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CERTIFICATION STATEMENT

RUMENSIN® (MONENSIN SODIUM) TYPE A MEDICATED ARTICLE

ENVIRONMENTAL ASSESSMENT FOR THE USE OF MONENSIN AT 40 G/TON (480 MG/HEAD/DAY) FOR CATTLE FED IN CONFINEMENT FOR SLAUGHTER

We certify the information furnished in the above titled environmental assessment report is true and accurate to the best of our knowledge.

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20 October 2005
Date

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A Division of Eli Lilly and Company

20 Oct 2005
Date

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Elanco Animal Health
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ENVIRONMENTAL ASSESSMENT FOR THE USE OF MONENSIN 40 G/TON (480 MG/HEAD/DAY) FOR CATTLE FED IN CONFINEMENT FOR SLAUGHTER

1. DESCRIPTION OF THE PROPOSED ACTION

Monensin granulated, USP is the active ingredient in RUMENSIN Type A Medicated Article. RUMENSIN is currently approved for feeding to cattle in confinement at a rate of 5-30 grams per ton to increase efficiency of feed utilization (21 CFR 558.355; December 16, 1975). When incorporated into cattle rations, monensin alters the production of volatile fatty acids in the rumen. As a result of increased production of propionic acid, the usable energy derived from the ration is increased. RUMENSIN is also approved (21 CFR 558.355; Federal Register, July 28, 1978) for use in the rations of growing cattle in pastures (up to 200 mg monensin/head/day). In 1983, approval for use of RUMENSIN in pastured cattle was expanded to include beef and dairy replacement heifers. In 1987, approval was granted for the use of RUMENSIN in reproducing beef cattle (21 CFR 558.355; Federal Register, December 15, 1988) and an Environmental Assessment was submitted for this use. In 1990, approval was granted for use of RUMENSIN in beef cattle for the prevention and control of coccidiosis (21 CFR 558.355; Federal Register, October, 1990). A supplemental new animal drug application was approved in October 2004 for the use of RUMENSIN in dairy cows for increased milk production efficiency (21CFR 558.335; Federal Register, April, 2005).

A new request has been provided to the U.S. Food and Drug Administration to allow for the use of RUMENSIN at an upper dose of 40 grams of feed per ton for cattle confined for slaughter. This increased level of RUMENSIN in the feed of beef cattle is for increased efficiency of feed utilization and for prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii.*

2. IDENTIFICATION OF CHEMICAL SUBSTANCE

A. RUMENSIN TYPE A MEDICATED ARTICLE

RUMENSIN Type A Medicated Article will be incorporated into rations of feedlot cattle. Monensin is the active ingredient in RUMENSIN and is produced in granular and crystalline forms. The raw material is mixed with diluents such as rice hulls, anti-dusting oil, and densifiers to concentrations of 45, 60, 80, and 90 grams monensin/lb. of medicated article.

B. MYCELIAL MONENSIN

Monensin is produced by the fermentation of a strain of *Streptomyces cinnamonensis*, an organism isolated from soil (Haney and Hoehn, 1968). The most economical procedure to prepare a usable form of monensin is to harvest the fermentation culture in such a way as to combine monensin with the mycelial cells of the producing organisms and the unused components of the feed-stock used in the fermentation to achieve growth of the organism. Thus, the granulated form of monensin contains dried mycelial biomass containing nutrients commonly found in cattle feedstuff, along with pelleting aids and limestone. Occasionally, crystalline monensin sodium may be added to the granules to adjust the monensin concentration.

C. MONENSIN

Monensin consists primarily of monensin factor A, but small amounts of monensin factor B, 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, 1968; Pressman, 1976).

Monensin Sodium:

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

Chemical Name (acid form):

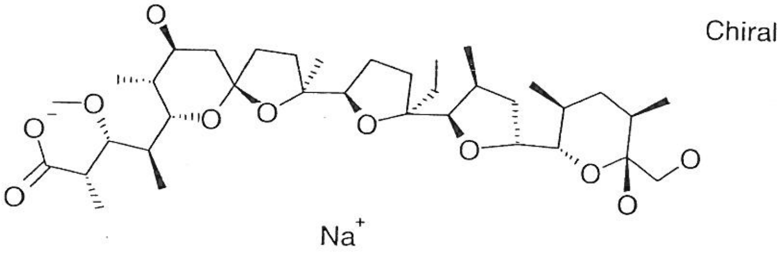
Stereoisomer of 2-[2-ethyloctahydro-3'-methyl-5'-tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl][[2,2'-bifuran]-5-yl]-9-hydroxy- ß-methoxy-α,γ'2,8-tetramethyl-1,6-dioxaspiro[4,5]decane-7-butanoic acid.

CAS Registry Number: Monensin 17090-79-8; Monensin Sodium 22373-78-0

Molecular Formula: $C_{36}H_{62}0_{11}$ (acid), $C_{36}H_{61}0_{11}Na$ (salt)

Molecular Weight: 670 (acid), 692 (sodium salt)

Structural Formula:



3. PHASE I ENVIRONMENTAL IMPACT ASSESSMENT

Final Guidance for Industry #89 (CVM, 2001) published by the FDA, Center for Veterinary Medicine and containing the VICH GL6 Phase I guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) was consulted to conduct the Phase I Environmental Assessment for this proposed action.

The total residue (monensin and metabolites) eliminated from beef cattle in confinement and applied to soil in manure can theoretically be calculated (Spaepen et al., 1997) to result in a concentration of 151 μg/kg soil. This is based on 480 mg of monensin ingested by each animal in confinement every day. If every animal that passed through confinement was fed monensin, then as much as 175,200 mg of monensin would be used over a year per animal space. If each animal space yields 9185 kg of manure (Spaepen et al., 1997), then the concentration of total residue in manure could be as high as 19.1 ppm. Assuming the nitrogen (52.64 kg N/animal space/year) in cattle manure is limited to use of no more than 170 kg N/ha/year, then about 29,663 kg of manure (9185 kg manure/animal space/yr x (170 kg N onto soil/ha/yr ÷ 52.64 kg N/animal space/yr)) would be used to fertilize one hectare of soil 25 cm deep each year. Total residues in soil would theoretically be no higher than 151 μg/kg ((29,663 kg manure/ha x 19,100 μg residues/kg manure) ÷ (1500 kg soil/m^3 x 10000 m^2/ha x 0.25 m deep)).

Since the $PEC_{soil\text{-}initial}$ of 151 μg/kg is higher than 100 μg/kg, a Phase II Tier A Environmental Impact Assessment has been conducted.

4. PHASE II TIER A ENVIRONMENTAL IMPACT ASSESSMENT

Final Guidance for Industry #166 (CVM, 2004) published by the FDA, Center for Veterinary Medicine and containing the VICH GL38 Phase II guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) was consulted to conduct the environmental impact assessment for this proposed action. This environmental impact assessment documents the results of the recommended tests, applies appropriate assessment factors, calculates risk quotients, and, where appropriate, refines the predicted environmental concentrations and recalculates the risk quotients.

A. RESULTS OF STUDIES APPLICABLE TO TIER A TESTING RECOMMENDED FOR PHYSICAL-CHEMICAL PROPERTIES

Solubility: HPLC method (Appendix A)

Milli-RO water	109 mg/L
pH4	Degraded
pH7	4.80 mg/L
pH9	8.91 mg/L

Melting Point:	103-105° C (acid) 267-269° C (sodium salt)
UV absorption:	None

pKa value: 6.65 (66% DMF)

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

N-octanol/Water Partition Coefficient at 25° C (Appendix B):
17329 at pH 5
567 at pH 7
6135 at pH 9

B. RESULTS OF STUDIES APPLICABLE TO TIER A TESTING RECOMMENDED FOR ENVIRONMENTAL FATE PROPERTIES

Hydrolysis (Appendix C): Little or no hydrolysis in 30 days at pH 5, 7, and 9.

Photolysis (Appendix C): Some photolysis at pH 7, with an apparent half-life of 43 days.

Soil Adsorption/Desorption (Appendices D and E): Log Koc is 5.45 to 5.68; Some leaching in sand and sandy loam soil; little leaching in loam and silty clay loam soils.

Soil Biodegradation (Appendices F, G, H and I):
Biodegradation half life in potting soil in a greenhouse: 5.8 days (with feces)
7.3 days (without feces)

Half life in field soil decline study: 7.5 days (with manure)
7.4 days (without manure)

Loss of ^{14}C labeled monensin from potting soil in a greenhouse: 48% in 5 weeks

Biodegradation of ^{14}C labeled monensin in sandy, silt and clay loam soils: More than 50% of the monensin disappeared from all three soils within 14 days. The principle degradation product was $^{14}CO_2$, accounting for 43 to 81% of the radiolabel by the end of the 12-week study.

C. RESULTS OF STUDIES APPLICABLE TO TIER A TESTING RECOMMENDED FOR ENVIRONMENTAL EFFECTS

AQUATIC EFFECTS STUDIES AND PREDICTED NO-EFFECT CONCENTRATIONS

Algal growth inhibition test (Appendix J):

A 72-hour static toxicity study was conducted to observe the effects of monensin on the green algae, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*). Based on mean measured concentrations, the EC_{50} for growth rate was 4.33 ppm with 95% confidence limits of 3.44 to 5.89 ppm. The NOEC for growth rate was 0.32 ppm. The EC_{50} for biomass was 0.98 ppm with 95% confidence limits of 0.81 to 1.17 ppm. The NOEC for biomass was 0.055 ppm. Biomass was the most sensitive endpoint in this study, thus the most conservative EC_{50} and NOEC values for this study were monensin concentrations of 0.98 and 0.055 ppm, respectively. Based on an application factor of 100 for the EC_{50}, the predicted no-effect concentration for algae is 9.8 ppb.

Daphnia immobilization test (Appendix K):

Based on daphnid immobility and mean measured concentrations of monensin sodium, the 48-hr EC_{50} and the corresponding 95% confidence limits for this acute study with *Daphnia magna* were 10.7 ppm and 9.8 to 11.7 ppm, repectively. The slope of the concentration-response curve was 0.280. No daphnids were found to be immobile nor did any daphnids display abnormal behavior (hypo-activity, prostration) in this study at a monensin concentration of ≤4.2 ppm. Abnormal behavior and/or immobility were noted for monensin concentrations ≥5.6 ppm. Based on an application factor of 1000 for the EC_{50}, the predicted no-effect concentration for daphnids is 10.7 ppb.

Fish acute toxicity tests:

A static toxicity test was conducted to determine the acute effects of monensin sodium (mycelial) on juvenile bluegill (Appendix L). Based on mean measured concentrations of monensin sodium, the 96-hr LC_{50}, the 95% confidence limits of the LC_{50}, and the slope of the concentration-response line were 16.6 ppm, 16.3 to 17.0 ppm, and 0.438, respectively. In this study, fish exposed to monensin concentrations ≥4.4 ppm displayed behavioral signs of toxicity (from hypoactivity to prostration). No mortalities or behavioral signs of toxicity were found for bluegill exposed to monensin sodium concentrations ≤ 3.1 ppm. Based on an application factor of 1000 for the LC_{50}, the predicted no-effect concentration for bluegill is 16.6 ppb.

A static toxicity test was conducted to determine the acute effects of monensin sodium (mycelial) on juvenile rainbow trout (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. Fish exposed to monensin concentrations ≥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 trout exposed to the monensin sodium concentration of 0.70 ppm.

Based on an application factor of 1000 for the LC_{50}, the predicted no-effect concentration for fish is 9.0 ppb.

TERRESTRIAL EFFECTS STUDIES AND PREDICTED NO-EFFECT CONCENTRATIONS

Earthworm Toxicity Tests:

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 (Appendix N). Six out of fifteen worms were dead by the end of the study at the highest monensin sodium concentration tested. No dead worms were found at the exposure concentration of 45 mg/kg. Normal physical condition and no mortalities were noted for worms exposed to monensin sodium concentrations ≤22.5 mg/kg. 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.

Earthworms (*Eisenia foitida foetida*) were also exposed for 14 days to mean calculated soil concentrations of 47.4, 121.8, 232.2, 467.4 and 1063.2 mg/kg monensin (Appendix O). Based on body weight and survival, the 14-day LC_{50} was estimated to be 264.2 mg/kg monensin. There were no significant differences in mean body weight between treatment groups and the controls. No unusual behavior or signs of toxicity were noted in any of the test or control vessels throughout the study. No statistically significant effects on survival or mean body weight were found at 47.4 ppm.

The predicted no-effect concentration (PNEC) for worms can be estimated by dividing the LC_{50} by 1000 (European Chemicals Bureau, 2003). So the PNEC for worms would be 264 ppb in soil.

Terrestrial plant tests:

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 (Appendix P). 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 annum*), 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 non-phytotoxic at monensin application rates of approximately 1 to 2 ppm. Monensin concentrations of 4 to 8 ppm in the soil caused moderate to severe injury to several plants. In another study, monensin was incorporated into soil with chicken litter at litter application rates of 1, 2, 4, and 8 tons of fresh litter per acre. Litter from monensin-fed chickens was no more phytotoxic than litter from control chickens. There was some phytotoxicity due just to the litter itself at an application rate of 8 tons/acre.

A field phytotoxicity study was conducted with 22 tons/acre (49.3×10^3 kg/ha) of manure from cattle fed monensin (Appendix Q). The cattle feed contained 20 g monensin/ton or 40 g monensin/ton. Cattle given feed with 40 g monensin/ton had an average of 4.4 ppm of monensin in their feces. The plot containing manure from cattle fed 40 g monensin/ton of feed had, therefore, a monensin sodium concentration of approximately 0.145 ppm ((49.3×10^3 kg/ha x 4.4 mg/kg) ÷ (4 inches x 375,000 kg/ha-inch soil)). The plant species tested were the same as those used in the greenhouse phytotoxicity study. Because of extensive rainfall, the plants in the plot treated with manure from cattle fed 20 g monensin/ton of feed could not be evaluated. The maturation, flowering, fruiting, or seed formation of oats, sorghum, soybeans, barley, sugar beets, corn, tomato, cotton, and cucumbers appeared to be the same in the control plot and the plot treated with manure from cattle fed 40 g monensin/ton feed. No differences between control and treatment plots were found for the growth or vigor of wheat, rice, pepper, alfalfa, and fescue.

A standard glasshouse phytotoxicity study was conducted in which one monocotyledonous (winter oats) and two dicotyledonous (radish, mung bean) plants were grown from seed in treated and untreated soils (Appendix R). Plants were exposed to measured monensin concentrations of 0.312, 4.347 and 35.970 mg/kg. In winter oats, an LC_{50} for emergence was greater than 35.970 mg/kg, the highest tested concentration, and an EC_{50} for growth was estimated to be 12.9 mg/kg monensin. The NOEC for winter oats was determined to be 4.347 mg/kg monensin. In radish, an LC_{50} for emergence was estimated to be 9.8 mg/kg, and an EC_{50} for growth was determined to be greater than 4.347 mg/kg monensin (no emergence at highest concentration). The NOEC for radishes was determined to be 4.347 mg/kg monensin. In mung bean, an LC_{50} for emergence was estimated to be 24.1 mg/kg, and an EC_{50} for growth was estimated to be 32.9 mg/kg monensin. A NOEC for mung bean was determined to be 4.347 mg/kg monensin. The test was terminated 14 days after 50% emergence was observed in the controls which corresponded to 19, 18 and 20 days for winter oats, radish and mung bean, respectively.

The overall predicted no-effect concentration for terrestrial plants has been determined based on a substantial amount of data. When manure from cattle fed monensin at the currently proposed use rate (40 g/ton) was used to fertilize soil at a rate (49.3 metric tons/ha), no effects on crop species were found. Waste from control chickens and those fed monensin affected crops in about the same fashion. When monensin was applied directly to soil, plants appeared to be more sensitive. The most definitive study was conducted with oats, the radish and mung beans according to recent testing guidelines. No adverse effects were found for any of these species at 4.3 ppm. Using an application factor of 100 on the most sensitive EC_{50} or LC_{50} value calculated for these 3 species would place the predicted no-effect concentration at 98 ppb. This level is clearly below the measured no-effect concentrations for any of the studies with terrestrial plants, but can be used as a conservative PNEC in order to calculate a risk quotient.

Soil microorganism and nitrogen transformation tests:

A soil microorganism study was conducted to evaluate the potential for monensin to inhibit respiration of soil microorganisms and their ability to transform nitrogen in soil under aerobic conditions (Appendix S). Mean exposure concentrations of monensin in the sandy loam soil were 3.2 and 18.3 mg/kg monensin. Deviations of 25 percent or more from control rates were not found in either evaluation in soil treated with 3.2 and 18.3 mg/kg monensin in this study. The predicted no-effect concentration for soil microorganisms and nitrogen transformation does not require an application factor and is at least 18.3 ppm.

D. PREDICTED ENVIRONMENTAL CONCENTRATIONS

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

Soil

The initial predicted environmental concentration used for the first calculations of risk quotients is the same as the $PEC_{soil\text{-}initial}$ used in the phase I environmental impact assessment shown in Section 3 of this document. This initial value of 151 ppb is considered to be the worst case concentration in that it combines the total of parent plus metabolites.

Several refinements to this calculation are possible. Monensin is extensively metabolized by cattle and actual elimination of parent, plus active metabolites above ten percent of the delivered daily dose is lower than the worst case calculation for $PEC_{soil\text{-}initial}$. Metabolites of monensin are far less active than the parent material. Finally, monensin has been documented to degrade within a few weeks in soil and mineralize within just a few months.

Monensin and its metabolites are quantitatively excreted in cattle feces (Donoho et al., 1978). Beef cattle fed a ration containing 40 g monensin/ton of feed had an average monensin concentration of 4.4 ppm in their feces (Appendix T). Dairy cows (Appendix U) were administered 918-1125 mg monensin/day intraruminally for 9 consecutive days, approximately double the proposed highest daily intake of 480 mg/day (40 g/ton). The average fecal concentration of monensin residues over the last 5 days of the study was as high as 12 ppm. Approximately 50% of the fecal residue was un-metabolized monensin, therefore monensin was present in the feces at a concentration of about 6 ppm. Since the highest expected daily intake for monensin is 480 mg/day and is about 50% of the dose used in this study with dairy cows, the concentration of monensin in the feces of dairy cows would be expected to be about 3 ppm (6 ppm x 0.50).

Monensin is extensively metabolized in cattle, dairy cows, rats, chickens, dogs, sheep, pigs, and turkeys (Donoho, 1984; Donoho et al., 1978, Kennington et al. 1995). The pattern of metabolism is qualitatively similar among species, although quantitatively different. By inference, the toxicology of monensin metabolites present in cattle feces has been evaluated in toxicology studies in which rats were exposed to monensin. More than 20 metabolites of monensin have been found for rats and cattle. About 50% to 60% of the monensin in an oral dose to cattle is metabolized (Donoho et al., 1978). The primary monensin metabolites, M-1, M-2, and M-6, are 0-demethylated. In dairy cows, the primary metabolites, M-1, M-2 and M-6,account for about 4%, 4%, and 5%, respectively, of the initial dose given to the animals (Appendix U). Metabolite M-1 is 20 times less biologically active than monensin, based on several test systems (Donoho, 1984). Thus, the first step in the metabolism of monensin (O-demethylation) appears to eliminate most of the biological activity of the compounds in this metabolic pathway (Donoho, 1984). Based on the number of metabolites, the fact that each represents a low percentage of the daily dose, and their low level of biological activity, metabolites of monensin were not considered in the first refined estimation of the environmental concentration of monensin. The measured concentration of monensin in cattle feces of 4.4 ppm is a realistic upper limit for monensin in the feces of beef cattle. Up to 50% of the monensin delivered to cattle on a daily basis can be used to calculate the worst-case concentration of monensin alone in manure from cattle kept in confinement.

The monensin eliminated from beef cattle in confinement and applied to soil in manure can theoretically be calculated (Spaepen et al., 1997) to result in a refined concentration of 75.5 μg/kg soil. This is based on 480 mg of monensin ingested by each animal in confinement every day. If every animal that passed through confinement was fed monensin, then as much as 175,200 mg of monensin would be used over a year per animal space, and up to 87,600 mg would be excreted as monensin into the manure. If each animal space yields 9185 kg of manure (Spaepen et al., 1997), then the concentration of total residue in manure could be as high as 9.54 ppm. Assuming the nitrogen (52.64 kg N/animal space/year) in cattle manure is limited to use of no more than 170 kg N/ha/year, then about 29,663 kg of manure (9185 kg manure/animal space/yr x (170 kg N onto soil/ha/yr ÷ 52.64 kg N/animal space/yr)) would be used to fertilize one hectare of soil 25 cm deep each year. Total monensin in soil would theoretically be no higher than 75.5 μg/kg ((29,663 kg manure/ha x 9540 μg monensin/kg manure) ÷ (1500 kg soil/m^3 x 10000 m^2/ha x 0.25 m deep)).

Based on the actual measured monensin concentration in cattle manure (4.4 ppm), the initial concentration of monensin in soil is likely to be no more than 35 μg/kg ((29,663 kg manure/ha x 4400 μg monensin/kg manure) ÷ (1500 kg soil/m^3 x 10000 m^2/ha x 0.25 m deep)).

Since monensin has been shown to substantially degrade within 12 weeks to carbon dioxide via a pathway that contains at least 27 components, the parent molecule is not expected to accumulate in soil with repeat applications of manure.

Water

Monensin adsorbs strongly to soil. As already noted in section 4B of this document, the organic carbon sorption coefficient (log K_{oc}) for monensin is up to a value of 5.68 (Appendix D). Except for sandy soils, very little leaching of monensin was measured in a soil column leaching study (Appendix E). Organic carbon content in soil averages about 1.8% (Appendix I). Normalizing for the percent organic carbon in the soils yields a calculated K_d value of 8615 ((antilog 5.68) x 0.018).

This K_d value can be used to calculate monensin concentrations in soil pore water (European Chemicals Bureau, 2003), which approximates groundwater concentrations.

$$PEC_{\text{soil pore water}} = ((PEC_{\text{soil}}) \text{ X } (\text{wet bulk density of soil}))/((1000) \text{ X } (K_{\text{soil-water}}))$$

Where:
Wet bulk density of soil = 1700
$K_{\text{soil-water}} = 0.2 + 1.5K_d$ where loss to air is considered to be zero

Since the highest calculated concentration of monensin and metabolites (total residues) in cropland soil is 151 ppb, this would result in an initial predicted environmental groundwater concentration ($PEC_{\text{initial groundwater}}$) for total monensin residues of about 0.020 ppb. This estimated concentration of total monensin residue is based on the assumption that the water would be in contact with cropland soil long enough to allow monensin concentrations in the soil and water to come to equilibrium. Concentrations extracted into surface waters ($PEC_{\text{initial surface water}}$) should be no higher than this level.

Refined calculations using only the active monensin molecule leads to even lower calculated concentrations. Using the fact that only 50% of the residues are the active monensin molecule, the highest concentration extracted into water would be about 0.010 ppb based on the theoretical concentration of monensin in manure. When based on the actual measured concentration of monensin in manure, the highest concentration calculated to be extracted into water would be 0.0046 ppb.

These calculated concentrations do not take into account dilution by surface waters, movement of soil from cropland into runoff, or direct loss from feedlots into runoff. So concentrations measured in grab samples from the field could vary substantially depending on the amount of dilution and suspended sediment in the samples. When detectable, monensin levels ranging from 0.005 to 1.2 ppb have been found in surface waters in southern Ontario (Lissemore, et. al., 2003) and from about 0.008 to 0.011 ppb in Colorado (Carlson, 2004).

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 less than 14 days) indicates that metabolism of monensin could occur in natural aquatic systems. Monensin does not hydrolyze but can be degraded by photolysis in a buffered (pH 7) solution, yielding a half-life of 43.9 days (Appendix C).

While not representative of concentrations expected in surface or groundwater, the highest measured concentration of monensin in surface waters (1.2 ppb) will be used to calculate risk quotients and evaluate the safety of aquatic organisms.

E. CALCULATED RISK QUOTIENTS FOR TERRESTRIAL AND AQUATIC ORGANISMS

Risk quotients for terrestrial organisms

The $PEC_{soil\text{-}initial}$ of 151 ppb provides risk quotients (PEC/PNEC) for earthworms and soil microorganism below 1. The risk quotient for earthworms is 0.57. The risk quotient for nitrogen transformation and respiration by soil microbes is at least as low as about 0.008. The risk quotient based on the conservative PNEC for terrestrial plants of 98 ppb and on the $PEC_{soil\text{-}initial}$ of 151 ppb is 1.5. This risk quotient for terrestrial plants requires futher evaluation based on refined PEC values, which exclude consideration of metabolites in low amounts with low biological activity (Section 4D). These refined calculations demonstrated that initial monensin concentrations in soil would be no higher than 75.5 ppb, and would likely be about 35 ppb. These refined initial PEC values for soil provide risk quotients of 0.77 and 0.36 for terrestrial plants. Both of these values are below 1. Based on this evaluation, Tier B testing and environmental impact assessment are not necessary. The refined predicted initial concentration of monensin in soil does not present a significant risk to representative terrestrial organisms.

Risk quotients for aquatic organisms

The highest measured concentration of monensin in surface waters is 1.2 ppb. This value is far higher than the predicted environmental concentration initially expected in surface water ($PEC_{sw\text{-}initial}$) of about 0.020 ppb or most of the other concentrations detected in surface waters. Yet even when the high measured value of 1.2 ppb is used to calculate the risk quotients for aquatic organisms, all the PEC/PNEC ratios are below 1. The risk quotient for algae is 0.12. The risk quotient for daphnids is 0.11. And the risk quotient for fish is 0.13. Based on this evaluation, Tier B testing and environmental impact assessment are not necessary. The $PEC_{sw\text{-}initial}$ and the highest measured monensin concentration in surface water do not present significant risks to representative aquatic organisms.

5. FATE OF MONENSIN IN AQUATIC ORGANISMS

Aquatic organisms could be exposed to very low levels of monensin when runoff occurs from surrounding agricultural fields. Moderate bioconcentration of monensin may occur based on the range of n-octanol/water partition coefficients that occur in the pH range of natural waters (pH 7 to pH 9). Neely, Branson, and Blau (1974) developed a regression equation for projected steady-state residue concentrations in trout muscle versus measured n-octanol/water partition coefficients for a variety of synthetic compounds:

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

Using this equation and the experimentally derived values for log K_{ow} (2.75 at pH 7; 3.79 at pH 9), the predicted BCF for monensin ranges from 41 to 151. This calculated BCF indicates that up to 151 times more monensin might be found in fish muscle than in the surrounding water. At the upper concentration of monensin (1.2 ppb) found by Lissemore et al. (2003), the theoretical concentration of monensin in fish woud be about 181 ppb. Dissipation of monensin from the environment would result in lower levels of this material in fish tissue.

6. GROUNDWATER

The measured sorption coefficient for monensin to organic carbon is large (Appendix D). The mobility of monensin in soil is expected to be low. Moderate mobility could occur in coarse soils such as sand and sandy loam (Appendix E). Given the moderately short half-life of monensin in field soil (less than 14 days), and its eventual mineralization, it is unlikely that significant concentrations of monensin would be found in groundwater. Calculations in Section 4D using an estimated soil sorption coefficient of 8615 demonstrates that the highest theoretical concentration of total monensin residues in groundwater should be no higher than about 0.020 ppb. Monensin itself should be no higher than 0.010 ppb. If it were possible for groundwater with 0.020 ppb monensin residues to surface and expose aquatic organisms, the risk quotient for this water would only be 0.002. There does not appear to be any significant risks from groundwater to aquatic organisms.

7. HUMAN HEALTH

Extensive chemistry and toxicology data have been developed to support the safe use of monensin in cattle relative to residues in edible tissues. The FDA published an Acceptable Daily Intake (ADI) for monensin of 12.5 µg/kg/day. If humans drank 2 liters of water containing the highest concentration of monensin found in surface water (1.2 ppb), exposure to a 70 kg person would only be 0.27% of the published ADI. If humans ate a 20-gram meal of fish with the highest possible residue levels of 181 ppb, exposure to a 70 kg person would only be 0.41% of the published ADI.

8. CONCLUSIONS

Monensin concentrations in the environment will be initially low (≤ 76 ppb in soil and ≤ 1.2 ppb in water) and will substantially decrease within weeks. Risk quotients are all below 1 for effects in soil microbes, earthworms, terrestrial plants, or aquatic organisms when the predicted environmental concentration in soil is based on the predicted monensin concentration alone. Environmental residues of monensin are expected to be safe to humans. The information provided in this environmental assessment demonstrates that no extraordinary circumstances exist for the safety of monensin in the human environment. This assessment demonstrates that the proposed action to increase the concentration of monensin to 40g/ton in feed of cattle confined for slaughter will not result in any expected effects in the environment.

9. INFORMATION ON ENVIRONMENTAL ASSESSMENT EXPERT

Name: Roger D. Meyerhoff

Degrees

Ph.D.	Fisheries/Pharmacology & Toxicology,	Oregon St. Univ.	1980
M.S.	Fisheries/Limnology and Water Pollution,	Oregon St. Univ.	1976
B.S.	Fisheries and Wildlife Biology,	Univ. Calif. at Davis	1974

Current Appointment
Senior Research Advisor
Health, Safety, and Environmental
Lilly Research Laboratories

Previous Appointments (Lilly Research Laboratories, Eli Lilly and Company)
1994 - 2003: Head, Environmental Sciences and Hazard Communications
1980 - 1994: Senior Toxicologist, Research Scientist, and Senior Research Scientist for Environmental Toxicology

Publications
Fifteen publications. Topics: Environmental toxicology, chemistry and risk assessment
More than one hundred regulatory reports. Topics: Environmental toxicology, chemistry and risk assessment

Professional Activities and Presentations to Learned Societies:
President (1991-1992), Society of Environmental Toxicology & Chemistry (SETAC)
Board of Directors (1987-1993), Society of Environmental Toxicology & Chemistry
Chairman (1993-1995), SETAC Foundation for Environmental Education
Member (1985-1987), Nat. Agri. Chem. Assoc. Env. Tox. & Chem. Subcommittee
Member (1987-Present), Animal Health Institute Environmental Working Group
Member (1991-Present), PhRMA Environmental Working Group
Member (2004-Present), ABPI Ad Hoc Working-Group for Environmental Assessment
Sixteen research presentations to scientific societies
Ten invited lectures at universities and government research laboratories

10. REFERENCES

CARLSON, K. (2004). Minimizing the environmental impact of agricultural antibiotic use at the watershed scale. Colorado Water. Newsletter of the Water Center of Colorado State University. pp. 22-24.

CENTER FOR VETERINARY MEDICINE (2001). Guidance for industry: Environmental impact assessments (EIA's) for veterinary medicinal products (VMP's). Phase I VICH GL6 Final Guidance. Food and Drug Administration CVM document 89. 10 pp.

CENTER FOR VETERINARY MEDICINE (2004). Guidance for industry: Environmental impact assessment (EIA's) for veterinary medicinal products (VMP's). Phase II VICH GL38. Food and Drug Administration. CVM document 166. 36 pp.

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

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

EUROPEAN CHEMICALS BUREAU. (2003). Technical guidance document on risk assessment. Part II. Environmental risk assessment. Institute for Health and Consumer Protection. European Commission Joint Research Center. 328 p.

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

LISSEMORE, L., HAO, C., YANG, P., SOLOMON, K., AND NGUYEN, B. (2003). An Exposure Assessment for Agricultural Antibiotics in Surface Waters within a Model Watershed in Southern Ontario.

KENNINGTON, A.S., DARBY, J.M., EHRENFRIED, K.M., KIEHL, D.E., MORAN, J.W., AND SWEENEY, D.J. (1995). ^{14}C Monensin Milk and Tissue Residues/Metabolism in Dairy Cows. Animal Science Chemical Research. Lilly Research Laboratories.

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

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

SPAEPEN, K.R.I., VAN LEEMPUT L.J.J., WISLOCKI, P.G., AND VERSCHUEREN C. (1997). A uniform procedure to estimate the predicted environmental concentration of the residues of veterinary medicines in soil. Env. Tox. and Chem. Vol. 16(9): 1977-1982.

Appendix A: Report Summary

Title: Physico-Chemical Testing with Monensin: Validation of Analytical Method and Determination of Water Solubility

Project Number: 341613

Report Number: 21247

Study Dates: Dec 17, 2001 to Feb 4, 2002

Name and Address of Investigator: A Hogg, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin sodium

Test System: Milli-RO water and Aqueous Buffered Media

Summary of Experimental Design: An analytical method for monensin sodium, based on high performance liquid chromatography, was developed for Assay Accuracy, System Precision, Stability, Specificity, Limit of Detection and Limit of Quantification.

The aqueous solubility of monensin sodium was determined in Milli-RO water, and pH 4, 7 and 9 aqueous buffers according to OECD Guideline 105. Monensin sodium (lot no. RS0234) was added to 60 ml amberlite jars with glass stoppers and diluted with 25 ml of Milli-RO water, or respective buffer solution. The jars were agitated on an orbital shaker with a water bath at 29.4 °C at 200 rpm for equilibration periods of 24, 48 and 72 hours. Upon completion of the equilibration period, samples were removed and placed in a water bath at 20° C for 24 hours. Duplicate samples were taken from each flask and centrifuged at 3,000 rpm for 5 minutes. Supernatents were passed through a 0.45 micron nylon filter, appropriately diluted (pH 4 not diluted), and analyzed for Monensin sodium.

Summary of Results: For Assay Accuracy, the overall mean percentage recovery from nominal was calculated to be 101.05% and a coefficient of variation (CoV) of 0.94% was achieved. For system precision, a CoV of 0.88% was calculated indicating that the system was suitably precise. Overall mean percent recoveries from nominal of 102.2% (CoV = 0.98%), 101.40% (CoV = 1.27%), and 103.74% (CoV = 0.89%) were calculated for the 24 h, 48 h, and 72 h time points, respectively, thus indicating that monensin sodium is stable in methanol/water (9:1 v/v) for at least 72 h at ambient laboratory temperature. For assay specificity, no potential interference with the test item peak from Milli-RO water or aqueous media buffered to pH 4, 7 or 9, or algae medium or acetone was noted. The LOQ was calculated to be 0.125 mg/L and the LOD was calculated to be 0.037 mg/L.

The solubility of monensin sodium in Milli-RO water and aqueous media buffered to pH 7 and pH 9 was determined to be 109 mg/L, 4.80 mg/L, and 8.91 mg/L, respectively. Monensin sodium degraded in aqueous medium buffered to pH 4 after 24 hour incubation at 30°C.

Medium	Mean water solubility of Monensin sodium at 30°C (mg/L)
Milli-RO water	109
pH 4	Degraded
pH 7	4.80
pH 9	8.91

APPENDIX B: Report Summary

Title: Octanol-Water Partition Coefficients for Monensin

Study: ABC-0438

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

Test Article: Crystalline ^{14}C Monensin Sodium

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

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

Summary of Results:

Results of the analyses are summarized in the following table:

Monensin Concentration	pH	K_{ow}	log K_{ow}
0.0002 M	5	17329	4.24
0.0002 M	7	567	2.75
0.0002 M	9	6135	3.79
0.00002 M	7	737	2.87

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

APPENDIX C: Report Summary

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

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:

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 show 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 (days)	43.9	
Rate Constant (day^{-1})	0.0158	
R^2	0.97	

Appendix D: Report Summary

Title: Physico-Chemical Testing with Monensin: Estimation of Adsorption Coefficient

Project Number: 341629

Report Number: 21246

Study Dates: Feb 11 to Mar 1, 2002

Name and Address of Investigator: A Hogg, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin sodium

Test System: Test 1: HPLC mobile Phase: methanol:0.01M citrate buffer (pH 6.0)
Test 2: HPLC mobile Phase: methanol:0.01M citrate buffer (pH 4.5)

Summary of Experimental Design (OECD Guideline 121):
The adsorption coefficient of Monensin sodium was estimated in two test systems (Test 1 and Test 2) using HPLC.

Test 1 Test Solutions were prepared by weighing duplicate aliquots (~10mg) of monensin sodium, lot no. RS0234, into separate 25 ml volumetric flasks and bringing to volume using methanol:0.01M citrate buffer (pH 6.0); 65:45, v/v. Dissolution was aided by sonication and each solution was analyzed, in duplicate, by HPLC. Test Solutions in Test 2 were prepared in the same manner, however, pH 4.5 buffer was used.

The following Individual Reference Standard Solutions were used for Test 1 and 2 and prepared with the respective methanol:0.01M citrate buffer (pH 6.0 – Test 1, pH 4.5 – Test 2).

> Reference Standards: N,N-Dimethylbenzamide, Naphthalene, 1,2,3-Trichlorobenzene, Phenanthrene and DDT, Carbendazim

For Test 1, reference standards, excluding DDT and Carbendazim were further diluted with respective mobile phase, and all reference standards were analyzed, in duplicate, by HPLC.

For Test 2, reference standards, excluding 1,2,3-Trichlorobenzene and DDT, were further diluted with respective mobile phase, and all reference standards were analyzed, in duplicate, by HPLC.

Sodium nitrate solution was prepared as an Unretained Standard Solution and used to determine the column dead time (t_0) of the HPLC column in both Test 1 and Test 2.

Mixed Standard Solutions were prepared for Tests 1 and 2 by taking appropriate aliquots of each Individual Reference Standard and the Unretained Standard Solution from respective test systems and adding them to a 50 ml volumetric flask. Solutions were brought to volume with respective buffer, sonicated to aid dissolution, and analyzed by HPLC.

Summary of Results:

Test 1: The dead time of the HPLC column was determined to be 2.49 minutes. Two peaks associated with monensin sodium were eluted. The first peak was considered to be a degradant and/or related substance of monensin sodium. Monensin sodium eluted in the second peak, following DDT, the reference standard with the highest log K_{oc} value quoted in OECD guideline 121. Log K_{oc} for Monensin was therefore determined to be Log K_{oc} > 5.63 (DDT value). A mean Log K_{oc} value was calculated by extrapolation of the reference standard plots to be 5.68. The pH of the test solutions and mobile phase ranged from 7.58 to 7.66.

Test 2: The dead time of the HPLC column was determined to be 3.38 minutes. Two peaks associated with monensin sodium were eluted. The first peak was considered to be a degradant and/or related substance of monensin sodium. Monensin sodium eluted in the second peak, following DDT, the reference standard with the highest log K_{oc} value quoted in OECD guideline 121. Log K_{oc} for Monensin was therefore determined to be Log K_{oc} > 5.63 (DDT value). A mean Log K_{oc} value was calculated by extrapolation of the reference standard plots to be 5.45. The pH of the test solutions and mobile phase ranged from 5.76 to 5.87.

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

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

Table 2

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

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

ND = not detectable

[1] Data are averages from three columns.

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 F: 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.

Table 1

Degradation of Monensin in Soil

	With Feces		Without Feces	
Sampling Time	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})		0.1190		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 G: 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 plat 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.

Table 1

ppm Monensin in Field Soil [a]

	Plot 1		Plot 2	
Sampling Time	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 H: Report Summary

Title: Monensin Biodegradation in Soil

Study Number: B77-3306

Study Dates: March 1 to November 1, 1974

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

Test Article: Crystalline ^{14}C Monensin

Test System: Soil maintained in the greenhouse

Summary of Experimental Design:

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

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

Summary of Results:

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

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

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 I: Report Summary

Title: The Degradation of [14C]-Monensin in Soil Under Aerobic Conditions

Project Number: 802395

Report Number: 21631

Study Dates: Jan 23 to April 17, 2002

Name and Address of Investigator: C Lowrie, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: [^{14}C]-Monensin sodium

Summary of Experimental Design: [^{14}C]-Monensin sodium was used to investigate the rate of breakdown of radiolabelled monensin in three fresh field soils at a nominal temperature of 20°C. Field soils consisted of Sandy Loam (route soil), Silt Loam (rate soil) and Clay Loam (rate soil). Upon collection and receipt, each soil was passed through a 2 mm sieve and the moisture content and maximum water holding capacity determined. Moisture content was adjusted to 45% MWHC prior to use and maintained at this level throughout the study. Soil characterization analysis revealed that the percent organic carbon content for sandy, clay and silt loam was 1.5%, 1.6% and 2.4%, respectively. Microbial biomass of each soil was determined prior to test article application and at study termination in control samples.

Treatment solutions were prepared for sandy loam, silt loam and clay loam soils from a [14C]-monensin stock concentration of 0.58 mCi/ml (equivalent to 89 mg/ml). The radiochemical purity of the test item was confirmed by HPLC and TLC. Treatment solutions were prepared corresponding to treatment application rates of 1.50, 1.53, and 1.52 mg/kg wet weight monensin in sandy, silt and clay loam soils, respectively. The radiochemical purity of the test solutions was assessed by HPLC prior to application to the test system and was deemed to be pure.

Single incubates from each soil were sampled at time zero, 7, 14, 21, 28, 42, 56, 70 and 84 days. Each soil sample was extracted with methanol:water (85:15, v/v) and chloroform by end-over-end shaker for approximately one hour. The extracts were separated from the residue by centrifugation (~ 1000 rpm; 15 minutes) and the radioactivity associated with the extract was quantified by liquid scintillation counting. Characterization of radioactivity in soil extracts was carried out using reverse-phase HPLC and normal-phase TLC.

Summary of Results:

Monensin degraded rapidly in sandy, silt and clay loam soils. Total levels of solvent extractable radioactivity in sandy loam declined from 98% to 4% over the 84 day study, while silt loam declined from 100% to 10%, and clay loam declined from 102% to 4%. The principle degradation product was $^{14}CO_2$ and accounted for 81%, 43%, and 63% of applied radioactivity at study termination in sandy, silt and clay loam, respectively. The degradation pathways to CO_2 included low levels of up to 27 unknown components. In the route soil (sandy loam), non-extractable residues increased to a maximum of 21% of applied radioactivity at Day 21, but declined for the remainder of the incubation period indicating complete mineralization in the soil. More than 50% of the monensin disappeared in all three soils in 14 days as shown in the following Table.

	Sandy Loam	Silt Loam	Clay Loam
Time (days)	% Monensin	% Monensin	% Monensin
0	97.93	100.21	101.64
7	25.78	71.40	58.19
14	13.40	36.77	6.74
21	2.18	21.25	3.02
28	2.75	8.63	1.32
42	1.88	3.47	0.71
56	1.78	2.00	1.89
70	1.84	1.53	0.59
84	2.19	1.37	2.21

Based on this data, silt Loam soil showed the most conservative DT_{50} value of 13 days for [^{14}C]-Monensin sodium with a dissipation rate of 0.055. Based on the results of this study, it is unlikely that monensin will persist in soil for a prolonged period of time.

Appendix J: Report Summary

Title: Monensin Sodium – Alga, Growth Inhibition (72h, EC50)

Project Number: 802594

Report Number: 21537

Study Dates: Mar 4 o Apr 12, 2002

Name and Address of Investigator: C R Kelly, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin Sodium (adjusted for purity of 96.5% Factor-A and expressed as mg/kg monensin).

Test Species: Green Alga, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum)*

Summary of Experimental Design: A static toxicity test was conducted to evaluate the effects of monensin sodium on the green alga, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*). There were six treatment levels containing monensin and three replicates at each treatment. There were six replicates for the control. To each replicate, approximately one million algal cells were added to 100 mL of appropriately treated Algal Growth Medium in sterile 250 mL flasks to give an initial cell concentration of 10,000 cells/mL. An uninoculated flask was prepared at the highest and second lowest concentration to indicate if monensin sorbed to algal cells during the exposure period. The test vessels were orbitally shaken at 100 rpm for 72 hours. Light intensity throughout the study ranged between 6,000 to 10,000 lux (spectral range 400 to 700 nm). The temperature and pH during the test ranged from 21.9 to 23.2°C and 7.4 to 8.2, respectively. At 24, 48, and 72 hours, a sample was removed from each flask and added to 100 µl of Lugols iodine in a scintillation vial and stored refrigerated before counting. Cell concentrations were determined using a compound light microscope and Improved Neubauer Counting Chambers. These measurements were used to calculate the growth rate and biomass for each replicate.

Summary of Results:
Initial measured concentrations in the treatments were 6.50, 2.62, 1.70, 0.781, and 0.336 mg/L. The lowest concentration of 0.055 mg/L was calculated by multiplying the lowest nominal concentration by the measured to nominal proportion of the highest dose. After 72 hours, measured concentrations of monensin were 6.36, 2.46, 1.32, 0.743, and 0.305, 0.055 (calculated) mg/L, resulting in mean measured concentrations of 6.43, 2.54, 1.51, 0.762, 0.321, and 0.055 mg/L, respectively. Additional replicates in the 6.50 and 0.35 mg/L treatments in which no cells were added resulted in 72 hr measured concentrations

of 6.19 and 0.265 mg/L, respectively, suggesting that monensin does not significantly sorb to algal cells. Over the duration of the study, the cell concentrations in the control flasks increased on average by more than 120 times the initial inoculated level, exceeding the minimum requirement of at least a 16-fold increase. The no-observed-effect concentration (NOEC) for growth rate was 0.32 mg/L. The median effective monensin concentration on reduction of growth rate (EC50) was 4.33 mg/L with 95% confidence limits of 3.44 to 5.89 mg/L. The NOEC for biomass was be 0.055 mg/L. The EC50 at 72 hours was calculated to be 0.98 mg/L with 95% confidence limits of 0.81 to 1.17 mg/L. Biomass was the most sensitive endpoint and, therefore, the most conservative EC50 and NOEC for this study were monensin concentrations of 0.98 and 0.055 mg/L, respectively.

APPENDIX K: Report Summary

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

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

Study Dates: May 25 to May 27, 1982

Study Number: C02382

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: *Daphnia magna*

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

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

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 ≥4.4 mg/L showed behavioral signs of toxicity in a concentration-related fashion, from hypoactivity to prostration. The 96-hr LC50, the 95% confidence limits for the LC50, and the slope of the concentration-response curve were 16.6 mg/L, 16.3 to 17.0 mg/L, and 0.438, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to monensin sodium concentrations ≤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 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: F10182

Test Article: Monensin sodium (mycelial)

Lot Number: X-30547

Species: Rainbow trout (*Oncorhynchus mykiss*)

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 ≥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 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 ≤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.

Appendix O: Report Summary

Title: Monensin Sodium – Determination of Acute Toxicity (LC50) to Earthworms

Project Number: 802589

Report Number: 21591

Study Dates: April 12 to April 26, 2002

Name and Address of Investigator: J M Hughes, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin Sodium (adjusted for purity of 96.5% Factor-A and expressed as mg/kg monensin).

Number of Animals: 40/treatment

Route: Incorporated into test media (industrial sand, kaolin clay, sphagnum moss peat)

Levels of Exposure: 0, 50, 125, 250, 500 and 1000 mg/kg monensin (nominal)

Length of Exposure: 14 days

Parameters Studied: Mean body weight gain per treatment group, mortality

Summary of Experimental Design: Four 1-L glass beakers containing 750 grams of soil were used for controls and for each exposure level. Nominal concentrations of 50, 125, 250, 500 and 1000 mg/kg monensin were chosen based on the results of a range finding test. A solvent control and untreated control were also included. Ten worms were placed onto the soil surface of each vessel at the beginning of the study. Prior to exposure, worms were rinsed with distilled water, blotted dry and weighed. The study was conducted in a temperature-controlled laboratory with the aim of maintaining a temperature range between 18 to 22°C. The concentration of monensin in the dosing solutions was analyzed by HPLC using a previously validated analytical method.

Summary of Results: Nominal concentrations of 50, 125, 250, 500 and 1000 mg/kg monensin resulted in mean exposure concentrations of 47.4, 121.8, 232.2, 467.4, and 1063.2 mg/kg, respectively.
All worms burrowed in the soil within 30 minutes of addition to the test vessels. No unusual behavior or signs of toxicity were noted in any of the test or control vessels. Temperature ranged from 20 to 22°C throughout the definitive test.

At Day 7, percent mortality was 0%, 7.5%, 22.5% and 80% for treatment concentrations of 47.4, 121.8, 232.2, 467.4 and 1063.2 mg/kg monensin, respectively. Control and solvent control vessels, at Day 7, showed 5% and 2.5% mortality, respectively. At Day 14, percent mortality was 17.5%, 45%, 32.5%, 65% and 95% for treatment concentrations of 47.4, 121.8, 232.2, 467.4 and 1063.2 mg/kg monensin, respectively. Control and solvent control vessels, at Day 14, showed 10% and 7.5% mortality, respectively. The LC50 was therefore estimated to be 690.3 and 264.2 mg/kg monensin for Day 7 and 14, respectively.

Even though the mean body weight loss was greater than the control value for all concentrations, there were no significant differences in mean body weight among treatment groups as compared to the control value.

No statistically significant effects were found for survival or body weight at an exposure concentration of 47.4 ppm.

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. Callendar, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

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

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

Summary of Experimental Design: Monensin or litter from monensin-fed chickens was incorporated into soil at concentrations shown in Table 1. A standard greenhouse phytotoxicity test was conducted in which fourteen mono- and dicotyledonous plants were grown from seed in the treated and untreated soils. The plant species were alfalfa (*Medicago sativa*), fescue (*Festuca elatior*), cucumber (*Cucumis sativus*), rice (*Oryza sativa*), cotton (*Gossypium hirsutum*), tomato (*Lycopersicon esculentum*), pepper (*Capsicum annum*), corn (*Zea mays*), sugar beet (*Beta vulgaris*), barley (*Hordem 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 2-1/2 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 non-phytotoxic 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.

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	2	3	1.5	0	0
Sugar Beets	0	0	3	4	0	0	0	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	1	0	0	0
Soybeans	0	0	0	1	0	0	0	1.5	0	0
	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
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.

APPENDIX Q: Report Summary

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

Study Number: B48-3273

Study Dates: February 1 to September 30, 1973

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

Test Article: Manure from Cattle fed Monensin

Test System: Crops grown in field plots

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

The plots were arranged in the following fashion:

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

Direction of rows→→→→→→→→→→

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

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

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

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

Table 1

Crop Injury Rating

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

Appendix R: Report Summary

Title: Monensin Sodium – Terrestrial Plant Growth Test

Project Number: 802464

Report Number: 21316

Study Dates: Feb 10 to Mar 4, 2002

Name and Address of Investigator: S Chapleo, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin Sodium (adjusted for purity of 96.5% Factor A and expressed as mg/kg monensin).

Test System: Plants grown from seed in environmental controlled glasshouse conditions.

Summary of Experimental Design: A standard glasshouse phytotoxicity test was conducted in which one monocotyledonous (oats) and two dicotyledonous (radish, mung bean) plants were grown from seed in treated and untreated soils. The plant species were winter oat (*Avena sativa*), radish (*Raphanus sativus*), and mung bean (*Phaseolus aureus*). The test was conducted at nominal concentrations of 0, 0.3, 3 and 30 mg/kg monensin. Appropriate amounts of test material were diluted with acetone and applied evenly to sand using a glass pipette to obtain the desired test concentration. The treated sand was mixed thoroughly by hand after allowing solvent to evaporate and transferred to a cement mixer containing the soil and mixed for approximately 10 minutes. Four pots were prepared for each treatment level for each species. Five seeds of appropriate species were sown in each pot and arranged in random block design. Pots were watered daily and covered with polythene until emergence, at which time it was removed. Pots were observed daily for shoot emergence and phytotoxic effects. An LC50 on emergence and an EC50 on growth was determined statistically for each species. Monensin concentrations were determined by HPLC analysis in each treatment solution used to administer the test item to the sand. Daily temperature and humidity were recorded.

Summary of Results: Nominal concentrations of 0.3, 3.0 and 30 mg/kg monensin corresponded to measured concentrations of 0.312, 4.347 and 35.970 mg/kg, respectively, as measured by HPLC. Monensin was not detected in control treatment solution.

The test was terminated 14 days after at least 50% emergence was observed in the control pots. This corresponded to day 19, 18 and 20 for winter oats, radish and mung beans, respectively. At termination, the stems of the plants were snipped at the soil surface, and the total fresh weight of seedlings from each pot was measured.

Winter oats

No reduction in emergence was seen at any treatment rate. An LC50 for emergence was therefore greater than 35.970 mg/kg monensin, the highest concentration tested.

Mean shoot weights did not differ significantly from control at 0.312 and 4.347 mg/kg. Mean shoot weights at 35.970 mg/kg, however, were significantly different from control. Plants at this concentration were smaller and emerged seedlings had darker coleoptiles than control plants. An EC50 for growth was estimated to be 12.9 mg/kg monensin.

No phytotoxic effects were seen at 0.312 and 4.347 mg/kg throughout the test. Therefore, a NOEC was determined to be 4.347 mg/kg monensin.

Radish

No emergence was seen at the 35.970 mg/kg treatment rate. An LC50 for emergence was estimated to be 9.8 mg/kg monensin.

Mean shoot weights at the 0.312 treatment rate were significantly greater than the control, however, this was not considered to be an adverse effect. Mean shoot weights at 4.347 mg/kg were not significantly different from the control and an EC50 for growth was therefore determined to be greater than 4.347 mg/kg monensin.

No adverse effects were seen at 0.312 and 4.347 mg/kg throughout the test. Therefore, a NOEC was determined to be 4.347 mg/kg monensin.

Mung Bean

Emergence was significantly reduced at the 35.970 mg/kg treatment rate. An LC50 for emergence was estimated to be 24.1 mg/kg monensin.

Mean shoot weights at the 0.312 treatment rate were significantly greater than the control, however, this was not considered to be an adverse effect. Mean shoot weights at 4.347 mg/kg were not significantly different from the control, however mean shoot weights at 35.970 mg/kg were significantly less than the control. An EC50 for growth was estimated to be 32.9 mg/kg monensin.

No adverse effects were seen at 0.312 and 4.347 mg/kg throughout the test. Therefore, a NOEC was determined to be 4.347 mg/kg monensin.

Appendix S: Report Summary

Title: Soil Microorganisms: Carbon and Nitrogen Transformation Tests with Monensin

Project Number: 802479

Report Number: 21530

Study Dates: Jan 30 to April 12, 2002

Name and Address of Investigator: S Chapleo, Inveresk Research, Tranent EH33 2NE, Scotland

Test Article: Monensin Sodium (adjusted for purity of 96.5% Factor-A and expressed as mg/kg monensin).

Summary of Experimental Design: A soil microorganism was conducted to evaluate the potential for monensin to inhibit the respiration of soil microorganisms and their ability to transform nitrogen in soil under aerobic conditions. The study was carried out using sandy loam soil collected from the upper 20 cm horizon by removing the surface vegetation and sampling the topsoil immediately below. The soil was passed through a 2mm sieve and kept in a moist, aerated condition until use. The microbial biomass of the soil was determined prior to test item application to confirm adequate microbial activity.

Approximately 1.2 kg soil (at 40% maximum water holding capacity) were weighed into 2.5 liter plastic containers. Containers were sealed with snap-tight lids containing holes to maintain aeration and incubated for 7 days at 20 ± 2°C to pre-equilibrate soil to laboratory conditions.

Three replicate soils were prepared for each treatment rate for each test (carbon transformation, nitrogen transformation). Each test was conducted at targeted concentration rates of 3.0 and 15.0 mg/kg monensin in soil. Soil samples in the nitrogen transformation test were amended with approximately 0.5% (w/w) Lucerne meal and thoroughly mixed. Soil samples were incubated at 20 ± 2°C in darkness under aerobic conditions for 28 days.

At intervals of 0-3 hour, 7, 14 and 28 days post-application, samples of soil were removed from each replicate and analyzed for soil respiration activity, and nitrogen determination.

Concentrations of monensin were determined in each treatment solution prior to addition to soil by HPLC analysis.

Summary of Results: Targeted concentrations of 3.0 and 15.0 mg/kg monensin resulted in mean exposure concentrations of 3.202 and 18.333 mg/kg monensin in soil, respectively. Monensin was not detected in the control treatment solution.

At 0-3 hr, 7, 14, and 28 days post-application, sub-samples of soil were amended with glucose and subsequent CO_2 evolution measured over an approximately 12 hour period. In soil treated with monensin at 3.202 and 18.333 mg/kg soil, neither treatment resulted in a deviation of more than 25% from the control at any time point over the test period.

At 0-3 hr, 7, 14, and 28 days post-application, sub-samples of soil amended with Lucerne were analyzed and levels of nitrate were determined. In soil treated with monensin at 3.202 and 18.333 mg/kg soil, neither treatment resulted in a deviation of more than 25% from the control at any time point over the test period.

This data indicates that monensin had no biologically significant effect on soil microbes at the two soil concentrations tested, 3.202 and 18.333 mg/kg monensin.

[redacted]

Appendix T: Report Summary

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

Study Number: C97-B47-215

Study Dates: February 9 to 21, 1973

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

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

Test System: Microbiological assay for monensin concentration

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

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

Table 1

Assay for Monensin in Cattle Feces

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

APPENDIX U: Report Summary

Title: ^{14}C Monensin Milk and Tissue Residues/Metabolism in Dairy Cows

Study Number: T1F749401

Study dates: August 2, 1994 to July 21, 1995

Name and Address of Investigator: A.S. Kennington, Animal Science Chemical Research, A Division of Eli Lilly and Company, Box 708, Greenfield, IN 46140

Test Article: ^{14}C Monensin

Test System: Lactating Dairy Cows

Summary of Experimental Design:

Five lactating dairy cows weighing 510 to 625 kg were administered gelatin capsules containing 14C monensin at a dose level of 1.8 mg/kg (918-1125 mg monensin/day) intraruminally for nine consecutive days. Animals were slaughtered 6 hours after the final dose for collection of tissues. Feces were collected daily and urine on days 2 and 6 from each animal for analysis of ^{14}C residues. Concentrations of residues were determined by LSC and residue identification was performed by LC/MS.

Summary of Results:

Results will only include those for feces and urine. Monensin residues were found in small quantities in the urine samples and averaged about 0.5 ppm on each day of sampling. Additional identification of urine metabolites was not performed. Residues were much higher in the feces. ^{14}C residues in the feces reached somewhat of a steady state by day 5 and averaged 8-12 ppm over the last 5 days of dosing. Additional analysis of the residues indicated that on average, parent monensin accounted for about 50% of the residues and the identified metabolites M-1, M-2, and M-6 accounted for about 4, 4, and 5%, respectively.