

ENVIRONMENTAL ASSESSMENT  
FOR THE USE OF RUMENSIN<sup>®</sup> PREMIXES IN THE FEED OF  
REPRODUCING BEEF CATTLE

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

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ENVIRONMENTAL ASSESSMENT FOR THE USE OF RUMENSIN<sup>®</sup> PREMIXES  
IN THE FEED OF REPRODUCING BEEF CATTLE

1. DATE July 1986
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<sup>®</sup> Premixes in the feed of reproducing beef cattle. Monensin sodium is the active ingredient in the RUMENSIN Premixes. Between 50 and 200 mg monensin per day would be fed to each cow. 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. In reproducing beef cattle, this effect is reflected in increased efficiency of feed utilization. Approval of the use of RUMENSIN Premixes in the feed of reproducing beef cattle would result in a small increase in the total amount of monensin sodium sold in the United States.

RUMENSIN Premixes are already approved for use in the rations of cattle (up to 360 mg monensin/head/day) fed in confinement for slaughter (21CFR 558.355; December 16, 1975). RUMENSIN Premixes are also approved

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RUMENSIN<sup>®</sup> (monensin sodium, Elanco)

(21CFR558.355; 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. An Environmental Impact Analysis Report (8) has been provided for RUMENSIN Premixes fed to cattle. The current Environmental Assessment provides updated information and specifically addresses the use of RUMENSIN Premixes in reproducing beef cattle.

Approval of the proposed action would authorize the use of RUMENSIN Premixes for pastured cattle to be expanded to include reproducing beef cattle. Approval of the proposed action would also 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 use in the rations of reproducing beef 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) Pastures and calving lots 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.

## 5. IDENTIFICATION OF CHEMICAL SUBSTANCE

### A. RUMENSIN PREMIXES

RUMENSIN Premixes will be incorporated into rations of reproducing beef 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 cinnamomensis, an organism isolated from soil (1). 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 feedstock 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 (References 1 and 2; Appendix G)

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 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  $\text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Li}^+ > \text{Cs}^+$ .

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- $\beta$ -methoxy- $\alpha,\gamma$ ,2,8-tetramethyl-1,6-dioxaspiro[4,5]decane-7-butanoic acid.

CAS Registry Number:

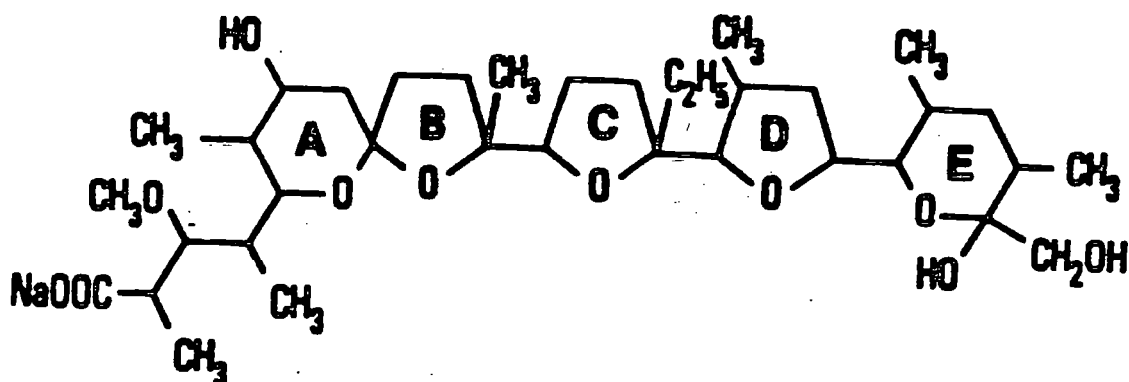
17090-79-8

Molecular Formula:

$\text{C}_{36}\text{H}_{62}\text{O}_{11}$  (acid),  
 $\text{C}_{36}\text{H}_{61}\text{O}_{11}\text{Na}$  (salt)

Molecular Weight:

670 (acid), 692 (sodium salt)

Structural Formula:Solubility

water

pH 7      63 mg/L  
 pH 9      0.85 mg/L

ethyl acetate  
 chloroform  
 acetone  
 benzene  
 methanol  
 hexane

very soluble  
 very soluble  
 very soluble  
 very soluble  
 very soluble  
 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.

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 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 (DEM)

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

<u>Location</u>	<u>Permit Identification No.</u>	<u>Issued</u>	<u>Expiration</u>
Clinton	83-09-87-0067	Dec. 13, 1983	Sept. 1, 1987
Clinton	83-09-87-0068	Dec. 13, 1983	Sept. 1, 1987
Clinton	83-09-87-0073	Dec. 13, 1983	Sept. 1, 1987
Lafayette	79-01-86-0264	Mar. 22, 1982	Jan. 1, 1986*
Lafayette	79-01-86-0277	Mar. 22, 1982	Jan. 1, 1986*

\*(These permits are being extended administratively by DEM until it issues the renewal permits, for which timely applications have been submitted by Eli Lilly and Company).

The following NPDES permits for the discharge of wastewaters from these plants to the Wabash River currently are administered by DEM's Office of Water Management.

<u>Location</u>	<u>NPDES Permit No.</u>	<u>Issued</u>	<u>Expiration</u>
Clinton	IN 0002852	September 23, 1985	August 31, 1990
Lafayette	IN 0002861	Issuance pending*	

\*(This permit is being extended administratively by DEM until it issues the renewal permit, for which a timely application has been submitted by Eli Lilly and Company).

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 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

The United States Department of Agriculture statistics indicate that there are about 33 million reproducing beef cows in the United States each year. The cow/calf industry exists in most areas of the United States, but is centered in the states of Texas, Oklahoma, Missouri, Kansas, Nebraska, Colorado, Montana, Wyoming, Washington, and Oregon. Direct marketing of RUMENSIN Premixes to major commercial feed mills will help to minimize environmental exposure during the product distribution process.

RUMENSIN would be used in the supplemental rations of reproducing beef cattle kept in pasture. RUMENSIN would also be used in the rations of reproducing beef cattle when they are confined for up to six months due to inclement weather during the late fall, winter, and early spring. Between 50 and 200 mg monensin/day will be fed to each cow in not less than one pound of supplemental feed. Suitable roughage fed at a rate of 15 to 20 lbs (air dried basis)/day would also be recommended. This recommendation yields 25 g/ton (200 mg/16 lbs) as the highest total concentration of monensin in the daily feed of reproducing beef cows. If all 33 million reproducing beef cows were fed a RUMENSIN Premix daily for up to six months, the maximum amount of monensin sodium that could be used annually would be  $1.20 \times 10^6$  kg (200 mg/head/day x 182 days x  $33 \times 10^6$  cows).

The actual average treatment level of monensin for these cows is expected to be about 110 mg/head/day. Only 10% of the 33 million reproducing beef cattle are expected to receive monensin in their diet. It is estimated that reproducing beef cows will actually be fed about  $66 \times 10^3$  kg (110 mg/head/day x 182 days x  $3.3 \times 10^6$  cows) of monensin sodium

in their diets each year. This is less than 10% 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 calving lot or into cropland soil by use of feces as fertilizer (Appendix A). Monensin and its metabolites are quantitatively excreted in cattle feces (4). Beef cattle fed a ration containing 40 g monensin/ton of feed (400 mg monensin/20 lbs of feed) had an average monensin concentration in their feces of 4.4 ppm. The highest recommended concentration of monensin in the feed of reproducing beef cows (200 mg/16 lbs of supplemental feed and roughage) is only 62.5% of the concentration (400 mg/20 lbs of feed) used in this study. The concentration of monensin in the feces of reproducing beef cattle would, therefore, be about 2.75 ppm ( $4.4 \text{ ppm} \times 0.625$ ).

Monensin is extensively metabolized in cattle, rats, chickens, dogs, sheep, pigs, and turkeys (3,4). 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 (4). About 50% to 60% of the monensin in an oral dose to cattle is metabolized (4). The primary monensin metabolite, 0-desmethyl monensin, is 20 times less biologically active than monensin, based on several test systems (3). This primary metabolite makes up about 5% of the total monensin and metabolite residue in cattle feces (4). 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 2.75 ppm is a realistic upper limit for monensin in the feces of reproducing beef cattle.

## 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 reproducing cows 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 calving lots.

### 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 ( $44.8 \times 10^3$  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  $2.25 \times 10^6$  kg. Wet manure from reproducing beef cows would contain, at most, 2.75 ppm of monensin. The highest expected concentration of monensin in cropland soil can then be calculated to be about 0.055 ppm ( $2.75 \text{ ppm} \times 44.8 \times 10^3 \text{ kg} / 2.25 \times 10^6 \text{ kg}$ ).

The concentration of monensin in soil would decline from the highest expected value of 0.055 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 B and C). 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 B). 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 C). 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  $^{14}\text{C}$  monensin (Appendix D). Extensive degradation of

monensin and its known metabolites would have had to occur to account for the apparent volatilization of  $^{14}\text{C}$ , perhaps as  $^{14}\text{CO}_2$ . 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.

## 2. Potential Monensin Concentration in Calving Lots

The highest asymptotic concentration of monensin in the soil of a calving lot can be calculated from information about the total amount of monensin excreted by cows and the dissipation rate constant of monensin in soil. A typical calving lot contains about 4 cows per acre. The highest consumption rate of monensin is 200 mg/head/day. About 50% of the monensin consumed by the cows could be excreted as the active parent compound (4). The highest daily excretion rate of monensin in a typical calving lot would be 400 mg/acre/day (200 mg/head/day X 0.5 X 4 hd/acre). To insure that the highest possible concentration of monensin in calving lot soil is calculated, it is assumed that all the monensin is located near the soil surface (top 1.36 inches). With this information, the initial concentration of monensin in the soil of a typical feedlot can be calculated as 0.0019 ppm as follows:

$$((400 \text{ mg/acre}) \div (1.36 \text{ in} \times 151763 \text{ kg soil/acre inch})).$$

Daily addition of monensin from excrement would be countered by loss of monensin from soil by degradation. The highest asymptotic concentration of monensin in the soil of a calving lot is represented by the following formula:

$$C_{\max} = \frac{C_0}{1 - e^{-r}}$$

where

$C_{\max}$  = asymptotic concentration of monensin in the calving lot soil,

$C_0$  = initial concentration of monensin in the calving lot soil, (0.0019 ppm from calculation above),

$r$  = average dissipation rate constant of monensin in field soil (0.093 day<sup>-1</sup>; from Appendix C).

The highest possible concentration of monensin in the soil of a typical calving lot was calculated to be 0.021 ppm.

## B. POTENTIAL CONCENTRATION OF MONENSIN IN AQUATIC SYSTEMS

### 1. Potential Monensin Concentration in Runoff from Cropland

The highest total loss of monensin from soil would probably occur from cropland since cropland contains the highest estimated concentration of monensin in soil (0.055 ppm). 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 feces into soil in order for monensin to reach surface water. If it were possible for all of the monensin in the cattle feces applied to one acre of cropland to be dissolved into runoff from one rainfall event, a two inch runoff event would carry 49.9 g of monensin, or 0.243 mg monensin/L ((20 tons of feces/acre x 907 kg/ton x 2.75 mg monensin/kg feces) ÷ (2 inches x 102794 L/acre-in)).

Since monensin adsorbs to moderately textured soils (calculated  $K_d$  of 24.1 for a loam soil, Appendix E), it is improbable that all the monensin in a field could be lost in one large runoff event. The calculated  $K_d$  value of 24.1 indicates that with equal weights of soil and runoff water, the ratio of the concentrations and total amounts of monensin in soil and water is 24.1 to 1. The weight of 2 acre-inches of runoff water (205588 L) is equal to the weight of 1.36 acre-inches of soil (205588 kg). The highest expected concentration of monensin in cropland soil is 0.055 ppm. The highest concentrations of monensin in the top 1.36 inches of cropland soil after a runoff event and in two inches of runoff would be 0.0528 ppm and 0.0022 ppm, respectively, providing a 24 to 1 ratio. This estimated concentration of monensin in runoff water (0.002 ppm) 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.

An estimate of the maximum expected concentration of monensin in runoff from cropland was calculated using the CREAMS (Chemical, Runoff, and Erosion from Agricultural Management Systems) model developed by the USDA Agricultural Research Service (Appendix E). The model estimates chemical yield in runoff from field-sized areas using daily rainfall records. Input parameters and the geographical locations used for the model were selected to maximize the potential for runoff from cropland where monensin might be found in the soil (Appendix E). The model

simulations were conducted for a twenty-year period, with 20 tons of wet cattle manure containing 2.75 ppm monensin incorporated into the soil each year. Conventional tillage, a high average field slope and row-cropped corn were used in the simulation to maximize runoff. In order to increase the opportunities for monensin to be lost into runoff, a 20-year simulation was conducted for annual manure application on April 1 (Julian Day 91). Since the degradation of monensin in field soil is moderately rapid, the date of the first runoff event after manure application was critical. Another date of manure application was selected for a second 20-year simulation to coincide with the date (April 30, Julian Day 120) of the second largest spring rainfall event (3.58 inches) and runoff event (2.0 inches) in 20 years for the selected site. The maximum concentration of monensin in the aqueous phase of runoff for both 20 year simulations was 0.0015 ppm. This maximum concentration was found in a low volume of runoff water the same days (Julian Day 91 and 120) that monensin was incorporated into the field. The maximum annual yield of monensin in runoff of sediment and water occurred in the year with the second largest spring runoff event in 20 years and was 0.81 % of the total applied on Julian Day 120 (April 30). If manure containing monensin was applied as early as April 1, when another smaller rainfall and runoff event occurred, the maximum annual yield of monensin in runoff of sediment and water was only 0.20 % of the total applied annually. Because of the relatively short half-life for monensin in soil, total annual yield monensin was only moderately impacted by a large number of runoff events in a single year. The calculated maximum annual yields

for monensin are within the annual yield values (<1.5%) suggested by Wauchope (5) and Willis and McDowell (6) for compounds with characteristics similar to those 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 F).

Based on the moderately short half-life of monensin in soil, runoff events would have to occur soon after application of cattle feces to field soil for monensin to be carried to natural aquatic systems. Dilution of the maximum expected concentration of monensin in runoff water (0.0015 ppm) by natural aquatic systems would result in nontarget organisms being exposed to low levels of monensin. These low concentrations of monensin may, however, 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. Because the n-octanol/water partition coefficient is not available for monensin and because the solubility of monensin in water appears to vary with pH, the extent of any accumulation of monensin in aquatic organisms is not easily estimated. Monensin is a large charged molecule that may not readily pass across a gill membrane. It is possible that these properties could

restrict the uptake rate of the chemical through a normal route for bioconcentration of a chemical by aquatic organisms (7). Monensin is metabolized and excreted by chickens, cattle, rats, dogs, sheep, pigs, and turkeys and does not appear to concentrate in fatty tissue (3,4). Monensin also rapidly dissipates from soil (Appendices B, C, and D). When monensin is absorbed by fish, the compound may be metabolized and excreted, as it is in higher vertebrates.

#### 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 through a soil column relative to the movement of water indicates that monensin adsorbs fairly strongly to loam soil ( $K_d$  estimated to be about 24.1, Appendix E). 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<sub>50</sub> 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<sup>3</sup> 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<sup>3</sup>.

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).

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 for reproducing beef cattle 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.

### 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<sub>50</sub>, 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 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 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 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 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  $\geq 4.4$  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  $\leq 3.1$  ppm.

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 (hypoactivity, 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 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 (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 ( $49.3 \times 10^3$  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 A). The plot containing manure from cattle fed 40 g monensin/ton of feed had, therefore, a monensin sodium concentration of approximately 0.145 ppm ( $(49.3 \times 10^3 \text{ kg/ha} \times 4.4 \text{ mg/kg}) \div (4 \text{ inches} \times 375,000 \text{ kg/ha-inch soil})$ ). The plant 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 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 0.0015 ppm. The 96-hr LC<sub>50</sub> values for rainbow trout and bluegill and the 48-hr EC<sub>50</sub> value for daphnids range from 9.0 to 16.6 ppm. These acute median lethal and acute median effect concentrations are about 6,000 to 11,067 times higher than the highest expected monensin concentration in runoff. 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 467 times higher than the maximum expected concentration of monensin in runoff. Even if it were possible for all of the monensin in the cattle manure used in a watershed to be extracted into runoff (Section 7B), the concentration of monensin in runoff would be 0.243 ppm. This concentration (0.243 ppm) is about three times lower than the concentration (0.70 ppm) at which no mortalities or behavior abnormalities were found for fish or daphnids.

The highest expected concentration of monensin in runoff (0.0015 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 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 60 and 111 times higher than the highest expected concentration (0.0015 ppm) of monensin in runoff. Even if it were possible for all of

the monensin in the cattle feces used in a watershed to be extracted into runoff, the concentration of monensin in that runoff (0.243 ppm) would only be about three times higher than the concentration of monensin (0.09 ppm) calculated to be chronically safe to the most sensitive species tested.

Based on the maximum expected monensin concentration (0.0015 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 maximum expected concentration of monensin in the soil of cropland was estimated to be 0.055 ppm (Section 7A). Monensin concentrations in soil decline relatively rapidly in the greenhouse ( $t_{1/2}$ =5.8 days) and in the field ( $t_{1/2}$ =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 182 times higher than the highest expected concentration of monensin in soil, earthworms should not be affected by monensin in cattle manure used as fertilizer.

### 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. Mean body weight gain was reduced somewhat for mallards and bobwhite at monensin dietary levels of 160 ppm (0.016%) and 200 ppm (0.02%), respectively. No physical signs of toxicity, mortalities or reduction in food consumption were found at these higher dietary levels for these two species of birds.

The highest recommended use rates of monensin sodium is 200 mg/lb of supplemental feed (400 g/ton or 441 ppm). The highest dietary concentration of monensin sodium in the total feed of reproducing beef cattle is 200 mg/16 lbs (25 g/ton or 27.6 ppm). If wild birds foraged only on supplemental feed for reproducing beef cattle with the highest recommended level of monensin for five days, reductions in body weight gain might occur. Monensin in supplemental feed is not freely available to large populations of birds. If wild birds foraged only on the total feed of reproducing beef cattle, reductions in body weight would not be expected. The proposed action would not be expected to substantially affect 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 18 times higher than

the highest expected monensin concentration of 0.055 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 2.6 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.

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 reproducing beef cattle 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.

#### 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.

12. LIST OF PREPARERS

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

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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

Merle E. Amundson, Ph.D.  
Executive Director  
Toxicology Division  
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August 4, 1986  
Date

14. REFERENCES

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APPENDIX A: 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.

## APPENDIX A (Continued)

Table 1

## Assay for Monensin in Cattle Feces

	<u>Replicate</u>	<u>Monensin Found (ppm)</u>			<u>Overall</u> $\bar{X} \pm \text{s.d.}$
		<u>Day 1</u>	<u>Day 2</u>	<u>Mean</u>	
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 $\pm$ .56
	2	3.9	4.8	4.4	

## 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<sup>2</sup> soil flat lined with plastic. The flat was maintained in the greenhouse 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.

## APPENDIX B: (Continued)

Table 1  
Degradation of Monensin in Soil

Sampling Time	With Feces		Without Feces	
	PPM	% of Initial	PPM	% of Initial
Zero	1.4 <sup>1,2</sup>	100	1.2 <sup>1,2</sup>	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 <sup>-1</sup> )	0.119		0.095	
R <sup>2</sup>	0.72		0.89	

<sup>1</sup>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.

<sup>2</sup>Test sensitivity was 0.1 to 0.2 ppm.

## APPENDIX C: 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<sup>2</sup> 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.

## APPENDIX C (Continued)

Table 1  
PPM Monensin in Field Soil <sup>a/</sup>

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 <sup>-1</sup> )	0.092		0.094	
R <sup>2</sup>	0.91		0.91	

<sup>a/</sup> 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 D: 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  $^{14}\text{C}$  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  $^{14}\text{C}$  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  $^{14}\text{CO}_2$ .

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  $^{14}\text{C}$  monensin puts the  $^{14}\text{C}$  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  $^{14}\text{C}$  is probably through volatilization, perhaps as  $^{14}\text{CO}_2$ . 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

## APPENDIX D (Continued)

agree with the studies conducted by microbiological assay. 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  $^{14}\text{C}$  Monensin.

<u>Time Interval</u>	<u>Radioactivity dpm/g Soil</u>	<u>% of Initial</u>
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

## APPENDIX E

Title: Use of the CREAMS (Chemicals, Runoff, and Erosion from Agricultural Management Systems) Model to Estimate the Maximum Concentration of Monensin in Runoff Water from Cropland

Authors: P. J. Cocke and R. D. Meyerhoff

Introduction:

Use of monensin in the feed of reproducing beef cattle could result in concentrations of monensin of up to 2.75 ppm in the wet feces from cattle. If this wet manure were applied to cropland as fertilizer, runoff occurring soon after application could transport small amounts of monensin into surface waters. The variable dilution available in receiving waters could make it difficult to estimate the highest expected monensin concentration to which aquatic organisms might be exposed. Aquatic organisms would certainly never be exposed to monensin concentrations greater than the maximum expected concentration in runoff water.

The highest expected concentration and total annual yield of monensin in runoff from cropland was estimated using the CREAMS (Chemicals, Runoff, and Erosion from Agricultural Management Systems) model developed by the USDA Agricultural Research Service. CREAMS is a daily simulation model that estimates runoff, erosion and sediment transport, and chemical yield from field-sized areas (1,2). The model uses the SCS curve number method to estimate surface runoff from daily rainfall data (3). Evapotranspiration and percolation algorithms are included to maintain a continuous water balance of the agricultural system. The Universal Soil Loss Equation is used in the model to incorporate the effects of topography, soil characteristics, crop cover, and meteorological conditions on soil erosion and sediment transport (4). In estimating the loss of a chemical from cropland, CREAMS accounts for the processes of sorption and degradation in the soil. A thorough discussion of the conceptual basis for the CREAMS model is provided in the user's manual (1).

Parameter selection, site selection, and model assumptions were generally made to maximize model estimates of the loss of monensin in runoff. Two 20-year simulations were run for the annual application of 20 tons per acre ( $4.48 \times 10^4$  kg/ha) of wet cattle manure containing 2.75 ppm monensin. The manure was incorporated into the soil each spring prior to planting straight-row corn. It was assumed that the corn was maintained on a field having a 5% slope using conventional tillage practices without contouring.

The first 20-year simulation was run for an annual manure application on April 1 (Julian day 091). This date was chosen to increase the opportunities for monensin loss in large spring runoff events. Since the half-life of monensin in field soil is short, the length of time between a

## APPENDIX E (Continued)

manure application and the first runoff event is critical. A second 20-year simulation was run for an annual manure application on April 30 (Julian day 120). This application date coincided with the second largest rainfall (9.1 cm) and runoff (5.2 cm) events to occur in the 20-year precipitation record selected for the site.

Methods:

The selection of the geographical location for the simulation was based on three criteria: 1) it must be representative of a major cattle production area; 2) it must be representative of major cropland to which cattle manure can be applied, and 3) it must have meteorologic and hydrologic conditions that facilitate runoff. The first two criteria were met by visually comparing U.S. maps showing the geographical distribution of major crops and cattle production (5,6). This comparison identified corn as the representative crop. Corn grown in straight rows using conventional tillage without contouring was selected to maximize the potential loss of monensin in runoff.

Cattle and corn production overlap in several states. Information on rainfall, hydrologic conditions, and soil type (6) was compared for the states where the largest production of corn and cattle occur (Table 1). Illinois was selected as the representative site for the runoff simulations because of high annual precipitation and slow infiltration rates which result in a moderate to high potential for runoff.

Operation of the CREAMS model required the estimation of parameters for three model components: hydrology, erosion/sediment transport, and chemistry. Once a particular geographical setting, soil type, and cropping pattern were selected, the CREAMS user's guide provided most of the parameters needed for the hydrology and erosion components (1,2). Parameters for the chemistry component of the model were obtained from environmental fate studies summarized for monensin in the adjoining appendices. Below is a description of the selection of model parameters.

**Hydrology:** Table 2 lists the general characteristics of the representative site (#3) in Illinois. Twenty years of daily rainfall data was obtained for meteorological station #8179 (Springfield, IL) from the National Center for Atmospheric Research database developed by EPA-Athens, GA. This database was also used to obtain monthly mean temperature values. Monthly mean solar radiation for Illinois was obtained from the CREAMS user's manual (2).

The physical setting for the simulation was a square 16.2 ha (40 acre) field planted in continuous corn with no winter cover crop. The field was assumed to have a high uniform 5% slope with a slope length of 402 m. The soil was a silt loam with 2.5% organic matter in the surface zone and

## APPENDIX E (Continued)

an average of 1.25% organic matter through the 90-cm root zone. The hydrologic soil group was C which represents a slow infiltration rate (3). The remaining hydrology parameters (leaf area index, soil drainage parameters) were taken from the user's guide for CREAMS. The specific set of hydrology parameters used for the April 30 (Julian day 120) application date is listed in Table 2. This data set corresponds to the format specified in the CREAMS user's manual (2).

Erosion: Parameters for the erosion/sediment yield component were obtained from the CREAMS user's manual (1,2). Soil loss ratios, contouring factors, and soil roughness factors were chosen to represent conventional tillage, moderate yields of corn, and partially shredded stalks. The particle size distribution assumed for the silt loam soil was 20% sand, 60% silt, and 20% clay. The simulation of manure application on April 1 assumed planting on May 1 and harvest on October 1. The simulation for application of manure on April 30 assumed planting on June 1 and harvest on October 30. The specific erosion parameters used in the model for the April 30 (Julian day 120) application date are listed in Table 3 in the format indicated by the program user's guide (2).

Chemistry: The chemistry component of the CREAMS model required parameters describing the use pattern and environmental behavior of monensin. Application of cattle manure with a monensin concentration of 2.75 ppm was assumed to occur at a rate of  $4.48 \times 10^4$  kg/ha (20 tons/acre) on April 1 or April 30 of each year of the 20-year simulations. This is equivalent to a monensin application rate of 0.202 kg/ha. The material was uniformly incorporated into the soil to a depth of 15 cm (6 inches). The decay rate constant of monensin in soil was obtained from a field study (Monensin Field Soil Decline Study, Study Number A22-B50-3270, Appendix C). The decay constant was  $0.093 \text{ day}^{-1}$ , which corresponds to a half-life of 7.5 days. The solubility of monensin in water was assumed to be 100 ppm. Model results are only affected by solubilities less than 1 ppm. The soil/water distribution coefficient ( $K_d$ ) for monensin was determined from the results of a column leaching study with a loam soil (Laboratory Soil Leaching Study with Monensin, Appendix G). The movement of monensin through the soil column relative to movement of water was expressed as a function of  $K_d$ , known as the retardation factor (7). The value of  $K_d$  calculated from the retardation factor was 24.1. The exact chemical parameters used in the model for the April 30 (Julian day 120) application date are listed in Table 4 in the format indicated by the program user's guide (2).

Results and Discussion:

The results of the 20-year runoff simulations are summarized in Table 5. Annual precipitation and the hydrologic response of the representative site are given in the first four columns of the table. Total annual precipitation ranged from 63.2 to 109.2 cm (24.5 to 43.0 in.) and averaged 86.4 cm (34.0 in.). Total annual runoff ranged from 3.3 to 21.4 cm (1.3 to 8.4 in.) and averaged 12.4 cm (4.9 in.). An average of 26 runoff events occurred each year; however, less than seven of these events contained monensin.

## APPENDIX E (Continued)

Since the half-life of monensin in soil is short, the annual loss of monensin in runoff was highly dependent on the amount of time between a manure application and the first runoff event. For the April 1 application, total annual monensin losses in runoff ranged from < 0.005 to 0.20 percent of the amount applied (Table 5). Annual losses ranged from < 0.005 to 0.81 percent of the amount applied for the April 30 application. In general, annual monensin losses greater than 0.10% only resulted when runoff occurred within one week of an application. The maximum annual loss of 0.81% occurred in a year when the application date (April 30, Julian day 120) coincided with the second largest spring rainfall (9.1 cm) and runoff (5.2 cm) events in the 20-year simulation period. This maximum annual monensin loss was within the annual yield values (< 1.5%) suggested by Wauchope (8) and Willis and McDowell (9) for soil-incorporated compounds with characteristics similar to those of monensin.

Tables 6 and 7 summarize the aqueous monensin concentrations in the simulated runoff for the April 1 and April 30 manure applications. Aqueous monensin concentrations in runoff ranged from < 0.0001 to 0.0015 mg/L for both application dates. The maximum concentration of 0.0015 mg/L was not associated with the largest runoff events, but rather with runoff events that occurred on the day of an application of manure containing monensin. Monensin concentrations in runoff greater than 0.0005 ppm resulted from runoff events that occurred within 10 days of an application.

## APPENDIX E (Continued)

TABLE 1.  
 METEOROLOGICAL AND SOIL CHARACTERISTICS OF  
 REPRESENTATIVE SITES ASSOCIATED WITH CORN<sup>a</sup> AND  
 MAXIMUM PRODUCTION OF CATTLE MANURE.

Site #	State	Meteoro- logical Station # <sup>b</sup>	Annual Precip (In)	Temp (°C)	Hydro- logic Soil Group <sup>c</sup>	% Organic Carbon	Predominant Soil Type
1	CO	834	16.3	10.6	C	1-2	Loamy sand
2	NE	3395	23.2	10.6	D	1-2(+)	Silty Clay loam
3	IL	8179	34.0	11.9	C	1-2(+)	Silt loam
4	MI	7690	34.6	9.4	B	1-2(+)	Loam
5	OH	1466	36.1	9.2	B	1-2	Silt Loam

<sup>a</sup>From Dean et al., 1984 (6).

<sup>b</sup>Selected from National Center for Atmospheric Research data base developed by EPA-Athens, GA.

<sup>c</sup>Defined in SCS, USDA, 1972 (3)

A - high infiltration rate - low runoff potential

B - moderate infiltration rate

C - slow infiltration rate

D - very slow infiltration rate - high runoff potential



## APPENDIX E (Continued)

TABLE 3.  
 PARAMETER SET FOR THE EROSION COMPONENT OF THE CREAMS MODEL USED TO  
 SIMULATE MONENSIN LOSS IN RUNOFF FOLLOWING AN APPLICATION ON APRIL 30.  
 THIS DATA SET CORRESPONDS TO THE FORMAT SPECIFIED IN THE CREAMS USER'S MANUAL (2).

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EROSION PARAMETERS - MONENSIN / CATTLE RUNOFF SIMULATION									
ILLINOIS / SILT LOAM									
CLIMATE STATION #8179, 1/54 - 12/73									
54	73	0	1	0	1	0			
0.20	0.60	0.20	0.025	20.0	4.0	0.05	1000.0		
40.0	1320.0	0.05	0.05	0.05	0.05	1320.0	0.0	1320.0	0.0
1	1.0	0.40							
1	MANAGEMENT PARAMETERS								
001	120	150	199	228	234	240	255	303	
1	1.0								
0.27	0.43	0.64	0.56	0.43	0.32	0.25	0.21	0.27	
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.03	

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## APPENDIX E (Continued)

TABLE 4.

PARAMETER SET FOR THE CHEMICAL COMPONENT OF THE CREAMS MODEL USED TO SIMULATE MONENSIN LOSS IN RUNOFF FOLLOWING AN APPLICATION ON APRIL 30. THIS DATA SET CORRESPONDS TO THE FORMAT SPECIFIED IN THE CREAMS USER'S MANUAL (2).

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CHEMICAL PARAMETERS - MONENSIN / CATTLE RUNOFF SIMULATION  
ILLINOIS / SILT LOAM  
CLIMATE STATION #8179, 1/54 - 12/73

54001	0	0	1	0	3	1				
0.430	0.320	1.250								
1	54119	73365								
1	MONENSIN		63.0	0.0	0.093	24.1	0.000	0.000	0.000	
1120	1366	1								
1	0.1232	15.24	1.000	0.000	1.000	0.000				
	0									

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APPENDIX E (Continued)

TABLE 5. ANNUAL SUMMARY OF RAINFALL, ESTIMATED RUNOFF, AND ESTIMATED MONENSIN LOSS.

Year	Rainfall (cm)/ No. Storms	Runoff (cm)/ No. Events	No. of Runoff Events Containing Monensin		Total Annual Monensin Loss (% of Applied)	
			April 1 Application	April 30 Application	April 1 Application	April 30 Application
1	63.2 / 101	3.3 / 18	6	4	< 0.005	< 0.005
2	91.2 / 101	15.1 / 30	9	7	0.02	0.02 <sup>b</sup>
3	76.9 / 99	8.2 / 23	5	9	0.01	0.02 <sup>b</sup>
4	104.1 / 117	12.6 / 34	15	10	0.16 <sup>a</sup>	0.01
5	81.5 / 104	12.4 / 21	6	8	0.04 <sup>a</sup>	0.03 <sup>a</sup>
6	89.7 / 98	19.4 / 22	1	4	< 0.005	< 0.005 <sup>b</sup>
7	99.9 / 99	16.0 / 35	12	13	0.03	0.03 <sup>b</sup>
8	95.5 / 102	12.6 / 28	5	8	0.03	0.09 <sup>a</sup>
9	78.6 / 101	12.0 / 26	5	5	< 0.005	0.03 <sup>a</sup>
10	74.1 / 80	11.1 / 23	5	7	< 0.005	0.01
11	78.3 / 90	12.5 / 24	6	2	0.20 <sup>a</sup>	< 0.005
12	95.3 / 98	17.1 / 30	8	5	0.08 <sup>a</sup>	0.05
13	82.1 / 97	9.6 / 26	5	2	0.05	0.03 <sup>a</sup>
14	84.8 / 117	8.6 / 24	2	6	0.01	0.09 <sup>a</sup>
15	82.4 / 105	11.8 / 30	6	7	0.15 <sup>a</sup>	0.06
16	92.2 / 107	13.5 / 30	2	4	0.12 <sup>a</sup>	< 0.005
17	96.6 / 115	15.3 / 24	10	7	0.14 <sup>b</sup>	0.81 <sup>c</sup>
18	70.6 / 94	7.5 / 25	5	6	< 0.005	0.01 <sup>a</sup>
19	81.6 / 116	7.5 / 24	3	4	0.02	< 0.005
20	109.2 / 119	21.4 / 28	7	5	0.07	0.03 <sup>a</sup>
Means:	86.4 / 103	12.4 / 26	6.2	6.2	0.06	0.07

<sup>a</sup> Runoff occurred within one week of the application of the manure containing monensin.

<sup>b</sup> Runoff occurred on the day manure containing monensin was applied to the soil.

<sup>c</sup> The second largest spring rainfall event (3.58 in ) and spring runoff event (2.0 in) in 20 years of precipitation data occurred on the day manure with monensin was applied to soil.

TABLE 6. SUMMARY OF AQUEOUS MONENSIN CONCENTRATIONS IN RUNOFF FOR THE APRIL 1 (JULIAN DAY 091) APPLICATION OF CATTLE MANURE CONTAINING MONENSIN.

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
14APR54	54104	1.70	0.05	0.0004
22APR54	54112	1.65	0.03	0.0002
31MAY54	54151	2.44	0.08	< 0.0001
02JUN54	54153	1.85	0.05	< 0.0001
08JUN54	54159	3.25	0.43	< 0.0001
13JUN54	54164	2.29	0.18	< 0.0001
11APR55	55101	2.59	0.20	0.0006 <sup>b</sup>
23APR55	55113	2.92	0.38	0.0002
10MAY55	55130	1.42	0.03	< 0.0001
12MAY55	55132	1.88	0.13	< 0.0001
13MAY55	55133	1.27	0.05	< 0.0001
21MAY55	55141	1.83	0.08	< 0.0001
28MAY55	55148	3.15	0.56	< 0.0001
11JUN55	55162	1.65	0.08	< 0.0001
14JUL55	55195	8.74	3.07	< 0.0001
28APR56	56119	3.84	0.79	0.0001
29APR56	56120	1.37	0.05	0.0001
26MAY56	56147	3.81	0.63	< 0.0001
31MAY56	56152	2.21	0.20	< 0.0001
22JUN56	56174	2.62	0.41	< 0.0001
03APR57	57093	4.42	0.94	0.0012 <sup>b</sup>
07APR57	57097	1.70	0.10	0.0008 <sup>b</sup>
20APR57	57110	2.03	0.10	0.0002
24APR57	57114	4.24	1.24	0.0001
25APR57	57115	1.47	0.13	0.0001
26APR57	57116	2.54	0.63	0.0001
27APR57	57117	1.83	0.30	0.0001
16MAY57	57136	1.68	0.08	< 0.0001
17MAY57	57137	1.70	0.18	< 0.0001
21MAY57	57141	1.42	0.05	< 0.0001
22MAY57	57142	1.07	0.03	< 0.0001
10JUN57	57161	3.33	0.51	< 0.0001
11JUN57	57162	2.46	0.51	< 0.0001
14JUN57	57165	2.06	0.36	< 0.0001
13JUL57	57194	4.50	0.84	< 0.0001

TABLE 6. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
05APR58	58095	3.15	0.38	0.0010 <sup>b</sup>
03MAY58	58123	1.90	0.05	0.0001
10JUN58	58161	11.40	5.92	< 0.0001
13JUN58	58164	3.15	0.74	< 0.0001
25JUN58	58176	2.46	0.18	< 0.0001
11JUL58	58192	4.01	0.58	< 0.0001
28MAY59	59148	1.80	0.03	< 0.0001
15APR60	60106	2.64	0.36	0.0004
16APR60	60107	2.01	0.33	0.0003
17APR60	60108	1.12	0.05	0.0003
20APR60	60111	1.27	0.03	0.0002
29APR60	60120	1.63	0.05	0.0001
16MAY60	60137	1.83	0.05	< 0.0001
19MAY60	60140	1.47	0.03	< 0.0001
25MAY60	60146	3.23	0.76	< 0.0001
05JUN60	60157	1.73	0.05	< 0.0001
11JUN60	60163	4.88	1.40	< 0.0001
20JUN60	60172	4.78	1.52	< 0.0001
23JUN60	60175	5.94	2.57	< 0.0001
24APR61	61114	4.98	1.35	0.0002
05MAY61	61125	2.62	0.23	< 0.0001
07MAY61	61127	2.95	0.56	< 0.0001
08MAY61	61128	2.31	0.43	< 0.0001
30JUN61	61181	4.04	0.53	< 0.0001
30APR62	62120	1.85	0.03	0.0001
01MAY62	62121	1.96	0.13	0.0001
08MAY62	62128	1.55	0.05	< 0.0001
09JUN62	62160	5.33	1.80	< 0.0001
13JUL62	62194	3.63	0.51	< 0.0001
29APR63	63119	1.78	0.03	0.0001
12MAY63	63132	2.49	0.15	< 0.0001
27MAY63	63147	1.90	0.03	< 0.0001
04JUL63	63185	3.38	0.23	< 0.0001
06JUL63	63187	6.58	2.31	< 0.0001

## APPENDIX E (Continued)

TABLE 6. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
02APR64	64093	2.84	0.30	0.0012 <sup>b</sup>
05APR64	64096	3.20	0.56	0.0009 <sup>b</sup>
19APR64	64110	6.45	2.92	0.0002
20APR64	64111	4.72	2.18	0.0002
27APR64	64118	2.87	0.58	0.0001
05JUN64	64157	2.29	0.10	< 0.0001
05APR65	65095	3.40	0.53	0.0010 <sup>b</sup>
11APR65	65101	2.13	0.23	0.0005 <sup>b</sup>
14APR65	65104	1.75	0.10	0.0004
25APR65	65115	1.90	0.10	0.0001
26MAY65	65146	2.29	0.13	< 0.0001
01JUN65	65152	9.83	4.90	< 0.0001
08JUN65	65159	2.21	0.23	< 0.0001
20JUN65	65171	2.11	0.10	< 0.0001
11APR66	66101	2.59	0.15	0.0006 <sup>b</sup>
20APR66	66110	4.57	1.45	0.0002
23APR66	66113	2.72	0.43	0.0002
17MAY66	66137	3.43	0.63	< 0.0001
21MAY66	66141	2.69	0.46	< 0.0001
06MAY67	67126	3.89	1.09	< 0.0001
30MAY67	67150	2.51	0.23	< 0.0001
03APR68	68094	4.75	1.32	0.0011 <sup>b</sup>
22MAY68	68143	3.51	0.51	< 0.0001
23MAY68	68144	5.00	2.06	< 0.0001
25MAY68	68146	3.02	0.76	< 0.0001
14JUN68	68166	4.55	1.02	< 0.0001
15JUN68	68167	2.31	0.46	< 0.0001
04APR69	69094	1.70	0.05	0.0011 <sup>b</sup>
18APR69	69108	8.18	3.94	0.0003
01APR70	70091	2.34	0.23	0.0015 <sup>c</sup>
18APR70	70108	5.13	1.57	0.0003
19APR70	70109	2.49	0.58	0.0002
30APR70	70120	9.09	5.21	0.0001
09MAY70	70129	1.55	0.03	< 0.0001
13MAY70	70133	1.52	0.05	< 0.0001
01JUN70	70152	2.49	0.18	< 0.0001
04JUN70	70155	3.53	0.71	< 0.0001

## APPENDIX E (Continued)

TABLE 6. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
05JUN70	70156	1.57	0.18	< 0.0001
14JUN70	70165	2.41	0.23	< 0.0001
06MAY71	71126	2.08	0.05	0.0001
11MAY71	71131	2.18	0.15	< 0.0001
23MAY71	71143	1.80	0.03	< 0.0001
24MAY71	71144	1.88	0.13	< 0.0001
10JUL71	71191	6.65	2.41	< 0.0001
19APR72	72110	3.25	0.53	0.0002
21APR72	72112	1.88	0.18	0.0002
14JUN72	72166	2.08	0.05	< 0.0001
09APR73	73099	1.57	0.05	0.0007 <sup>b</sup>
16APR73	73106	1.93	0.10	0.0004
21APR73	73111	5.36	1.96	0.0002
22APR73	73112	2.82	0.86	0.0002
07MAY73	73127	2.44	0.30	< 0.0001
18JUN73	73169	6.65	3.02	< 0.0001
26JUN73	73177	2.13	0.15	< 0.0001

<sup>a</sup> First two digits represent the year and the last three digits represent the day of the year.

<sup>b</sup> Runoff event that occurred within 10 days of a monensin application (Julian day 091).

<sup>c</sup> Runoff event that occurred on the day of a monensin application (Julian day 091).

## APPENDIX E (Continued)

TABLE 7. SUMMARY OF AQUEOUS MONENSIN CONCENTRATIONS IN RUNOFF FOR THE APRIL 30 (JULIAN DAY 120) APPLICATION OF CATTLE MANURE CONTAINING MONENSIN.

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
31MAY54	54151	2.44	0.10	0.0001
02JUN54	54153	1.85	0.08	0.0001
08JUN54	54159	3.25	0.53	< 0.0001
13JUN54	54164	2.29	0.20	< 0.0001
10MAY55	55130	1.42	0.03	0.0006 <sup>b</sup>
12MAY55	55132	1.88	0.13	0.0005
13MAY55	55133	1.27	0.05	0.0004
21MAY55	55141	1.83	0.08	0.0002
28MAY55	55148	3.15	0.63	0.0001
11JUN55	55162	1.65	0.10	< 0.0001
14JUL55	55195	8.74	3.58	< 0.0001
29APR56	56120	1.37	0.05	0.0015 <sup>c</sup>
26MAY56	56147	3.81	0.66	0.0001
31MAY56	56152	2.21	0.20	0.0001
21JUN56	56173	1.83	0.08	< 0.0001
22JUN56	56174	2.62	0.48	< 0.0001
03JUL56	56185	2.49	0.23	< 0.0001
12JUL56	56194	2.59	0.28	< 0.0001
31JUL56	56213	5.54	1.45	< 0.0001
01AUG56	56214	6.63	3.51	< 0.0001
16MAY57	57136	1.68	0.08	0.0003
17MAY57	57137	1.70	0.18	0.0003
21MAY57	57141	1.42	0.05	0.0002
22MAY57	57142	1.07	0.03	0.0002
10JUN57	57161	3.33	0.61	< 0.0001
11JUN57	57162	2.46	0.58	< 0.0001
14JUN57	57165	2.06	0.36	< 0.0001
28JUN57	57179	2.11	0.23	< 0.0001
13JUL57	57194	4.50	1.17	< 0.0001
15JUL57	57196	2.54	0.56	< 0.0001

TABLE 7. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
03MAY58	58123	1.90	0.05	0.0011 <sup>b</sup>
01JUN58	58152	1.47	0.03	0.0001
10JUN58	58161	11.40	6.30	< 0.0001
13JUN58	58164	3.15	0.76	< 0.0001
25JUN58	58176	2.46	0.20	< 0.0001
11JUL58	58192	4.01	0.79	< 0.0001
14JUL58	58195	1.73	0.08	< 0.0001
27JUL58	58208	4.14	0.86	< 0.0001
28MAY59	59148	1.80	0.05	0.0001
18JUL59	59199	2.62	0.08	< 0.0001
23JUL59	59204	2.92	0.25	< 0.0001
06AUG59	59218	7.21	3.28	< 0.0001
29APR60	60120	1.63	0.05	0.0015 <sup>c</sup>
16MAY60	60137	1.83	0.08	0.0003
19MAY60	60140	1.47	0.03	0.0002
25MAY60	60146	3.23	0.81	0.0001
05JUN60	60157	1.73	0.08	< 0.0001
11JUN60	60163	4.88	1.57	< 0.0001
16JUN60	60168	1.45	0.05	< 0.0001
20JUN60	60172	4.78	1.60	< 0.0001
23JUN60	60175	5.94	2.59	< 0.0001
30JUN60	60182	2.16	0.20	< 0.0001
10JUL60	60192	3.02	0.43	< 0.0001
13JUL60	60195	2.46	0.38	< 0.0001
25JUL60	60207	3.30	0.48	< 0.0001
05MAY61	61125	2.62	0.23	0.0009 <sup>b</sup>
07MAY61	61127	2.95	0.53	0.0007 <sup>b</sup>
08MAY61	61128	2.31	0.43	0.0006 <sup>b</sup>
30JUN61	61181	4.04	0.66	< 0.0001
19JUL61	61200	3.78	0.53	< 0.0001
21JUL61	61202	4.75	1.45	< 0.0001
22JUL61	61203	1.75	0.23	< 0.0001
01AUG61	61213	4.19	1.19	< 0.0001
01MAY62	62121	1.96	0.10	0.0013 <sup>b</sup>
08MAY62	62128	1.55	0.05	0.0007 <sup>b</sup>
09JUN62	62160	5.33	2.01	< 0.0001
11JUL62	62192	1.83	0.05	< 0.0001
13JUL62	62194	3.63	0.74	< 0.0001

## APPENDIX E (Continued)

TABLE 7. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MGNENSIN CONC. (mg/L)
12MAY63	63132	2.49	0.13	0.0005
27MAY63	63147	1.90	0.03	0.0001
04JUL63	63185	3.38	0.30	< 0.0001
06JUL63	63187	6.58	2.46	< 0.0001
13JUL63	63194	2.69	0.28	< 0.0001
28JUL63	63209	3.10	0.28	< 0.0001
12AUG63	63224	4.37	0.69	< 0.0001
05JUN64	64157	2.29	0.15	< 0.0001
21JUN64	64173	1.73	0.05	< 0.0001
26MAY65	65146	2.29	0.15	0.0001
01JUN65	65152	9.83	5.18	<del>0.0001</del>
08JUN65	65159	2.21	0.25	< 0.0001
20JUN65	65171	2.11	0.13	< 0.0001
07JUL65	65188	1.73	0.05	< 0.0001
17MAY66	66137	3.43	0.58	0.0003
21MAY66	66141	2.69	0.43	0.0002
06MAY67	67126	3.89	1.07	0.0008 <sup>b</sup>
30MAY67	67150	2.51	0.28	0.0001
12JUN67	67163	1.63	0.05	< 0.0001
16JUN67	67167	1.50	0.03	< 0.0001
23JUL67	67204	4.01	0.63	< 0.0001
08AUG67	67220	4.19	0.61	< 0.0001
22MAY68	68143	3.51	0.56	0.0002
23MAY68	68144	5.00	2.16	0.0001
25MAY68	68146	3.02	0.76	0.0001
14JUN68	68166	4.55	1.17	< 0.0001
15JUN68	68167	2.31	0.51	< 0.0001
17JUL68	68199	2.29	0.13	< 0.0001
27JUL68	68209	3.58	0.53	< 0.0001
05JUL69	69186	1.75	0.05	< 0.0001
06JUL69	69187	2.59	0.43	< 0.0001
07JUL69	69188	2.21	0.43	< 0.0001
08JUL69	69189	1.60	0.20	< 0.0001

## APPENDIX E (Continued)

TABLE 7. (Continued)

DATE	JULIAN DATE <sup>a</sup>	RAINFALL (cm)	RUNOFF (cm)	AQUEOUS MONENSIN CONC. (mg/L)
30APR70	70120	9.09	5.16	0.0014 <sup>c</sup>
09MAY70	70129	1.55	0.03	0.0005 <sup>b</sup>
13MAY70	70133	1.52	0.05	0.0003
01JUN70	70152	2.49	0.23	0.0001
04JUN70	70155	3.53	0.84	< 0.0001
05JUN70	70156	1.57	0.18	< 0.0001
14JUN70	70165	2.41	0.25	< 0.0001
06MAY71	71126	2.08	0.03	0.0008 <sup>b</sup>
11MAY71	71131	2.18	0.13	0.0005
23MAY71	71143	1.80	0.03	0.0002
24MAY71	71144	1.88	0.10	0.0001
08JUL71	71189	2.26	0.05	< 0.0001
10JUL71	71191	6.65	2.77	< 0.0001
14JUN72	72166	2.08	0.08	< 0.0001
28JUN72	72180	2.51	0.15	< 0.0001
09JUL72	72191	2.29	0.10	< 0.0001
12AUG72	72225	4.75	0.76	< 0.0001
07MAY73	73127	2.44	0.30	0.0008 <sup>b</sup>
18JUN73	73169	6.65	3.51	< 0.0001
26JUN73	73177	2.13	0.18	< 0.0001
27JUN73	73178	1.55	0.13	< 0.0001
19JUL73	73200	3.51	0.48	< 0.0001

<sup>a</sup> First two digits represent the year and the last three digits represent the day of the year.

<sup>b</sup> Runoff event that occurred within 10 days of a monensin application (Julian day 120).

<sup>c</sup> Runoff event that occurred on the day of a monensin application (Julian day 120).

## APPENDIX E (Continued)

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## APPENDIX F: 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  $^{14}\text{C}$  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  $\mu$  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:

pH	Monensin Concentration ( $\mu\text{g/ml}$ )			Average
	24 hr	48 hr	72 hr	
7.0	64	62	not tested	63
9.0	<2.5	0.8	0.9	0.85

## APPENDIX F (continued)

-2-

## 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:

Day	Monensin Concentration ( $\mu\text{g/ml}$ )		
	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

## 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.

Day	Monensin Concentration ( $\mu\text{g/ml}$ )	
	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 ( $\text{day}^{-1}$ )	0.0158
R <sup>2</sup>	0.97

## 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.

Table 1

Monensin Standard Recovery Data

<u>Sample</u>	<u>Monensin (<math>\mu</math>g)</u>		<u>% of Theory</u>
	<u>Amount Added</u>	<u>Amount Found</u>	
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

## APPENDIX G (Continued)

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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<sup>1</sup>

<u>Leachate (ml applied)</u>	<u>Sand</u>	<u>Sandy Loam</u>	<u>Loam</u>	<u>Clay Loam</u>
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
<u>Soil Section (in)</u>				
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

<sup>1</sup>Data are averages from three columns.

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.

## 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 Four-  
teen-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.

Summary of Results:

Study A03680: The LD<sub>50</sub>, 95% confidence interval for the LD<sub>50</sub>, and the  
slope of the dose-response curve for bobwhite dosed with monensin sodium

## APPENDIX H (Continued)

Summary of Results Study A03680 (continued):

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.

## 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.

Results:

Study A03782: The 8-day  $LC_{50}$ , the 95% confidence limits for the  $LC_{50}$  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  $LD_{50}$ , the 95% confidence limits for the  $LD_{50}$ , and the slope of the dose-response curve for monensin sodium in this dietary study

## APPENDIX I (Continued)

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.

## 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 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.

## 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 LC<sub>50</sub>, the 95% confidence limits for the LC<sub>50</sub>, 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  $< 3.1$  mg/L.

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 LC<sub>50</sub>, the 95% confidence limits for the LC<sub>50</sub>, 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.

## 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,  $\leq$  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 EC<sub>50</sub>, 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  $\geq$  5.6 mg/L.

## 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: 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 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 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

## APPENDIX N (Continued)

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.

## APPENDIX 0: 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 annum), 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.

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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 <sup>a/</sup> on Chicken Litter Treatments

Treatment <sup>b/</sup> Rate(tons/acre)	Litter from Monensin Treated Chickens				Litter from Control Chickens				No Litter	
	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

<sup>a/</sup> Rating scale was 0 to 10. A rating of 0 represents no injury and 10 represents complete kill.

<sup>b/</sup> Monensin treated chickens received 110 g monensin per ton of feed.

## 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

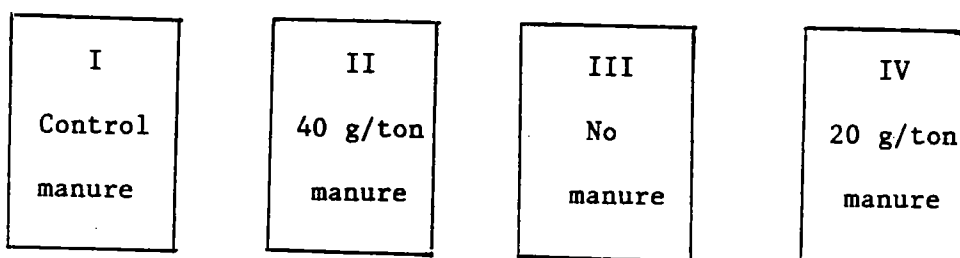
Test Article: Manure from Cattle fed Monensin

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:



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.

## 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 ( <i>Avena sativa</i> )	There were no observable differences in maturation, flowering, fruiting, or seed formation between untreated, blank manure plot and the plot with monensin in the manure.
Sorghum ( <i>Sorghum bicolor</i> )	
Soybean ( <i>Glycine max</i> )	
Barley ( <i>Hordeum vulgare</i> )	
Sugar Beet ( <i>Beta vulgaris</i> )	
Corn ( <i>Zea mays</i> )	
Tomato ( <i>Lycopersicon esculentum</i> )	
Cotton ( <i>Gossypium hirsutum</i> )	
Cucumber ( <i>Cucumis sativus</i> )	
Wheat ( <i>Triticum aestivum</i> )	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.
Rice ( <i>Oryza sativa</i> )	
Pepper ( <i>Capsicum annuum</i> )	
Alfalfa ( <i>Medicago sativa</i> )	
Fescue ( <i>Festuca elatior</i> )	