plant sites, these washwaters would be treated by wastewater concentration and pyrolysis, by land application or by microbiological degradation.

Residual biodegradable fermentation nutrients from the manufacture of other fermentation products at each of the plant sites are discharged to receiving rivers at rates significantly below permitted limitations. Since monensin will not be the only fermentation-based product manufactured at these plant sites, it will account for a small portion of the permitted discharge of residual nutrients expressed as biological oxygen demand (BOD).

Essentially no other wastewater pollutants or liquid, solid or gaseous pollutants from the manufacture of monensin will be allowed to

11

enter the environment. Therefore, the manufacture of monensin will have a minimal effect on the environment at these plant sites.

Limitations for atmospheric pollutant emissions and wastewater pollutant discharges, and disposal practices for other liquid and solid wastes applicable to these plant sites, are defined by regulations administered by the U.S. Environmental Protection Agency and, in certain instances by Indiana's Department of Environmental Management (IDEM).

The following operating permits for those manufacturing and emission control facilities which would produce monensin at these plants currently are administered by the Office of Air Management in the IDEM.

Location	Permit Identification No.	Expiration
Clinton	83-09-91-0082	September 1, 1991
Clinton	83-09-91-0083	September 1, 1991
Clinton	83-09-91-0085	September 1, 1991
Lafayette	79-04-90-0372	April 1, 1990
Lafayette	79-04-90-0386	April 1, 1990

The following NPDES permits for the discharge of wastewaters from these plants are administered by the Office of Water Management in the IDEM.

Location	NPDES Permit No.	Expiration
Clinton	IN 0002852	August 31, 1990
Lafayette	IN 0002861	September 30, 1992

No hazardous wastes and essentially no solid wastes will be generated in these manufacturing operations. Processes which use organic solvents provide for recovery and reuse of solvents, and those operations where solvents are present are served by condensers, carbon

12

adsorbers or scrubbers to prevent solvent emissions from being discharged to the atmosphere. Those manufacturing operations which use dry procedures are served by dust control facilities to prevent particulate matter emissions from being discharged to the atmosphere. Packaging materials, non-recyclable tailings and floor sweepings from these plants either are incinerated at the Clinton plant with industrial and domestic trash from other sources or are landfilled.

Based on the information above, any atmospheric emissions, wastewater pollutant discharges and disposal practices for other wastes from the manufacturing processes for monensin will comply with appropriate statutes, regulations, and permits.

B. INTRODUCTION OF SUBSTANCE FROM FEED MIXING LOCATIONS

Most of the feed mixing will be done at commercial feed mills. These feed mills have to meet Good Manufacturing Practice Standards for feeds. With the required manufacturing controls for feed, inventory accountability, and quality assurance procedures, the potential for release of monensin sodium into the environment at these locations should be minimal.

C. INTRODUCTION OF SUBSTANCE AT THE USE SITE

Statistics from the United States Department of Agriculture indicate that there were about 26 million beef cattle fed for slaughter in the United States in 1987 (National Agricultural Statistics Service, 1988). Production was centered in the states of Arizona, California, Colorado, Illinois, Idaho, Iowa, Kansas, Minnesota, Nebraska, Oklahoma,

13

South Dakota, Texas, and Washington. Direct marketing of RUMENSIN Premixes to major commercial feed mills will help to minimize environmental exposure during the product distribution process.

RUMENSIN is currently used in the supplemental rations of feedlot cattle to improve efficiency of feed utilization. Prevention and control of coccidiosis is an additional reason to use RUMENSIN in the rations of feedlot cattle. Between 100 and 360 mg monensin/day can be fed to each animal for three weeks to prevent or control coccidiosis. The highest concentration of monensin in the feed would be 30 g/ton. If all 26 million beef cattle were fed a RUMENSIN Premix for three weeks to prevent or control coccidiosis, the maximum amount of monensin sodium that could be used annually would be 1.97×10^5 kg (360 mg/head/day x 21 days x 26×10^6 cattle).

The actual average treatment level of monensin for these cattle is expected to be about 200 mg/head/day. Only about 5% of the 26 million beef cattle are expected to receive monensin for prevention and control of coccidiosis. It is estimated that beef cattle will actually be fed about 5.46×10^3 kg (200 mg/head/day x 21 days x 1.3×10^6 cattle) of monensin sodium in their diets each year. This is less than 1% of the monensin sodium already sold in the United States.

Monensin is found in cattle feces and may be introduced into the soil of a feedlot or into cropland soil by use of feces as fertilizer (Appendix C). Monensin and its metabolites are quantitatively excreted in cattle feces (Donoho et al., 1978). Beef cattle fed a ration containing 40 g monensin/ton of feed had an average monensin concentration in their feces of 4.4 ppm. The highest recommended

14

concentration of monensin in the feed of beef cattle (30 g monensin/ton) is only 75% of the concentration used in this study. The concentration of monensin in the feces of beef cattle would, therefore, be about 3.3 ppm (4.4 ppm x 0.75).

Monensin is extensively metabolized in cattle, rats, chickens, dogs, sheep, pigs, and turkeys (Donoho, 1984; Donoho et al., 1978). The pattern of metabolism is qualitatively similar among species, although quantitatively different. By inference, the toxicology of monensin metabolites present in cattle feces has been evaluated in toxicology studies in which rats were exposed to monensin. More than 20 metabolites of monensin have been found for rats and cattle. About 50% to 60% of the monensin in an oral dose to cattle is metabolized (Donoho et al., 1978). The primary monensin metabolite, O-desmethyl monensin, is 20 times less biologically active than monensin, based on several test systems (Donoho, 1984). This primary metabolite makes up about 5% of the total monensin and metabolite residue in cattle feces. Thus, the first step in monensin metabolism appears to eliminate most of the biological activity of this compound. Based on this low level of biological activity, metabolites of monensin were not considered in the estimation of the environmental concentration of monensin. Biologically inactive metabolites and the measured concentration of monensin in cattle feces support the conclusion that 3.3 ppm is a realistic upper limit for monensin in the feces of beef cattle.

15

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

The primary manner in which measurable amounts of monensin would be introduced into the environment is through cattle feces collected from confined cattle and applied to cropland. Based on its large molecular weight, relatively high melting point and thermogravimetric analysis, measurable concentrations of free monensin will not occur in the atmosphere. Monensin may be found in cropland soil to which it is applied with cattle feces and in adjacent aquatic systems. It may also be possible to find measurable concentrations in the soil and runoff from feedlots.

A. POTENTIAL CONCENTRATION OF MONENSIN IN SOIL

1. Potential Monensin Concentration in Cropland Soil

The highest expected initial concentration of monensin sodium in cropland soil can be estimated from the concentration of monensin sodium in wet feces and the use rate of wet feces on cropland. A reasonable estimate of the application rate of wet cattle manure as fertilizer is 20 tons/A (44.8×10^3 kg/ha). It is standard practice to incorporate manure into the top six inches of the soil to avoid loss of nutrients in runoff. A six inch deep soil layer in one hectare weighs approximately 2.25×10^6 kg. Wet manure from beef cattle would contain, at most, 3.3 ppm of monensin. The highest expected concentration of monensin in cropland soil can then be calculated to be about 0.066 ppm ($3.3 \text{ ppm} \times 44.8 \times 10^3 \text{ kg} / 2.25 \times 10^6 \text{ kg}$).

16

The concentration of monensin in soil would decline from the highest expected value of 0.066 ppm, which could occur directly after application of cattle feces to soil. Studies with crystalline monensin mixed in soil show a moderately rapid decline in monensin activity (Appendices D and E). The half-life of crystalline monensin in soil under greenhouse conditions was 7.3 days. The half-life of crystalline monensin mixed with steer manure and soil in the greenhouse was 5.8 days. Monensin was considered to have degraded under the greenhouse conditions because dissipation by leaching was not possible in this study and monensin activity declined in the soil, as measured by microbiological assay (Appendix D). When crystalline monensin was mixed in soil and exposed to field conditions, the dissipation half-life was 7.5 days with manure and 7.4 days without steer manure (Appendix E). Dissipation of monensin in this study also appeared to result from degradation because the rates of loss were very similar to those found in the greenhouse study. Monensin seems to be extensively degraded in soil. In five weeks under greenhouse conditions, almost 48% of the radioactivity was lost from soil treated with crystalline ^{14}C monensin (Appendix F). Extensive degradation of monensin and its known metabolites would have had to occur to account for the apparent volatilization of ^{14}C, perhaps as $^{14}CO_2$. Because of the moderately rapid decline of monensin in agricultural soil, nontarget terrestrial organisms would presumably be exposed to monensin for a short period of time.

17

2. Potential Monensin Concentration in Feedlots

The highest levels of monensin in a feedlot can be calculated from the levels excreted daily by cattle treated for coccidiosis. Each animal would be fed a maximum of 360 mg of monensin each day. About 50% (180 mg) of the monensin fed to an animal would be excreted as the parent material. The metabolites are substantially less biologically active than monensin. The monensin excreted over the 21-day treatment period would also degrade, with a half life of about 7.5 days. The highest level of monensin accumulated from each animal in a feedlot after 21 days would be 1.75 gms, based on the following equation.

$$\text{Total monensin after 21 days} = \sum_{t=0}^{20} M_0 e^{-rt}$$

where: M_0 is the amount of monensin excreted daily (180 mg)

r is the dissipation rate, constant (0.092; Appendix E)

t is the number of days over which monensin has degraded.

A typical outdoor feedlot pen contains about 200 cattle with 200 ft^2 of pen space per animal. The highest concentration of monensin in the soil of a feedlot would be no higher than the concentration of monensin in cattle manure, 3.3 ppm.

18

B. POTENTIAL CONCENTRATION OF MONENSIN IN AQUATIC SYSTEMS

1. Potential Monensin Concentration in Runoff from Cropland and Feedlots

Runoff water from rainfall could carry some monensin from cropland into surface waters containing aquatic organisms. Because monensin concentrations decline at a moderately rapid rate in soil, a runoff event would have to occur soon after application of cattle manure to soil in order for monensin to reach surface water. If it were possible for all of the monensin in the cattle manure applied to one acre of cropland to be dissolved into runoff from one rainfall event, a two inch runoff event would carry 59.9 g of monensin, or 0.291 ppm ((20 tons of feces/acre x 907 kg/ton x 3.3 mg monensin/kg feces) ÷ (2 inches x 102794 L/acre-in)).

Monensin adsorbs to moderately textured soils. It is improbable that all the monensin in a field could be lost in one large runoff event. The calculated Kd value of 9.3 (Appendix G) indicates that the concentration of monensin in runoff water would be, at most, 10.8% (1 ÷ 9.3) of the concentration of monensin in cropland soil. Since the concentration of monensin in cropland soil is 0.066 ppm this would result in a monensin concentration of 0.007 ppm in runoff water. This estimated concentration of monensin in runoff water is based on the assumption that the runoff water would be in contact with the cropland soil long enough to allow monensin concentrations in the soil and water to come to equilibrium. Wauchope (1978) and Willis and McDowell (1982) indicate that the annual yield of soil-incorporated pesticides into runoff water is normally less than 1.5%.

19

Runoff water from a feedlot could also carry monensin to surface water. The highest concentration of monensin in feedlot soil would be no higher than 3.3 ppm. The calculated Kd value of 9.3 indicates that the concentration of monensin in runoff water would be, at most, 10.8% of the concentration of monensin in feedlot soil. The highest concentration of monensin in runoff water from a feedlot would be 0.36 ppm.

Dilution of the maximum possible concentration of monensin in runoff water by natural aquatic systems would result in nontarget organisms being exposed to low levels of monensin. Monensin is expected to degrade in natural bodies of water, although the process may take several weeks to occur. Moderately rapid metabolism of monensin in field soil (half-life of about 7.5 days) indicates that metabolism of monensin may occur in natural aquatic systems. Monensin does not hydrolyze but can be photolytically degraded in a buffered (pH 7) solution, with a half-life of 43.9 days (Appendix A).

Low concentrations of monensin may persist in the aquatic systems for several weeks.

2. Fate of Monensin in Aquatic Organisms

Aquatic organisms could be exposed to low levels of monensin when runoff occurs from surrounding agricultural fields. Moderate bioconcentration of monensin may occur based on the range of n-octanol/water partition coefficients that occur in the pH range of natural waters (pH 7 to pH 9). Neely, Branson, and Blau (1974)

20

developed a regression equation for projected steady-state residue concentrations in trout muscle versus measured n-octanol/water partition coefficients for a variety of synthetic compounds.

$$\text{Log BCF (bioconcentration factor)} = 0.542 \ (\log K_{ow}) + 0.124$$

Using this equation and the experimentally derived values for log K_{ow} (2.75 at pH 7; 3.79 at pH 9), the predicted BCF for monensin ranges from 41 to 151. This calculated BCF indicates that up to 151 times more monensin might be found in fish muscle than in the surrounding water. If fish only lived in cropland runoff water containing the highest expected concentration of monensin (0.007 ppm), the theoretical concentration of monensin in fish tissue would range from 0.29 to 1.06 ppm. Dilution of runoff in surface waters would rapidly reduce the actual concentration of monensin to which fish could be exposed. Dilution and dissipation of monensin would result in substantially lower levels of this material in fish tissue. The calculated BCF does not allow for metabolism of monensin. Monensin is readily metabolized by food-producing animals and does not accumulate in edible tissues. There is no reason to expect accumulation in fish tissue.

C. OCCURRENCE OF MONENSIN IN GROUNDWATER

The mobility of monensin is moderate in coarse-textured soils such as sand and sandy loam, but mobility is lower in soils such as loam and silty clay loam (Appendix G). Monensin was leached somewhat through coarse soils by the equivalent of about six inches of rain and was moderately mobile when exposed to the equivalent of 25 inches of rain (Appendix G). The retardation factor for the movement of monensin

21

through a soil column relative to the movement of water indicates that monensin adsorbs fairly strongly to loam soil (Kd estimated to be about 9.3). Given the moderately short half-life of monensin in field soil (7.5 days), it is likely that monensin would degrade before enough rainfall occurred to leach significant amounts in even coarse-textured soils.

8. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY TESTS

An in-depth testing program has been completed with various laboratory animal species to determine the toxicological properties of monensin. Complete reports of all of these studies have been submitted to support the proposed action. Studies which are important for determining the safety of monensin to the public and to the producers and users of RUMENSIN Premixes are briefly described below.

Hazard Evaluation Studies

Acute Oral LD_{50} with Rats: Fifty to 80 mg mycelial monensin/kg of body weight in male rats and 15 to 30 mg mycelial monensin/kg body weight in female rats.

Inhalation: No signs of toxicity found for rats exposed to an aerosol of 10 mg of monensin sodium/M^3 one hour a day for 14 days. No signs of toxicity in dogs exposed for six hours a day for 90 days to 0.15 mg of monensin sodium/M^3.

22

Ocular Irritation in Rabbits: Mycelial monensin causes severe irritation when placed in the eyes of rabbits. Rinsing eyes immediately after exposure was effective in preventing permanent damage.

Dermal Irritation in Rabbits: No irritation and no signs of dermal toxicity occurred when 500 mg of mycelial monensin/kg body weight was applied to shaved and abraded skin.

Chronic, Reproduction and Teratology Studies

One-Year Dog Study: No effects at a daily oral dose of 1.25 mg monensin sodium activity (mycelial form)/kg body weight.

Two-Year Mouse Study: No-effect level at a dietary concentration of 10 ppm monensin sodium activity (mycelial form), or a time weighted average daily dose of 1.2 mg/kg for males and 1.4 mg/kg for females. Not carcinogenic at the highest dietary concentration tested, 150 ppm (22 to 25 mg/kg/day).

Two-Year Rat Study: No-effect level at a dietary concentration of 33 ppm monensin sodium activity (mycelial form), or a time weighted average daily dose of 1.40 mg/kg for males and 1.72 mg/kg for females. No carcinogenic effects at the highest dietary concentration tested, 80 ppm (3.6 to 5.0 mg/kg/day).

Rat Multigeneration Reproduction Study: No evidence of reproductive impairment or effect on the offspring at a dietary level of 80 ppm monensin sodium activity (mycelial form).

23

Rabbit Teratology Study: No evidence of maternal toxicity with daily oral doses as high as 0.76 mg monensin sodium/kg body weight during gestation days 6 through 18 and no evidence of dose-related teratogenic effects up to this same dose, the highest tested in this study.

B. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON HUMAN HEALTH

1. Production of Monensin and Manufacture of RUMENSIN Premixes

Monensin used for prevention and control of coccidiosis would be produced in the same two plants that produce RUMENSIN for cattle. Engineering controls, personal hygiene precautions, and respiratory protection are effective in minimizing exposure of workers. Safety glasses or other eye protection is worn by workers. If accidental eye contact occurs with monensin, a worker can immediately rinse the eye thoroughly at available eye-wash stations. Precautionary labeling would advise people mixing and handling RUMENSIN Premixes to wear protective clothing, impervious gloves, and a dust mask. Immediate and thorough rinsing is advised if eye contact occurs. Thorough washing with soap and water is also advised after handling RUMENSIN Premixes. Considering these measures and the fact that in laboratory animals monensin is not a teratogen, carcinogen, or a reproductive toxin, it is concluded that workers producing RUMENSIN Premixes and users of the premixes would not be adversely affected by the proposed action.

24

2. Human Exposure to Monensin Via the Food Supply

Extensive chemistry and toxicology data have been developed to support the safe use of monensin in cattle relative to residues in edible tissues. Based on toxicology and residue data, preslaughter withdrawal is not required for RUMENSIN by the Food and Drug Administration. It may, therefore, be concluded that any small quantity of residual monensin in food would not cause any adverse effect. It is highly improbable that measurable concentrations of monensin would occur in ground or surface water-derived potable water supplies (Sections 7B and 7C).

C. EFFECTS OF MONENSIN ON NONTARGET ORGANISMS

Studies have been conducted to determine the effects of monensin on nontarget organisms. The results of these studies are summarized below and are listed in detail in the referenced appendices.

Avian Species

Bobwhite quail 14-day acute oral toxicity studies (Appendix H):

Two acute oral studies with mycelial monensin and bobwhite quail (Colinus virginianus) have been conducted. In one study with monensin sodium doses ranging from 45 to 250 mg/kg body weight, the 14-day LD_{50}, 95% confidence interval, and slope of the dose-response curve for adult bobwhite were 85.7 mg/kg, 64.4 to 114.2 mg/kg, and 2.915, respectively. No sex-related differences in mortality were evident within treatment groups. No mortalities

25

were found in the second study with monensin sodium doses ranging from 5 to 45 mg/kg body weight. Physical signs of toxicity noted in the first study were dose-related and included loose feces, ataxia, lethargy, emaciation, and prostration. No physical signs of toxicity were noted in the second study. Food consumption and body weight gain were reduced down to the lowest dose tested, 45 mg/kg, in the first study. Body weight gain was also reduced at the 45 mg/kg dose in the second study. A dose of 27.5 mg/kg was the highest level of monensin sodium tested which did not result in mortalities, signs of toxicity, or treatment-related reductions in food consumption and body weight.

Bobwhite quail five-day dietary studies (Appendix I): Two five-day dietary studies were conducted with 11 and 14-day old bobwhite quail (Colinus virginianus). Nominal mycelial monensin sodium concentrations from 0.0365 to 0.125% (w/w) and from 0.005 to 0.0365% (w/w) were used in the first and second studies, respectively. The birds were observed while being fed treated diets for five days, followed by three days of basal diet. Based on nominal dietary concentrations of monensin (assayed levels ranged from 94 to 105% of nominal) in the first study conducted with bobwhite, the eight-day LC_{50}, the 95% confidence interval, and the slope of the concentration-response curve were 0.109%, 0.081 to 0.147%, and 4.285, respectively. Based on estimates of total food consumed, average body weight during the 5-day exposure period, and

26

nominal monensin concentrations, the LD_{50}, the 95% confidence interval for the LD_{50}, and the slope of the dose-response curve were 980 mg monensin sodium/kg body weight, 717 to 1340 mg/kg body weight, and 4.098, respectively. In the first study, physical signs of toxicity (ataxia, lethargy, wing droop, prostration) or reduced weight gain and food consumption were found at all dietary levels of monensin tested, down to 0.0365%. No mortalities were found in the second study and physical signs of toxicity were only found at the highest dietary level of monensin tested, 0.0365%. In the second study, body weight gain was also reduced in birds exposed to dietary monensin levels of 0.0365% and 0.02%. The test level of 0.01% was the highest dietary concentration of monensin sodium tested which resulted in no mortalities, no physical signs of toxicity, and no reductions in food consumption or body weight gain.

Mallard duck five-day dietary study (Appendix J): A five-day dietary study was conducted with 10-day old mallard ducks (Anas platyrhynchos) and monensin sodium (mycelial) at nominal dietary concentrations of 0.0, 0.0062, 0.016, 0.0365, 0.09, 0.225, and 0.5% (w/w). Assayed values ranged from 98 to 103% of nominal. The birds were observed while being fed treated diets for five days, followed by three days of basal diets. One duckling in the 0.09% treatment group died during this study. No physical signs of

27

toxicity (lethargy, ataxia, loose feces, hyperactivity and prostration) were found for birds in this study. Mean body weight gain was reduced at dietary concentrations ≥0.016%. Food consumption was reduced for birds fed diets containing ≥0.09% of monensin sodium. The test level of 0.0062% was the highest dietary concentration of monensin sodium tested which resulted in no mortalities, no physical signs of toxicity, and no reductions in food consumption or body weight gain.

Aquatic Species

Bluegill 96-hour toxicity study (Appendix K): A static toxicity test was conducted to determine the acute effects of monensin sodium (mycelial) on juvenile bluegill. Based on mean measured concentrations of monensin sodium, the 96-hr LC_{50}, the 95% confidence limits of the LC_{50}, and the slope of the concentration-response line were 16.6 ppm, 16.3 to 17.0 ppm, and 0.438, respectively. In this study, fish exposed to monensin concentrations ≥4.4 ppm displayed behavioral signs of toxicity (from hypoactivity to prostration). No mortalities or behavioral signs of toxicity were found for fish exposed to monensin sodium concentrations ≤3.1 ppm.

28

Rainbow trout 96-hour toxicity study (Appendix L): Based on mean concentrations of monensin sodium, the 96-hr LC_{50}, the 95% confidence limits for the LC_{50}, and the slope of the concentration-response curve were 9.0 ppm, 7.8 to 10.2 ppm, and 0.366, respectively. Fish exposed to monensin concentrations $\geq$1.12 ppm showed behavioral signs of toxicity in a concentration-related fashion from hypoactivity to prostration. No mortalities and no behavioral signs of toxicity were found for fish exposed to the monensin sodium concentration of 0.70 ppm.

Daphnia 48-hour toxicity study (Appendix M): Based on daphnid immobility and mean measured concentrations of monensin sodium, the 48-hr EC_{50} and the corresponding 95% confidence limits for the acute study with Daphnia magna were 10.7 ppm and 9.8 to 11.7 ppm. The slope of the concentration-response curve was 0.280. No daphnids were found to be immobile nor did any daphnids display abnormal behavior (hypo-activity, prostration) in this study at a monensin concentration of $\leq$4.2 ppm. Abnormal behavior and/or immobility were noted for monensin concentrations $\geq$5.6 ppm.

Terrestrial Species

Earthworm 14-day toxicity study (Appendix N):

Earthworms (Lumbricus terrestris) were exposed for 14 days to nominal soil concentrations of 0.0, 10.0, 22.5, 45.0, and 100.0 ppm of monensin sodium. Six out of fifteen worms were dead

29

by the end of the study at the highest monensin sodium concentration tested. The rest of the worms exposed to the highest concentration tested were flaccid, soft and flaccid, and moribund. Although no worms died at the exposure concentration of 45 mg/kg, one worm was moribund, one worm was soft and flaccid, and two worms were flaccid. Normal physical condition and no mortalities were noted for worms exposed to monensin sodium concentrations $\leq$22.5 mg/kg. Worms exposed to the two highest concentrations of monensin sodium lost weight during the experiment. Worms exposed to the 22.5 mg/kg treatment level gained less weight than control worms, but the reduced weight gain was not significant. All worms exposed to the monensin sodium concentration of 10 mg/kg in soil were alive, had a normal physical appearance, and gained as much weight as control worms by the end of the 14-day study.

Phytotoxicity of Monensin (Appendices O and P): A greenhouse phytotoxicity test was conducted in which fourteen mono- and dicotyledonous plants were grown from seed in untreated soils and soils treated with monensin alone, or monensin in chicken litter. The plant species tested were alfalfa (Medicago sativa), fescue (Festuca elatior), cucumber (Cucumis sativus), rice (Oryza sativa), cotton (Gossypium hirsutum), tomato (Lycopersicon esculentum), pepper (Capsicum annuum), corn (Zea mays), sugar beets (Beta vulgaris), barley (Hordeum vulgare), soybean (Glycine max), wheat

30

(Triticum aestivum), grain sorghum (Sorghum bicolor), and oats (Avena sativa). Plants were rated for phytotoxic injury (0 = no injury, to 10 = complete kill) and injury, described as chlorosis, burning, stunting, or reduced germination. Ratings were made 18 to 21 days after planting. High levels of control chicken litter in a pilot study caused severe phytotoxicity alone. Monensin-treated soil without chicken litter in the pilot study was relatively nonphytotoxic at monensin application rates of approximately 1 to 2 ppm. Monensin concentrations of 4 to 8 ppm in the soil caused moderate to severe injury to several plants. In another study, monensin was incorporated into soil with chicken litter at litter application rates of 1, 2, 4, and 8 tons of fresh litter per acre. Litter from monensin-fed chickens was no more phytotoxic than litter from control chickens. There was some phytotoxicity due just to the litter itself at an application rate of 8 tons/acre.

A field phytotoxicity study was conducted with 22 tons/acre (49.3 x 10^3 kg/ha) of manure from cattle fed monensin. The cattle feed contained 20 g monensin/ton or 40 g monensin/ton. Cattle given feed with 40 g monensin/ton had an average of 4.4 ppm of monensin in their feces (Appendix C). The plot containing manure from cattle fed 40 g monensin/ton of feed had, therefore, a monensin sodium concentration of approximately 0.145 ppm ((49.3 x 10^3 kg/ha x 4.4 mg/kg) ÷ (4 inches x 375,000 kg/ha-inch soil)). The plant

31

species tested were the same as those used in the greenhouse phytotoxicity study. Because of extensive rainfall, the plants in the plot treated with manure from cattle fed 20 g monensin/ton of feed could not be evaluated. The maturation, flowering, fruiting, or seed formation of oats, sorghum, soybeans, barley, sugar beets, corn, tomatoes, cotton, and cucumbers appeared to be the same in the control plot and the plot treated with manure from cattle fed 40 g monensin/ton feed. No differences between control and treatment plots were found for the growth or vigor of wheat, rice, pepper, alfalfa, and fescue.

D. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON AQUATIC AND WILDLIFE ORGANISMS

1. Potential Adverse Effects on Aquatic Organisms

The influx of monensin into surface water systems is expected to be acute and episodic, depending on runoff from watersheds fertilized with cattle manure containing monensin. The half-life of monensin in soil is relatively short (7.5 days), so runoff events would have to occur soon after application of monensin in cattle manure to cropland. Because monensin does not undergo rapid photolysis or hydrolysis in water and because the microbial degradation rate of monensin in natural waters is unknown, it should be assumed that aquatic organisms could be exposed acutely and chronically to monensin. The acute safety of aquatic

32

organisms should then be assessed by comparing the maximum expected concentration of monensin in runoff from cropland to the results of acute studies with aquatic organisms. The chronic safety of aquatic organisms could initially be assessed by comparing the maximum expected concentration of monensin in runoff to the concentrations calculated to be chemically safe to aquatic organisms.

In Section 7B, the maximum expected monensin concentration in runoff from cropland was calculated to be about 0.007 ppm. The 96-hr LC_{50} values for rainbow trout and bluegill and the 48-hr EC_{50} value for daphnids range from 9.0 to 16.6 ppm. These acute median lethal and acute median effect concentrations are about 1,286 to 2,371 times higher than the highest expected monensin concentration in runoff from cropland. In acute laboratory studies, no mortalities or behavioral abnormalities were found for fish or daphnids at 0.70 ppm. This concentration (0.70 ppm) is approximately 100 times higher than the maximum expected concentration of monensin in runoff from cropland. The highest possible concentration of monensin in runoff water from the feedlot (0.36 ppm) is also lower than 0.70 ppm.

The highest expected concentration of monensin in runoff (0.007 ppm) is substantially below concentrations which can be calculated to have no chronic effects on aquatic organisms. An application factor of 100 can be used with the results from acute studies to extrapolate the concentrations which have no observed effects on the test organisms during chronic exposure. The calculated chronic no-observed-effect concentrations for bluegill, rainbow trout, and daphnids are

33

0.166 ppm (16.6 ppm ÷ 100), 0.09 ppm (9.0 ppm ÷ 100), and 0.107 ppm (10.7 ppm ÷ 100), respectively. These calculated concentrations are between 12.9 and 23.7 times higher than the highest expected concentration (0.007 ppm) of monensin in runoff from cropland.

Based on the maximum expected monensin concentration (0.007 ppm) in runoff from cropland, the dilution of runoff in receiving waters, and the eventual dissipation of monensin from water, the proposed action would not be expected to have a significant acute or chronic effect on aquatic organisms.

2. Potential Adverse Effects on Earthworms

The concentration of monensin in cattle manure is expected to be 3.3 ppm (Section 6C) and the highest concentration of monensin in cropland soil was estimated to be 0.066 ppm (Section 7A). Monensin concentrations in soil decline relatively rapidly in the greenhouse ($t_{½}$=5.8 days) and in the field ($t_{½}$=7.5 days). All earthworms tested for 14 days in soil containing 10 ppm of monensin were alive, had normal physical appearance, and gained as much weight as control worms. Since this test concentration is 3 times higher than the concentration of monensin in cattle manure and is 152 times higher than its concentration in soil, earthworms should not be affected by monensin in cattle manure used as fertilizer.

34

3. Potential Adverse Effects on Avian Species

No mortality, no significant reduction in body weight gain or food consumption, no change in appearance, and no change in behavior occurred for mallard ducks or bobwhite quail fed diets containing 62 ppm (0.0062%) and 100 ppm (0.01%) of monensin, respectively. The highest recommended dietary concentration of monensin in the feed of beef cattle is 33 ppm (30 g/ton). If wild birds foraged only on the feed of beef cattle being treated for coccidiosis, significant effects on body weight, food consumption, or survival would not be expected. Use of monensin for prevention and control of coccidiosis in beef cattle would have no adverse effect on populations of wild avian species.

4. Potential Adverse Effects on Plants

Soil with monensin at 1 to 2 ppm was relatively nonphytotoxic to alfalfa, fescue, cucumber, rice, cotton, tomato, pepper, corn, sugar beets, barley, soybean, wheat, grain sorghum, and oats in a pilot greenhouse study. This soil concentration is at least 15 times higher than the highest expected monensin concentration of 0.066 ppm in cropland. In another study, monensin in chicken litter was found to be only as phytotoxic as the control chicken litter. No phytotoxicity was found in a field study at a calculated monensin concentration of 0.145 ppm in the soil, a concentration 2.2 times that expected in cropland soil. Monensin concentrations dissipate relatively rapidly ($t_{1/2}$= 7.5 days) in field soil. Based on information from these phytotoxicity studies and the relatively short half-life of monensin in field soil, adverse affects from monensin on crops are not expected.

35

9. USE OF RESOURCES AND ENERGY

Manufacturing RUMENSIN Premixes requires an amount of energy similar to that used to produce and package any conventional fermentation product for animals. RUMENSIN is already approved and produced for use in cattle. Disposal of washwater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources. Manufacture of RUMENSIN Premixes for use with beef cattle for prevention and control of coccidiosis will occur at facilities already producing RUMENSIN for use with cattle. Unusual levels of noise, odors, construction, or other disruptions should not be required for any increase in total production of RUMENSIN Premixes.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. The label for RUMENSIN Premixes will instruct users to wear protective clothing, impervious gloves, and a dust mask when mixing and handling RUMENSIN Premixes. Immediate and thorough rinsing is advised if eye contact occurs. The user will also be instructed to wash thoroughly with soap and water after handling RUMENSIN Premixes. The label will also indicate that horses and other equines must not be allowed access to RUMENSIN Premixes. Ingestion of RUMENSIN by equines has been fatal. Other than these precautions listed on the label, no mitigation measures are necessary for RUMENSIN Premixes.

36

11. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

37

12. LIST OF PREPARERS

The following Lilly personnel are responsible for the preparation of this Environmental Assessment:

Roger D. Meyerhoff, Ph.D.
Research Scientist
Environmental Toxicology

July 31, 1989
Date

Alvin L. Donoho, Ph.D.
Research Advisor
Agricultural Biochemistry

August 2, 1989
Date

Raymond H. Hoefer, Ph.D.
Head, Agricultural Analytical Chemistry

August 3, 1989
Date

Stanley J. Parka, Ph.D.
Consultant, Environmental Affairs

August 1, 1989
Date

Meliton N. Novilla, D.V.M., Ph.D.
Senior Research Scientist
Toxicology Project Leader

August 4, 1989
Date

38

13. CERTIFICATION

The undersigned official certifies that the information presented in the Environmental Assessment is true, accurate, and complete to the best of his knowledge.

Merle E. Amundson, Ph.D.
Executive Director
Toxicology Division
Lilly Research Laboratories

August 18, 1989
Date

39

14. REFERENCES

DONOHO, A. L. (1984). Biochemical studies on the fate of monensin in animals and in the environment. J. An. Sci. 58 (6), 1528-1539.

DONOHO, A., MANTHEY, J., OCCOLOWITZ, J., AND ZORNES, L. (1978). Metabolism of monensin in the steer and rat. Ag. and Food Chem. 26 (5), 1090-1095.

HANEY, Jr., M. E. AND HOEHN, M. M. (1968). Monensin, a new biologically active compound. I. Discovery and isolation. In Antimicrobial Agents and Chemotherapy-1967. pp. 349 - 352. American Society for Microbiology, Ann Arbor, MI.

NATIONAL AGRICULTURAL STATISTICS SERVICE. (1988). Cattle. United States Dept. of Agriculture, Agricultural Statistics Board. February 5, 1988. 14 p.

NEELY, W. B., BRANSON, D. R., AND BLAU, G. E. (1974). Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Env. Sci. and Tech. 8 (13), 113-115.

PRESSMAN, B. C. (1976). Biological applications of ionophores. Ann. Rev. Biochem. 45, 925.

WAUCHOPE, R. D. (1978). The pesticide content of surface water draining from agricultural fields-a review. J. Env. Qual. 7 (4), 459-472.

WILLIS, G. H. and McDOWELL, L. L. (1982). Pesticides in agricultural runoff and their effects on downstream water quality. Env. Tox. and Chem. 1, 267-279.

40

APPENDIX A: Report Summary

Title: The Solubility, Hydrolysis, and Photolysis of Monensin in Aqueous Solutions

Study Number: S-AAC-81-13

Study Dates: March 27 to June 11, 1981

Name and Address of Investigators: G. M. Poole, S. D. West, and A. L. Donoho, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Monensin Sodium

Test System: Aqueous Solutions

Summary of Experimental Design:

Solubility

The aqueous solubility of the antibiotic, monensin, was determined turbidimetrically following sterile filtration of buffer solutions containing a visible excess of monensin through a 0.2 μ filter. Triplicate assays were performed on samples taken at 24 hour intervals.

Hydrolysis

The stability of monensin in aqueous solution at pH 5.0, 7.0, and 9.0 was determined turbidimetrically in sterile buffer solutions stored in the dark at 25°C. Assays were performed in triplicate.

Photolysis

The stability of monensin in pH 7.0 aqueous solution was determined turbidimetrically in a sterile buffer solution exposed to a laboratory irradiation apparatus which simulated natural summer sunlight.

Summary of Results

Solubility

The results of the solubility studies with monensin at pH 7 and 9 are summarized below:

41

APPENDIX A: (Continued)

	Monensin Concentration (µg/ml)			
pH	24 hr	48 hr	72 hr	Average
7.0	64	62	not tested	63
9.0	<2.5	0.8	0.9	0.85

Hydrolysis

The hydrolysis of monensin was slow at pH 5.0, 7.0, and 9.0. Little or no degradation was noted within 30 days as shown below:

	Monensin Concentration (µg/ml)		
Day	pH 5.0	pH 7.0	pH 9.0
1	0.384	1.240	0.779
7	0.263	1.158	0.789
15	0.374	1.312	0.906
30	0.343	1.270	0.794

42

APPENDIX A (Continued)

Photolysis

The photolytic degradation of monensin at pH 7.0 was moderate. The half-life appears to be longer than 30 days. Microbiological assay data are presented below. These data show a gradual decline of approximately 40 percent over a 30-day observation period. The positive control samples held in the dark were stable during this period.

	Monensin Concentration (µg/ml)	
Day	pH 7.0	pH 7.0 (Dark Control)
1	1.180	1.240
7	1.028	1.158
15	0.979	1.312
30	0.729	1.270
Half-life (days)	43.9	
Rate Constant (day^{-1})	0.0158	
R^2	0.97	

43

APPENDIX B: Report Summary

Title: Octanol-Water Partition Coefficients for Monensin

Study: ABC-0438

Names and Address of Investigators: A. L. Donoho and D. E. Ruggles, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Monensin Sodium

Test System: n-Octanol and water buffered to pH levels of 5, 7, and 9.

Summary of Experimental Design: The n-octanol to water partitioning coefficient of ^{14}C monensin was determined at pH 5.0, pH 7.0, and pH 9.0 at 25°C and at a monensin concentration of 0.0002 M. It was also determined at pH 7.0 at a concentration of 0.00002 M. The samples were prepared in triplicate in 50-ml glass centrifuge tubes which were mixed by tumbling on a mixing wheel for 24 hours. Duplicate aliquots of octanol and aqueous phases were assayed by liquid scintillation counting. The method was the shake flask procedure set forth in the FDA ENVIRONMENTAL TECHNICAL ASSISTANCE DOCUMENT, Section 3.02, March 1987.

Summary of Results:

Results of the analyses are summarized in the following table:

Monensin Concentration	pH	Kow	log Kow
0.0002 M	5	17329	4.24
0.0002 M	7	567	2.75
0.0002 M	9	6135	3.79
0.00002 M	7	737	2.87

These results indicate a greater partitioning into octanol at both pH 5 and pH 9 than at pH 7. The good agreement between the 0.0002 M and 0.00002 M sets at pH 7 indicate that the test concentrations were sufficiently low for accurate K_{ow} determination.

44

APPENDIX C: Report Summary

Title: Monensin Levels in Feces of Cattle Fed Monensin at a Level of 40 g per Ton of Feed.

Study Number: C97-B47-215

Study Dates: February 9 to 21, 1973

Name and Address of Investigator: A. L. Donoho, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Feces from cattle being fed 40 g monensin per ton of feed.

Test System: Microbiological assay for monensin concentration

Summary of Experimental Design:

A pooled feces sample of approximately 3 kg was obtained from beef cattle which were being fed a ration containing 40 g/ton monensin. The wet feces sample was mixed thoroughly and sampled for analysis. The sample was assayed for monensin by the quantitative microbiological plate assay method described by Kline et. al., JAOAC 53:49 (1970) after sample purification by silica gel column chromatography.

Summary of Results:

Four independent samples were prepared and the extracts were assayed on two different days giving eight determinations. The mean monensin concentration was 4.4 ppm in the wet feces sample. Data are presented in Table 1. Contemporary control excreta samples were negative and the recovery sample gave a value of 87% of theory. Monensin values are corrected for % recovery.

Table 1

Assay for Monensin in Cattle Feces

	Monensin Found (ppm)				
	Replicate	Day 1	Day 2	Mean	Overall $\bar{X} \pm$ s.d.
Set A	1	4.6	4.8	4.7	
	2	3.7	4.0	3.9	
Set B	1	4.1	5.3	4.7	4.4 ± .56
	2	3.9	4.8	4.4	

45

APPENDIX B: Report Summary

Title: Monensin Greenhouse Soil Decline Study

Study Number: A22-B47-3264

Study Dates: April 15 to June 15, 1973

Name and Address of Investigator: L. L. Zornes, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Monensin

Test System: Soil flats maintained in the greenhouse

Summary of Experimental Design:

Crystalline monensin was incorporated into approximately 6 kg air dried potting soil at a nominal concentration of 1 ppm. The monensin was added in a small volume of methanol and the sample was blended and then air dried to remove the methanol. The soil was placed in a nominal 0.07 m^2 soil flat lined with plastic. The flat was maintained in the green house at approximately 27°C. A similar flat was prepared in which feces from steers fed 40 g monensin/ton of feed were incorporated into the soil at 20 tons per acre equivalent along with the nominal 1 ppm monensin. Periodically, samples were taken and air dried, and then portions were assayed for monensin by the microbiological plate assay. Appropriate control and recovery samples were run with the experimental samples.

Summary of Results:

Results from the decline study are shown in Table 1. Degradation of monensin was relatively rapid. In the feces-fortified treated sample, the monensin had declined to less than 20 percent of initial in about a week and was not detectable after two weeks. The decline rate in soil without feces was somewhat slower but was still relatively rapid. This decline of monensin is due to degradation rather than to loss of compound by leaching because the flats were not watered sufficiently to cause leaching.

46

APPENDIX D. (Continued)

Table 1

Degradation of Monensin in Soil

Sampling Time	With Feces		Without Feces	
	PPM	% of Initial	PPM	% of Initial
Zero	1.4[1,2]	100	1.2[1,2]	100
3 days	1.0	71	1.1	92
5 days	0.3	21	0.6	50
8 days	0.2	14	0.4	33
12 days	0.1	7	0.2	17
14 days	0.0	--	0.2	17
28 days	0.0	--	0.0	--
Half-life (days)	5.8		7.3	
Rate Constant (day^{-1})	0.119		0.095	
R^2	0.72		0.89	

[1]Zero-time values are the means of five determinations, and subsequent values are the means of duplicates. All values are on an air-dry basis.

[2]Test sensitivity was 0.1 to 0.2 ppm.

47

APPENDIX E: Report Summary

Title: Monensin Field Soil Decline Study

Study Number: A22-B50-3270

Study Dates: May 1 to June 30, 1973

Name and Address of Investigators: L. L. Zornes and A. L. Donoho, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Monensin

Test System: Field soil plots

Summary of Experimental Design:

Two 9 ft^2 field soil plots at Greenfield, Indiana, were fortified with monensin at a concentration of approximately 1.25 ppm. One of the plots was also fortified with cattle manure equivalent to 20 tons per acre fresh weight. The top 3-inch soil layer was removed from each plot then air dried and screened. Monensin was added in a small volume of methanol while the soil was tumbling in a small concrete mixer. The methanol was evaporated and the soils were returned to the field plots. Periodically, soil cores of the 0-3 inch soil layer were taken for assay. Samples were assayed by quantitative microbiological plate assay using five replicates for zero-time samples and triplicate assays for later samples. When monensin had declined to approximately 0.2 ppm, the plate assay gave negative results and the samples were then monitored by semi-quantitative thin-layer bioautography until concentrations dropped below 0.05 ppm.

Summary of Results:

Results from this study are presented in Table 1. Monensin degradation was relatively rapid over the period of one month. Monensin did not decline rapidly during the first two weeks. This was probably due to the cool weather. The measured soil temperature was approximately 10-12°C during this time. As the soil temperature increased to 15-20°C at about 3 weeks, the degradation rate increased. The plots were negative at 20 days by the plate assay, indicating that 80% or more of the monensin had degraded. The plots were negative by bioautographic assay at 33 days indicating 95% or more degradation.

These data alone do not demonstrate that loss of monensin activity was due to degradation rather than leaching. Therefore, at 42 days, a plate assay was performed on a 0 to 9 inch core sample and this assay was negative. These results, along with the data from greenhouse soil studies, support the conclusion that decline in monensin is due to degradation and not to leaching.

48

APPENDIX E (Continued)

Table 1

PPM Monensin in Field Soil [a/]

Sampling Time	Plot 1		Plot 2	
	Plate	TLB	Plate	TLB
Zero	1.08		1.04	
5 days	1.08		1.01	
12 days	0.86		0.80	
20 days	Neg.	Pos.	Neg.	Pos.
26 days	Neg.	Pos.	Neg.	Pos.
33 days		Neg.		Neg.
Half-life (days)	7.5		7.4	
Rate Constant (day^{-1})	0.092		0.094	
R^2	0.91		0.91	

[a/] Plot 1 contained manure while Plot 2 did not. The plate assay and the thin-layer bioautographic (TLB) assay had limits of detection of approximately 0.2 ppm and 0.05 ppm, respectively.

APPENDIX F: Report Summary

Title: Monensin Biodegradation in Soil

Study Number: B77-3306

Study Dates: March 1 to November 1, 1974

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline ^{14}C Monensin

Test System: Soil maintained in the greenhouse

Summary of Experimental Design:

An aliquot of regular greenhouse potting soil (ca. 6 kg) was fortified with ^{14}C monensin (activity ca. 75,000 dpm/mg) to a level of 10 ppm in the soil giving about 750 dpm/g. The mixture was placed in a plastic-lined flat and placed in the greenhouse to age. The depth of soil in the flat was approximately 3 inches.

Ambient soil temperature ranged between 20-30°C. The soil was maintained in a moist condition. Periodically, soil samples were taken for determination of radioactivity. The samples were air dried, and aliquots were combusted for recovery of $^{14}CO_2$.

Summary of Results:

The results are shown in Table 1. The rate of decline of radioactivity was rapid during the first few weeks and somewhat slower after nine weeks. The labeling procedure for producing the ^{14}C monensin puts the ^{14}C label in each ring except one. Therefore, the fact that such a considerable proportion of the radioactivity is lost from the soil indicates that the molecule is being extensively degraded. The loss of ^{14}C is probably through volatilization, perhaps as $^{14}CO_2$. Monensin and its known metabolites are completely non-volatile and would have to be extensively degraded to be lost through volatilization.

In a companion study, a flat of soil was prepared as above except the monensin used was not radioactive. Samples were taken at weekly intervals and processed to separate monensin from its degradation products. The fractions were examined by TLC and by colorimetric measurement at 520 nm of the acid-vanillin reaction product. Results of this study showed that after three weeks the monensin level was only about 10% of initial and after six weeks was less than 3% of initial. These results agree with the studies conducted by microbiological assay.

50

APPENDIX F: (Continued)

The results of this study also showed that there is no buildup of vanillin positive degradation products in soil. Together the radiochemical and colorimetric data from the soil show that monensin is biodegradable in soil and that the degradation of the molecule is extensive.

Table 1. Decline of Radioactivity in Soil Treated with ^{14}C Monensin.

Time Interval	Radioactivity dpm/g Soil	% of Initial
Start	800	100
2 weeks	635	79
5 weeks	413	52
9 weeks	249	31
15 weeks	247	31
23 weeks	187	23
29 weeks	188	23

51

APPENDIX G: Report Summary

Title: Laboratory Soil Leaching Study with Monensin

Test Article: Crystalline monensin

Name and Address of Investigators: O. D. Decker and E. W. Day, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, IN 46140.

Test System: Laboratory Soil Leaching

Summary of Experimental Design:

The design follows protocols as described in Guidelines for Registering Pesticides in the U.S., published in the Federal Register, Vol. 40, No. 123, June 25, 1975, pages 26884-26886. Monensin was applied at a rate equivalent to 10 pounds (10 ppm) activity per acre in 100 g on top of 30 cm high by 6.35 cm I.D. columns of four different textures of soil. One control and three treatment columns were prepared from each soil type and leached with the water equivalent of 25 inches of rainfall. The leachates were collected in four increments and analyzed for monensin. At the end of the experiment each soil column was divided into sections for monensin analysis.

Summary of Results:

Some recovery data for monensin from water and the various soils are presented in Table 1. The direct standard used to fortify the samples assayed 76.2 - 88.8% of theory by the microbiological assay. Varying standards in 400 ml of 1:1 water:methanol when extracted and assayed gave excellent recoveries with the exception of one low value. Recoveries from soils fortified at 10 ppm were from 62-85%. Because of this variability in recoveries, the observed values from the leachates and soil segments were not corrected for recovery efficiency.

52

APPENDIX G4 (Continued)

Table 1

Monensin Standard Recovery Data

Sample	Monensin (µg) Amount Added	Amount Found	% of Theory
Standard in 1.0 ml methanol	50	38.1	76.2
Water:Methanol (1:1), 400 ml	50	49.1	98.2
	100	67.2	67.2
	250	238.8	95.5
Sand, 25 g	250	156.5	62.6
Sandy Loam, 25 g	250	195.0	78.0
Loam, 25 g	250	158.7	63.5
Silty Clay Loam, 25 g	250	212.2	84.9

The results of the laboratory leaching study are summarized in Table 2.

Table 2

Percent of Monensin Applied to the Column in a Laboratory Soil Leaching Study

Leachate (ml applied)	Sand	Sandy Loam	Loam	Clay Loam
0 - 500	0.5	0.4	ND	ND
500 - 1000	7.5	8.0	1.6	ND
1000 - 1500	38.9	37.4	3.4	6.3
1500 - 2000	27.7	34.6	5.1	17.2
Soil Section (in)				
0 - 4	13.3	1.1	78.0	54.8
4 - 8	8.5	5.7	10.3	17.9
8 - 12	3.7	12.8	1.8	3.7

ND = not detectable

[1] Data are averages from three columns.

53

APPENDIX G (Continued)

Under the conditions of this experiment, the application of the equivalent of 25 inches of rain caused substantial leaching of monensin from a sand and a sandy loam soil while there was very little leaching from a loam and a silty clay loam. Substantial losses of monensin (presumably due to degradation) were observed during the leaching process, the greater losses occurring in soils which required longer time periods for leaching. The results of this experiment indicate that monensin is moderately mobile in coarse textured soils.

The soil sorption coefficient (Kd) for monensin can be calculated from the results of the column leaching study with sandy loam soil. The velocity of water movement through the soil column relative to the velocity of monensin was 26.14. The Kd value for monensin is related to this ratio of velocities by the following equation:

$$26.14 = 1.0 + \frac{\rho \, Kd}{\theta_{fc}}$$

where ρ is the bulk density of sandy loam soil (1.32 g/cm^3)

and

θ_{fc} is the water content of soil at field capacity (0.486 ml/cm^3)

The Kd value for monensin calculated from this equation is 9.3.

54

APPENDIX H: Report Summary

Titles: The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Fourteen-Day Acute Oral Study

and

The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Fourteen-Day Acute Oral Study: Determination of the No-Observed-Effect Dose

Name and Address of Investigator: C. C. Kehr, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Study Numbers: A03680
A01882

Study Dates: A03680 - November 4 to November 18, 1980
A01882 - September 14 to September 28, 1982

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Bobwhite quail (Colinus virginianus)

Age: A03680 - 18 weeks
A01882 - 20 weeks

Number of Animals: A03680 - 5/sex/group
A01882 - 6/sex/group

Dose Levels: A03680 - 0.0, 45, 62, 90, 125, 180, and 250 mg monensin sodium/kg body weight

A01882 - 0.0, 5, 9, 16, 27.5 and 45 mg monensin sodium/kg body weight.

Route: Oral (gavage)

Length of Observation: 14 days

Parameters Studied:

Food consumption, body weight, physical signs of toxicity (loose feces, lethargy, ataxia, hyperactivity emaciation, prostration) and mortality.

55

APPENDIX H (Continued)

Summary of Results:

Study A03680: The LD50, 95% confidence interval for the LD50, and the slope of the dose-response curve for bobwhite dosed with monensin sodium were 85.7 mg/kg, 64.4 to 114.2 mg/kg, and 2.915, respectively. No sex-related differences in mortality were evident within treatment groups. Dose-related toxic effects included loose feces, ataxia and lethargy. Some birds given the highest doses appeared emaciated or prostrate. Bobwhite given the lowest dose appeared hyperactive and had loose feces. A dose-related decline in mean body weight values occurred at all monensin treatment levels and treated birds consumed less food than control birds during the first seven days of the test.

Summary of Results Study A01882:

No mortalities or treatment-related signs of toxicity were found for any treatment group. No treatment-related effects were found for food consumption. Mean body weights of males were slightly reduced on days three and seven in the 45 mg/kg treatment group. No treatment-related physical abnormalities (hyperactivity, loose feces, ataxia, lethargy, emaciation and prostration) no treatment-related effects on body weight or food consumption, and no mortalities were found for bobwhite dosed at $\leq$27.5 mg monensin sodium/kg body weight.

56

APPENDIX I- Report Summary

Titles: The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Five-Day Dietary Study.

and

The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Five-Day Dietary Study: Determination of the No-Observed-Effect Concentration.

Name and Address of Investigator: C. C. Kehr, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Numbers: A03780
A01982

Study Dates: November 13 to November 21, 1980

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Bobwhite Quail (Colinus virginianus)

Age: A03780 - 11 days old
A01982 - 14 days old

Number of Animals: 10/treatment

Levels of Exposure: Study A03782: 0.0, 0.0365, 0.056, 0.09, 0.125% w/w (nominal). Assayed values ranged from 94 to 105% of nominal values.

Study A01982: 0.0, 0.005, 0.02, 0.0365% w/w (nominal). Assayed values ranged from 95 to 99% of nominal values.

Route: Dietary

Length of Exposure: Treated diets, 5 days; basal diets, 3 days.

Parameters Studied: Food consumption, body weight, physical signs of toxicity (ataxia, lethargy wing droop, prostration) and mortality.

APPENDIX I (Continued)

Results:

Study A03782: The 8-day LC50, the 95% confidence limits for the LC50 and the slope of the concentration-response curve for bobwhite exposed to monensin sodium in fed were 0.109%, 0.081 to 0.147%, and 4.285, respectively. Based on food consumption, average body weight during the 5-day exposure period, and nominal concentrations of monensin sodium in the diet the LD50, the 95% confidence limits for the LD50, and the slope of the dose-response curve for monensin sodium in this dietary study were 980 mg monensin sodium/kg body weight, 717 to 1340 mg monensin sodium/kg body weight, and 4.098, respectively. No mortality or physical signs of toxicity occurred in the control group or in the group that received the lowest dietary concentration of monensin sodium. At higher dietary levels of monensin sodium, physical signs of toxicity (ataxia, lethargy, wing droop, and prostration) appeared to be concentration-related. Significant reductions in body weight gain or body weight loss occurred at all dietary levels of monensin tested in this study. Slight reductions in food consumption also occurred at all treatment levels.

Study A01982: No mortalities were found in this study. Lethargy was seen in all birds tested at the highest treatment level and one bird at this level was ataxic and had wing droop. Food consumption and body weight gain were reduced at the highest treatment level, 0.0365%, and body weight gain was reduced slightly at the 0.02% treatment level. The test level of 0.01% was the highest dietary concentration of monensin sodium tested which resulted in no mortalities, no physical signs of toxicity, and no reductions in food consumption or body weight gain.

58

APPENDIX J: Report Summary

Title: The Toxicity of Mycelial Monensin Sodium to Mallards in a Five-Day Dietary Study.

Name and Address of Investigator: C. C. Kehr, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P.O. Box 708, Greenfield, Indiana 46140

Study Dates: August 19 to August 27, 1982

Study Number: A01782

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Mallard Duck (Anas platyrhynchos)

Age: 10 days

Number of Animals: 10/treatment

Levels of Exposure: 0.0, 0.0062, 0.016, 0.0365, 0.09, 0.225, and 0.5% w/w (nominal). Assayed values ranged from 98 to 103% of nominal.

Length of Exposure: Treated diets, 5 days; basal diets, 3 days.

Route: Dietary

Parameters Studied: Food consumption, body weight gain, physical signs of toxicity (ataxia and lethargy), and mortality.

Results:

One duckling in the 0.09% treatment group died during this study. No physical signs of toxicity (lethargy, ataxia, loose feces, hyperactivity and prostration) were found for birds in this study. Mean body weight gain was reduced at dietary concentrations $\geq$ 0.016%. Food consumption was reduced for birds fed diets containing $\geq$ 0.09% of monensin sodium. The test level of 0.0062% was the highest dietary dietary concentration of monensin sodium tested which resulted in no mortalities, no physical signs of toxicity, and no reductions in food consumption or body weight gain.

59

APPENDIX K: Report Summary

Title: The Acute Toxicity of Mycelial Monensin Sodium to Bluegill in a Static Test System.

Name and Address of Investigators: D. W. Grothe and P. C. Francis, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Dates: August 23 to August 27, 1982

Study Number: F10082

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Bluegill (Lepomis macrochirus)

Experimental Design:

Groups of ten juvenile bluegill (mean weight, 0.93 g) were exposed to average assayed monensin sodium concentrations of 0.0, 1.15, 1.65, 3.1, 4.4, 7.6, 12.1, 14.2, 14.6, 17.0, and 17.6 mg/L for 96 hours. Jars with 15 L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Behavioral signs of toxicity (hypoactive, minimal swimming behavior, disorientation, labored respiration, and prostration) and mortality were monitored for fish in each jar on a daily basis.

Results:

The temperature of the test solutions averaged 20°C, pH values ranged from 8.2 to 8.7 and dissolved oxygen concentrations were above 89% of saturation. Fish exposed to monensin sodium concentrations $\geq$4.4 mg/L showed behavioral signs of toxicity in a concentration-related fashion, from hypoactivity to prostration. The 96-hr LC50, the 95% confidence limits for the LC50, and the slope of the concentration-response curve were 16.6 mg/L, 16.3 to 17.0 mg/L, and 0.438, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to monensin sodium concentrations $\leq$3.1 mg/L.

60

APPENDIX L: Report Summary

Title: The Acute Toxicity of Mycelial Monensin Sodium to Rainbow Trout in a Static Test System.

Name and Address of Investigator: D. W. Grothe and P. C. Francis, Toxicology Division, Lilly Research Laboratories, A Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Dates: August 23 to August 27, 1982

Study Number: F10182

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Rainbow trout (Salmo gairdneri)

Experimental Design:

Groups of ten juvenile rainbow trout (mean weight, 1.14 g) were exposed to average assayed monensin sodium concentrations of 0.0, 0.70, 1.12, 1.48, 4.3, 5.2, 6.6, 8.2, 10.6, 12.5, and 15.7 mg/L. Jars with 15 L of test or control solution were used to contain each group of ten fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior, disorientation, labored respiration, and prostration) and mortality were monitored for fish in each jar on a daily basis.

Results:

The temperature of the test solutions averaged 12.0°C, pH values ranged from 8.0 to 8.4 and dissolved oxygen concentrations were above 95% saturation. Fish exposed to monensin sodium concentrations $\geq$1.12 mg/L showed behavioral signs of toxicity in a concentration-related fashion, from hypoactivity to prostration. The 96-hr LC50, the 95% confidence limits for the LC50, and the slope of the concentration-response curve were 9.0 mg/L, 7.8 to 10.2 mg/L and 0.366, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to the monensin sodium concentration of 0.70 mg/L.

61

APPENDIX M: Report Summary

Title: The Acute Toxicity of Mycelial Monensin Sodium to Daphnia magna in a Static Test System

Name and Address of Investigators: P. C. Francis and D. W. Grothe, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Dates: May 25 to May 27, 1982

Study Number: C02382

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Daphnia magna

Summary of Experimental Design:

Groups of 30 Daphnia, ≤24 hours old, were exposed to average assayed monensin sodium concentrations of 0.0, 2.6, 4.2, 5.6, 7.1, 10.8, 14.4, and 18.1 mg/L for 48 hours. Each of three beakers with 200 ml of solution were used to contain 10 Daphnia for each treatment or control solution. Test solutions were maintained at 20°C and pH values ranged from 8.2 to 8.6 in all of the test and control solutions. Dissolved oxygen concentration remained above 66% saturation in all test solutions.

Results:

Based on immobility, the 48-hour EC50, the 95% confidence interval, and the slope of the concentration-response curve for monensin sodium were 10.7 mg/L, 9.8 to 11.7 mg/L, and 0.280, respectively. The highest monensin sodium concentration tested which did not result in physical signs of toxicity (hypoactivity or prostration) and did not result in immobilization was 4.2 mg/L. Hypoactivity and immobilization were concentration-related at monensin sodium concentrations ≥5.6 mg/L.

62

APPENDIX N: Report Summary

Title: The Toxicity of Soil-Incorporated Mycelial Monensin Sodium to Earthworms in a 14-Day Test.

Name and Address of Investigators: . C. Francis and D. W. Grothe, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Study Dates: May 12 to May 26, 1982

Study Numbers: W01082

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Lumbricus terrestris

Average Initial Wet Weight: 3.67 g

Number of Animals: 15/treatment

Route: Incorporated into test media (rabbit feces, water, and loamy sand soil)

Levels of Exposure: 0.0, 10.0, 22.5, 45.0, and 100 ppm (nominal)

Length of Exposure: 14 days

Parameters Studied: Body weight gain, mortality, and physical appearance (flaccid, soft and flaccid, moribund).

Experimental Design:

Test media was placed in 2-L cylindrical glass jars. Three jars were used for controls and three jars were used for each exposure level. Five worms were placed into each jar at the beginning of each study. The study was conducted at 12°C.

Results:

Six out of fifteen worms were dead by the end of the study at the highest monensin sodium concentration tested. The rest of the worms exposed to the highest concentration tested were flaccid, soft and flaccid, and moribund. Although no worms died at the exposure concentration of 45 mg/kg, one worm was moribund, one worm was soft and flaccid, and two worms were flaccid. Normal physical condition and no mortalities were noted for worms exposed to monensin sodium

63

APPENDIX N: (Continued)

concentrations ≤22.5 mg/kg. Worms exposed to the two highest concentrations of monensin sodium lost weight during the experiment. Worms exposed to the 22.5-mg/kg treatment level gained less weight than control worms, but the reduced weight gain was not significant. All worms exposed to the monensin sodium concentration of 10 mg/kg in soil were alive, had a normal physical appearance, and gained as much weight as control worms by the end of the 14-day study.

64

APPENDIX O: Report Summary

Title: Greenhouse Test for Monensin Phytotoxicity

Study Numbers: WB71-1 and WB1-31

Study Dates: January 2 to July 1, 1971

Name and Address of Investigators: R. B. Bevington and M. E. Callendar, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Crystalline Monensin and Litter from Monensin-Fed Chickens

Test System: Plants grown from seed in greenhouse soil flats.

Summary of Experimental Design:

Monensin or litter from monensin-fed chickens was incorporated into soil at concentrations shown in Table 1. A standard greenhouse phytotoxicity test was conducted in which fourteen mono- and dicotyledonous plants were grown from seed in the treated and untreated soils. The plant species were alfalfa (Medicago sativa), fescue (Festuca elatior), cucumber (Cucumis sativus), rice (Oryza sativa), cotton (Gossypium hirsutum), tomato (Lycopersicon esculentum), pepper (Capsicum annuum), corn (Zea mays), sugar beet (Beta vulgaris), barley (Hordeum vulgare), soybean (Glycine max), wheat (Triticum aestivum), grain sorghum (Sorghum bicolor), and oats (Avena sativa). Plants were rated for phytotoxic injury (0 = no injury, to 10 = complete kill) and injury, described as chlorosis, burning, stunting, or reduced germination, was noted 18 to 21 days after planting.

Summary of Results

A pilot experiment (WB71-1) was conducted in which chicken litter was applied at rates equivalent to 2½ to 10 tons per acre on a dry matter basis. This exposure level proved to be too high because of severe phytotoxicity even with the control litter treatment. Monensin itself without any litter present was relatively nonphytotoxic at application rates of approximately 1-2 ppm (lb/acre equivalent). However, rates of 4-8 ppm caused moderate to severe injury on several plant species.

A second experiment (WB1-31) was conducted in which litter from control chickens and monensin-treated chickens was applied at rates equivalent to 1, 2, 4, and 8 tons of fresh litter per acre. Litter samples were weighed, dried, and milled, and the litter was incorporated into the test soils at the appropriate rates.

65

APPENDIX O (Continued)

Results are shown in Table 1. Litter from monensin-fed chickens was no more phytotoxic than litter from control chickens. There was some phytotoxicity due just to the litter itself at an application rate of 8 tons/acre.

Table 1

Phytotoxicity Ratings [a] on Chicken Litter Treatments

Treatment [b]	Litter from Monensin Treated Chickens				Litter from Control Chickens				No Litter	
Rate(tons/acre)	1	2	4	8	1	2	4	8	0	0
Cotton	0	0	0	1.5	0	0	0	1.5	0	0
Sugar Beets	0	0	3	4	0	2	3	10	0	0
Tomatoes	0	0	0	1.5	0	0	0	1.5	0	0
Alfalfa	0	0	0	0	0	0	0	2	0	0
Peppers	0	0	0	0	0	0	0	0	0	0
Cucumbers	0	0	0	0	0	0	0	0	0	0
Soybeans	0	0	0	1	0	0	0	1.5	0	0
Wheat	0	0	0	0	0	0	0	0	0	0
Barley	0	0	0	0	0	0	0	1	0	0
Rice	0	0	0	0	0	0	0	0	0	0
Corn	0	0	0	0	0	0	0	0	0	0
Fescue	0	0	0	0	0	0	0	0	0	0
Oats	0	0	0	0	0	0	1	2	0	0
Sorghum	0	0	0	0	0	0	0	2	0	0

[a] Rating scale was 0 to 10. A rating of 0 represents no injury and 10 represents complete kill.

[b] Monensin treated chickens received 110 g monensin per ton of feed.

66

APPENDIX P: Report Summary

Title: Field Phytotoxicity Study of Manure from Monensin-Treated Cattle

Study Number: B48-3273

Study Dates: February 1 to September 30, 1973

Name and Address of Investigator: J. A. Manthey, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: Manure from Cattle fed Monensin

Test System: Crops grown in field plots

Summary of Experimental Design:

During the winter of 1973, manure was collected from the pens of cattle which were fed with feed that contained monensin. The dosing levels of monensin were 20 and 40 g/ton of feed. On June 1, the manure from the piles was weighed and spread on, the test plots at the rate of 22 tons/acre. Each plot was 23 x 54'. Such plots were large enough to accommodate the rows of 14 selected crop plants. The manure was disked into the upper 4 inches of the soil. During the next three weeks, the plots were made fallow by disking.

The plots were arranged in the following fashion:

I	II	III	IV
Control Manure	40 g/ton Manure	No Manure	20 g/ton Manure

Direction of rows ---------->

On June 25, 1973, the field plots were seeded with the crops shown in Table 1. Subsequently, weeds were controlled by cultivation, and insecticide sprays were used as needed to maintain the seedlings in good condition.

Extreme rainfall washed out part of a test plot. It became necessary to reseed the tomatoes and peppers. This was done on July 11, 1973.

67

APPENDIX P: (Continued)

Summary of Results

The evaluation of crop injury from this test is shown in Table 1. There were no adverse effects from the manure of animals fed the highest level of monensin (40 g/ton). No evaluation of the lower (20 g/ton) monensin level plot could be made. This plot was in a poorly drained area of the field. The very wet season of 1973 caused extensive water damage to all crops in that plot.

There were no indications of monensin-related phytotoxicity to any of the crops.

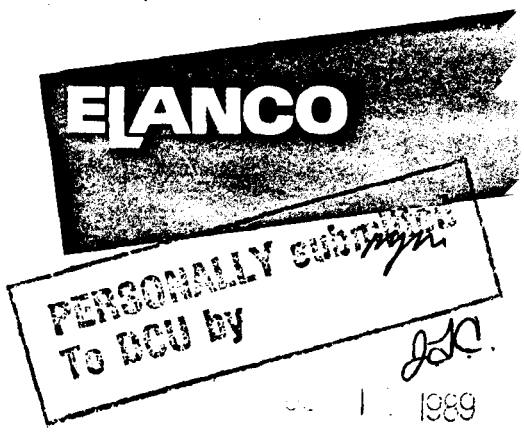
Table 1

Crop Injury Rating

Oats (Avena sativa) Sorghum (Sorghum bicolor) Soybean (Glycine max) Barley (Hordeum vulgare) Beet (Beta vulgaris) Corn (Zea mays) Tomato (Lycopersicon esculentum) Cotton (Gossypium hirsutum) Cucumber (Cucumis sativus)	There were no observable differences in maturation, flowering, fruiting, or seed formation between Sugar untreated, blank manure Corn plot and the plot with monensin in the manure.
Wheat (Triticum aestivum) Rice (Oryza sativa) Pepper (Capsicum annuum) Alfalfa (Medicago sativa) Fescue (Festuca elatior)	No observable differences in growth or vigor of these plants between treatments. Due to short duration of this trial, no fruit or seeds were formed to date.

Elanco Regulatory Services
Elanco Products Company
A Division of Eli Lilly and Company

Lilly Corporate Center
Indianapolis, Indiana 46285
Telephone (317) 467-4872



October 17, 1989

PHASING NADA

Donald A. Gable, D.V.M.
Director, Division of Therapeutic Drugs
for Food Animals (HFV-130)
Center for Veterinary Medicine
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Dear Dr. Gable:

Re: NADA 95-735 - RUMENSIN - COCCIDIOSIS IN CATTLE

We refer to Dr. A. Gabuten's letter of February 18, 1986. The letter described several issues which required resolution prior to the Agency granting approval for the use of RUMENSIN for the control of coccidiosis in cattle. The required environmental assessment was submitted on August 31, 1989, and the revised FOI Summary was submitted on September 14, 1989. Attached are the revised Type A and Blue Bird labels.

We certify that the nonclinical laboratory studies contained in the application were conducted in compliance with good laboratory practice regulations.

This submission completes the supplemental application. We would appreciate a timely review of this application.

Please inform me if further information is needed.

Sincerely,

ELANCO PRODUCTS COMPANY

Michael J. McGowan, Ph.D.
Manager, Animal Science Regulatory Affairs
(317) 467-4124

MJM:mw

Attachment

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION	**NEW ANIMAL DRUG APPLICATION** (Drugs for Animal Use) (Title 21, CFR 514)	*Form Approved; OMB No. 0910-0032* *Expiration Date: February 29, 1991* **NADA** 95-735

DRUG PRODUCT

ESTABLISHED NAME *(e.g., USP/USAN)*	PROPRIETARY NAME:
monensin	RUMENSIN

DOSAGE FORM:	PROPOSED INDICATIONS FOR USE:	SPECIES:
Type A Medicated Article	Prevention of coccidiosis	Bovine

PROPOSED MARKETING STATUS: ☐ PRESCRIPTION PRODUCT *(Rx)* ☒ OVER - THE - COUNTER PRODUCT *(OTC)*

NAME OF APPLICANT	ADDRESS *(Street Number, City, and Zip Code)*
Elanco Products Company A Division of Eli Lilly and Company	Lilly Corporate Center Indianapolis, Indiana 46285

NOTE: No application may be filed unless a completed application form has been received.

TYPE OF SUBMISSION *(Check one)*

- ☐ ORIGINAL APPLICATION *(21 CFR 514.1(a))*
- ☐ AMENDMENT TO AN UNAPPROVED ORIGINAL APPLICATION *(21 CFR 514.6)*
- ☒ SUPPLEMENT TO AN APPROVED APPLICATION *(21 CFR 514.8 (a))*
- ☐ AMENDMENT TO AN UNAPPROVED SUPPLEMENT TO AN APPROVED APPLICATION *(21 CFR 514.6)*
- ☐ SPECIAL SUPPLEMENT TO AN APPROVED APPLICATION - CHANGES BEING EFFECTED *(21 CFR 514.8 (e))*

REASON FOR SUPPLEMENTAL APPLICATION:

INSTRUCTIONS FOR PREPARING AND SUBMITTING THE NEW ANIMAL DRUG APPLICATION

i. Prepare three identical copies of the submission.

ii. Identify front cover of each copy with the name of the applicant, the proprietary name (if available), the name of the new animal drug and the dosage form.

iii. Use separate pages for each numbered heading consistent with the subparagraphs of this application form. *(See reverse side of this page)*.

iv. **Number the pages of the new animal drug application**. Each copy should bear the same page numbering.

v. Submit separate applications for each different dosage form of the proposed drug.

vi. Basic information pertinent to a dosage form should be made by reference to volume and page of the application containing such information. Include in each application information applicable to the specific dosage form, such as, labeling, composition, stability data, efficacy data, method of manufacture and references to appropriate investigational new animal drug applications and master file(s).

vii. Submit applications to: **Food and Drug Administration**
Center for Veterinary Medicine (HFV-16)
5600 Fishers Lane
Rockville, MD 20857

viii. Prepare amendments, supplements, reports and other correspondence in the above format. Identify the submission with the assigned NADA number.

ix. If the submission is a supplemental application, full information shall be provided on each proposed change concerning any statement made in the approved application.

x. Submit page 1 and 2 of this form with each submission.

FOR FDA USE ONLY

AF0360-50B

Elanco

Rumensin®

Monensin Sodium

60

TM

Net Weight **50 lbs**

(22.68 kg)

Type A Medicated Article

Do Not Feed Undiluted

Feedlot Cattle:
A. For improved feed efficiency (cattle fed in confinement for slaughter)
B. For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii.*

Pasture Cattle: Slaughter, stocker, feeder, dairy and beef replacement heifers weighing more than 400 pounds--For increased rate of weight gain.

Mature Reproducing Beef Cows: For improved feed efficiency when receiving supplemental feed.

Goats: For the prevention of coccidiosis caused by *Eimeria crandallis, E. christenseni,* and *E. ninakohlyakimovae* in goats maintained in confinement.

Important: Must be thoroughly mixed in feeds before use. This product should be further diluted before mixing in the final feed. See back of bag for directions.

CAUTION: Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal.

Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.

Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions..

Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.

For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.

Do not feed to lactating dairy cows.

Do not feed to lactating dairy goats.

WARNING: When mixing and handling Rumensin 60, use protective clothing, impervious gloves and a dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse with water.

Store in a cool, dry place. Not to be used after date printed on side of bag.

ELANCO PRODUCTS COMPANY
A DIVISION OF ELI LILLY AND COMPANY
INDIANAPOLIS, IN 46285, U.S.A.

Rumensin® 60

DIRECTIONS FOR USE

Read All Directions Carefully Before Mixing and Feeding

Active Drug Ingredients: Monensin (as monensin sodium) 60 g per pound

I. A. **Feedlot Cattle:** For improved feed efficiency.
Goats: For prevention of coccidiosis.

> **CAUTION:** Do not feed undiluted. Feed only to cattle being fed in confinement for slaughter. Feed only to goats maintained in confinement.

1. Complete Feed Mixing and Feeding Directions:

Thoroughly mix the following amounts of Rumensin 60 to make one ton of complete feed that provides either 5 to 30 g/ton monensin sodium for cattle or 20 g/ton monensin sodium for goats on a 90% dry matter basis (Table 1). **An intermediate blending step should be performed to insure an adequate mix.** Feed complete feed (5-30 g/ton) continuously to growing finishing beef cattle to provide not less than 50 nor more than 360 mg monensin per head per day. Feed complete feed (20 g/ton) continuously to goats as the sole ration.

Table 1.

Lbs. Rumensin 60 Per Ton of Complete Feed	Rumensin Concentration in Complete Feed 90% Dry Matter Basis (Grams Per Ton)
.08	5
.33	20[a]
.50	30[b]

[a]Cleared level for goats.
[b]Maximum approved concentration for cattle.

2. Type B or C Medicated Feed Mixing Directions (Dry and Liquid)

a) Dry or Liquid
Thoroughly mix the following amounts of Rumensin 60 to make one ton of Type B or C medicated feed to provide the levels shown in Table 2. Dry Only - **An intermediate blending step should be performed to insure an adequate mix.**

b) Liquid Limitations
1) The supplement pH must be between 4.3-7.1.
2) Stored Rumensin Liquid Type B or C Medicated Feeds should be recirculated or agitated for 10 minutes daily.

> **CAUTION:** Inadequate mixing (recirculation or agitation) of Rumensin Liquid Type B or C Medicated Feeds has resulted in increased Rumensin concentration which has been fatal to cattle and could be fatal in goats.

B. **Feedlot Cattle:** For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*. Feeding Directions: Feed continuously at a rate of not less than 10 grams nor more than 30 grams of monensin per ton of feed to provide not less than 100 nor more than 360 mg monensin per head per day depending on body weight during periods of exposure to coccidia or when coccidiosis is likely to be a hazard.

II. **Pasture Cattle (slaughter, stocker, and feeder, dairy and beef replacement heifers weighing more than 400 pounds):** For increased rate of weight gain. Feeding Directions: Feed at the rate of not less than 50 nor more than 200 mg per head per day in not less than one pound of Type C Medicated Feed or after the fifth day feed at the rate of 400 mg per head per day every other day in not less than 2 pounds of Type C Medicated Feed as monensin sodium. The concentration of Rumensin in the pasture Type C Medicated Feed must be between 25 and 400 grams per ton.

Mature Reproducing Beef Cows (on pasture or in dry lot): For improved feed efficiency when receiving supplemental feed. Feeding Directions: Feed 50 to 200 mg/hd/day. Blend into a minimum of 1 lb. of Type C Medicated Feed and either hand feed or mix into the total ration. Feed (other than the Type C Medicated Feed containing Rumensin) can be restricted to 95% (of normal requirements) when 50 mg of Rumensin is fed, and to 90% at 200 milligrams.

Cows on pasture or in dry lot must receive a minimum of 1 lb. of Type C Medicated Feed per head per day. Additionally, a minimum of 16 pounds (air-dry basis) of roughage such as silage, haylage, ammoniated straw, hay or equivalent feedstuffs should be fed in order to meet NRC recommendations for mature reproducing beef cows to gain 0.25 to 0.75 pounds per head per day. Standing, dried winter range forage may not be of adequate quality to result in improved efficiency when supplemented with Rumensin.

CAUTION: During the first 5 days, cattle should receive no more than 100 mg per day contained in not less than 1 lb. of feed.

Do not self feed.

Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.

For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.

Do not feed to lactating dairy cows.

Do not feed to lactating dairy goats.

1. **Type B or C Medicated Feed Mixing Directions:**
Thoroughly mix the following amounts of Rumensin 60 to make one ton of Type B or C Medicated Feed to provide the levels shown in Table 2. Dry Only - **An intermediate blending step should be performed to insure an adequate mix.**

CAUTION: Do not allow horses or other equines access to feeds containing monensin.

Table 2.

Amount of Rumensin 60 per ton of Unmedicated Feed (lbs.)	Rumensin Concentration in Type B or C Medicated Feed (g/ton)
.33	20[a]
.83	50
3.33	200
20.00	1200

[a]Cleared level for goats.

NEW WEIGHT ON BAG OR BULK

ELANCO

Agriculture Partner Worldwide

RUMENSIN TYPE B MEDICATED FEED

FEED ONLY TO CATTLE MAINTAINED IN CONFINEMENT

For the prevention and control of coccidiosis due to *Eimeria bovis* and *E. zuernii* in cattle maintained in confinement.

DO NOT FEED UNDILUTED

ACTIVE DRUG INGREDIENT

Rumensin (as Monensin Sodium) . . . . . 30 - 1200 g/ton

GUARANTEED ANALYSIS

Nutritional Ingredients . . . . . xxxx%

INGREDIENTS

As listed in AAFCO Handbook.

MIXING & FEEDING DIRECTIONS

Thoroughly mix the appropriate amount of Rumensin with grain and roughage to provide a Type B medicated feed as follows:

LEVEL OF RUMENSIN TYPE B MEDICATED FEED IN G/TON	DESIRED LEVEL OF RUMENSIN IN GRAMS PER TON IN COMPLETE FEED	NO. OF LBS OF TYPE B MEDICATED FEED TO BE MIXED WITH OTHER FEEDSTUFF TO MAKE A TON OF COMPLETE FEED	POUNDS OF GRAIN AND/OR ROUGHAGE	MILLIGRAMS RUMENSIN PER POUND OF COMPLETE FEED
100	10	200	1800	5
	20	400	1600	10
	30	600	1400	15
300	10	67	1933	5
	20	133	1867	10
	30	200	1800	15
600	10	33	1967	5
	20	67	1933	10
	30	100	1900	15
1200	10	17	1983	5
	20	33	1967	10
	30	50	1950	15

Feed complete feed (10 - 30 g/ton) continuously to cattle maintained in confinement to provide not less than 100 mg nor more than 360 mg of Rumensin per head daily.

CAUTION:

1. Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal
2. Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.
3. Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions.
4. Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.
5. For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.
6. Do not feed to lactating dairy cows.
7. Do not feed to lactating dairy goats.

MANUFACTURED BY

TAKE TIME TO OBSERVE LABEL DIRECTIONS

Expiration Date _____

Lot Number _____

9/89

ELANCO

Agriculture's Partner Worldwide

NET WEIGHT ON BAG OR BULK

RUMENSIN TYPE C MEDICATED FEED

FEED ONLY TO CATTLE MAINTAINED IN CONFINEMENT

For the prevention and control of coccidiosis due to *Eimeria bovis* and *E. zuernii* in cattle maintained in confinement.

ACTIVE DRUG INGREDIENT

Monensin (as Monensin Sodium) . . . . . 10 - 30 g/ton

GUARANTEED ANALYSIS

Nutritional Ingredients . . . . . xxxx%

INGREDIENTS

As listed in AAFCO Handbook.

MIXING & FEEDING DIRECTIONS

Feed complete feed (10 - 30 g/ton) continuously to cattle maintained in confinement.

CAUTION:

1. Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal
2. Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.
3. Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions.
4. Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.
5. For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.
6. Do not feed to lactating dairy cows.
7. Do not feed to lactating dairy goats.

MANUFACTURED BY

Expiration Date _____ Lot Number _____

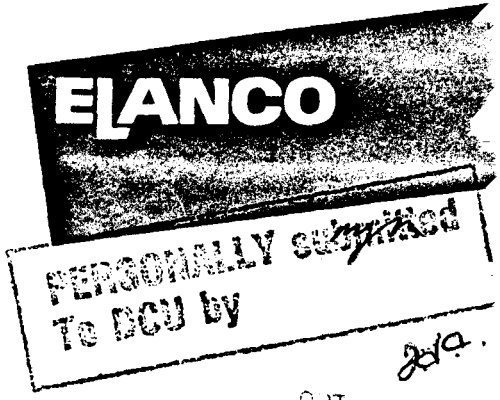
9/89

TAKE TIME

OBSERVE LABEL

Elanco Regulatory Services
Elanco Products Company
A Division of Eli Lilly and Company

Lilly Corporate Center
Indianapolis, Indiana 46285
Telephone (317) 467-4872



October 17, 1989

PHASING NADA

Donald A. Gable, D.V.M.
Director, Division of Therapeutic Drugs
for Food Animals (HFV-130)
Center for Veterinary Medicine
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Dear Dr. Gable:

Re: NADA 95-735 - RUMENSIN - COCCIDIOSIS IN CATTLE

We refer to Dr. A. Gabuten's letter of February 18, 1986. The letter described several issues which required resolution prior to the Agency granting approval for the use of RUMENSIN for the control of coccidiosis in cattle. The required environmental assessment was submitted on August 31, 1989, and the revised FOI Summary was submitted on September 14, 1989. Attached are the revised Type A and Blue Bird labels.

We certify that the nonclinical laboratory studies contained in the application were conducted in compliance with good laboratory practice regulations.

This submission completes the supplemental application. We would appreciate a timely review of this application.

Please inform me if further information is needed.

Sincerely,

ELANCO PRODUCTS COMPANY

Michael J. McGowan, Ph.D.
Manager, Animal Science Regulatory Affairs
(317) 467-4124

MJM:mw

Attachment

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION	**NEW ANIMAL DRUG APPLICATION** (Drugs for Animal Use) (Title 21, CFR 514)	*Form Approved; OMB No. 0910-0032* *Expiration Date: February 29, 1991* **NADA** 95-735

DRUG PRODUCT

ESTABLISHED NAME *(e.g., USP/USAN)*	PROPRIETARY NAME:
monensin	RUMENSIN

DOSAGE FORM:	PROPOSED INDICATIONS FOR USE:	SPECIES:
Type A Medicated Article	Prevention of coccidiosis	Bovine

PROPOSED MARKETING STATUS: ☐ PRESCRIPTION PRODUCT *(Rx)* ☒ OVER - THE - COUNTER PRODUCT *(OTC)*

NAME OF APPLICANT	ADDRESS *(Street Number, City, and Zip Code)*
Elanco Products Company A Division of Eli Lilly and Company	Lilly Corporate Center Indianapolis, Indiana 46285

NOTE: No application may be filed unless a completed application form has been received.

TYPE OF SUBMISSION *(Check one)*

- ☐ ORIGINAL APPLICATION *(21 CFR 514.1(a))*
- ☐ AMENDMENT TO AN UNAPPROVED ORIGINAL APPLICATION *(21 CFR 514.6)*
- ☒ SUPPLEMENT TO AN APPROVED APPLICATION *(21 CFR 514.8 (a))*
- ☐ AMENDMENT TO AN UNAPPROVED SUPPLEMENT TO AN APPROVED APPLICATION *(21 CFR 514.6)*
- ☐ SPECIAL SUPPLEMENT TO AN APPROVED APPLICATION - CHANGES BEING EFFECTED *(21 CFR 514.8 (e))*

REASON FOR SUPPLEMENTAL APPLICATION:

INSTRUCTIONS FOR PREPARING AND SUBMITTING THE NEW ANIMAL DRUG APPLICATION

i. Prepare three identical copies of the submission.

ii. Identify front cover of each copy with the name of the applicant, the proprietary name (if available), the name of the new animal drug and the dosage form.

iii. Use separate pages for each numbered heading consistent with the subparagraphs of this application form. *(See reverse side of this page).*

iv. **Number the pages of the new animal drug application.** Each copy should bear the same page numbering.

v. Submit separate applications for each different dosage form of the proposed drug.

vi. Basic information pertinent to a dosage form should be made by reference to volume and page of the application containing such information. Include in each application information applicable to the specific dosage form, such as, labeling, composition, stability data, efficacy data, method of manufacture and references to appropriate investigational new animal drug applications and master file(s).

ii. Submit applications to: **Food and Drug Administration**
Center for Veterinary Medicine (HFV-16)
5600 Fishers Lane
Rockville, MD 20857

viii. Prepare amendments, supplements, reports and other correspondence in the above format. Identify the submission with the assigned NADA number.

ix. If the submission is a supplemental application, full information shall be provided on each proposed change concerning any statement made in the approved application.

x. Submit page 1 and 2 of this form with each submission.

FOR FDA USE ONLY

AF0360-50B

Elanco

Rumensin®

Monensin Sodium

60

TM

Net Weight **50 lbs**

(22.68 kg)

Type A Medicated Article

Do Not Feed Undiluted

Feedlot Cattle:
A. For improved feed efficiency (cattle fed in confinement for slaughter)
B. For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii.*

Pasture Cattle: Slaughter, stocker, feeder, dairy and beef replacement heifers weighing more than 400 pounds--For increased rate of weight gain.

Mature Reproducing Beef Cows: For improved feed efficiency when receiving supplemental feed.

Goats: For the prevention of coccidiosis caused by *Eimeria crandallis, E. christenseni,* and *E. ninakohlyakimovae* in goats maintained in confinement.

Important: Must be thoroughly mixed in feeds before use. This product should be further diluted before mixing in the final feed. See back of bag for directions.

CAUTION: Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal.

Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.

Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions..

Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.

For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.

Do not feed to lactating dairy cows.

Do not feed to lactating dairy goats.

WARNING: When mixing and handling Rumensin 60, use protective clothing, impervious gloves and a dust mask. Operators should wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse with water.

Store in a cool, dry place. Not to be used after date printed on side of bag.

ELANCO PRODUCTS COMPANY
A DIVISION OF ELI LILLY AND COMPANY
INDIANAPOLIS, IN 46285, U.S.A.

Rumensin® 60

DIRECTIONS FOR USE

Read All Directions Carefully Before Mixing and Feeding

Active Drug Ingredients: Monensin (as monensin sodium) 60 g per pound

I. A. **Feedlot Cattle:** For improved feed efficiency.
Goats: For prevention of coccidiosis.

> **CAUTION:** Do not feed undiluted. Feed only to cattle being fed in confinement for slaughter. Feed only to goats maintained in confinement.

1. **Complete Feed Mixing and Feeding Directions:**

Thoroughly mix the following amounts of Rumensin 60 to make one ton of complete feed that provides either 5 to 30 g/ton monensin sodium for cattle or 20 g/ton monensin sodium for goats on a 90% dry matter basis (Table 1). **An intermediate blending step should be performed to insure an adequate mix.** Feed complete feed (5-30 g/ton) continuously to growing finishing beef cattle to provide not less than 50 nor more than 360 mg monensin per head per day. Feed complete feed (20 g/ton) continuously to goats as the sole ration.

Table 1.

Lbs. Rumensin 60 Per Ton of Complete Feed	Rumensin Concentration in Complete Feed 90% Dry Matter Basis (Grams Per Ton)
.08	5
.33	20[a]
.50	30[b]

[a]Cleared level for goats.
[b]Maximum approved concentration for cattle.

2. **Type B or C Medicated Feed Mixing Directions (Dry and Liquid)**

a) Dry or Liquid
Thoroughly mix the following amounts of Rumensin 60 to make one ton of Type B or C medicated feed to provide the levels shown in Table 2. Dry Only - **An intermediate blending step should be performed to insure an adequate mix.**

b) Liquid Limitations
1) The supplement pH must be between 4.3-7.1.
2) Stored Rumensin Liquid Type B or C Medicated Feeds should be recirculated or agitated for 10 minutes daily.

> **CAUTION:** Inadequate mixing (recirculation or agitation) of Rumensin Liquid Type B or C Medicated Feeds has resulted in increased Rumensin concentration which has been fatal to cattle and could be fatal in goats.

B. **Feedlot Cattle:** For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*. Feeding Directions: Feed continuously at a rate of not less than 10 grams nor more than 30 grams of monensin per ton of feed to provide not less than 100 nor more than 360 mg monensin per head per day depending on body weight during periods of exposure to coccidia or when coccidiosis is likely to be a hazard.

II. **Pasture Cattle (slaughter, stocker, and feeder, dairy and beef replacement heifers weighing more than 400 pounds):** For increased rate of weight gain. Feeding Directions: Feed at the rate of not less than 50 nor more than 200 mg per head per day in not less than one pound of Type C Medicated Feed or after the fifth day feed at the rate of 400 mg per head per day every other day in not less than 2 pounds of Type C Medicated Feed as monensin sodium. The concentration of Rumensin in the pasture Type C Medicated Feed must be between 25 and 400 grams per ton.

Mature Reproducing Beef Cows (on pasture or in dry lot): For improved feed efficiency when receiving supplemental feed. Feeding Directions: Feed 50 to 200 mg/hd/day. Blend into a minimum of 1 lb. of Type C Medicated Feed and either hand feed or mix into the total ration. Feed (other than the Type C Medicated Feed containing Rumensin) can be restricted to 95% (of normal requirements) when 50 mg of Rumensin is fed, and to 90% at 200 milligrams.

Cows on pasture or in dry lot must receive a minimum of 1 lb. of Type C Medicated Feed per head per day. Additionally, a minimum of 16 pounds (air-dry basis) of roughage such as silage, haylage, ammoniated straw, hay or equivalent feedstuffs should be fed in order to meet NRC recommendations for mature reproducing beef cows to gain 0.25 to 0.75 pounds per head per day. Standing, dried winter range forage may not be of adequate quality to result in improved efficiency when supplemented with Rumensin.

CAUTION: During the first 5 days, cattle should receive no more than 100 mg per day contained in not less than 1 lb. of feed.

Do not self feed.

Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.

For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.

Do not feed to lactating dairy cows.

Do not feed to lactating dairy goats.

1. **Type B or C Medicated Feed Mixing Directions:**
Thoroughly mix the following amounts of Rumensin 60 to make one ton of Type B or C Medicated Feed to provide the levels shown in Table 2. Dry Only - **An intermediate blending step should be performed to insure an adequate mix.**

CAUTION: Do not allow horses or other equines access to feeds containing monensin.

Table 2.

Amount of Rumensin 60 per ton of Unmedicated Feed (lbs.)	Rumensin Concentration in Type B or C Medicated Feed (g/ton)
.33	20[a]
.83	50
3.33	200
20.00	1200

[a]Cleared level for goats.

NEW WEIGHT ON BAG OR BULK

RUMENSIN TYPE B MEDICATED FEED

FEED ONLY TO CATTLE MAINTAINED IN CONFINEMENT

ELANCO
Agriculture Partner Worldwide

For the prevention and control of coccidiosis due to *Eimeria bovis* and *E. zuernii* in cattle maintained in confinement.

DO NOT FEED UNDILUTED

ACTIVE DRUG INGREDIENT
Rumensin (as Monensin Sodium) . . . . . 30 - 1200 g/ton

GUARANTEED ANALYSIS
Nutritional Ingredients . . . . . xxxx%

INGREDIENTS
As listed in AAFCO Handbook.

MIXING & FEEDING DIRECTIONS

Thoroughly mix the appropriate amount of Rumensin with grain and roughage to provide a Type B medicated feed as follows:

LEVEL OF RUMENSIN TYPE B MEDICATED FEED IN G/TON	DESIRED LEVEL OF RUMENSIN IN GRAMS PER TON IN COMPLETE FEED	NO. OF LBS OF TYPE B MEDICATED FEED TO BE MIXED WITH OTHER FEEDSTUFF TO MAKE A TON OF COMPLETE FEED	POUNDS OF GRAIN AND/OR ROUGHAGE	MILLIGRAMS RUMENSIN PER POUND OF COMPLETE FEED
100	10	200	1800	5
	20	400	1600	10
	30	600	1400	15
300	10	67	1933	5
	20	133	1867	10
	30	200	1800	15
600	10	33	1967	5
	20	67	1933	10
	30	100	1900	15
1200	10	17	1983	5
	20	33	1967	10
	30	50	1950	15

Feed complete feed (10 - 30 g/ton) continuously to cattle maintained in confinement to provide not less than 100 mg nor more than 360 mg of Rumensin per head daily.
CAUTION:

1. Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal
2. Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.
3. Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions.
4. Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.
5. For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.
6. Do not feed to lactating dairy cows.
7. Do not feed to lactating dairy goats.

MANUFACTURED BY

TAKE TIME

ELANCO

Agriculturas Partner Worldwide

NET WEIGHT ON BAG OR BULK

RUMENSIN TYPE C MEDICATED FEED

FEED ONLY TO CATTLE MAINTAINED IN CONFINEMENT

For the prevention and control of coccidiosis due to *Eimeria bovis* and *E. zuernii* in cattle maintained in confinement.

ACTIVE DRUG INGREDIENT

Monensin (as Monensin Sodium) . . . . . 10 - 30 g/ton

GUARANTEED ANALYSIS

Nutritional Ingredients . . . . . xxxx%

INGREDIENTS

As listed in AAFCO Handbook.

MIXING & FEEDING DIRECTIONS

Feed complete feed (10 - 30 g/ton) continuously to cattle maintained in confinement.

CAUTION:

1. Do not allow horses or other equines access to feeds containing Rumensin. Ingestion of Rumensin by horses has been fatal
2. Feeding undiluted or mixing errors resulting in high concentrations of Rumensin has been fatal to cattle and could be fatal to goats.
3. Rumensin medicated feed is intended for use in cattle or in goats. Consumption by unapproved species may result in toxic reactions.
4. Do not exceed the levels of Rumensin recommended in the feeding directions as reduced average daily gains may result.
5. For control of coccidiosis during an outbreak, medication with monensin should be initiated as soon as the diagnosis is established. The efficacy of monensin treatment of individual animals exhibiting clinical signs of coccidiosis has not been established.
6. Do not feed to lactating dairy cows.
7. Do not feed to lactating dairy goats.

MANUFACTURED BY

Expiration Date _____ Lot Number _____

9/89

TAKE TIME