ENVIRONMENTAL ASSESSMENT

FOR THE USE OF

COBAN®45 IN THE FEED OF TURKEYS

GREATER THAN 10 WEEKS OF AGE

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

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TABLE OF CONTENTS

	Page
TITLE PAGE	1
TABLE OF CONTENTS	2-4
1.2.3. DATE AND APPLICANT INFORMATION	5
4. DESCRIPTION OF THE PROPOSED ACTION	5-6
5. IDENTIFICATION OF CHEMICAL SUBSTANCE	7-9
A. COBAN	7
B. Mycelial Monensin	7-8
C. Monensin	7-9
6. INTRODUCTION OF SUBSTANCE INTO THE ENVIRONMENT	10-14
A. Introduction of Substances from the Manufacturing Site	10-12
B. Introduction of Substance from Feed Mixing Locations	12
C. Introduction of Substance at the Use Site	12-14
7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT	14-19
A. Potential Concentration of Monensin in Soil	15-16
B. Potential Concentration of Monensin in Aquatic Systems	16-19
1. Potential Monensin Concentration in Runoff from Cropland	16-18
2. Fate of Monensin in Aquatic Organisms	18-19
C. Occurrence of Monensin in Groundwater	19
8. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES	20-33
A. Mammalian Toxicity Tests	20-21
B. Potential Adverse Effects of the Proposed Action on Human Health	22
1. Production of Monensin and Manufacture of COBAN 45	22
2. Human Exposure to Monensin via the Food Supply	22
C. Effects of Monensin on Nontarget Organisms	23-28
D. Potential Adverse Effects of the Proposed Action on Aquatic and Wildlife Organisms	28-33
1. Potential for Adverse Effects on Aquatic Organisms	28-30

TABLE OF CONTENTS CONTINUED

		Page
2.	Potential Adverse Effects on Earthworms	30-31
3.	Potential for Adverse Effects on Avian Species	31
4.	Potential for Adverse Effects on Plants	31-32
9.	USE OF RESOURCES AND ENERGY	32
10.	MITIGATION MEASURES	32-33
11.	ALTERNATIVES TO THE PROPOSED ACTION	33
12.	LIST OF PREPARERS	34
13.	CERTIFICATION	35
14.	REFERENCES	36
15.	APPENDICES	37-75
A.	^{14}C Monensin Metabolism Study in Turkeys	37-38
B.	Monensin Levels in Excreta of Broiler Chickens Fed Monensin at a Level of 120 ppm	39
C.	Monensin Greenhouse Soil Decline Study	40-41
D.	Monensin Field Soil Decline Study	42-43
E.	Monensin Biodegradation in Soil	44-45
F.	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	46-58
G.	The Solubility, Hydrolysis, and Photolysis of Monensin in Aqueous Solutions	59-60
H.	Laboratory Soil Leaching Study with Monensin	61-62
I.	The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Fourteen-Day Acute Oral Study and The Toxicity of Mycelial Monensin Sodium to Bobwhite in a Fourteen-Day Acute Oral Study: Determination of the No-Observed-Effect-Dose	63-64
J.	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	65-66
K.	The Toxicity of Mycelial Monensin Sodium to Mallards in a Five-Day Dietary Study	67
L.	The Acute Toxicity of Mycelial Monensin Sodium to Bluegill in a Static Test System	68

TABLE OF CONTENTS CONTINUED

Page

APPENDICES (Continued)

M. The Acute Toxicity of Mycelial Monensin Sodium to Rainbow Trout in a Static Test System 69

N. The Acute Toxicity of Mycelial Monensin Sodium to Daphnia magna in a Static Test System 70

O. The Toxicity of Soil-Incorporated Mycelial Monensin Sodium to Earthworms in a 14-day Test 71-72

P. Greenhouse Test for Monensin Phytotoxicity 73-74

ENVIRONMENTAL ASSESSMENT FOR THE USE OF COBAN®45 IN THE FEED OF TURKEYS GREATER THAN 10 WEEKS OF AGE

1. DATE — March 1989

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

3. ADDRESS — Lilly Corporate Center
Indianapolis, Indiana 46285

4. DESCRIPTION OF THE PROPOSED ACTION

A new Animal Drug Approval has been requested for use of COBAN®45 in the feed of turkeys greater than 10 weeks of age. COBAN 45 is a substance which prevents coccidiosis when incorporated into the feed of turkeys. Between 60 and 99 ppm (54 to 90 grams per ton) of monensin sodium, the active ingredient, would be used continuously in the feed of turkeys 10 weeks of age or older. COBAN 45 is already approved (21 CFR 558.355) for use in chicken feed and the feed of young turkeys for the prevention of coccidiosis. Approval of COBAN 45 for use in turkeys 10 weeks of age or older would result in a small increase in the total amount of monensin sold in the United States.

Approval of the proposed action would authorize the fermentation and processing plants of Eli Lilly and Company at Clinton and Lafayette, Indiana, to manufacture and package the COBAN 45 to be sold in the United States for use in the feed of turkeys 10 weeks of age or older.

COBAN®45 (monensin sodium, Elanco)

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 COBAN 45 with feed.

c) Turkey farms where residues may be found in turkey excreta.

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

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

5. IDENTIFICATION OF CHEMICAL SUBSTANCE

A. COBAN 45

COBAN 45 will be incorporated into the feed of turkeys. Monensin sodium is the active ingredient in COBAN 45 and is produced in a dried mycelial biomass form. This dried mycelial biomass is added to the formulated product to achieve a monensin concentration of 45 g/lb. COBAN 45 may contain diluents such as rice hulls.

B. MYCELIAL MONENSIN

Monensin is produced by the fermentation of a strain of Streptomyces cinnamonensis, an organism isolated from soil (Haney and Hoehn, 1967). The most economical procedure to prepare a usable form of monensin is to harvest the fermentation culture in such a way as to combine monensin with the mycelial cells of the producing organisms and the unused components of the feed-stock used in the fermentation to achieve growth of the organism. Thus, the dried mycelial or biomass form of monensin contains nutrients which can commonly be found in turkey feedstuff.

C. MONENSIN

Monensin consists primarily of monensin factor A, but small amounts of monensin factor B and very small amounts of factors C and D do occur. Monensin factor A accounts for at least 90 percent of the microbiologically active material of mycelial monensin. The characteristics of monensin factor A are discussed in this section. Monensin is a monocarboxylic polyether compound which complexes with monovalent alkali

cations and shows ionophorous activity with a selectivity of $Na^+ > K^+ > Rb^+ > Li > Cs^+$ (Haney and Hoehn, 1967; Pressman, 1976; Appendix G).

Monensin Sodium:

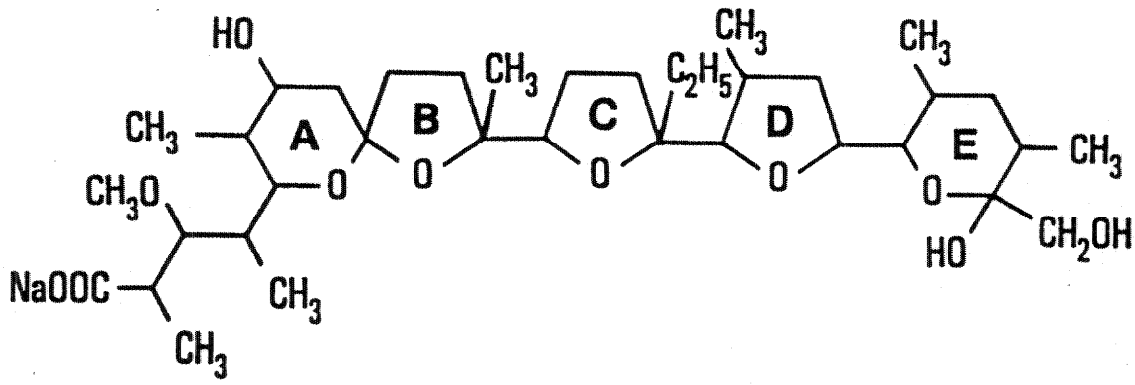
During the manufacturing process, monensin is exposed to sodium ions during a pH adjustment giving rise to monensin sodium which is the chemical form in the product.

Chemical Name (acid form):

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

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

Structural Formula:



Solubility

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

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

UV absorption: None

pKa value: 6.65 (66% DMF)

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

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

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 (IDEM).

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

Location	Permit Identification No.	Issued	Expiration
Clinton	83-09-91-0082	Dec. 8, 1987	Sept. 1, 1991
Clinton	83-09-91-0083	Dec. 8, 1987	Sept. 1, 1991
Clinton	83-09-91-0085	Dec. 8, 1987	Sept. 1, 1991
Lafayette	79-04-90-0372	Oct. 9, 1986	April 1, 1990
Lafayette	79-04-90-0386	Oct. 9, 1986	April 1, 1990

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

Location	NPDES Permit No.	Issued	Expiration
Clinton, IN	0002852	September 23, 1985	August 31, 1990
Lafayette, IN	0002861	September 30, 1987	September 30, 1992

No hazardous wastes and essentially no solid wastes will be generated in these manufacturing operations. Processes which use organic solvents provide for recovery and reuse of solvents, and those operations where solvents are present are served by condensers, carbon absorbers 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 or at feed mills of large integrated turkey operations. These feed mills do 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 show that there were about 242 million turkeys produced in the United States in 1988 (USDA, 1989). Most of these animals were heavy turkeys. Most turkeys were produced in Arkansas, California, Indiana, Iowa, Minnesota, Missouri, North Carolina, Pennsylvania, South Carolina, and Virginia. Almost all turkey production is concentrated in approximately 20 companies. COBAN 45 would be marketed directly to these companies. This should minimize potential environmental exposure during the product distribution process.

Approval of the use of COBAN 45 in the feed of turkeys 10 weeks of age or older, in addition to the currently approved use for young

turkeys, will allow COBAN 45 to be used continuously for turkeys from 1 day of age to maturity. Between 54 and 90 grams of monensin sodium would be used in each ton of final feed. Heavy male and female turkeys eat, on average, 62 pounds of final feed from 1 day of age up to maturity. Approximately 45 pounds of this feed is consumed after 10 weeks of age. If all the turkeys 10 weeks of age or older in the United States were fed the maximum concentration of COBAN 45 in their feed, the maximum amount of monensin sodium that could be used annually would be 5.06×10^5 kg (2.5×10^8 turkeys x 45 lbs feed/turkey x 1 ton/2000 lbs x 0.090 kg monensin/ton of feed). This amount of monensin sodium is equivalent to 5.10×10^6 kg of COBAN 45 (45 g monensin sodium/lb of COBAN 45). It is assumed that monensin sodium could be used for as many as 15% of the turkeys greater than 10 weeks old produced in the United States, so up to 7.59×10^4 kg of monensin sodium, or 7.65×10^5 kg of COBAN 45 could be used each year.

Monensin is found in turkey excreta and may be introduced into soil by use of turkey litter as fertilizer (Appendix A). Seven-week old turkeys fed a ration containing 110 ppm ^{14}C monensin (highest recommended rate is 99 ppm) for five days produced excreta which contained radioactivity equivalent to about 75 ppm monensin in wet excreta. Only about 4 ppm of the radioactivity was parent monensin in the wet excreta. Approximately the same concentration of the parent monensin (3.6 ppm) was found in the litter of chickens fed a ration with 120 ppm of ^{14}C-monensin (Appendix B). Since turkeys older than 10 weeks of age would be fed a diet with the same concentration of COBAN 45 as younger birds, it is reasonable to assume that the concentration of monensin in their excreta would be no higher than 4 ppm.

Monensin is extensively metabolized in cattle, rats, chickens, dogs, sheep, pigs, and turkeys (Donoho, 1984). The pattern of metabolism is qualitatively similar among species, although quantitatively different. By inference then, the toxicology of monensin metabolites present in turkey excreta have been evaluated in toxicology studies in which rats were exposed to monensin. More than 25 metabolites of monensin have been found for rats and turkeys (Donoho et al., 1978 Appendix A). The primary monensin metabolite, O-desmethyl monensin, is 20 times less biologically active than monensin, based on several test systems (Donoho, 1984). This primary metabolite makes up less than 10% of the radioactivity in turkey excreta (Appendix A). 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 ^{14}C-monensin excretion studies in turkeys and chickens support the conclusion that 4 ppm is a realistic upper limit for monensin in turkey litter.

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 turkey excreta collected from a turkey production facility 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 turkey excreta and in adjacent aquatic systems.

A. POTENTIAL CONCENTRATION OF MONENSIN IN SOIL

The highest expected initial concentration of monensin in soil can be calculated from the concentration of monensin in wet turkey excreta and the use rate of wet excreta on cropland. The highest expected concentration of monensin in wet turkey excreta is 4 mg/kg. A reasonable estimate of the application rate of fresh turkey excreta as fertilizer is 10 tons/A (22.4×10^3 kg/ha). It is standard practice to incorporate fertilizer into the top six inches of soil to avoid loss of nutrients in runoff. A six inch deep soil layer in one hectare weighs about 2.25×10^6 kg. The highest initial concentration of monensin in soil is, therefore, about 0.04 ppm ((4 mg/kg x 22.4×10^3 kg/ha) ÷ 2.25×10^6 kg of soil/ha).

The concentration of monensin in soil would decline from the highest expected value of 0.04 ppm, which could occur directly after application of turkey excreta to soil. Studies with crystalline monensin mixed in soil show a moderately rapid decline in monensin activity. 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 C). When crystalline monensin was mixed in soil and exposed to field conditions, the dissipation half-life was 7.5 days without manure and 7.4 days with steer manure (Appendix D). Dissipation of monensin in this study also appeared to result from degradation because the rates of loss were very similar to those found in the greenhouse study. Monensin seems to be extensively

degraded in soil. In five weeks under greenhouse conditions, almost 48% of the radioactivity was lost from soil treated with crystalline ^{14}C monensin (Appendix E). Extensive degradation of monensin and its known metabolites would have had to occur to account for the apparent volitilization of ^{14}C, perhaps as $^{14}CO_2$. Because of the moderately rapid decline of monensin in agricultural soil, nontarget terrestrial organisms would presumably be exposed to monensin for a short period of time.

B. POTENTIAL CONCENTRATION OF MONENSIN IN AQUATIC SYSTEMS

1. Potential Monensin Concentration in Runoff from Cropland

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 turkey excreta into soil in order for monensin to reach surface water. If it were possible for all of the monensin in the turkey excreta applied to cropland to be dissolved into runoff from one rainfall event, a two inch runoff event would carry 36.28 g of monensin, or 0.18 mg monensin/L ((10 tons of excreta/acre x 907 kg/ton x 4 mg monensin/kg excreta) ÷ (2 inches x 102794 L/acre-in)). Since the half-life of monensin in field soil is about 7.5 days (Appendix D) and since monensin adsorbs to moderately textured soils (calculated Kd of 24.1 for loam, Appendix F), it is improbable that all the monensin in a field could be lost in one large runoff event.

A better estimate of the highest 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 F). The model

estimates chemical yield in runoff from field-sized areas using daily rainfall records. Input parameters and the geographical location (North Carolina) used for the model were selected to maximize the potential for runoff from cropland where monensin might be applied to the soil (Appendix F). The model simulation was conducted for a thirty-year period, with 10 tons of wet turkey excreta containing 4 ppm monensin incorporated into each acre of soil every year. Conventional tillage, a high average field slope and row-cropped corn were used in the simulation to maximize runoff. In order to maximize the opportunities for monensin to be lost into runoff, the 30-year simulation was conducted for annual applications of excreta on April 1 (Julian Day 91). The third highest spring rainfall (3.3 in.) and second highest spring runoff (1.7 in.) events in the 30-year simulation occurred on Julian Day 91 at the selected site. The short half-life of monensin in the field was not, therefore, critical in the estimation of a near-maximum yield of monensin in a single event. Over the thirty-year simulation many runoff events occurred close to the date of application (Julian Day 91). The short half-life of monensin in the field did preclude any build up of monensin in the soil from one year to the next.

The 30-year CREAMS simulation was used to estimate the maximum concentration of monensin in the aqueous phase of runoff, the maximum loss of monensin in a single runoff event, the maximum annual yield of monensin in runoff and the average annual yield of monensin. The maximum concentration of monensin in runoff was 0.0011 ppm and was found for a 1.3-inch rainfall event on the same day the turkey excreta was applied to the field. The maximum loss of monensin in a single runoff event was 0.69% of the original amount applied. The maximum and average annual

yields of monensin for the 30-year simulation were 0.81% and 0.09%, respectively, of the original amount applied. Because of the relatively short half-life of monensin in soil, annual yield of monensin was not significantly increased by a large number of runoff events throughout the year. The calculated maximum annual yield of monensin (0.81%) in the CREAMS simulation is within the actual annual yield values (<1.5%) suggested by Wauchope (1978) and Willis and McDowell (1982) for soil-incorporated 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 G).

Based on the moderately short half-life of monensin in soil, runoff events would have to occur soon after application of turkey excreta to field soil for monensin to be carried to natural aquatic systems. Dilution of the highest expected concentration of monensin in runoff water (0.0011 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 (Spacie and Hamelink, 1985). Monensin is metabolized and excreted by chickens, cattle, rats, dogs, sheep, pigs, and turkeys and does not appear to concentrate in fatty tissue (Donoho, 1984; Donoho et al., 1984). Monensin also rapidly dissipates from soil (Appendices C, D, and E). 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 clay loam (Appendix H). 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 H). 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 (Kd estimated to be about 24.1, Appendix F). 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 soil.

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 the producers and users of COBAN 45 are briefly described below.

Hazard Evaluation Studies

Acute Oral LD_{50} with Rats: 219 mg of COBAN 45/kg of body weight.

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

Ocular Irritation in Rabbits: Severe irritation when COBAN 45 was placed in the eyes of rabbits. Rinsing eyes immediately after exposure was effective in preventing immediate damage and eyes appeared normal 72 hours after treatment.

Dermal Irritation in Rabbits: Slight irritation, but no signs of systemic toxicity occurred when 2000 mg of COBAN 45/kg body weight was applied to shaved and abraded skin.

Chronic, Reproduction and Teratology Study

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 Multigeneraton 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 COBAN 45

Monensin would be produced in only two plants. 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 throughly at available eye-wash stations. Precautionary labeling would advise people mixing and handling COBAN 45 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 COBAN 45. 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 COBAN 45 and users of this premix would not be adversely affected by the proposed action.

2. Human Exposure to Monensin Via the Food and Water Supply

Extensive chemistry and toxicology data have been developed to support the safe use of monensin in turkeys and chickens relative to residues in edible tissues. Based on this information it may 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.

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 I):

Two acute oral studies with mycelial monensin and bobwhite quail (Colinus virginianus) have been conducted. In one study with monensin sodium doses ranging from 45 to 250 mg/kg body weight, the 14-day LD_{50}, 95% confidence interval, and slope of the dose-response curve for adult bobwhite were 85.7 mg/kg, 64.4 to 114.2 mg/kg, and 2.915, respectively. No sex-related differences in mortality were evident within treatment groups. No mortalities 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 J): 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 K): 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 L): A static toxicity test was conducted to determine the acute effects of monensin sodium (mycelial) on juvenile bluegill (Lepomis macrochirus). 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 M): 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. Rainbow trout (Salmo gairdneri) 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 N): 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 O):

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 (Appendix 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. Monensin-fed chickens produced litter with a monensin concentration approximately equivalent to the concentration of monensin in turkey excreta (Appendices A and B). 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.

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 turkey excreta 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 turkey excreta 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 can 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 highest expected monensin concentration in runoff from cropland was calculated to be 0.0011 ppm. The 96-hr LC_{50} values for rainbow trout and bluegill and the 48-hr EC_{50} value for daphnids range from 9.0 to 16.6 ppm. These acute median lethal and acute median effect concentrations are about 8180 to 15,090 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 636 times the highest expected concentration of monensin in runoff. Even if it were possible for all of the monensin in the turkey excreta used in a watershed to be extracted into runoff (Section 7B), the concentration of monensin in runoff would be 0.18 ppm. This concentration (0.18 ppm) is about four 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 (0.0011 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 82 and 151 times higher than the highest expected concentration (0.0011 ppm) of monensin in runoff. If it were possible for all of the monensin in the turkey excreta used in a watershed to be extracted into runoff, the concentration of monensin in that runoff (0.18 ppm) would be two times higher than the concentration of monensin (0.09 ppm) calculated to be chronically safe to the most sensitive species tested.

Based on the highest expected monensin concentration (0.0011 ppm) and highest possible monensin concentration (0.18 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 0.04 ppm (Section 7A). Monensin concentrations in soil decline relatively rapidly in the greenhouse ($t_{\frac{1}{2}}$=5.8 days) and in the field ($t_{\frac{1}{2}}$=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 250 times higher than the highest expected concentration

of monensin in soil, earthworms should not be affected by monensin in turkey excreta 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 recommended use rates of COBAN 45 in turkey feed would result in a maximum dietary monensin concentration of 99 ppm (90g/ton). If wild birds foraged only on turkey feed with the highest expected concentration of monensin for five days, reductions in body weight gain might occur. The proposed action would not, however, 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 25 times higher than the highest expected monensin concentration of 0.04 ppm in cropland. In another study, monensin in chicken litter was found to be only as phytotoxic as the control chicken litter. 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 COBAN 45 requires an amount of energy similar to that used to produce and package any conventional fermentation product for animals. COBAN 45 is already approved and produced for use in chickens. Disposal of washwater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources. Manufacture of COBAN 45 for use in turkey feed will occur at facilities already producing COBAN 45 for use in chicken feed. Unusual levels of noise, odors, construction, or other disruptions should not be required for any increase in total production of COBAN 45.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. The COBAN 45 label will instruct users to wear protective clothing, impervious gloves, and a dust mask when mixing and handling COBAN 45. 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 COBAN 45. The label will also indicate that horses, other equines, and guinea fowl must not be allowed direct access to COBAN 45. Ingestion of

COBAN 45 by horses and guinea fowl has been fatal. Other than these precautions listed on the label, no mitigation measures are necessary for COBAN 45.

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.

John L. Emmerson, Ph.D.
Lilly Research Fellow and
~~Director~~ of Toxicology
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March 23, 1989
Date

14. REFERENCES

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APPENDIX A: Report Summary

Title: ^{14}C Monensin Metabolism Study in Turkeys

Study Number: ABC-0151

Study Dates: February 15, 1982 to May 3, 1982

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

Test Article: Crystalline ^{14}C Monensin

Test System: Young turkeys

Summary of Experimental Design:

Turkey chicks were reared from one day of age on unmedicated ration. At approximately 7 weeks of age, three turkeys were fed a ration containing 110 ppm ^{14}C monensin for five days and were then killed at a practical zero withdrawal (6 hrs. off treatment). The ^{14}C monensin had a radiochemical purity of 98% or higher. Edible tissues and excreta were assayed for radioactivity concentrations. Metabolite patterns in excreta and liver were determined chromatographically and comparisons were made with similar data from chickens.

Summary of Results:

The residue pattern in turkey tissues was very similar to that found in chickens fed 120 ppm ^{14}C monensin as shown in Table 1. Total radioactivity in turkey liver was slightly higher than in chicken liver, but all other edible tissues were well within the range of values from the chicken data.

The metabolite pattern in turkeys from both the liver and excreta agreed very well with similar chromatographic data from chickens. Metabolites which were found were hydroxylation and O-demethylation products of monensin which were observed in rats and cattle, and have been reported in the literature (Donoho et. al., J. Agric. Food Chem 26:1090 (1978).

Turkey excreta contained radioactivity equivalent to approximately 75 ppm monensin equivalents in the wet excreta. Only about 5.6% of the radioactivity was parent monensin. The remainder was a mixture of more than 25 metabolites, none of which exceeded 10% in relative abundance. The primary metabolite, O-desmethyl monensin, has lost most of its biological activity as measured by a variety of systems (Donoho, J. An. Sci. 58:1528 (1984). This metabolite accounted for less than 10% of the excreta radioactivity. The metabolite pattern and monensin concentrations in turkey excreta are very similar to the data from chickens. The parent monensin concentration in wet excreta was calculated to be approximately 4 ppm.

APPENDIX A (continued)

Table 1

Comparison of Radioactivity Concentrations (PPM)
in Tissues of Turkeys and Chickens

	Liver	Kidney	Muscle	Skin	Fat
Turkeys	.909	.159	<.03	.092	.135
Chickens [a/]	.495	.128	<.03	.078	.095

[a/] Data from 13 chickens fed 120 ppm ^{14}C monensin, Donoho, J. An. Sci. 58:1528 (1984) and Donoho et. al., J. Agric. Food Chem. 30:909 (1982).

APPENDIX B: Report Summary

Title: Monensin Levels in Excreta of Broiler Chickens Fed Monensin at a Level of 120 ppm.

Study Number: C97-Q06-60

Study Dates: June 1 to August 31, 1975

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

Test Article: Excreta from chickens being fed 120 ppm monensin

Test System: Microbiological assay for monensin concentration

Summary of Experimental Design:

A pooled excreta sample of 1000 g was obtained from Hubbard strain broiler chickens which were being fed a ration containing 120 ppm monensin. The pooled sample was air dried and milled 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:

The excreta sample after drying contained 299 g giving a moisture content of 70%. Four independent samples were prepared and the extracts were assayed on two different days giving eight determinations. The mean monensin concentration was 12.1 ppm in the air dry sample which is equivalent to 3.6 ppm in the wet excreta as received. Data are presented in Table 1. Contemporary control excreta samples were negative and recovery samples ranged from 94 to 102% of theory.

Table 1. Monensin Concentratins in Air Dried Excreta from Chickens Fed 120 ppm Monensin

	PPM Monensin		
Replicate	1st assay	2nd assay	Sample Mean
1	11.9	11.8	11.85
2	12.9	13.4	13.15
3	11.2	12.1	11.65
4	11.1	12.4	11.75
		Mean	12.1

APPENDIX C: Report Summary

Title: Monensin Greenhouse Soil Decline Study

Study Number: A22-B47-3264

Study Dates: April 15 to June 15, 1973

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

Test Article: Crystalline Monensin

Test System: Soil flats maintained in the greenhouse

Summary of Experimental Design:

Crystalline monensin was incorporated into approximately 6 kg air dried potting soil at a nominal concentration of 1 ppm. The monensin was added in a small volume of methanol and the sample was blended and then air dried to remove the methanol. The soil was placed in a nominal 0.07 m^2 soil flat lined with plastic. The flat was maintained in the 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 C (continued)

Table 1

Degradation of Monensin in Soil

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

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

[2]Test sensitivity was 0.1 to 0.2 ppm.

APPENDIX D: Report Summary

Title: Monensin Field Soil Decline Study

Study Number: A22-B50-3270

Study Dates: May 1 to June 30, 1973

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

Test Article: Crystalline Monensin

Test System: Field soil plots

Summary of Experimental Design:

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

Summary of Results:

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

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

APPENDIX D (continued)

Table 1

PPM Monensin in Field Soil [a/]

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

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

APPENDIX E: Report Summary

Title: Monensin Biodegradation in Soil

Study Number: B77-3306

Study Dates: March 1 to November 1, 1974

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

Test Article: Crystalline ^{14}C Monensin

Test System: Soil maintained in the greenhouse

Summary of Experimental Design:

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

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

Summary of Results:

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

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

APPENDIX E (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}C Monensin.

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

APPENDIX F: Report Summary

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 turkeys could result in concentrations of monensin of up to 4 ppm in the wet feces from turkeys. 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. A thirty-year simulation was run for the annual application of 10 tons per acre (2.24×10^4 kg/ha) of turkey manure containing 4 ppm monensin to cropland in North Carolina. It was assumed that the manure was incorporated each spring (April 1, Julian day 091) into the soil prior to the planting of straight-row corn. To maximize the loss of monensin in runoff, it was assumed that the corn was maintained on a 5% field slope under conventional tillage practices without contouring. The following section details the selection of parameters for the runoff simulation.

APPENDIX F: (continued)

Methods:

The selection of the geographical location for the simulation was based on three criteria: 1) it must be representative of a major turkey production area; 2) it must be representative of major cropland to which turkey 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 turkey 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.

Turkey and corn production overlap in several areas of the United States including the eastern Great Plains, parts of the Midwest, and the central Atlantic states. Because of its relatively high annual precipitation, and hence a higher potential for runoff, North Carolina was selected as the representative site for this assessment.

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 was 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 1 lists the general characteristics of the representative site in North Carolina. Thirty years of daily rainfall data were obtained for meteorological station #5177 (Lumberton, NC) from the National Center for Atmospheric Research database developed by EPA-Athens, GA. Monthly mean temperature and solar radiation for the site were selected from Arnold and Williams (7).

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 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 hydrology component of CREAMS is listed in Table 2. This data set corresponds to the format specified in the user's manual for CREAMS (2).

APPENDIX F: (continued)

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 for the silt loam soil was 20% sand, 60% silt, and 20% clay. It was assumed that corn was planted on May 1 (Julian day 121) and harvested on October 1 (Julian day 274) for each year of the simulation. The specific set of parameters used for the erosion/sediment yield component of CREAMS is listed in Table 3. This data set corresponds to the format specified in the program's documentation (2).

Chemistry: The chemistry component of the CREAMS model required parameters describing the use pattern and environmental behavior of monensin. Application of turkey manure with a monensin concentration of 4 ppm was assumed to occur at a rate of 2.24×10^4 kg/ha (10 tons/acre) on April 1 (Julian day 091) of each year of the 30-year simulation. This is equivalent to a monensin application rate of 0.0896 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 D). The decay constant was 0.093 day^{-1}, which corresponds to a half-life of 7.5 days. The solubility of monensin in water was assumed to be 63 ppm. Model results are only affected by solubilities less than 1 ppm. The soil/water distribution coefficient (Kd) for monensin was determined from the results of a column leaching study with loam soil (Laboratory Soil Leaching Study with Monensin, Appendix H). The movement of monensin through the soil column relative to the movement of water was expressed as a function of Kd, known as the retardation factor (8). The value of Kd calculated from the retardation factor was 24.1. The specific chemical parameters used in the model are given in Table 4. This data set corresponds to the format specified in the program's documentation (2).

Results and Discussion:

The results of the thirty-year runoff simulation is given in Table 5. Annual precipitation and the hydrologic response of the representative field are summarized in the first three columns of the table. Total annual precipitation ranged from 70.3 to 146.9 cm (27.7 to 57.8 in) and averaged 116.8 cm (46.0 in). Total annual runoff ranged from 2.9 to 27.8 cm (1.1 to 10.9 in) and averaged 15.0 cm (5.9 in). An average of 24 runoff events occurred each year of the simulation. On the average, however, less than 4 runoff events per year contained monensin.

The fourth column of Table 5 shows for each year the maximum aqueous monensin concentration in runoff produced in a single event. These maximum single-event concentrations ranged from 0.0 to 1.11 ppb. The concentration of 1.11 ppb resulted from a 3.4-cm (1.3 in) rainfall event

APPENDIX F: (continued)

and a 1.1-cm runoff event on the day of turkey manure application. All of the maximum annual concentrations greater than 1 ppb occurred within 1 day of the application of manure containing monensin. Maximum annual concentrations in runoff greater than 0.5 ppb resulted from runoff events occurring within 8 days of the application. These results indicated that the aqueous concentration of monensin in runoff was dependent upon the time interval between manure application and the first significant rainfall event. As this time interval increased, monensin concentrations in runoff became greatly reduced due to the short half-life of the compound in soil.

The last two columns in Table 5 show the maximum single-event loss of monensin and the total annual loss of monensin in runoff for each year of the simulation. Both quantities were expressed as a percent of the total mass of the annually applied monensin (0.0896 kg/ha). The maximum single-event losses ranged from 0.0 to 0.69% of the annual application. The highest value of 0.69% resulted from a 8.1 cm (3.3 in) rainfall event that occurred on the day of manure application. This storm produced 4.4 cm (1.7 in) of runoff. These were the third largest rainfall event and second largest runoff event in the spring during the 30-year simulation. In general, maximum annual single-event losses greater than 0.1% were associated with storms that occurred within 5 days of the manure application. The only exception to this was year # 7 in which a 6.1 cm (2.4 in) rainfall event producing 2.7 cm (1.1 in) of runoff occurred 13 days post-application.

Total annual monensin losses in runoff ranged from 0.0 to 0.81% and averaged 0.09%. In most years, the maximum single-event loss accounted for over 60% of the total annual monensin loss in runoff. The highest total annual yield of monensin for the thirty-year simulation (0.81%) was within the annual yield values (<1.5%) suggested by Wauchope (9) and Willis and McDowell (10) for soil-incorporated compounds with characteristics similar to those of monensin.

The information in Table 5 was used to construct annual frequency distributions for the maximum single-event monensin concentration in runoff and for the maximum single-event monensin loss. This type of analysis allows a frequency, or a return period, to be associated with the major runoff events. The method used to construct the cumulative frequency distributions is described in the SCS National Engineering Handbook (3). For example, the values for maximum single-event loss given in Table 5 were log-transformed and arranged in descending order. A "plotting percentile" was then assigned to each value according to its relative position in the order. Each plotting position was associated with its standardized Z-scores from the normal distribution. Regressing the log-transformed single-event loss values against their Z-scores resulted in a straight line from which return periods were estimated. The maximum single-event monensin loss with a 10-year frequency was 0.18%. In other words, there is a 10% chance that more than 0.18% of the applied monensin

APPENDIX F: (continued)

will be transported from the treated field in runoff in any given year. The maximum single-event loss of 0.69% reported in Table 5 was found to have a frequency of approximately 55 years.

A similar analysis was performed with the maximum single-event monensin concentrations given in Table 5 (In this case, the best-fit straight line was determined without log-transforming the concentration values). The maximum annual single-event monensin concentration with a 10-year frequency was determined to be 1.02 ppb. The highest concentration of 1.11 ppb given in Table 5 was found to have a frequency of approximately 16 years.

APPENDIX F: (continued)

References:

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APPENDIX F: (continued)

TABLE 1.
METEOROLOGICAL AND SOIL CHARACTERISTICS OF
REPRESENTATIVE SITE ASSOCIATED WITH TURKEY
AND CORN PRODUCTION

State	North Carolina
Meteorological Station #[a]	5177
Average Annual Precipitation	116.8 cm (46.0 in)
Soil Type	Silt loan
Hydrolic Soil Group[b]	C

[a]Selected from National Center for Atmospheric Research data base developed by EPA-Athens, GA.

[b]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 F: (continued)

TABLE 2.
PARAMETER SET FOR THE HYDROLOGY COMPONENT OF THE CREAMS MODEL USED TO SIMULATE MONENSIN LOSS IN RUNOFF.
THIS DATA SET CORRESPONDS TO THE FORMAT SPECIFIED IN THE CREAMS USER'S MANUAL (2).

HYDROLOGY PARAMETERS - MONENSIN / TURKEY
NORTH CAROLINA / SILT LOAM
CLIMATE STATION #5177, 1/49 - 12/78

49001	0	1	1						
40.0	0.10	0.64	0.50	4.50	0.43	0.12			
0.2	86.0	0.05	.1.0	36.0					
0.31	1.55	1.86	1.86	1.86	1.86	1.86			
41.6	42.9	49.5	59.3	67.6	75.1	77.9	76.9	71.2	60.5
50.0	41.9								
235.0	302.0	360.0	466.0	494.0	564.0	535.0	476.0	379.0	307.0
235.0	199.0								
1.0									
001	0.00								
121	0.00								
136	0.09								
151	0.19								
166	0.23								
181	0.49								
196	1.16								
211	2.97								
226	3.00								
241	2.72								
256	1.83								
274	0.00								
366	0.00								
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							
0	0	0							

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APPENDIX F: (continued)

TABLE 2. (continued)
PARAMETER SET FOR THE HYDROLOGY COMPONENT OF THE CREAMS MODEL USED TO SIMULATE MONENSIN LOSS IN RUNOFF.
THIS DATA SET CORRESPONDS TO THE FORMAT SPECIFIED IN THE CREAMS USER'S MANUAL (2).

0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
-1	0	0

APPENDIX F: (continued)

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

EROSION PARAMETERS - MONENSIN / TURKEYS
NORTH CAROLINA / SILT LOAM
CLIMATE STATION #5177, 1/49 - 12/78

49	78	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	091	121	170	199	205	211	226	274	
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	1.00	
0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.03	

APPENDIX F: (continued)

TABLE 4.
PARAMETER SET FOR THE CHEMICAL COMPONENT OF THE CREAMS MODEL USED TO SIMULATE MONENSIN LOSS IN RUNOFF.
THIS DATA SET CORRESPONDS TO THE FORMAT SPECIFIED IN THE CREAMS USER'S MANUAL (2).

CHEMICAL PARAMETERS - MONENSIN / TURKEYS
NORTH CAROLINA / SILT LOAM
CLIMATE STATION #5177, 1/49 - 12/78

49001	0	0	1	0	3	1			
0.430	0.320	1.250							
1	49090	78365							
1	MONENSIN		63.0	0.0	0.093	24.1	0.0	0.0	0.0
1091	1366	1							
1	0.0896	15.24	1.0	0.0	1.0	0.0			
	0								

APPENDIX F: (continued)

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

Year	Total Rainfall (cm)/ No. of Storms	Total Runoff (cm)/ No. of Events	Number of Runoff Events Containing Monensin	Maximum Single-Event Aqueous Monensin Concentration (ppb)/ Days After Application	Maximum Single-Event Loss (% of Applied)/Days After Application	Total Annual Loss (% of Applied)
1	120.3/110	13.4/25	8	0.84/3	0.02/3	0.08
2	130.5/105	9.3/21	2	0.07/29	<0.005/29	<0.005
3	70.3/99	2.9/10	3	0.93/1	0.04/7	0.06
4	133.1/114	13.7/32	6	1.09/a	0.16/a	0.23
5	98.8/97	8.3/19	5	0.07/29	0.01/31	0.02
6	84.7/70	12.0/13	3	1.11/a,b	0.17/a	0.26
7	115.0/110	17.6/22	4	0.92/2	0.12/13	0.14
8	108.1/93	10.0/21	3	0.39/11	0.03/11	0.04
9	114.3/104	10.0/23	1	0.05/33	0.007/33	0.01
10	125.3/108	18.3/29	6	0.49/9	0.03/21	0.08
11	120.5/127	16.0/23	3	1.01/1	0.14/1	0.15
12	117.8/96	17.0/26	4	0.69/5	0.05/5	0.06
13	116.6/105	9.3/25	8	1.09/a	0.17/a	0.21
14	143.5/123	27.8/26	1	0.39/11	<0.005/11	<0.005
15	104.2/101	13.2/20	5	0.64/6	0.01/6	0.01
16	135.4/120	17.8/33	2	0.52/8	0.06/8	0.06
17	124.7/112	14.9/27	3	0.64/6	0.01/6	0.01
18	122.4/108	16.6/28	6	0.09/26	0.02/26	0.03
19	127.4/108	22.3/25	2	0.10/26	<0.005/26	<0.005
20	87.1/94	10.0/13	1	0.63/6	0.03/6	0.03
21	131.2/95	20.3/31	3	0.68/5	0.12/5	0.13
22	95.4/106	9.9/15	0	0.0	0.0	0.0
23	139.9/119	20.0/30	4	0.70/5	0.01/5	0.02

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APPENDIX F: (continued)

TABLE 5. (continued)
ANNUAL SUMMARY OF RAINFALL, ESTIMATED RUNOFF, ESTIMATED MONENSIN LOSS, AND MAXIMUM ESTIMATED MONENSIN CONCENTRATION IN RUNOFF.

Year	Total Rainfall (cm)/ No. of Storms	Total Runoff (cm)/ No. of Events	Number of Runoff Events Containing Monensin	Maximum Single-Event Aqueous Monensin Concentration (ppb)/ Days After Application	Maximum Single-Event Loss (% of Applied)/Days After Application	Total Annual Loss (% of Applied)
24	109.0/125	6.5/22	2	0.05/34	<0.005/34	<0.005
25	124.0/115	18.3/28	5	1.07/a	0.69/a	0.81
26	135.2/119	21.4/30	6	0.22/17	0.01/48	0.01
27	146.9/134	19.0/34	6	0.93/2	0.05/2	0.06
28	103.3/110	8.4/23	2	0.06/31	<0.005/46	<0.005
29	129.1/109	27.6/23	3	0.01/51	0.01/54	0.01
30	119.1/97	16.7/25	5	0.37/12	0.01/25	0.05

[a] Rainfall and runoff occurred on the day manure containing monensin was applied to the soil.

[b] Highest monensin concentration estimated from the thirty-year simulation.

APPENDIX G: Report Summary

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

Study Number: S-AAC-81-13

Study Dates: March 27 to June 11, 1981

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

Test Article: Crystalline ^{14}C Monensin Sodium

Test System: Aqueous Solutions

Summary of Experimental Design:

Solubility

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

Hydrolysis

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

Photolysis

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

Summary of Results

Solubility

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

APPENDIX G: (continued)

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

Hydrolysis

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

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

Photolysis

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

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

APPENDIX H: 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

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

APPENDIX H: (continued)

-2-

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[1]

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

ND = not detectable

[1]Data are averages from three columns.

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 I: Report Summary

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

and

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

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

Study Numbers: A03680
A01882

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

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Bobwhite quail (Colinus virginianus)

Age: A03680 - 18 weeks
A01882 - 20 weeks

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

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

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

Route: Oral (gavage)

Length of Observation: 14 days

Parameters Studied:

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

Summary of Results:

Study A03680: The LD_{50}, 95% confidence interval for the LD_{50}, and the slope of the dose-response curve for bobwhite dosed with monensin sodium

APPENDIX I: (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 J: 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

APPENDIX J: (continued)

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 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 K: Report Summary

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

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

Study Dates: August 19 to August 27, 1982

Study Number: A01782

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Mallard Duck (Anas platyrhynchos)

Age: 10 days

Number of Animals: 10/treatment

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

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

Route: Dietary

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

Results:

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

APPENDIX L: 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_{50}, the 95% confidence limits for the LC_{50}, and the slope of the concentration-response curve were 16.6 mg/L, 16.3 to 17.0 mg/L, and 0.438, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to monensin sodium concentrations $\leq$ 3.1 mg/L.

APPENDIX M: 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_{50}, the 95% confidence limits for the LC_{50}, 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 N: 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_{50}, 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 O: 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 O: (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 P: 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. Callender, 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 greenhouse phytotoxicity test was conducted in which fourteen mono- and dicotyledonous plants were grown from seed in the treated and untreated soils. The plant species were alfalfa (Medicago sativa), fescue (Festuca elatior), cucumber (Cucumis sativus), rice (Oryza sativa), cotton (Gossypium hirsutum), tomato (Lycopersicon esculentum), pepper (Capsicum annuum), corn (Zea mays), sugar beet (Beta vulgaris), barley (Hordeum vulgare), soybean (Glycine max), wheat (Triticum aestivum), grain sorghum (Sorghum bicolor), and oats (Avena sativa). Plants were rated for phytotoxic injury (0 = no injury, to 10 = complete kill) and injury, described as chlorosis, burning, stunting, or reduced germination, was noted 18 to 21 days after planting.

Summary of Results

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

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

APPENDIX P: (continued)

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

Table 1. Phytotoxicity Ratings [a/] on Chicken Litter Treatments

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

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

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