(1) ENVIRONMENTAL ASSESSMENT REPORT

1. **DATE**

July, 1993

2. and 3. **NAME OF APPLICANTS AND ADDRESSES**

Syntex Animal Health
Division of Syntex Agribusiness, Inc.
3401 Hillview Avenue
Palo Alto, California 94304

4. **DESCRIPTION OF PROPOSED ACTION**

A new animal drug approval has been requested for the use of Cattlyst™ in cattle. Cattlyst™ is a feed premix that contains 11% (50 g/lb) laidlomycin propionate potassium as active ingredient, and is to be incorporated in cattle rations at 0.10 lb/ton for improved average daily weight gain, or at 0.20 lb/ton for improved average daily weight gain and feed efficiency. Approval of this new animal drug would authorize the production of laidlomycin propionate potassium at Syntex Nutritional and Chemicals Division at Springfield, Missouri, for sale within and outside of the United States. The formulation and packaging of Cattlyst™ premix will be done by Glatt Air Techniques, located at Ramsey, New Jersey.

Based on the proposed action, laidlomycin propionate potassium could potentially be introduced into the following environments:

a. The environment adjacent to the Syntex Nutritional and Chemical Division, at Springfield, MO.

b. The environment adjacent to the Glatt Air Techniques formulating and packaging facility at Ramsey, NJ.

Laidlomycin, primarily as the sodium salt, which is the major metabolite of laidlomycin propionate potassium excreted by cattle, could potentially enter the following environments:

a. Cattle feedlots where laidlomycin sodium may be found in accumulated animal manure.

b. Agricultural lands where cattle waste from the cattle feedlot may be applied as a means of waste disposal.

c. Aquatic ecosystems where runoff may collect from sites receiving animal manures.

d. The atmosphere surrounding feedlots, as a result of volatilization and from fugitive dust suspended above dry feedlots.

The manufacture of the drug laidlomycin propionate potassium will occur at the following site:

Syntex Agribusiness, Inc.
Nutrition and Chemical Division
1915 West Sunshine St.
Springfield, MO 65805

The manufacture of the drug product, Cattlyst™, will occur at the following site:

Glatt Air Techniques, Inc.
20 Spear Road
Ramsey, NJ 07446

Returned drug product goods in the United States will be sent to a facility in Des Moines, Iowa. They will then be packaged and shipped for incineration as nonhazardous material at an approved incinerator.

The types of environments present and adjacent to the production facilities are described below by site:

Syntex Agribusiness, Inc. (Springfield, MO)

This facility is located within the city limits of Springfield, Missouri. It is situated within an industrial area with the nearest residential area approximately 1/4 mile away. The climate is seasonal, with an average rainfall of approximately 40 inches.

Glatt Air Techniques (Ramsey, New Jersey)

This facility is located in northeastern New Jersey in Ramsey, a suburban community, in an area zoned for light industrial and commercial uses.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

A. Laidlomycin Propionate Potassium

Laidlomycin propionate potassium is a polyether antibiotic derived from laidlomycin sodium, a material prepared by microbial fermentation with *Streptoverticillium eurocidicum*. The chemical name is: 9-Hydroxy-beta-(1-oxopropoxy)-2-[5-methyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-[(1-oxopropoxy)methyl]-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-alpha,gamma,2,8-tetramethyl-1,6-dioxaspiro-[4,5]decane-7-butyric acid potassium salt.

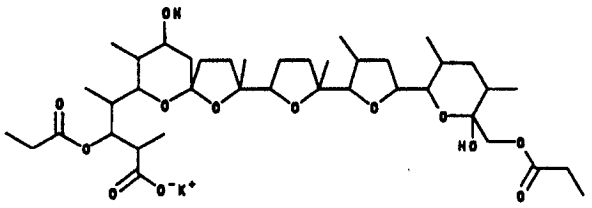
Laidlomycin propionate potassium is a mixture of approximately 90% propionyl ester and 10% butyryl ester at position C-3, and is prepared by C-26 propionation of laidlomycin sodium.

The chemical formula is $C_{40}H_{65}O_{13}K$.

The molecular weight is 793.04.

The CAS Registry No. is 84799-02-0

The structural formula is:



6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. Introduction Of Substances From The Manufacturing Sites

The identities of components, reagents, and solvents used in the manufacture of laidlomycin propionate potassium are described elsewhere in this New Animal Drug Application. Whenever possible, the compounds used and/or byproducts of manufacturing are recycled back into the process or, when recycling is infeasible, disposed of in accordance with appropriate laws and regulations. Each facility has a spill prevention and control plan which specifies the handling of an accidental release of any hazardous material into the environment.

B. Compliance Statements

Manufacturing controls and permit information are described below for each facility. Appendix A lists the applicable environmental laws and regulations, and includes the compliance statements for each site.

(1) Syntex Agribusiness, Inc.- Springfield, MO

Laidlomycin propionate potassium will be manufactured for approximately the first year in an interim facility at the Springfield plant site, then transferred to a permanent facility at the same site. The following description covers both facilities, and quantities identified represent emissions during the projected fifth year of production.

(a) Air Emissions

All emissions of volatile organic compounds (VOC's) generated during the production process are controlled at the source by conservation vents or condensers. In addition, process equipment vents in the permanent facility will be manifolded and ducted to a regenerative thermal oxidizer. Consequently, the treatment of emissions reduces or eliminates the discharge of VOC's into the environment.

(b) Wastewater

Aqueous streams are pretreated in the process and pumped to a wastewater tank prior to discharge to the City of Springfield's publicly owned treatment works (POTW). Approximately 1,650,000 liters of treated wastewater is expected to be discharged annually. In discussions concerning the production of laidlomycin propionate potassium, the POTW (the Springfield Public Works) requested that Syntex conduct Whole Effluent Toxicity (WET) tests using effluent from the Springfield POTW spiked with laidlomycin propionate potassium. These tests are the same as those the Public Works must run on their effluent to show compliance with the State of Missouri wastewater discharge permit requirements.

The tests used, standard EPA methods commonly applied to POTW' effluents, were taken from the EPA handbook: Methods for Measuring the Aquatic Toxicity of Effluent to Freshwater and Marine Organisms (600-4-85-013). Specifically, the WET tests using *Ceriodaphnia dubia* (water fleas) and *Pimephales promelas* (fathead minnows) were employed. Springfield POTW effluent was spiked with laidlomycin propionate potassium to 1.0 mg/L, then saturated with ozone to simulate the ozonation operations used by the POTW to treat wastewater. The spiked–ozonated sample was then diluted to 20% with Jordan Creek receiving water, in accordance with standard procedure for these tests, and 100% survival was shown for both species.

In addition to the WET tests, an ozonation destructionefficiency test was performed on an effluent samples containing 1000mg/L laidlomycin propionate potassium. Subsequent chemical analysis indicated that 50% of the laidlomycin propionate potassium was degraded by ozonation. WET tests on this sample demonstrated that ozonation of laidlomycin propionate potassium did not produce toxic by–products (no mortality was seen).

In order to calculated the maximum allowable discharge from Syntex Agribusiness, the POTW low flow rate of 20,000,000 gallons per day was used as follows:

(20,000,000 gal/day)(8.345 lbs/gal) = 166.9×10^6 lbs/day water
and (166.9×10^6 lbs/day)(1.0 ppm) = 167 lbs/day laidlomycin propionate potassium

This is based on the WET test results and a no observed effect level (NOEL) of 1.0 mg/L laidlomycin (1 ppm) for various aquatic species. The colculation does not account for any destruction of laidlomycin propionate potassium in the POTW due to biodegradation of ozonation, an assumption that adds a satisfactory margin of safety to the 167 lbs/day limit. This limit was incorporated into the Syntex Agribusiness discharge permit issued by the Springfield Public Works. Controls within the site will ensure that discharge will remain well below that limit. The potential risk of an adverse impact on teh POTW or downstream environment is negligible.

(c) Bulk Solvents

Spent methanol and methylene chloride streams generated in the process are recovered and analyzed to determine compliance with appropriate specifications. If they comply, the solvents are reused in the process. Unusable solvents will be sent off-site to a permitted hazardous waste treatment facility for either thermal oxidation or fuel blending

(d) Solid Wastes

Approximately 200,000 pounds per year of solid waste in the form of filters, gloves, liners, dust suits, dust bags, process samples, product returns, and production losses will be sent off site to a permitted waste treatment facility.

(e) Occupational Exposure

Personnel working in the plant are provided with safety helmets, safety glasses, goggles or face shields, uniforms, safety shoes, and gloves. If conditions warrant, the operators have at their disposal respirator protection, dust suits, aprons, and boots. Directions are written at each appropriate step of the operating procedure advising the operators which safety equipment must be used during that step of the

operation. Each operating procedure includes a safety and health section advising the operator of potential hazards of all chemicals used in that operation. In addition, Material Safety Data Sheets are available for all the chemicals used in the manufacture of laidlomycin propionate potassium.

(f) Summary

The pollution control devices in use and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of laidlomycin propionate potassium. Emissions from this facility are in compliance with the laws and regulations listed for the Springfield site in Appendix A.

(2) Glatt Air Techniques - Ramsey, New Jersey

(a) Air Emissions

All regulated air discharges are permitted by the New Jersey Department of Environmental Protection (NJDEP). The permits for equipment to be used for Cattlyst™ production are: APC Plant ID 01054; Certificate Number 095974. During operation of the equipment, particulates in the process air stream will be controlled by baghouses. The resulting emissions will be within NJDEP limits and will be below the allowable limits of the permit.

(b) Wastewater

During cleanup, all visible material is swept or vacuumed. Any remaining solid material that is washed off the equipment, or floors and walls, is collected by the sewer system and discharged to the Northwest Bergen County Utility Authority Treatment Plant. The sewer permit number is 87018. Additionally, internal laidlomycin discharge limits have been established that are considered to be protective of the treatment plant. Adherence to these levels should prevent adverse effects to POTW organisms and aquatic species downstream from the POTW, due to laidlomycin propionate potassium.

(c) Hazardous Wastes

Any hazardous waste generated will be collected and labeled, then disposed of by a licensed hazardous waste facility, in accordance with applicable local, state, and federal regulations. The material is manifested and transported to the disposal site using an approved carrier.

(d) Solid Wastes

Solid, non-hazardous waste resulting from Cattlyst™ production is collected into marked fiber drums and transported to a site licensed to incinerate pharmaceutical wastes.

(e) Occupational Exposure

Personnel working in the plant have been trained in the use of personal protective equipment which would typically be used for the manufacture of Cattlyst™. Employees also receive training in understanding the potential hazards in handling the various materials used at this facility. They are aware of the location of binders containing MSDS information.

(f) Summary

The pollution control devices in use and the solid waste disposal methods used by this facility serve to minimize release of environmental emissions resulting from the production of Cattlyst™. A review of the various activities related to the production of laidlomycin was undertaken and no apparent violation or exceedance of allowable emissions was revealed. Emissions from this facility are in compliance with the laws and regulations listed for the Ramsey site in Appendix A.

C. Introduction And Concentration Of Substances Introduced Into The Environment From Product Use

(1) Introduction Of Substances At Feed Manufacturing Sites

Laidlomycin propionate potassium may be introduced into the environment during the feed manufacturing process when the Cattlyst™ premix is combined with feed. However, laidlomycin propionate potassium is encapsulated during the drug product manufacturing process, thereby minimizing any exposure by dust inhalation. To protect workers, Syntex has established an occupational exposure limit (OEL) of 0.050 mg/m^3 laidlomycin propionate potassium in air. The OEL is the time–weighted average concentration for a normal 8–hour workday and 40–hour workweek to which nearly all workers may be exposed repeatedly without harmful effects. Air monitoring at a feed mixing operation indicated that levels of laidlomycin propionate potassium were well below the established OEL for laidlomycin propionate potassium (for discussion, see Section 8.B.(2)). The MSDS's for laidlomycin propionate potassium premix are sent, as required by law, to the feed mixing operations. These MSDS's outline the necessary precautions and personal safety equipment to be used in handling the premix.

(2) Estimated Concentration of Laidlomycin Sodium in Fresh Cattle Excreta

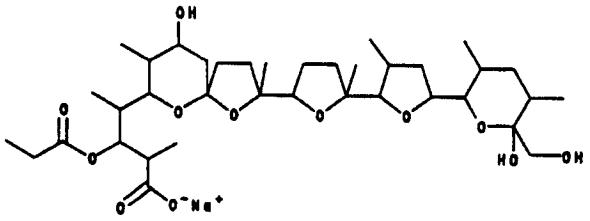
The maximum daily amount of laidlomycin propionate potassium expected to be ingested by each animal is no more than 120 mg. Metabolism and route of excretion of residues were determined in two studies in which ^{14}C-laidlomycin propionate potassium was administered orally to cattle at a dose of 1 mg/kg/day. Laidlomycin propionate potassium is extensively metabolized in cattle, and the residues are primarily excreted in the feces(1). Residues in feces were characterized by extraction into organic solvents and analysis by high performance liquid chromatography(2). Laidlomycin propionate potassium accounted for <2% of the extracted radioactivity. All salt forms of laidlomycin averaged 33.2% (range of 21.1-39.7 %) of extracted radioactivity, but other residues, (including C-3 despropionyl laidlomycin), individually accounted for no more than 10%.

Important characteristics of cattle manures that impact the chemical form of laidlomycin in cattle waste were reported from a study at seven North Carolina dairy farms(3). The main mineral components in "as-excreted" manure were potassium, sodium, and chlorine (as chloride). The pH of manure during storage varies, but stayed within the range of 5.4 to 7.7. Based on these observations, and the known acid dissociation constant, laidlomycin (and any metabolite containing the carboxylic acid function) is expected to be present in the potassium and sodium salt forms in cattle waste. For purposes of this environmental assessment, the Center for Vetinary Medicine has agreed that the sodium salt may be used as representative form for evaluating environmental release mechanisms and estimating environmental concentrations.

The chemical name of laidlomycin sodium is: 9-Hydroxy-beta-(1-oxopropoxy)-2-[5-methyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-[hydroxymethyl]-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-alpha,gamma,2,8-tetramethyl-1,6-dioxaspiro-[4,5]decane-7-butyric acid sodium salt. The antibiotic activity of this ionophore is markedly reduced from the parent laidlomycin propionate potassium, as demonstrated by reduced propionic acid production by *in vitro* rumen fermentation.(4)

The chemical formula is $C_{37}H_{61}O_{12}Na$, and the molecular weight is 720.86.

The structure of laidlomycin sodium is:



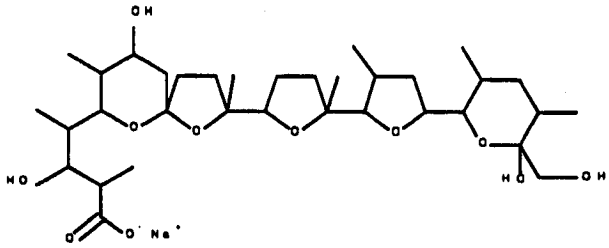
The concentration of laidlomycin sodium in cattle feces depends primarily upon the initial percent roughage in the ration and the water content. As indicated in "Development Document for Effluent Limitations Guidelines and New Source Performance Standards-Feedlots Point Source Category"(5), the average wet solids production of beef cattle weighing 360 kg (800 lbs.) is 21.8 kg/head/day. The maximum nominal concentration of laidlomycin in fresh cattle excreta, $C_{N\text{-}L}$, based on the fraction of radioactivity recovered (33.2/100) and the molecular weight ratio (720.9/793.0), is estimated to be

$C_{N\text{-}L}$ = (120 mg/day)(33.2/100 percent excreted)(720.9/793.0)/(21.8 kg excreta/day)

or 1.66 mg/kg excreta as laidlomycin sodium.

(3) Estimated Concentration of C-3 Despropionyl Laidlomycin Sodium in Fresh Cattle Excreta

The second most abundant metabolite of laidlomycin propionate potassium in cattle is C-3 despropionyl laidlomycin sodium, (<9.6%). The chemical formula is $C_{34}H_{57}O_{11}Na$. The molecular weight is 664.80. The structure of C-3 despropionyl laidlomycin sodium is given below.



This metabolite is not formed in the aerobic degradation of laidlomycin sodium in feedlot waste, *vide infra*. The maximum initial concentration of C-3 despropionyl laidlomycin sodium in feedlot waste is equivalent to the excreted fraction of radioactivity (9.6/100), corrected for conversion to the sodium salt (664.8/793.0) . The nominal concentration of C-3 despropionyl laidlomycin sodium in fresh cattle excreta, $C_{N\text{-}D}$, is

$C_{N\text{-}D}$ = (120 mg/head/day)(9.6/100)(664.8/793.0) / (21.8 kg-waste/head/day),

or 0.443 mg/kg. By analogy to an ionophore of similar structure, monensin, the biological activity of C-3 despropionyl laidlomycin is expected to be markedly less than that of laidlomycin sodium (6).

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

A. Summary of Laidlomycin Environmental Fate Tests

(1) Acid Dissociation Constant

The pK_A of laidlomycin was determined by the solubility method. After each pH adjustment, the dissolved laidlomycin was separated from the solid by membrane filtration. Laidlomycin recovered from the filtrate was determined by a spectrophotometric method based on the reaction with vanillin-sulfuric acid reagent in methanol. Solubility data, fit to a least squares linear regression, gave a pK_A of 5.45. The reported pK_A was 5.5, with an uncertainty of 0.1 unit. An expanded summary of this study is given in Appendix B.

(2) Water Solubility/Solubility Products

Laidlomycin sodium is sparingly soluble in water, and the measured solubility depends upon the sodium ion concentration in solution according to the mass-action law of equilibrium. Solubility products for the sodium and potassium salts of laidlomycin were determined by a solubility method which measured the amount of laidlomycin that dissolved in suspensions of laidlomycin sodium or laidlomycin potassium. The solubility products of the sodium and potassium salts are 1.17×10^{-7} $(mol/L)^2$ and 5.21×10^{-7} $(mol/L)^2$.

The water solubilities of the sodium and potassium salts at an ionic strength of 0.200 are 3.42×10^{-4} mol/L (145 mg/L) and 7.22×10^{-4} mol/L (314 mg/L), respectively. An expanded summary of this study is given in Appendix C.

(3) n-Octanol-Water Partition

The octanol/water distribution coefficient for laidlomycin sodium at a pH of 9.00 was determined by measuring the concentrations of laidlomycin sodium in octanol and water at weekly intervals over a four week equilibration. The K_{OW} values for three replicates ranged from 1.69×10^4 - 1.90×10^4, and the average value was 1.88×10^4. An expanded summary of this study is given in Appendix D.

(4) Vapor Pressure of Laidlomycin Sodium

A preliminary estimate of the vapor pressure of laidlomycin sodium was made by measuring the maximum rate of volatilization from a solid 57.2 µg deposit maintained at

60°C. A nitrogen gas stream was used to sweep away the material that volatilized. After a period of 20 hours, 100% of the residual laidlomycin sodium was recovered from the deposit, indicating that the vapor pressure was significantly less than 10^{-6} Torr.

The vapor pressure of laidlomycin sodium was estimated by a method which utilized electron impact mass spectrometry. Five µg of laidlomycin sodium were placed in the sample cup of a temperature-controlled direct-insertion probe. When the operating pressure of the spectrometer was reached, about 2×10^{-6} Torr, repetitive mass spectra were obtained as the temperature was increased in steps of 30°C. Repetitive scans were used to obtain the molecular ion current at each temperature. A significant ion current for laidlomycin sodium was achieved at 270°C. The measured vapor pressure at 270°C was 3.2×10^{-7} Torr. The estimated vapor pressure at 20°C is 3×10^{-12} Torr. An expanded summary of this study is given in Appendix E.

(5) Determination of Soil Adsorption/Desorption Constants for Laidlomycin (Soduim)

A soil adsorption/desorption test with laidlomycin sodium was conducted with four different types of soil: California sandy loam (pH 6.1, cation exchange capacity (CEC) 7.9 meq/100 g, and 1.6% OM); Mississippi clay loam (pH 7.4, CEC 12.8 meq/100 g, 1.4% OM); Missouri silt loam, (pH 7.2, CEC 7.5 meq/100 g, 1.8% OM); and Texas silt loam (pH 7.2, CEC 11.4 meq/ 100g, 1.1% OM). The water solubility of laidlomycin sodium is so low at pH values below 5.5 that the existing analytical methods can not measure equilibrium concentrations in either water or soil phases. For this reason, acidic soils were not included in this study.

A screening test showed that the presence of 0.01 **M** $CaCl_2$ did not affect the absorption of laidlomycin sodium. A kinetic test indicated that absorption equilibrium was reached within 4 hours after the start of the equilibration. Approximately 64% of laidlomycin sodium in solution was adsorbed on CA sandy loam, 95% on MS clay loam, 46% on MO silt loam, and 69% on TX silt loam. Subsequent desorption was 43%, 9%, 64% and 45%, respectively. Mass balance studies showed quantitative recoveries ranging between 92.5 and 99%.

A definitive study was conducted to define the Freundlich adsorption isotherms. The distribution coefficients, K_d (expressed as [µg/g soil]/[µg/g solution]), are estimated from the slopes of the Freudlich plot. Four soil types (CA sandy loam, MS clay loam, MO silt loam, and TX silt loam) were used in the definitive study. From 50 to 700 µg laidlomycin sodium was added to each equilibration flask containing 5 g soil and 25 mL 0.01 **M** $CaCl_2$ solution. The K_d values for CA, MS, MO and TX soils were 7.4, 105, 3.8,

and 8.7, respectively. The corresponding K_{OC} values were 8.0 x 10^2, 1.3 x 10^4, 3.6 x 10^2, and 1.4 x 10^3. An expanded summary of this study is given in Appendix F.

(6) Hydrolysis of Laidlomycin and Potassium Laidlomycin in Aqueous Solution

Laidlomycin and/or laidlomycin salts in strongly alkaline solution hydrolyze at position C-3 to produce C-3 despropionyl laidlomycin. However, a preliminary study of the rate of hydrolysis at 25°C in solutions of pH 5.0, 7.3, 8.7 and 9.0 over a period of 41 days gave no indication of hydrolysis. The data indicate that the free acid and salt forms of laidlomycin are reasonably stable to hydrolysis in the pH range of 5 to 9.

(7) Distribution of ^{14}C-Laidlomycin Between Cattle Feces and Water in Equilibrated Fecal Suspensions

A limited study was performed to characterize the distribution of ^{14}C-laidlomycin between feces and water. Fresh homogenized feces were obtained from a study in which cattle were administered ^{14}C-laidlomycin propionate potassium at 1 mg/kg/day for seven days. After equilibration with water, centrifugation and membrane filtration, ^{14}C-laidlomycin and metabolites in the filtrate were extracted with methylene chloride. Radioactive laidlomycin was separated from other metabolites by thin layer chromatography, then measured by counting in liquid scintillation fluid. The mean values of triplicate distribution coefficients for samples of feces from two animals ranged from 35 to 71. This study indicates that upon equilibration of fecal waste with rainfall, most of the excreted laidlomycin is expected to be retained by the waste.

(8) ^{14}C-Laidlomycin-Biodegradation in Soils

The extent of microbial mineralization of ^{14}C-laidlomycin sodium in three soil types was determined by measuring the production of ^{14}C-CO_2 and ^{14}C-volatiles over a 65 day period. Mississippi silty clay (pH 5.6, CEC 21.6 meq/100 g, and 0.8% OM), Texas silty clay loam (pH 7.8, CEC 15.0 meq/100 g, and 1.6% OM), and California sandy loam (pH 7.7, CEC 11.3 meq/100 g, and 0.7% OM), were used in this study. Cumulative conversion of ^{14}C-laidlomycin sodium to ^{14}C-CO_2 was 0.1% for MS soil, 4.9% for TX soil, and 1.0 % for CA soil. Conversion of glucose to carbon dioxide in the same time period ranged from 51 to 69%. It was concluded that indigenous soil organisms have a low capacity to extensively metabolize laidlomycin sodium. An expanded summary of this work is given in Appendix G.

(9) Measurement of ^{14}C-Laidlomycin Remaining in Soils Following the Test for Aerobic Biodegradation

Upon completion of the soil biodegradation test, all soil samples were frozen and shipped to Syntex Research.

The recovery of laidlomycin sodium from fortified soils by methanol extraction was found to range from 92.2 to 96.8%. After a total elapsed time of six months, residual ^{14}C-laidlomycin was extracted from incubated samples, concentrated and separated from radioactive metabolites by thin layer chromatography on silica gel GF. Recovery of ^{14}C-laidlomycin in MS, TX and CA soils ranged from 43.3-48.2%, 4.94-13.0%, and 36.3-57.3% respectively. This preliminary study indicates that laidlomycin undergoes biotransformation in soils within six months, but it is not extensively mineralized.

(10) ^{14}C-Laidlomycin Propionate Potassium Biodegradation in Cattle Feedlot Waste

The potential for aerobic mineralization of ^{14}C-laidlomycin propionate in a representative feedlot waste (Texas) was evaluated by the quantity of ^{14}C-CO_2 and ^{14}C-volatile products released over a period of 84 days. While no mineralization occurred, (the amount of ^{14}C-volatiles and ^{14}C-CO_2 evolved during the study was insignificant), chemical specific analysis did indicate that the biotransformation was extensive. Residual test compound and biotransformation products were extracted from the samples with methanol. The analytes were concentrated, purified, and analyzed by reverse phase HPLC. Biotransformation is initiated by day 5, and increases through day 84. The least squares linear regression of Log % Remaining against Time was linear, and indicated a half-life of 18 days. Biotransformations are often more complex than the abiotic chemical transformations that are studied by conventional methods. In this case, the decrease in laidlomycin propionate concentration is typical of a simple first-order reaction.

The major product formed on biotransformation of ^{14}C-laidlomycin propionate was ^{14}C-laidlomycin, in one or more salt forms. For the purpose of calculation, the total laidlomycin formed was expressed as the sodium salt. The percent laidlomycin sodium in the extractable radioactivity increased up to day 28, and slowly declined to study termination at day 84. The residual concentration of ^{14}C-laidlomycin propionate ranged from 0.7% to 7.7% of the nominal starting concentration. The distribution of radioactivity in extracts indicated that from 1.3% to 12.5% was present as laidlomycin propionate. Variable amounts of ^{14}C-radioactivity was recovered as laidlomycin and 7 (or more) very polar products. An expanded summary of this study is given in Appendix H.

(11) ^{14}C-Laidlomycin Sodium Biodegradation in Cattle Feedlot Waste

The potential for aerobic mineralization of ^{14}C-laidlomycin sodium in a typical feedlot waste (Texas) was evaluated by the quantity of ^{14}C-CO_2 and ^{14}C-volatile products released over a period of 98 days. Insignificant mineralization of the test compound occurred (the amount of ^{14}C-volatiles and ^{14}C-CO_2 generated during the study was

<0.7%) over the course of the study, but chemical specific analysis did indicate that the biotransformation of ^{14}C-laidlomycin sodium was extensive. Radioactive residues were extracted from the samples with methanol. The analytes were concentrated, purified, and analyzed by reverse phase HPLC. No biodegradation or biotransformation was evident up to day 28 of the study. At day 56, however, only 52.2 to 33.7% of the ^{14}C-laidlomycin sodium remained, with the balance of the radioactivity collectively recovered as 4 or more polar metabolites. At the end of 98 days, the fraction of residual radioactivity present in waste as ^{14}C-laidlomycin sodium ranged from 6% to 7% of the nominal amount added. The biotransformation of laidlomycin sodium is consistent with logistic growth of organisms capable of utilizing laidlomycin sodium. An expanded summary of this study is given in Appendix I.

(12) ^{14}C-Laidlomycin Propionate Potassium Biodegradation in Manure Amended Agricultural Soil

The biodegradation of laidlomycin propionate potassium in manure amended soil was undertaken to better understand the soil dynamics of this ionophore. The information presented here is not used in subsequent calculations of metabolite concentrations in environmental compartments, and is presented for auxiliary information on the compound.

The rate at which radiolabeled ^{14}C-laidlomycin propionate undergoes aerobic biodegradation was investigated in CA, MS, and TX soils amended by the addition of 10% cattle feedlot waste. The quantities of ^{14}C-CO_2 and ^{14}C-volatile products released as a result of mineralization was determined over incubation periods of 120 to 179 days. Between 0.3 to 1.3% of the initial ^{14}C-laidlomycin propionate added to the soil was recovered as ^{14}C-CO_2 depending upon soil type. Production of volatile products did not account for more than 0.0% (detection level 0.005%) of the initial radioactivity in any soil type.

Chemical specific analysis of ^{14}C-laidlomycin propionate was performed by high performance liquid chromatography of extracts from the soil samples to determine residual test chemical. Analyses were performed on all replicates that were sacrificed throughout the study. Radiometric detection was used to monitor the loss of ^{14}C-laidlomycin propionate as well as the formation of ^{14}C-biotransformation products. On day 120, the amount of residual ^{14}C-laidlomycin propionate in CA, MS, and TX soils averaged 0%, 1.8%, and 0.3% of nominal, respectively. Mississippi soil samples were kept on test until day 179, at which point the residual radioactivity in waste as ^{14}C-laidlomycin propionate averaged 12.5% of nominal.

The distribution of extractable radioactivity as ^{14}C-laidlomycin propionate, ^{14}C-laidlomycin sodium, C-3 despropionyl-^{14}C-laidlomycin, and ^{14}C-polar metabolites was determined for each replicate at all time points. The formation of ^{14}C-laidlomycin sodium and C-3 despropionyl-^{14}C-laidlomycin was confirmed by MS-FAB of isolates from HPLC separations. Although the method of applying ^{14}C-laidlomycin propionate to the soil samples was constant, the distribution into different metabolites as the samples aged was not always consistent among replicates. In general, laidlomycin sodium (loss of C-26 propionyl group) is the major product initially formed in CA and TX soils. The amount of polar ^{14}C-metabolites generally exceeds the amount of ^{14}C-laidlomycin sodium formed in MS soil.

Average values for the percent of radioactivity as ^{14}C-laidlomycin sodium peak at 21 days in CA soil, at 56 days in TX soil, and at 179 days in MS soil. The formation of C-3 despropionyl-^{14}C-laidlomycin was spurious, and only observed at small levels in CA and MS soils between days 56 and 179.

Reference experiments in each of the three soil types was performed with radiolabeled ^{14}C-glucose, resulting in ^{14}C-CO_2 evolution in the range of 38.3% to 43.4% of the applied material. It was concluded that the soils contained a metabolically active microbial population. An expanded summary of this study is given in Appendix J.

B. Potential Concentration Of Laidlomycin Propionate Potassium And Metabolites In Environmental Compartments

(1) Estimated Concentration of Laidlomycin Sodium in Accumulated Feedlot Waste

Feces excreted onto the feedlot quickly lose water and organic matter begins the process of decomposition. The amount of accumulated waste generated by beef cattle weighing 360 kg (800 lbs) was reported to average 3.6 kg/head/day at a moisture content of 28.6%(7). Another study performed at Texas Tech University Experimental Facilities,(8) reported that 1.81 to 2.27 kg dry waste was produced per head per day (4 to 5 lbs/head/day) from groups of cattle averaging 295 to 352 kg that were fed rations containing 10%-12% roughage for periods of 127 to 178 days. The corresponding amount of air-dry waste that would be generated at a 30% moisture level is 2.58 to 3.24 kg/head/day. Based on all reported information, a 180 day air-dry waste accumulation averaging 3.0 kg/head/day at a 30% moisture content was selected for estimating environmental concentrations of laidlomycin sodium and metabolites in accumulated feedlot waste.

The nominal concentration of laidlomycin sodium in aged feedlot waste ($C_{N\text{-}AW}$) may be estimated from the fraction of radioactivity excreted as laidlomycin, (33.2/100), and the ratio of molecular weights, (720.9/793.0), by the equation

$C_{N\text{-}AW}$ = (120 mg/day/head)(33.2/100)(720.9/793.0)/(3.0 kg/head/day).

$C_{N\text{-}AW}$ = 12.1 mg/kg

This is the potential concentration if laidlomycin sodium did not biotransform in feedlot waste. Compensation for biotransformation is made in the following way. The concentration of laidlomycin sodium in waste that has not aged for 56 days is assumed to be 12.1 mg/kg.

The laidlomycin sodium concentration in waste older than 56 days is assumed to be zero. This calculation is based on the biotransformation profile, which indicates that as soon as the organisms acclimate to the media, (between 28 and 56 days), laidlomycin sodium is rapidly transformed. The estimated concentration of laidlomycin sodium in air-dry feedlot waste ($C_{A\text{-}180}$) that has accumulated for 180 days is given by

$$C_{A\text{-}180} = \frac{(56\text{ day})(3.0\text{ kg/head/day})(12.1\text{mg/kg}) + (124\text{ day})(3.0\text{ kg/head/day})(0\text{mg/kg})}{(180\text{ day})(3.0\text{ kg/head/day})}$$

$C_{A\text{-}180}$ = 3.76 mg/kg

(2) Potential Release of Laidlomycin Sodium in Fresh Cattle Waste to Rainfall Runoff

The maximum concentration of laidlomycin sodium in rainfall runoff may be estimated from the nominal concentration of laidlomycin sodium in feedlot waste and the fecal solids/water partition coefficient. A preliminary study indicated that the partition coefficient ranged from 35 to 71 [see **7** A (7)]. The nominal concentration in fresh excreta, $C_{N\text{-}L}$, is 1.66 mg/kg. The concentration of laidlomycin sodium in equilibrated water is given by the relation $C_a = C_s/K_{feces/water}$. The corresponding maximum aqueous concentration is (1.66 mg/kg)/(35), or 0.047 mg/kg.

The importance of rainfall runoff as a mechanism for transport of laidlomycin sodium from the feedlot to the aquatic environment may be evaluated by estimating the amount removed per acre by a major rainfall event. The total amount in runoff may be obtained from the concentration in runoff and the volume of runoff per acre. The volume of runoff is less than the actual rainfall, owing to absorption of water by the accumulated solids. Examples of amount and quality of runoff from a feedlot after fifteen rainfall events are reported for a study performed at Texas Tech University(9). Rainfall amounts of 2.72 and 2.86 inches produced 0.61 and 0.796 inches of runoff from dirt surfaced lots. Similar results are reported for a study performed at Colorado State University (10). More than 2.8 inches of rain (applied artificially at 1.45"/hr) are required to initiate runoff from a feedlot having only 4.3 cm of accumulated waste.

The potential for runoff as a release mechanism for laidlomycin sodium is evaluated by assuming one inch of runoff results from three inches of rain on a dirt surface lot having a 180 day accumulation of waste. The corresponding per cent laidlomycin sodium removed from a lot containing 200 head/ac is given by

$$\%\text{Removed} = \frac{(\text{volume of runoff per acre})(\text{concentration in runoff})}{\text{total laidlomycin sodium excreted on one acre}}$$

$$\%\text{Removed} = \frac{100(1/12 \text{ ac ft})(1.233\text{x}10^{6} \text{ kg - water / ac ft})(0.047 \text{ mg / kg})}{(120 \text{ mg / head / day})(33.2/100)(720.9/793.0)(200 \text{ head})(180 \text{ day})}$$

Feedlot runoff is not an important release mechanism, as only 0.37% of the laidlomycin sodium is expected to be removed from the feedlot by a major rainfall.

(3) Potential Release of Laidlomycin Sodium in Fresh Cattle Waste to the Atmosphere

Laidlomycin sodium is used as a representative salt for calculation of the transport from feedlot waste to the atmosphere. The estimation of the concentration and amount in air is permissible as the sodium salt of laidlomycin may volatilize intact.

The upper limit of the potential for atmospheric transport is evaluated from the maximum rate of evaporation. The maximum evaporation rate can be derived from the kinetic theory of gases(11) starting with the fundamental relation $\mathbf{n} = (1/4)\mathbf{n_1}\,\mu$, where **n** is the number of molecules per square centimeter striking a gas boundary, $\mathbf{n_1}$ is the number of molecules per cubic centimeter in the gas phase, and μ is the mean velocity of the gas phase molecules. The number of molecules leaving the condensed phase is independent of the number in the gas phase, so that if there is no reflection of molecules from the gas to the condensed phase, the number **n** may be used to determine the maximum rate of transport across the phase boundary. The mean velocity, μ may be obtained from the relation $\mu = [(8RT/\pi M)]^{1/2}$, where R is the molar gas constant, T the absolute temperature, and M is the molecular weight. The gas phase concentration, $\mathbf{n_1}$, may be expressed as N_aP/RT, where P is the vapor pressure in dyne/cm^2, and N_a is Avagadro's number. Substitution on $\mathbf{n_1}$ and μ into the fundamental relation gives

$$\mathbf{n} = (N_aP/4RT) / (8RT/\pi M)^{1/2} .$$

When the pressure is expressed in Torr (mm mercury), and **n** in grams (N), this equation becomes

$$N = 3.52 \text{ x } 10^{22} (P)(M)/N_a (MT)^{1/2} \text{ grams/cm}^2\text{/sec} .$$

When only part of a physical surface is covered with aggregates that contribute to the mass transport, the equation above is modified to include the fraction covered, F_{cov}, to give

$$N = 3.52 \times 10^{22} (P)(M)F_{cov}/N_a (MT)^{1/2} \text{ grams/cm}^2\text{/sec} .$$

The actual rate of evaporation in most circumstances will be less than the maximum rate, owing to non–zero values for reflection.

The feedlot is normally completely covered with organic matter containing interstitial water, so that F_{cov} may be set equal to unity. The vapor pressure of laidlomycin sodium is estimated from the concentration of laidlomycin sodium in interstitial water, and the maximum solubility in interstitial water by application of Raoult's law (12). The vapor pressure of sodium laidlomycin above a saturated solution at 145 mg/L can be no greater than the ambient vapor pressure of solid laidlomycin sodium, 3×10^{-12} Torr, a value that was obtained by the mass spectrometric method [See 7 A (4)]. The vapor pressure of sodium laidlomycin above a solution in equilibrium with fresh cattle excreta containing a nominal concentration of 1.66 mg/kg laidlomycin sodium is reduced by the factor (1.66 /145) to give a maximum ambient vapor pressure of 3.4×10^{-14} Torr.

The corresponding maximum rate of evaporation of laidlomycin sodium at 22°C is

$$N = (3.52 \times 10^{22})(3.4 \times 10^{-14})(720.9) / (6.023 \times 10^{23})[(295)(720.9)]^{1/2}$$

$$N = 3.11 \times 10^{-15} \text{ g/cm}^2\text{/sec} .$$

The corresponding loss per acre by volatilization is

$$N = (3.11 \times 10^{-15} \text{ g/cm}^2\text{/sec})(4.047 \times 10^3 \text{ m}^2\text{/ac})(10^4 \text{ cm}^2\text{/m}^2)(8.64 \times 10^4 \text{ sec/day})$$

$$N = 0.011 \text{ g/ac/day}$$

The importance of volatilization as a release mechanism may be evaluated by comparing the loss to the amount of laidlomycin sodium introduced into the feedlot per day. Assuming a loading of 200 head/acre, the daily amount of excreted laidlomycin sodium is (120 mg/day/head)(33.2/100)(720.9/793.0)(200 head/ac), or 7.23 g/day/ac.

The maximum rate of volatilization, (0.011 g/day/ac)(100)/(7.23 g/day/ac), or 0.15%, is not sufficient to be a significant release mechanism for laidlomycin sodium in feedlot waste.

Fugitive dust from a cattle feedlot operation is a potential contributor to atmospheric contamination. Dust in air over feedlots is a cyclical phenomenon, with a daily maximum occurring several hours before sunset(13). The maximum amount of

particulate matter may reach 9.0 mg/m^3 over dry feedlots containing aged wastes. The corresponding concentration of laidlomycin sodium in a typical plume is estimated to be (0.0090)(3.76 μg/g) or 0.0338 μg/m^3. This level is very much less than the laidlomycin propionate potassium occupational exposure level (0.05 mg/m^3).

The significance of fugitive dust as a release mechanism may be evaluated by comparing the daily amount of laidlomycin sodium removed in a typical plume to the amount excreted onto the feedlot. A plume 20 meters high over a feedlot in which the laidlomycin sodium concentration in aged waste ($C_{A\text{-}180}$) is 3.76 mg/kg (0.0376 mg/g) has the potential of removing

$$\frac{(100)(1\text{ ac})(4.047\text{x}10^3\text{ m}^2/\text{ac})(20\text{ m/day})(0.0090\text{ g dust/m}^3)(0.00376\text{ mg/g dust})}{(120\text{ mg/day/head})(33.2/100)(720.9/793.0)(200\text{ head})},$$

or 0.038% of the nominal daily amount of laidlomycin sodium introduced into the feedlot. The estimated atmospheric loading from fugitive dust is far below the level needed for atmospheric transport to be an important release mechanism.

(4) Potential Release of Laidlomycin Sodium in Accumulated Cattle Waste by Disposal on Agricultural Land

The concentration of laidlomycin sodium in agricultural soils that results from the disposal of feedlot waste may be estimated from 1) the concentration of laidlomycin sodium in aged waste ($C_{A\text{-}180}$), 2) the amount of waste applied per acre, and 3) the weight of soil per acre in which the waste is incorporated. Feedlot waste is normally applied at a range of 5 to 10 tons per acre to dryland crops and at a range of 5 to 20 tons per acre to irrigated crops, at a 25-40% moisture basis (as received from the feedlot)(14). When the maximum amount of waste, 20 tons/acre, is applied and plowed into the top six inches of soil weighing approximately 0.909 x 10^6 kg/ac, the maximum expected concentration of laidlomycin sodium, C_{soil}, is

C_{soil} = (3.76 mg/kg)(40,000 lbs/ac)(0.454 kg/lb) / (0.909 x 10^6 kg/ac)

or C_{soil} =0.0752 mg/kg.

Studies following the test for aerobic biodegradation of laidlomycin sodium in soil show that the biotransformation of laidlomycin sodium in soil is much faster than expected from the rate of mineralization (CO_2 generation). More than 50% of the laidlomycin sodium is biotransformed in all soil types within a six month period [see 7 A (9)]. The normal frequency of repeat applications of manure is not expected to result in the accumulation of laidlomycin sodium in agricultural soils.

(5) Potential Release of Laidlomycin Sodium in Amended Soil to Surface and Ground Waters

The maximum concentration of laidlomycin sodium in water retained by surface soils after feedlot waste is applied may be estimated from the laidlomycin sodium soil/water

distribution coefficients (obtained in the soil adsorption/desorption study), using the relation $C_W = C_{SOIL}/ K_d$. Missouri soil, $K_d = 3.78$, is the least retentive of the soils in the definitive test, and is used to estimate a maximum water concentration. For a soil concentration of 0.0752 mg/kg, the corresponding maximum equilibrium concentration in the surface water is (0.0752 mg/kg)/(3.78), or 0.0199 mg/kg.

The transport of water from surface to subsurface horizons will lead to a decrease in laidlomycin sodium concentrations, owing to the adsorption of laidlomycin sodium by underlying soil components. No migration is expected to occur in Texas or Mississippi soils, since subsurface transport is considered to be insignificant(15) for compounds having organic carbon distribution ratios greater than 1000. Transport to ground water is not expected to be a significant release mechanism for laidlomycin sodium.

(6) Potential Release of C-3 Despropionyl Laidlomycin Sodium in Cattle Waste to Rainfall Runoff

The fecal-waste/water partition coefficient for C-3 despropionyl laidlomycin sodium is expected to be less than the corresponding coefficient for laidlomycin sodium, owing to the less polar nature of C-3 propionyl ester(2). The potential maximum concentration in feedlot runoff is therefore estimated from the total amount of C-3 despropionyl laidlomycin sodium excreted over a period of 180 days. The total amount of C-3 despropionyl laidlomycin sodium in fresh waste (C_{N-D}) accumulating on 1 acre from 200 head/acre is (21.8 kg/head/day)(200 head/ac)(180 days)(0.443 mg/kg), or 3.48×10^5 mg/ac. Since a three inch rainfall will produce only 1 inch of runoff, or 1.02×10^5 L/ac, the estimated concentration of C-3 despropionyl laidlomycin sodium is

$$C_{runoff} = (3.40 \times 10^5 \text{ mg/ac})/(1.02 \times 10^5 \text{ L/ac})$$

or 3.41 mg/L.

(7) Estimated Concentration of C-3 Despropionyl Laidlomycin Sodium in Accumulated Feedlot Waste

The nominal concentration of C-3 despropionyl laidlomycin sodium in aged feedlot waste is based on the accumulation of 3.0 kg/head aged waste per day at a water content of 30%. This is the same basis as used for the nominal concentration of laidlomycin sodium [see B (1)]. Biotransformation of laidlomycin sodium in cattle waste does not generate additional C-3 despropionyl laidlomycin, so that the maximum nominal concentration in aged waste is given by

$$C_{180} = \frac{(120 \text{ mg/head/day})(9.6/100)(664.8/793.0)}{3.0 \text{ kg/head/day}}$$

$C_{180} = 3.22$ mg/kg.

Biotransformation during residence in the feedlot is expected to lower the actual concentration in feedlot waste at the time of disposal.

(8) Estimated Concentration of C-3 Despropionyl Laidlomycin Sodium in Atmosphere from Fugitive Dust

The amount of C-3 despropionyl laidlomycin sodium in the air resulting from fugitive dust above cattle feedlots is estimated in the same way as the amount of laidlomycin sodium. The corresponding maximum amount in air is given by the equation

C_{air} = (amount suspended)(concentration in suspended matter)

C_{air} = (9.0 mg/m^3)(0.0032 µg/mg)

C_{air} = 0.029 µg/m^3

(9) Potential Release of C-3 Despropionyl Laidlomycin Sodium by Disposal of Accumulated Cattle Waste on Agricultural Land

The rate and frequency of application of feedlot waste to agricultural soil that was used to estimate the concentration of laidlomycin sodium in soil is also used to estimate the concentration of 3-despropionyl laidlomycin sodium. For an application of 20 tons/acre dry waste containing (120 mg/head/day) (9.6/100)(664.5/793.0)/3 kg/head/day, or 3.22 mg/kg, of C3-despropionyl laidlomycin sodium, the resulting concentration in soil is estimated to be

C_{soil} = (40,000 lbs-waste/ac)(3.22 mg/kg)/(2.20 lbs/kg)(0.909 x 10^6 kg-soil/acre) or 0.0644 mg/kg.

(10) Summary of Potential Concentrations of Laidlomycin Sodium and C-3 Despropionyl Laidlomycin Sodium in Environmental Compartments

The normal use of laidlomycin propionate potassium as a cattle feed additive may result in the following concentrations of laidlomycin and C-3 despropionyl laidlomycin sodium in the environment:

	Maximum Feedlot Concentrations			Concentration in Soil	
Excreted Metabolite	Aged waste, mg/kg	Atmosphere, µg/m^3	Runoff, mg/L	Solids, mg/kg	Runoff, mg/L
Laidlomycin Sodium	3.76	0.0338*	0.047	0.0752	0.0199
C-3 Despropionyl Laidlomycin Sodium	3.22	0.0290*	3.41	0.0644	**

* Dust loading

** No estimate available

(11) Potential Release of Laidlomycin Propionate Potassium Into the Atmosphere at Feed Manufacturing Sites

Laidlomycin propionate potassium may be introduced into the environment during the feed manufacturing process when the Cattlyst™ premix is combined with feed. However, laidlomycin propionate potassium is encapsulated during the drug product manufacturing process, thereby minimizing any exposure by dust inhalation. To protect workers, Syntex has established an occupational exposure limit (OEL) of 0.050 mg/m^3 laidlomycin propionate potassium in air. The OEL is the time–weighted average concentration for a normal 8–hour workday and 40–hour workweek to which nearly all workers may be exposed repeatedly without harmful effects. Air monitoring at a feed mixing operation indicated that levels of laidlomycin propionate potassium were well below the established OEL for laidlomycin propionate potassium (for discussion, see Section 8.B.(2)). The MSDS's for laidlomycin propionate potassium premix are sent, as required by law, to the feed mixing operations. These MSDS's outline the necessary precautions and personal safety equipment to be used in handling the premix.

(12) Potential Release of Laidlomycin Propionate Potassium at Cattle Feedlots

Laidlomycin propionate potassium may be introduced into the atmosphere at the cattle feedlot sites when the feed incorporating Cattlyst™ is fed to the cattle. Laidlomycin propionate potassium may then enter the atmosphere through fugitive dust generated from the feedlot operation. It has been shown that airborne particulate concentrations in dust over dry feedlots can be as high as 9.0 mg/m^3 (see 7.B.(3)). Airborne laidlomycin propionate potassium concentrations can be estimated from:

(50 g laidlomycin propionate potassium/lb Cattlyst™)(0.20 lb Cattlyst™/ton feed)(ton/1016.0469 kg) = 0.0098 g/kg

or 9.8 parts laidlomycin propionate potassium per million parts feed.

Assuming a worst–case scenario, and assuming that the fugitive dust is composed solely of uneaten feed, then the maximum amount of laidlomycin propionate potassium present in fugitive dust over a cattle feedlot is:

(9.8×10^{-6} mg laidlomycin propionate potassium/mg feed)(9.0 mg/m^3) = 8.8×10^{-5} mg laidlomycin propionate potassium/m^3 air

or 0.088 µg/m^3. This is approximately three orders of magnitude below the established occupational exposure level (OEL) of 0.050 mg/m^3 and the nuisance dust level of 10 mg/m^3. Exposure to fugitive dust containing laidlomycin propionate potassium is not expected to result in adverse health effects in feedlot operators.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

A. Mammalian Toxicity Studies

Acute, subchronic and chronic toxicity and reproduction studies were conducted in laboratory animals. A subchronic tolerance study and a chronic safety study were conducted in cattle. In all studies, laidlomycin propionate potassium was administered orally (gavage or diet). In addition, four short-term *in vitro* genotoxicity tests were conducted. The results of these studies, which are summarized below, provide information on the safety of laidlomycin propionate potassium to the public and the environment. Expanded summaries of these studies are presented in Appendix K.

(1) Acute studies (Single Dose) with Laidlomycin Propionate Potassium

24–hour Oral Median Lethal Dose for Mice: 133 mg/kg for males and females, combined.

48–hour Oral Median Lethal Dose for Rats: 63 mg/kg for males and females, combined.

Oral Minimum Lethal Dose for Dogs: between 20 to 60 mg/kg for males and females combined

(2) Subchronic and Chronic Studies with Laidlomycin Propionate Potassium

Subchronic (3-month) oral toxicity studies were performed in the rat and dog. In the 3-month toxicity study in rats, animals were dosed orally once daily. The no-observed effect level (NOEL) was 4 mg/kg/day for males and 8 mg/kg/day for females. The adverse effects in the male rats consisted of a slight decrease in food intake and body weight gain. In the 3-month toxicity study in dogs, animals were dosed orally twice daily. The NOEL was 2 mg/kg/day. At the highest dose level, 4 mg/kg/day, peripheral neuropathy and poor clinical condition were present.

A chronic (1-year) toxicity study was conducted starting with beagle dogs. Dogs were dosed orally twice daily. The NOEL was 0.75 mg/kg/day. At 1.5 and 3.0 mg/kg/day, dose-related neurological deficits and histopathological changes in heart, liver, nerve, and skeletal muscle were present

(3) Reproductive Toxicity

No evidence of teratogenicity was present when laidlomycin propionate potassium was administered to rats and rabbits at doses up to and including 4 mg/kg/day.

A two-generation rat reproduction study was conducted in which males and females were administered 0, 50, 100, or 300 parts per million (ppm) of laidlomycin in the diet. Treatment with laidlomycin did not effect reproduction and fertility parameters in either generation. The NOEL for reproductive effects was 300 ppm which provided averaged doses of approximately 25 and 37 mg/kg/day of laidlomycin for the first and second generations, respectively.

(4) Genotoxicity

Laidlomycin propionate potassium had no genotoxic activity in the following studies: 1) Ames/Salmonella Mutagenicity Assay; 2) the Yeast Mitotic Recombination Assay; 3) the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay; and 4) the Chinese Hamster Ovary Cell/Hypoxanthine-guanine Phosphoribosyl Transferase (HGPRT) Mutation Assay.

(5) Target Animal Studies

Laidlomycin propionate potassium administered to cattle in seven daily oral doses of 1500 mg/animal/day caused anorexia, watery diarrhea, rumen atony, abnormal eating behavior, dullness and/or listlessness, bradycardia, myocardial fiber degeneration, and death (1/4 animals) due to cardiopathy.

In the 265-day safety study in cattle, steers and heifers received dietary concentrations of 0, 10, 30, and 50 g/ton of laidlomycin propionate potassium, up to 5 times the intended field use level. Average daily weight gains essentially paralleled the dry matter intake. The lowest intake and gain was shown by the steers that received the 50 g/ton laidlomycin propionate potassium diet. Laidlomycin propionate potassium feeding did not result in improved feed efficiency. Increased incidence of intermittent loose feces were present in heifers that received the diet containing 50 g/ton of laidlomycin propionate potassium. No clinical pathologic or anatomic pathologic changes attributable to laidlomycin propionate potassium were present.

(6) Special Toxicity Studies

Laidlomycin propionate potassium powder was a slight dermal irritant and a severe ocular irritant in the rabbit. Because of these effects, laidlomycin powder was tested. Laidlomycin was not considered to be a dermal irritant and only a slight, reversible ocular irritant. The release of propionic acid in the rabbit eye is considered the cause of the ocular irritation from solid unformulated laidlomycin propionate potassium. The decreased eye irritancy of laidlomycin compared with laidlomycin propionate potassium supports this conclusion.

B. Potential Effects of the Proposed Action on Human Health

(1) Human Exposure to Laidlomycin Propionate Potassium During Production Operations

The potential human health effects resulting from exposure to laidlomycin propionate potassium are limited, due to the manufacturing process, to occupational exposure to airborne material. Direct contact may cause eye injury and skin irritation based on animal studies with laidlomycin propionate potassium. Laidlomycin propionate potassium has not been shown to be teratogenic, mutagenic, or carcinogenic in appropriate animal models. However, overexposure may cause gastrointestinal and nervous system effects, e.g. salivation, labored breathing, and loss of reflexes. Syntex Agribusiness, Inc. has adopted an Occupational Exposure Level (OEL), based on

currently available data, of 0.050 mg/m^3 laidlomycin propionate potassium in air. The OEL is the time–weighted average concentration for a normal 8-hour workday and 40-hour workweek to which nearly all workers may be repeatedly exposed without harmful effects. Appropriate engineering controls and personal protective equipment have been selected, based on extensive industrial hygiene monitoring, to maintain exposures below the OEL during Cattlyst™ manufacture. MSDS sheets for laidlomycin sodium, laidlomycin propionate potassium, and laidlomycin propionate potassium premix 11% are given in Appendix L.

(2) Potential Effects of Laidlomycin Propionate Potassium To Users at the Site of Feed Mixing and Use

Airborne exposure levels of laidlomycin propionate potassium during manufacture of cattle feed were determined from an air monitoring survey conducted at Kansas State University in March, 1992. Seven lots of laidlomycin premix or Medicated B mix were combined with feed in ratios of 5 g/ton, 200 g/ton or 2000g/ton in a Forberg (paddle) or Ribbon mixer in 1000 pound batches. The mixed feed, meal or pellets, was discharged into 25 pound feed bags which were then sewn shut.

Area (6) and personal (13) air samples were collected using DuPont Alpha I air samplers calibrated at approximately 3 liters per minute. The samples were collected on glass fiber filters which were analyzed for laidlomycin propionate potassium by Syntex using high pressure liquid chromatography (HPLC). The limit of detection was 0.4 µg/filter.

Laidlomycin propionate potassium exposure levels were found to range from 0.669 µg/m^3 to 3.88 µg/m^3 with one spurious sample of 21.8 µg/m^3. None of the exposures were above the Syntex derived Occupational Exposure Level (OEL) of 50 µg/m^3. Thus, respiratory protection for future operations involving similar concentrations and volumes of laidlomycin sodium and laidlomycin propionate potassium is not required. Respiratory protection, e.g. 3M 8710 Dust/Mist respirator, is suggested to minimize nasal irritation from nuisance dust. The operations at Kansas State were similar to standard feedlot mixing operations in that the same basic processing steps were completed and similar mixers were used.

C. Potential Toxicity Effects on Non-Target Organisms

(1) Aquatic Ecosystems

(a) Laidlomycin - Determination of Microbial Growth Inhibition.

In a preliminary study, suspensions of laidlomycin (free acid) were used to prepare 100 to 0.1 mg/L concentrations in agar media to evaluate the minimum inhibitory concentrations (MIC) for 14 species of soil bacteria and fungi. MIC values were ≥100 mg/L for Aspergillus bicolor, Chaetomium piluliferoides, Fusarium chlamydosporum, Penicillium inflatum, and Trichoderma harzianum species of

fungi. The gram positive bacteria Arthrobacter terregens, Streptomyces galilaeus, Bacillus mycoides, Cellulomonas flavigena, and Clostridium absonum, had average MIC values of 100, 23, 3, 1, and 100 mg/L, respectively. The gram negative bacteria Azotobacter chroococcum, Cytophaga johnsonae, Psuedomonas facilus, and Flavobacterium species had average MIC values of 53, >100, >100, and >100 mg/L.

Microbial inhibition of laidlomycin sodium was examined in a definitive study performed at Springborn Laboratories, Inc. (Syntex Report #RS-11988 CH0268). In order to avoid the formation of suspensions, each stock solution of laidlomycin sodium was prepared in 40/60 acetone/water solvent. Test concentrations for range finding varied from 1000 to 0.1 mg/L. The following species were used in this study: Aspergillus niger, Trichoderma viride, Clostridium perfringens (Gram positive), Bacillus subtilis (Gram positive), and Nostoc. The corresponding MIC values were >1,000, >1,000, 0.4, 0.4, and 40 mg/L.

(b) Acute Toxicity of Laidlomycin Sodium to *Daphnia magna.*

The results of the 48-hour static acute *Daphnia magna* toxicity study are summarized below. Based on the absence of immobility and other abnormal effects after 48-hours, the no-observed effect level was reported to be 5.5 mg/L and the dose-response slope was reported to be 4.2.

Summary Table

EC_{50} (mg/L)	
24-hour[a]	48-hour[a]
>33	21
(na)	(17-26)

a 95% confidence interval in parentheses

An expanded summary of this study is given in Appendix M.

(c) Acute Toxicity of Laidlomycin Sodium to *Hyalella azteca.*

The acute toxicity of laidlomycin sodium to *Hyalella azteca* was evaluated in a 96 hour exposure to nominal concentrations ranging from 17 to 270 mg/L. Actual concentrations ranged from 15 to 54 mg/L at test termination, owing to a lower than expected solubility in the dilution water used in this study. The no-observed effect level was 25 mg/L. An expanded summary of this study is given in Appendix N.

(d) Laidlomycin Sodium - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions.

The acute toxicity of laidlomycin sodium to Bluegill (*Lepomis macrochirus*,) a warm-freshwater fish was assessed in a well controlled study. Twenty fish per group (ten per replicate) were exposed in duplicate test aquaria to mean measured test concentrations of 19, 9.5, 5.9, 3.5, 2.1, and 1.2 mg/L and a dilution water control. Biological observations were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Following 96 hours of exposure, 100 and 50% mortality were reported in the two highest concentrations, 19 and 9.5 mg/L, respectively. Mortality ranging from 0-5% was reported among fish exposed to the remaining concentrations tested. Sub-lethal effects were reported among surviving fish in the 9.5 mg/L treatment level. No mortalities were reported among the control groups. LC_{50} values and the corresponding confidence intervals are given in the following Table. The no-observed effect concentration was 5.9 mg/L. An expanded summary of this study is given in Appendix O.

Summary Table

LC_{50} , (mg A.I./L)[a,b]

24-hour[c]	48-hour[d]	72-hour[d]	96-hour[d]
11	10	9.9	9.5
(9.3-13)	(5.9-19)	(5.9-19)	(5.9-19)

a Based on mean measured concentrations
b Corresponding 95% confidence interval in parentheses
c LC_{50} value calculated by probit analysis
d LC_{50} value estimated by non-linear interpolation; 95% confidence interval calculated by binomial probability

(e) Laidlomycin Sodium - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-through Conditions.

The acute toxicity of laidlomycin sodium to rainbow trout (*Oncorhynchus mykiss*), a cold-freshwater fish was assessed in a well controlled study. In a definitive test, the pH and dissolved oxygen concentration of the exposure solutions remained within acceptable ranges for the survival of rainbow trout. Twenty organisms, (ten per replicate) were exposed in duplicate test aquaria to each of six concentrations of laidlomycin sodium and a dilution water control. During the test, the mean measured test concentrations were reported to be 11, 6.7, 3.8, 2.2, 1.2 and 0.95 mg/L. Biological observations were made and recorded at test initiation and every 24 hours thereafter until test termination. Following 96 hours exposure, 100, 100, and 75% mortality was reported in the three highest concentrations tested, 11, 6.7 and 3.8 mg/L, respectively. Mortality ranging from 5-25% was reported among fish exposed to the remaining concentrations tested, 2.2- 0.95 mg/L. Sub-lethal effects

were reported among surviving fish in the 2.2 and 1.2 mg/L treatment levels. Based on these data, it was reported that the effects observed during this study were concentration dependent. No mortalities were reported among the control groups. LC_{50} values and the corresponding confidence intervals are reported in the following table. The no-observed effect concentration was reported to be 1.2 mg/L. An expanded summary of this study is given in Appendix P.

Summary Table

LC_{50}, (mg A.I./L)[a,b]

24-hour[c]	48-hour[c]	72-hour[c]	96-hour[c]
5.7	3.9	3.1	2.7
(4.9-6.7)	(3.3-4.5)	(2.7-3.6)	(2.3-3.2)

a Based on mean measured concentrations
b Corresponding 95% confidence interval is in parentheses
c LC_{50} calculated by probit analysis

(f) C-3 Despropionyl Laidlomycin Sodium (RS 78585-037) - Acute Toxicity to *Daphnia magna.*

The toxicity of C-3 despropionyl laidlomycin sodium to *Daphnia magna* was determined in a well controlled study at nominal test concentrations ranging from 39 to 500 mg/L. Actual test concentrations ranges from 95 to 105% of nominals. Test temperature during the 48 hour exposure ranged from 19.9 - 20.5^{O}C. The 48-hour EC_{50} was 239 mg/L (based on measured concentrations), with a 95 percent confidence range of 202-283 mg/L. The no-observed effect concentration was 109 mg/L. An expanded summary of this study is given in Appendix Q.

(2) Terrestrial Ecosystems

(a) Procedure for Determining the Effects of Laidlomycin Sodium on seed Germination and Root Elongation.

Preliminary range finding tests at 300, 100, 10, and 1 mg/L laidlomycin sodium were conducted with cabbage, corn, cucumber, pinto bean, perennial ryegrass, and wheat. Based on the results, definitive studies were used to determine the following no-observed effect concentrations.

Summary Table

Species	NOEC, mg/L Germination	NOEC, mg/L Elongation
Cabbage	10	1.0
Corn	300	14
Cucumber	300	10
Pinto bean	10	1.5
Perennial ryegrass	4.9	2.7
Wheat	27	10

An expanded summary of this study is given in Appendix R.

(b) Laidlomycin Sodium - Determination of Effects on Seedling Growth of Six Plant Species.

Preliminary range finding tests at 100, 10, 1.0 and 0.1 mg/L laidlomycin sodium per liter were conducted with corn, cucumber, perennial ryegrass, soybean, wheat, and tomato as test species. Based on the results, definitive tests were conducted according to guidelines in the FDA-Environmental Assessment Technical Assistance Document using the following nominal concentrations of test material:

Corn	4.0,	2.0,	1.0,	0.50,	0.25,	0.13 mg/L	
Cucumber		2.0,	1.0,	0.50,	0.25,	0.13 mg/L	
Ryegrass*			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Soybean			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Wheat			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Tomato				0.50,	0.25,	0.13,	0.063, 0.031 mg/L

* Lolium perenne

Chemical analyses were performed to establish the concentration of laidlomycin sodium in the nutrient solutions. From the statistical effects observed, a lowest-observed effect concentration and the no-observed effect concentration were determined for each species as shown in the following Table.

Summary Table. Plant Species Grown in a Sand Root Support

Species	Concentration Laidlomycin Sodium			
	LOEC[a]		NOEC[a]	
	Nutrient, mg/L[b]	Root Support, mg/kg[c]	Nutrient[b]	Root Support[c]
Corn	0.25[d]	1.85	0.12	0.89
Cucumber	1.4[e]	10.39	0.70	5.19
Ryegrass*	0.21[f]	1.56	0.11	0.82
Soybean	0.11[g]	0.82	0.056	0.42
Tomato	0.056[f]	0.42	0.022	0.16
Wheat	0.70[h]	5.19	0.36	2.67

a LOEC and NOEC values were based on the most sensitive parameter(s).
b Based on measured concentration in mg/L of laidlomycin sodium in the nutrient solution.
c Based on an average 742% accumulation of laidlomycin sodium in the top half of sand root support, mg/kg. See Section 8.D.(2) and Appendix S.
d Based on statistically significant effects on shoot and root weight.
e Based on statistically significant effects on shoot length, shoot weight, and root weight.
f Based on statistically significant effects on root weight.
g Based on statistically significant effects on shoot weight.
h Based on statistically significant effects on shoot length and shoot weight.
* *Lolium perenne*

An expanded summary of this work is given in Appendix S.

(c) Laidlomycin Sodium - Determination of Effects on Seedling Growth of Seven Plant Species in a Natural Field Soil.

In a preliminary study, we determined that the unexpected sensitivity of some plant species might have been due to an increase in the concentration of laidlomycin sodium in the sand root support over the course of the 21 day experiment. A preliminary study indicated that the concentration in the top halves of pots increased by an average of 742%. The increase was due to water loss by both evaporation and plant transpiration. In another preliminary study, perennial ryegrass seedlings were grown in a CA sandy loam soil initially treated with nutrient solutions containing from 1 to 100 mg/L of laidlomycin sodium. Nutrient solution without test chemical was used to replenish water lost from the pots. Ryegrass was used for this test, owing to the sensitivity to laidlomycin sodium, and the narrow range of NOEL's (shoot length, shoot and root weight) in the previous study. As expected, the soil components greatly reduced the laidlomycin sodium toxicity to ryegrass, as the NOEL's increased from 0.11 mg/L to 5 mg/L.

The phytotoxicity of laidlomycin sodium incorporated into blended California sandy loam soil (pH 7.8-8.2, CEC 5.3-12.4 meq/g, and 1.4% OM) was evaluated in

a well controlled study using corn, cucumber, annual ryegrass, perennial ryegrass, soybeans, tomato, and wheat seedlings. Laidlomycin sodium was dissolved in acetone, and added to the soil to achieve concentrations of 0.2 to 50 mg/kg. The solvent was removed by air drying. A solvent control was used for all plant species. Water lost from the pots (by evaporation and transpiration) over the course of the study was replaced by bottom watering with nutrient solution that did not contain the test compound.

Chemical analyses were performed to establish the laidlomycin sodium concentration in each of the stock solutions used to treat the soil. An attempt was made to confirm the laidlomycin sodium concentrations in the treated soils. However, interferences in the analytical procedure related to the soil matirx, gave lower than expected recoveries. Test results are expressed as nominal test concentrations with acceptable analytical confirmation of the stock concentrations used to treat the soil. From the statistical effects observed, a lowest-observed effect concentration and the no-observed effect concentration were determined for each species as shown in the following Table.

Summary Table. Plant Species Grown in a Natural Field Soil

Species	Concentration Laidlomycin Sodium, mg/kg			
	LOEC[a]		NOEC	
	Nominal[b]	Measured[c]	Nominal[b]	Measured[c]
Corn	3.3[d]	2.57	1.6[h]	1.25
Cucumber	6.5[e]	5.07	< 6.5	< 5.07
Annual Ryegrass*	3.3[e]	2.57	1.6	1.25
Perrenial Ryegrass**	3.3[f]	2.57	1.6	1.25
Soybean	3.3[g]	2.57	1.6	1.25
Tomato	13[d]	10.14	6.5	5.07
Wheat	6.5[d]	5.07	3.3	2.57

a LOEC and NOEC values were based on the most sensitive parameter(s).
b Based on nominal concentration of laidlomycin sodium.
c Based on an average 78% recovery of laidlomycin sodium in all test levels. See Section 8.D.(2) and Appendix T.
d Based on statistically significant effects on shoot length and shoot weight.
e Based on statistically significant effects on shoot length, shoot weight, and root weight.
f Based on statistically significant effects on shoot weight.
g Based on statistically significant effects on shoot weight and root weight.
h The two–tailed Dunnett's Test, as required by the study protocol, indicates that the 25, 13, and 3.3 mg A.I./kg treatment levels are significantly different from the solvent control. Consequently, a conservative NOEC of 1.6 mg A.I./kg has been selected.
* *Lolium multiflorum*
** *Lolium perenne*

Mean values for growth parameters for plants grown in control soil were compared to those for plants grown in untreated sand, where nutrients were obtained from bottom watering. Shoot lengths for annual and perennial ryegrass, tomatoes, and

wheat were significantly larger (18% or more) for plants grown in soil. Shoot weights for corn, annual ryegrass, perennial ryegrass, and tomatoes were significantly greater for plants grown in soil. Root weights for tomatoes, annual ryegrass, perennial ryegrass, and wheat were more than 15% smaller for plants grown in soil. In this study, cucumbers and soybeans were the only species whose growth characteristics were not appreciably affected by the change in growing medium. An expanded summary of this study is given in Appendix T.

D. Potential Effects of Proposed Action on Non-Target Organisms

(1) Aquatic Organisms

The potential concentration of laidlomycin sodium in groundwater, 0.0199 mg/L, is not expected to have an effect on microbial organisms in soil. Concentrations inhibiting microbial growth are greater than environmental levels, even for the most sensitive microorganisms *Bacillus subtilis* (MIC = 0.4 mg/L) and *Clostridium perfringens* (MIC = 0.4 mg/L). Changes in mycelial morphology observed at 10 mg/L for *Aspergillus niger* and *Trichodermia viride* are of no significance when compared to potential environmental concentrations in either groundwater or rainfall runoff.

The no-observed effect levels for laidlomycin sodium in the *Daphnia magna*, *Hyalella azteca*, rainbow trout and bluegill sunfish studies ranged from 1.2 to 5.9 mg/L. This range is from 60 to 296 times higher than the maximum expected concentration of laidlomycin sodium in surface or groundwater (0.0199 mg/L) that might enter freshwater streams as a result of disposal of feedlot waste on agricultural soils. Even though runoff from a cattle feedlot is subject to impound, the concentration of laidlomycin sodium is well below the effect levels for aquatic organisms.

The no-observed effect level for C-3 despropionyl laidlomycin sodium toxicity to *Daphnia magna*, 109 mg/L, is substantially greater than the corresponding NOEC for laidlomycin sodium, 5.5 mg/L. Aquatic levels of C-3 despropionyl laidlomycin sodium in feedlot runoff, 3.41 mg/L, are not expected to have an adverse environmental impact, even if runoff should be directly discharged to a water body that supports aquatic life.

(2) Terrestrial Organisms

As expected, Gram positive organisms are much more strongly affected by laidlomycin than are Gram negative organisms. The incorporation of laidlomycin sodium to soil at a concentration of 0.0752 mg/kg is not expected to have an adverse effect on the population of soil microorganisms, since Gram negative organisms predominate in soils(16).

The effect of laidlomycin sodium on seed germination and root elongation was expected, since monensin, a structurally related ionophore(17), is known to strongly inhibit both germination of annual ryegrass and plant development at test concentrations as low as 7 ppm. The laidlomycin sodium NOEC for the germination of perennial ryegrass (4.9

ppm) was the lowest of the monocot species tested. The NOEC's for cabbage and pinto bean (10 ppm) were the lowest of dicot species tested. The germination of both monocots and dicots is not expected to be influenced at the concentration of laidlomycin sodium in soil, 0.0752 mg/kg, resulting from the incorporation of cattle waste at a maximum rate of 20 tons/acre.

As expected, the root elongation NOEC's of both mono- and dicots are lower than the corresponding seed germination NOEC's. However, the range of NOEC's, 1.0 to 10 ppm, is still much greater than the maximum expected concentration of laidlomycin in agricultural soil. Accordingly, the application of feedlot cattle waste to cropland is not expected to have an adverse effect on primary root development.

The effect of laidlomycin sodium on seedling growth was evaluated by periodic measurement of the shoot length, shoot and root weights of growing plants. Both monocots and dicots were used in this first 21-day test. Sand was used for a root support, and nutrient solutions were applied to assure proper plant growth. Exposure levels were established by incorporating laidlomycin sodium into the nutrient solutions at known concentrations.

Unlike the results for root elongation, the NOEC's for plant growth, 0.022 mg/L to 0.96 mg/L, exhibited a much wider and lower range. Further, these levels were much lower than would be expected from the known phytotoxicity of monensin. When it was realized that a substantial amount of nutrient solution needed to be added to replace the moisture lost by evaporation over the 21-day growing period, we recognized the potential for laidlomycin sodium to accumulate in the sand root support. A preliminary experiment, using cucumber for the test species, was performed under the same conditions as the earlier experiment with six plant species. At various time points the plants were carefully removed and the sand sectioned into top and bottom halves. Laidlomycin sodium was recovered from sand and analyzed by colorimetric analysis. On average, there was a 742% increase in nominal laidlomycin sodium concentrations in the top section of the sub–irrigated pots compared to the nominal concentrations of laidlomycin sodium in the nutrient solution. Accordingly, the summary table giving NOEC's for plants grown in sand (Section 8.C.(2)(b)) was expanded to include NOEC's after compensating for accumulation in the root support.

A preliminary test in a CA sandy loam soil using ryegrass as a test species resulted in a large increase in NOEC levels relative to those measured in sand. This is consistent with the known difference in the toxicities of DDT to legumes grown in sand and soil[18]. Similar effects on plant growth are also known for the soil-applied herbicides pyrazon, benzthiazuron, cycloate, delachlor[19], and pronamide[20]. A definitive study was undertaken to determine the phytotoxicity of laidlomycin sodium to plants grown in California sandy loam soil using levels of laidlomycin based on the preliminary ryegrass study in soil. The test chemical was incorporated into the soil at known levels prior to

planting. Although seedling growth in CA soil can not be expected to be representative of all soils, the information should provide a more realistic estimate of plant toxicity.

Growing conditions for plants in soil were maintained as close as possible to those used for plants grown in sand. The number of test species was increased to seven to accommodate annual ryegrass. Control plants (no exposure to laidlomycin sodium) were grown in three root supports: soil, solvent treated soil, and sand. Root supports were kept moist by sub irrigation.

Measurements were made after the 21 day growing period to establish the no-observed effect levels. Control plants for the species tomato, soybean, annual ryegrass, and corn did not grow as well in sand as in the soil root supports when root and shoot weights are used for comparison. The nominal NOEC levels ranged from 1.6 to 6.5 mg/kg. When corrected for the average 78% recovery of laidlomycin sodium from treated soils, all NOEC's for the seven plant species ranged from 1.25 to 5.07 mg/kg.

There may be several reasons for the differences between the NOEC values for plants grown in sand (range from 0.022 mg/L to 0.70 mg/L as measured in the nutrient solutions) and for plants grown in soil (range from 1.6 mg/kg to 6.5 mg/kg, based on the nominal starting concentrations). The root development of plants grown in sand may be stressed, particularly for tomatoes, soybeans, annual ryegrass, and corn. This may have led to an increased sensitivity to laidlomycin sodium. The accumulation of laidlomycin sodium in the sand in the top half of the pots, owing to water loss, may also be expected to lead to variable effects on plant development depending on the type of root growth. For a more accurate comparison between the NOEC values found in sand and those found in soil, the sand NOEC values must be adjusted to compensate for this accumulation of laidlomycin sodium (on average, there was a 742% accumulation in the top half of the pots). Overall, the compensated NOEC values for plants grown in sand (range from 0.42 to 5.19 mg/kg) are fairly comparable to the NOEC values for plants in soil (range from 1.25 to 5.07 based on an average recovery of 78%). These results are now consistent with the results of the seed germination and root elongation study, Appendix R. In addition, these data are more in agreement with the phytotoxicity of related antibiotics ionophores, monensin[17] and maduramicin[21].

Regardless of the way a phytotoxic effect of laidlomycin sodium is evaluated, there is a substantial difference between the maximum expected levels in agricultural soils resulting from an application of 20 tons of manure per acre, 0.0752 ppm, and the lowest no-observed effect levels, 0.16 (sand) and 1.25 ppm (soil), from the plant studies. Taking into account the biotransformation of both laidlomycin propionate potassium and laidlomycin sodium in soils amended with cattle waste, no adverse plant effects are expected when cattle feedlot waste from treated animals is applied to agricultural land.

9. **USE OF RESOURCES AND ENERGY**

The raw materials used in the manufacture of laidlomycin propionate potassium are readily available. The production of the drug substance and product and the energy use involved therein do not cause a depletion of any natural resources that are in critically short supply.

10. **MITIGATION MEASURES**

Syntex Agribusiness and Glatt Air Techniques, Inc. take all necessary measures (described in item 6) to achieve compliance with the regulations governing the proposed manufacture of laidlomycin propionate potassium. In light of the information presented, no mitigation measures are necessary.

No other potential adverse environmental impact is associated with the manufacture of laidlomycin propionate potassium.

11. **ALTERNATIVES TO THE PROPOSED ACTION**

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

12. **PREPARERS**

The following personnel at Syntex Development Research and Syntex Discovery Research are responsible for the preparation of the Environmental Assessment

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13. CERTIFICATION

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

Anthony A. Bourdakis
Vice President - Corporate Regulatory Affairs and Compliance
Division of Product Safety and Compliance

14. REFERENCES

1. Kushinsky, S., "Depletion of (^{14}C) from the Principal Edible Tissues of Cattle Following Cessation of a Twice-daily Dosing Regimen of (^{14}C)-Laidlomycin Propionate Potassium", Syntex Report RS-11988, ATv 3437 (1985)

2. Lee, G., Massey, I.J., Rogers, J., "Characterization of the ^{14}C-Residues Present in the Feces of Cattle Following Oral Administration of ^{14}C-Laidlomycin Propionate Potassium", Syntex Report RS-11988, ATv 5172 (1989)

3. Safley, L.M. Jr., Westerman, P.W., Barker, J.C., "Fresh Dairy Manure Characteristics and Barnlot Nutrient Losses", in *Characteristics of Agricultural Waste Utilization and Management*, Proc. 5th Internat. Symposium on Agric. Wastes, Dec 16-17 (1985)

4. Clark, R.D., Hedden, G.L., Kluge, A.F., Maddox, M.L., "Enhancement of the Activity of the Antibiotic Laidlomycin by Acylation and the ^{13}C NMR Spectra of Laidlomycin and its Esters", J. Antibiotics, **35**,(11), 1527-1537, (1982)

5. Train, R.E., Sansom, R.L., Cywin, A., Denit, J.D., "Development Document for Effluent Limitations Guidelines and New Source Performance Standards - Feedlots Point Source Category", p. 58, U.S.E.P.A., Wash. D.C. (1974)

6. Donoho, A.L., "Biochemical Studies on the Fate of Monensin in Animals and in the Environment", J. An. Sci. **58**, (6), 1528-1539 (1984)

7. Train, R.E., *et. al., loc. cit.*, p.60

8. Wells, D., Albin, R., Grub, W., Coleman, E., Meenaghan, G., "Characteristics of Wastes from Southwestern Cattle Feedlots", Texas Tech University Water Resources Center, Lubbock, TX, p. 22, U.S.E.P.A. Project #13040 DEM (1971)

9. Ibid., p. 32

10. Hansen, R., Harper, J., Stone, M., Ward, G., Kidd, R., "Manure Harvesting Practices: Effects on Waste Characteristics and Runoff", p. 103, Report # EPA-600/2-76-292, Robert S. Kerr Env. Res. Lab., Office of Res. and Dev., U.S.E.P.A., Ada Oklahoma 74820 (1976)

11. De Boer, J.H., "The Dynamical Character of Adsorption", p. 7, Oxford University Press, (1953)

12. Daniels, F., Alberty, R.A., "Physical Chemistry", 4th ed., p. 109, John Wiley and Sons, New York, (1975)

13. Simpson, F.M. Jr., "How to control feedlot pollution", Bulletin C, California Cattle Feeders Association (1971). See also Hart, S.A., Bunger, R.E., "Manure Management", Chapter 12, in *"The Feedlot"*, p. 195, I.A. Dyer and C.C. O'Mary, eds., Lea & Febiger 2 ed., Philadelphia, (1977)

14. Sweeton, J.M., Withers, R.E. Jr., "Feedlot Manure Drops in Texas", Beef, p. 54, Oct. 1990

15. Hamaker, J.W., "The Interpretation of Soil Leaching Experiments", p.115-133, in *Environmental Dynamics of Pesticides,* R. Haque and V.H. Freed, Eds., Plenum Press (1975)

14. REFERENCES

16. Alexander, M.,"Introduction to soil Microbiology", p. 25, Krieger Publ. Co., Malabar, FL (1991)

17. Mollenhauer, H.H., Morre, D.J., Droleskey, R.E., "Monensin Inhibition of Growth of Ryegrass Seedlings", Bot. Gaz. **147**,(4), 432-436 (1986)

18. Appleman, M.D., Sears, O.H., "Effect of DDT Upon Nodulation of Legumes", J. Am. Soc. Agron., **38**, 545-550 (1946)

19. Horowitz, M., Blumenfeld, T., "Phytotoxicity for Herbicides in Different Synthetic Soils", Phytoparasitica J., **2**, 101-110 (1973)

20. Mitideri, A., Crabtree, G., "The Effect of Pronamide on Lettuce Growth and Weeds as Affected by Different Soil Types", HortScience, **8**, (3), 224-225 (1973)

21. "Cygro 1% Type A Medicated Article - EIAR", p. 21, Document Control # 95352, Food and Drug Administration, Rockville MD 02857

15. EXPANDED REPORT SUMMARIES

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Appendix A: Report Summary

Title: Applicable Laws and Regulations and Compliance Statements.

SPRINGFIELD, MISSOURI

FEDERAL LAWS	STATUTE	REGULATION
1. Clean Air Act, as amended.	42 U.S.C. § 7401 et. seq.	40 CFR 52
2. Resource Conservation and Recovery Act, as amended.et. seq.	42 U.S.C § 6901	40 CFR 260
3. Water Pollution Control Act, as amended (Clean Water Act).et. seq.	33 U.S.C. § 1251	40CFR 403,439
4. Occupational Safety and Health Act, as amended.et. seq.	29 U.S.C. § 651	29CFR 1900-1910
5. Emergency Planning and Community Right-to-Know Act.	42 U.S.C. § 11001 et. seq.	40 CFR 372
6. Toxic Substance Control Act	15 U.S.C. § 2601 et. seq.	40 CFR 720
7. Hazardous Materials Transportation Act.	49 U.S.C. § 1801 et. seq.	49 CFR 171-177
8. Pollution Prevention Act of 1990	42 U.S.C. § 13101 et seq.	None Issued

MISSOURI LAWS	STATUTE	REGULATION
1. Missouri Clean Water Law	Chapter 644 R.S. et. seq	
2. Missouri Air Regulations		10 CSR 6
3. Hazardous Waste Management		10 CSR 25
4. Solid Waste Management		10 CSR 80

LOCAL RULES	STATUTE	REGULATION
1. Springfield City Code	Section 30-30 Section 30-31	

SYNTEX agribusiness inc NUTRITION AND CHEMICAL DIVISION P.O. BOX 1246 SPRINGFIELD, MISSOURI 65801 417-866-72

May 22, 1992

Eleanor Alderette
Syntex Corporation
3401 Hillview Avenue
Palo Alto, CA 94304

Dear Ms. Alderette:

Syntex Agribusiness, Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of laidlomycin propionate at its facilities in Springfield, Missouri, as well as emission requirements set forth in applicable federal, state, and local statutes and regulations, applicable to the production of laidlomycin propionate at its facilities in Springfield, Missouri.

Sincerely,

Patrick J. Lee
Vice President & General Manager

PL/tje

RAMSEY, NEW JERSEY

FEDERAL LAWS	STATUTE	REGULATION
1. Clean Air Act, as amended.	42 U.S.C. § 7401 et. seq.	40 CFR 52
2. Resource Conservation and Recovery Act, as amended.	42 U.S.C. § 6901 et. seq.	40 CFR 260
3. Water Pollution Control Act, as amended (Clean Water Act).	33 U.S.C. § 1251 et. seq.	40 CFR 403,439
4. Occupational Safety and Health Act, as amended.	29 U.S.C. § 651 et. seq.	29 CFR 1900-1910
5. Emergency Planning and Community Right-to-Know Act.	42 U.S.C. § 11001 et. seq.	40 CFR 372
6. Toxic Substance Control Act.	15 U.S.C. § 2601 et. seq.	40 CFR 720
7. Hazardous Materials Transportation Act.	49 U.S.C. § 1801 et. seq.	49 CFR 171-177
8. Pollution Prevention Act of 1990	42 U.S.C. § 13101 et seq.	None Issued

NEW JERSEY LAWS	STATUTE	REGULATION
1. Solid Waste Management Act	NJSA 13:1E-1 et. seq.	NJAC 7:26-1 et. seq.
2. Air Pollution Control Act	NJSA 26:2G-1 et. seq.	NJAC 7:27-1 et. seq.
3. Water Pollution Control Act, as amended (Clean Water Enforcement Act)	NJSA 58:10A-1 et. seq.	NJAC 7:14A-1 et. seq.
4.Motor Vehicle Law	NJSA 39:5B-1 et. seq.	NJAC 16:49-1 et. seq.

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Glatt Air Techniques, Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees, and administrative orders applicable to the production of laidlomycin propionate premix (11%) at its facilities in Ramsey, NJ, as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the production of laidlomycin propionate premix (11%) at its facilities in Ramsey, NJ.

John Niemiec
Manager, Facility Engineering

Kenneth Olsen
Executive, V.P.

6/24/92
Date

Appendix B: Report Summary

Title: Measurement of the pK_A of Laidlomycin from the Solubility-pH Profile.

Investigator: Marple, L.W., Syntex Report #RS-11988 CH 0252 dated June 7, 1989

The pK_A of laidlomycin was determined by the solubility method. The study is done by measuring the total solubility as a function of the pH above and below the pK_A of the free acid. When activity coefficients are ignored, the pK_A is numerically equivalent to the pH when the concentration of the free acid is equal to the conjugate base. The solubility of the free acid is determined by lowering the pH of the suspension until the total laidlomycin in solution exhibits no further decline.

The analysis of laidlomycin in many fate studies was based on the work of Golab, et al.* who developed a sensitive colorimetric method for monensin, a structurally related ionophore. The method is based on the pink color produced in methanol at 60 ^{O}C by the reaction with vanillin in the presence of sulfuric acid. A linear relation between absorbance at 518 nm and monensin was observed over the concentration range of 1 - 8 µg/mL. The color development is sensitive to time of development, order of reagent addition, and source of vanillin. The inclusion of standards with every set of samples to calibrate the response minimizes these experimental problems.

In the study approaching solubility from undersaturation, suspensions of the acid form of laidlomycin were prepared in water and tetrabutylammonium chloride (TBACl) to give a salt concentration of 0.0106 molar. After equilibration, the pH was adjusted to pH 4 with 0.01 M HCl-0.0106 M TBACl solution. The pH of the suspension was measured, then increased slightly by adding tetrabutylammonium hydroxide-0.105 M TBACl. Separately, the solubility was approached from supersaturation by acidifying solutions that had been brought to pH 6. For this purpose, 0.10 M HCl in 0.0106 M TBACl was used to avoid changing ionic strength.

Samples of the suspensions taken for analysis of dissolved laidlomycin were filtered through 0.2 µ membrane filters. The laidlomycin in each sample of filtrate was recovered by extraction with methylene chloride. After evaporating the solvent, the residue was dissolved in methanol, and the pink color characteristic of laidlomycin was developed at 60^{O}C by reaction with vanillin-sulfuric acid reagent. The colors developed by a set of known laidlomycin standards, containing 0 to 100 µg, were developed concurrently and used to calibrate the response at 510 nm. The slope of the least squares linear regression for the solubility data gave a pK_A of 5.45 for laidlomycin. The reported pK_A is 5.5, with an uncertainty of 0.1 unit.

* Golab, T., Barton, S., Scroogs, R., "Colorimetric Method for Monensin", J.O.A.C., 56, p. 171-173 (1973).

Appendix C: Report Summary

Title: Measurement of Formation Constants for Sodium Laidlomycin and Potassium Laidlomycin in Water.

Investigator: Marple, L., Syntex Report #RS-11988 CH 0255 dated January 11,1990

The solubility products of the sodium and potassium salts of laidlomycin were determined by measuring the amount of laidlomycin in the filtrates of suspensions produced by adding an excess of the sodium or potassium salt to solutions containing known amounts of NaCl or KCl. The suspensions were weakly buffered at pH 9. The ionic strength of the suspensions was adjusted to 0.200 by the addition of tetramethylammonium chloride (TMACl). The total sodium and potassium concentrations were varied from 0.0012 to 0.0501 M by substituting the corresponding chloride salts for TMACl. After equilibration, portions of the suspension were filtered through 0.2 μ membrane filters. A spectrophotometric method was used to measure the dissolved laidlomycin in each filtrate. A characteristic color was developed by reaction with vanillin-sulfuric acid reagent in methanol at 60^{O}C. The colors developed by a set of known laidlomycin standards, containing 0 to 100 μg, were developed concurrently and used to calibrate the response at 510 nm.

Values of the water solubility and solubility product of laidlomycin sodium is 3.42×10^{-4} mol/L (145 mg/L) and 1.17×10^{-7} $(mol/L)^2$, respectively, Values of the water solubility and solubility product of laidlomycin potassium is 7.22×10^{-4} mol/L (314 mg/L) and 5.21×10^{-7} $(mol/L)^2$.

Appendix D: Report Summary

Title: Octanol/Water Partition Coefficient of Sodium Laidlomycin.

Investigator: Marple, L.W., Syntex Report #RS-11988 CH 0263, dated May 10, 1990.

The slow stirring method of Brooke, D.N., Dobbs, A.J., Williams, N.* was used for the measurement of the distribution of laidlomycin sodium between 1-octanol and water. This method is more suitable than the shake flask method when the test chemical is stable and is expected to have a high distribution coefficient. Here, equilibrium between the two phases is attained by transport of the test chemical across a relatively static interface.

In this study, the concentration of laidlomycin sodium in both 1-octanol and water phases was measured weekly for 4 weeks. Analysis of laidlomycin sodium in the octanol phase started by dissolving a 100 µL 1-octanol sample in 50 mL of methylene chloride, then evaporating a 2 mL aliquot to dryness. The residual laidlomycin sodium was dissolved in methanol and the characteristic pink color developed by reaction with vanillin-sulfuric acid reagent in methanol. Laidlomycin sodium in an aliquot of aqueous phase, was extracted with methylene chloride. After evaporation of the solvent, the residue was dissolved in methanol, and the color developed with vanillin-sulfuric acid reagent. The colors developed by a set of known laidlomycin standards, containing 0 to 100 µg, were developed concurrently and used to calibrate the response at 510 nm.

The mean value of K_{OW} for the sodium salt of laidlomycin at pH 9.00 was reported to be 1.88×10^4, and the range of values was 1.69 to 1.90×10^4 at the conclusion of the study.

* Brooke, D., Dobbs, A., Williams, N., "Octanol:Water Partition Coefficients (P): Measurement, Estimation, and Interpretation, Particularily for Chemicals with $P > 10^5$", Ecotoxicology and Environ. Safety, **11**, p. 251-260 (1986).

Appendix E: Report Summary

Title: Vapor Pressure of Sodium Laidlomycin.

Investigator: Marple, L.W., Spilkin, A., Syntex Report #RS-11988 CH 0265.

This study reports a vapor pressure of laidlomycin sodium using a method developed by Syntex which utilized electron impact mass spectrometry. Approximately five µg of laidlomycin sodium were placed in a sample cup and inserted into the mass spectrometer. The measured operating pressure was 2×10^{-6} Torr. The temperature of the sample was increased in steps of $30^{O}C$ until a mass spectrum was obtained. A constant parent-ion current for laidlomycin sodium was achieved at $270^{O}C$, and maximum ion currents was observed at $300^{O}C$. The temperature at which the ion current is maximum is the temperature at which the vapor pressure of the sample is essentially equivalent to the operating pressure of the spectrometer. The measured vapor pressure at $270^{O}C$ was 3.2×10^{-7} Torr. Using the approximation that a change of $50^{O}C$ produces a 10 fold lowering of the vapor pressure, the estimated vapor pressure at ambient temperature ($20^{O}C$) is 3×10^{-12} Torr.

The vapor pressure of a reference compound, di(2-ethyl hexyl) phthalate, was also estimated by this procedure, and was found to be within a factor of two of the known vapor pressure determined by the vapor pressure balance.

Appendix F: Report Summary

Title: Determination of Soil Adsorption/Desorption Constants for Laidlomycin.

Investigator: Nielsen, B., Marple, L.W., Syntex Report #RS-11988 CH 0251

A soil sorption/desorption test with laidlomycin sodium was conducted in triplicate for four different types of soil. The characteristics of the soils are summarized in Table 1.

For the advanced testing, the amount of laidlomycin sodium in the soil (5g)/water (25g) suspensions was adjusted for each soil type in order to achieve measurable concentrations of laidlomycin sodium in the aqueous phase. A screening test showed that the presence of $CaCl_2$ did not interfere. Approximately 64% of laidlomycin sodium in solution was adsorbed to CA sandy loam, 95% to MS clay loam, 46% in MO silt loam, and 69% in TX silt loam soil. Mass balance studies showed quantitative recoveries ranging between 92.5 and 99%. A summary of distribution measurements for CA, MS, MO and TX soils is given in Table 2. Table 3 provides summary data from the screening study for K_d and K_{oc} for all soils tested. Table 4 provides the advanced test summary data for all soils.

TABLE 1

SOIL CHARACTERIZATION

origin:	California	Mississippi	Missouri	Texas[1]
type:	sandy loam	clay loam	silt loam	silt loam
% sand[2]:	56.0	20.0	27.9	5
% silt[2]:	27.0	45.0	64.7	69
% clay[2]:	17.0	35.0	7.4	26
% OM:	1.6	1.4	1.8	1.1
P(ppm):	24	52	58	1
K(ppm):	198	240	155	266
Mg(ppm):	232	477	100	156
Ca(ppm):	830	1620	1240	1880
Na(ppm):	41	20	18	13
pH:	6.1	7.4	7.2	7.2
CEC:	7.9	12.8	7.5	11.4
% BASE SATURATION				
K:	6.4	4.8	5.3	6.0
Mg:	24.6	31.1	11.1	11.4
Ca:	52.7	63.4	82.6	82.2
H:	14.0	0.0	0.0	0.0
Na:	2.3	0.7	1.0	0.5

Notes:

1 - Representative analysis from same deposit
2 - Textural analysis performed on original soil sample prior to soil particle size reduction

% OM : % Organic Matter
CEC : Cation Exchange Capacity; meq/100 g

TABLE 2

ISOTHERM DETERMINATION; 0.01 M $CaCl_2$ SOLUTION

μg_i		100		150		200		250	
SAMPLE		Ce	x/m	Ce	x/m	Ce	x/m	Ce	x/m
CA	A	1.421	12.89	2.254	18.73	3.265	23.78	4.332	28.34
	B	1.478	12.61	2.321	18.40	3.388	23.06	4.394	28.03
	C	1.512	12.44	2.445	17.78	3.593	22.04	4.413	27.93

μg_i		400		500		600		700	
SAMPLE		Ce	x/m	Ce	x/m	Ce	x/m	Ce	x/m
MS	A	0.741	76.30	0.995	95.02	1.033	114.8	1.264	133.7
	B	0.726	76.37	0.819	95.90	1.086	114.6	1.264	133.7
	C	0.752	76.24	0.857	95.71	1.051	114.7	1.424	132.9

μg_i		50		100		150		200	
SAMPLE		Ce	x/m	Ce	x/m	Ce	x/m	Ce	x/m
MO	A	1.164	4.179	2.301	8.497	3.540	12.30	4.535	17.33
	B	1.042	4.788	2.356	8.219	3.382	13.09	4.712	16.44
	C	1.080	4.602	2.261	8.697	3.411	12.95	4.684	16.58

μg_i		100		150		200		250	
SAMPLE		Ce	x/m	Ce	x/m	Ce	x/m	Ce	x/m
TX	A	1.640	11.80	2.359	18.21	3.217	23.92	3.556	32.22
	B	1.505	12.47	2.143	19.29	2.847	25.76	3.287	33.56
	C	1.408	12.96	2.172	19.14	2.778	26.11	3.492	32.54

μg_i : μg of analyte initially added to soil sample; applies to data beneath entry

Ce : concentration of the analyte in the supernatant (μg/g)

x/m : Concentration of the analyte in the soil (μg/g)

TABLE 3

SCREENING TEST SUMMARY TABLE
(AVERAGED DATA AND CALCULATED VALUES)

	% SORBED	% DESORBED	K_d	K_{oc}	% RECOVERY
CA	63.6	43.12	8.72	9.4×10^2	98.6
dev.1.5	0.96	0.57			
MS	94.90	8.78	93.2	1.1×10^4	92.5
dev.0.25	0.32	4.7			
MO	45.8	64.3	4.09	3.9×10^2	98.6
dev.1.9	4.8	0.31			
TX	69.49	45.29	11.39	1.8×10^3	98.8
dev.0.95	0.17	0.51			

% SORBED : Percentage of initial analyte in system retained on soil.
% DESORBED : Percentage of amount retained that is released in two rinses.
K_d : The distribution adsorption coefficient, concentration in the soil divided by concentration in the aqueous phase.
K_{oc} : K_d / % organic carbon x 100, where % organic carbon = % organic matter/1.724.
% RECOVERY : Percentage of initial analyte in system recovered at the end of the experiment.

TABLE 4

ADVANCED TEST SUMMARY TABLE
(VALUES CALCULATED FROM LINEAR REGRESSION)

	R	K	1/n	K_d	K_{oc}
CA	0.977	9.69	0.718	7.40	8.0×10^2
dev.	0.10	0.030	0.88		
MS	0.982	104.96	0.971	105.4	1.3×10^4
dev.	0.84	0.092	3.0		
MO	0.998	4.10	0.91	3.78	3.6×10^2
dev.	0.08	0.03	0.21		
TX	0.993	7.58	1.153	8.73	1.4×10^3
dev	.0.16	0.067	0.49		

R : Correlation coefficient from linear regression.
K : Y-intercept from the Freundlich equation:

$$\text{Ln}(x/m) = \text{Ln}(K) + (1/n)\,\text{Ln}(Ce)$$

1/n : Describes the exponential nature of the isotherm.
K_d : Average distribution adsorption coefficient calculated using all isotherm samples.
K_{oc} : K_d / % organic carbon x 100, where % organic carbon = % organic matter/1.724.

Appendix G: Report Summary

Title: ^{14}C-Laidlomycin Biodegradation in Soils

Investigator: P. Fackler, Springborn Laboratories, Inc., Syntex Report #RS-37728 CH 0259

The extent of microbial mineralization of ^{14}C-laidlomycin sodium in three soil types was determined by measuring the production of ^{14}C-CO_2 and ^{14}C-volatiles over a 65 day period. Mississippi silty clay (p 5.6, CEC 21.6 meq/100g, and 0.8% OM), Texas silty clay loam (pH 7.8, CEC 15.0 meq/100g, and 1.6% OM), and California sandy loam (pH 7.7, CEC 11.3 meq/100g, and 0.7% OM) were used in this study. Incubation flasks were prepared for the following test conditions: 1) 10 mg C as ^{14}C-laidlomycin sodium; 2) 10 mg C as ^{14}C-laidlomycin sodium + 25 mg glucose; and 3) 10 mg ^{14}C-glucose.

Cumulative conversion of ^{14}C-laidlomycin sodium to ^{14}C-CO_2 was 0.1% for MS soil, 4.9% for TX soil, and 1.0% for CA soil. Conversion of glucose to carbon dioxide in the same time period ranged from 51-69%. The addition of glucose to soils containing laidlomycin sodium did not result in a substantial increase in the rate of mineralization. The production of ^{14}C-CO_2 for the test soils is given in the following graphs. The ^{14}C-laidlomycin accounted for in various forms as within reasonable error, and it is concluded that indigenous soil organisms acclimate slowly to the test compound.

Figure 1

Cumulative Mean Percent of Theoretical CO_2 vs. Time for Mississippi Silty Clay Dosed With ^{14}C-Laidlomycin

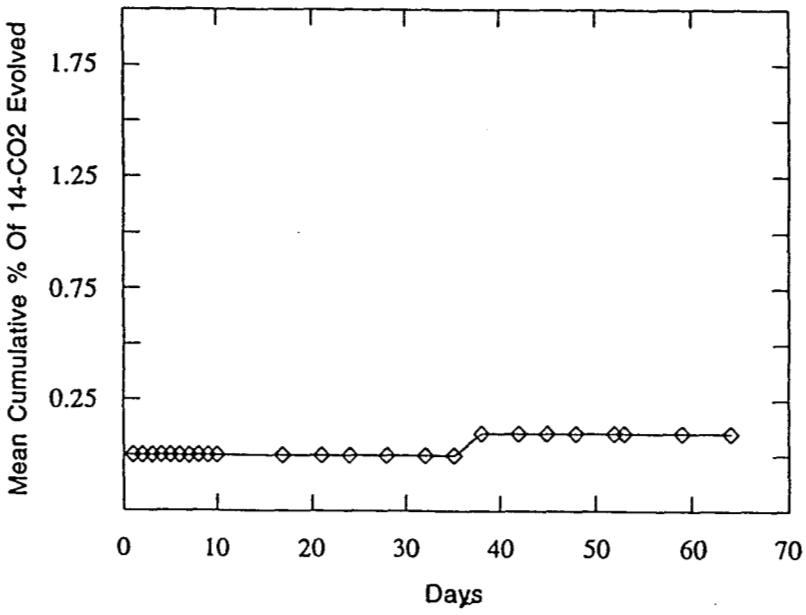


Figure 2

Cumulative Mean Percent of Theoretical CO_2 vs. Time for Texas Silty Clay Loam Dosed With ^{14}C-Laidlomycin

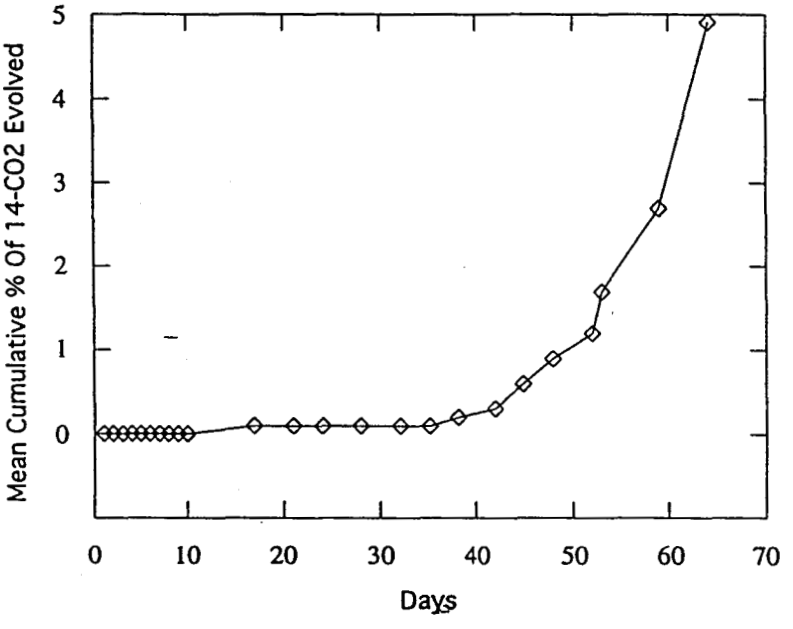
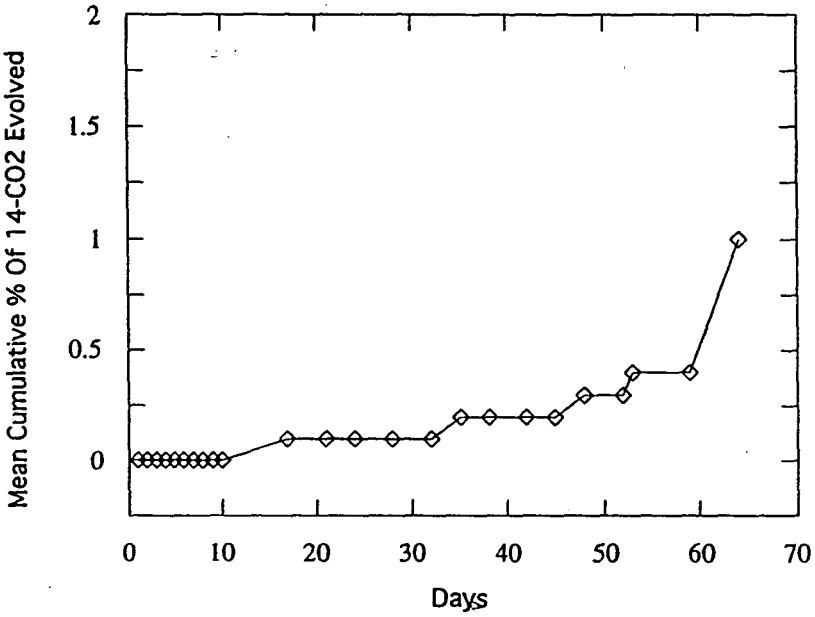


Figure 3

Cumulative Mean Percent of Theoretical CO_2 vs. Time for California Sandy Loam Dosed With ^{14}C-Laidlomycin



Appendix H: Report Summary

Title: Laidlomycin Propionate: Biodegradation in Cattle Feedlot Waste

Investigator: D. Weeden, Springborn Laboratories, Inc., Syntex Report #RS-11988 CH 0274

The potential for aerobic mineralization of ^{14}C-laidlomycin propionate in a representative feedlot waste (Texas) was evaluated by the quantity of ^{14}C-CO_2 and ^{14}C-volatile products released over a period of 84 days. The absence of significant gas production indicated that no mineralization occurred. However, chemical specific analysis did indicate that laidlomycin propionate was extensively biotransformed during the 84 day period. Residual radioactive test compound and biotransformation products were extracted from the samples with methanol. The analytes were concentrated, purified, and quantitatively measured by reverse phase HPLC using radiometric detection. Laidlomycin propionate degradation was evident by day 5, and continued through day 84. Biotransformation is often more complex than the abiotic chemical transformation that is studied by conventional methods. In this case, the decrease in laidlomycin propionate concentration was typical of a simple first-order reaction. The least squares linear regression of Log % Remaining against Time, shown in Figure 1, indicated a first-order half-life of 18 days.

The major product formed on biotransformation of ^{14}C-laidlomycin propionate was ^{14}C-laidlomycin, in one or more salt forms. For the purpose of calculating mass balance, the total laidlomycin formed was expressed as the sodium salt. The percent laidlomycin sodium in the extractable radioactivity increased rapidly up to day 28, but then slowly declined to study termination at day 84. The residual radioactivity in the form of laidlomycin propionate ranged from 0.7% to 7.7% of the nominal starting amount. The distribution of ^{14}C-radioactivity at the end of 84 days gave from 1.3% to 12.5% laidlomycin propionate, and from 8.3 to 63.7% laidlomycin sodium. The balance of the radioactivity was recovered as 7 or more very polar products. None of the HPLC retention times for these biotransformation products was the same as the known retention time for C-3 despropionyl laidlomycin. The biotransformation of laidlomycin propionate, as well as the formation and decline of laidlomycin sodium, is shown in Figure 2.

A mineralization reference experiment was performed with ^{14}C-glucose. Evolution of ^{14}C-CO_2 was 33.7% of the theoretical amount over a period of 64 days. The evolution of carbon dioxide, and the results of microbial plate counts, indicate that the feedlot waste contained a metabolically active microbial population.

Figure 1.

Concentrations of Laidlomycin Propionate as
Determined by HPLC-RAM and % Radioactivity
Distribution Remaining

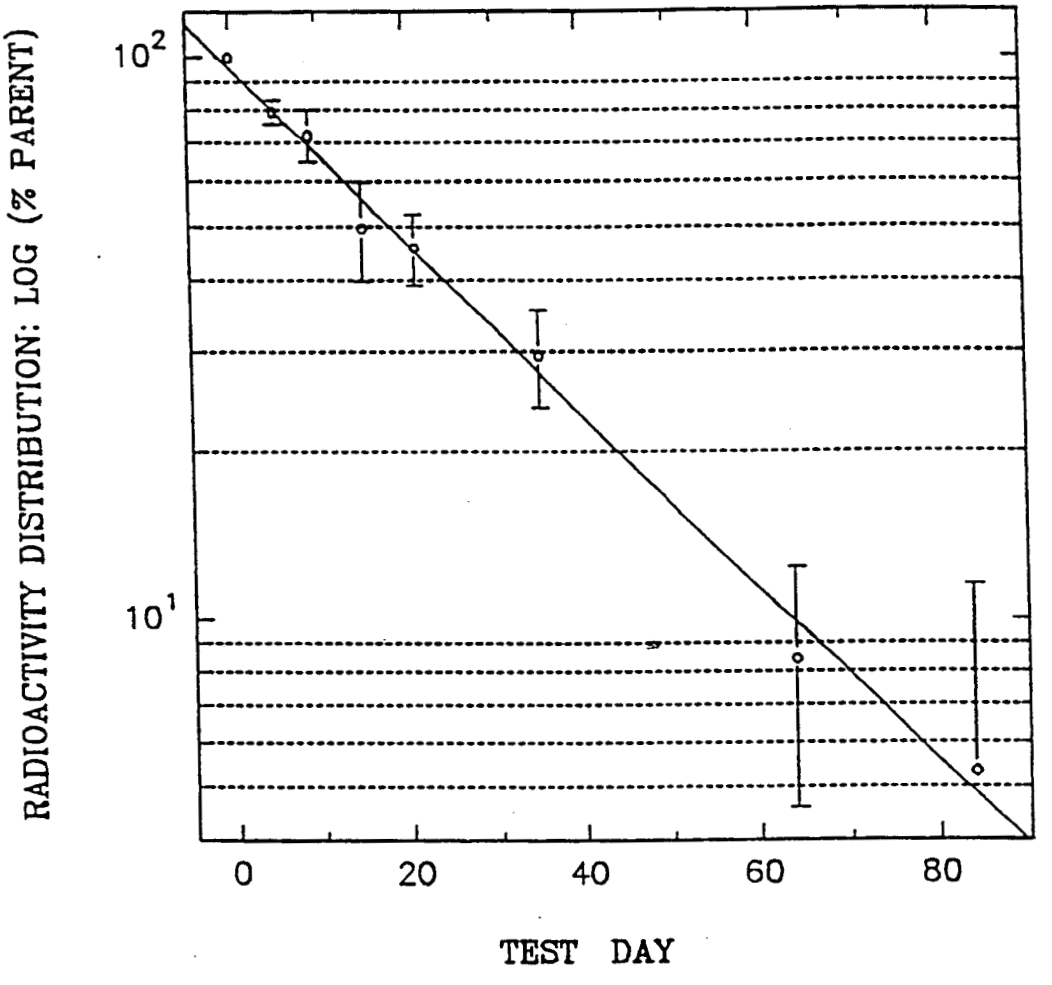
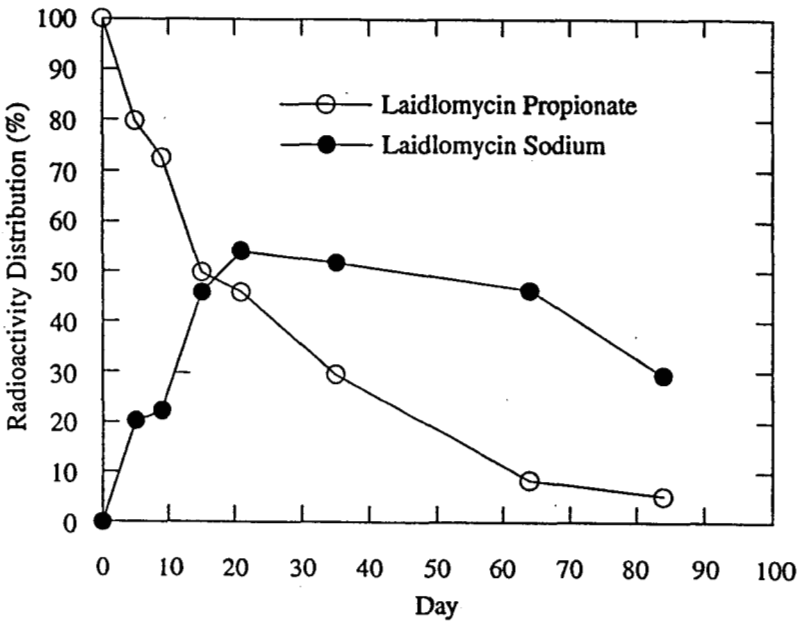


Figure 2

Average Percentage of Radioactivity in Cattle Waste Extract as ^{14}C-Laidlomycin Propionate and ^{14}C-Laidlomycin Sodium



Appendix I: Report Summary

Title: Laidlomycin Sodium: Biodegradation in Cattle Feedlot Waste

Investigator: D. Weeden, Springborn Laboratories, Inc., Syntex Report #RS-37728 CH 0273

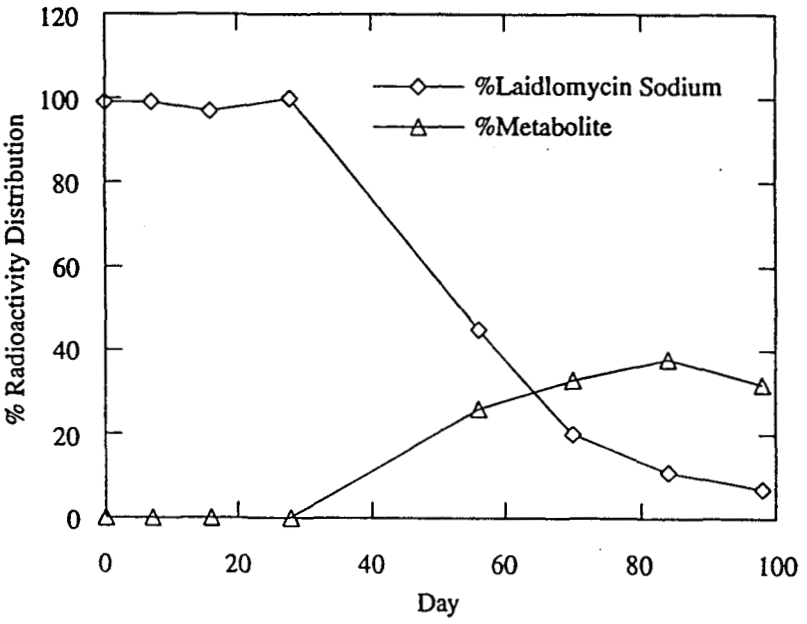
The potential for aerobic mineralization of ^{14}C-laidlomycin sodium in a typical feedlot waste (Texas) was evaluated by the quantity of ^{14}C-CO_2 and ^{14}C-volatile products released over a period of 98 days. The absence of significant volatile gas production indicated that no mineralization of the test compound occurred over the course of the study (the amount of ^{14}C-volatiles and ^{14}C-CO_2 generated during the study was <0.7%). However, chemical specific analysis did indicate that extensive biotransformation of ^{14}C-laidlomycin sodium took place. Radioactive residues in waste samples were repeatedly extracted with methanol. The analytes were concentrated, purified, and analyzed by reverse phase HPLC. Degradation up to 28 days was not significant. However, at 56 days, only 52.2 to 33.7% of the ^{14}C-laidlomycin sodium remained, with the balance of the radioactivity collectively recovered as 4 or more polar metabolites. At the end of 98 days, the amount of radioactivity recovered from the waste as ^{14}C-laidlomycin sodium ranged from 6% to 7% of the nominal amount added.

Variable amounts of ^{14}C-radioactivity were extracted from wastes as 4 or more very polar products. However, the HPLC retention times for these materials did not correspond to the retention time for C-3 despropionyl laidlomycin. The decrease in concentration of ^{14}C-laidlomycin sodium, along with the formation of the major ^{14}C-metabolite is shown in Figure 1. The biotransformation of laidlomycin sodium is consistent with logistic growth of organisms capable of utilizing laidlomycin sodium.

A mineralization reference experiment in feedlot waste was performed with ^{14}C-glucose. Evolution of $^{14}CO_2$ was 57.8% of the theoretical amount over a period of 107 days. The evolution of carbon dioxide, and the results of microbial plate counts, indicate that the feedlot waste contained a metabolically active microbial population.

Figure 1

Percent of Radioactivity in Cattle Waste Extract
as ^{14}C-Laidlomycin and an unidentified ^{14}C-Metabolite



Appendix J: Report Summary

Title: Biodegradation of Laidlomycin Propionate in Soils Amended with Cattle Feedlot Waste

Investigator: D. Weeden, Springborn Laboratories, Inc., Syntex Report #RS-11988 CH 0277

The rate at which radioactive ^{14}C-laidlomycin propionate undergoes aerobic biodegradation was investigated in CA, MS, and TX soils amended by the addition of 10% cattle feedlot waste. The quantities of ^{14}C-CO_2 and ^{14}C-volatile products released as a result of mineralization was determined over incubation periods of 120 to 179 days. Overall, between 0.3% to 1.3% of the initial ^{14}C-laidlomycin propionate added to the soil was recovered as ^{14}C-CO_2 depending upon soil type. Production of volatile products did not account for more than 0.0% (detection limit 0.005%) of the initial radioactivity in any soil type.

Biotransformation of the test chemical in soil was monitored by repeated extraction of radioactive materials with methanol. The extracts were combined, the analytes concentrated and separated by high performance liquid chromatography using radiometric detection. Analyses were performed on all replicates that were sacrificed throughout the study. The loss of ^{14}C-laidlomycin propionate as well as the formation of ^{14}C-biotransformation products was subject to some variability, even though a constant amount of ^{14}C-laidlomycin propionate was added to each replicate. On day 120, ^{14}C-laidlomycin propionate levels in CA, MS, and TX soils averaged 0%, 1.8%, and 0.3% of nominal, respectively. Mississippi soil samples were kept on test until day 179, at which point the ^{14}C-laidlomycin propionate in residual soil replicates averaged 12.5% of nominal.

The distribution of extractable radioactivity as ^{14}C-laidlomycin propionate, ^{14}C-laidlomycin sodium, C-3 despropionyl-^{14}C-laidlomycin, and ^{14}C-polar metabolites was determined for each replicate at all time points. The formation of ^{14}C-laidlomycin sodium and C-3-despropionyl-^{14}C-laidlomycin was confirmed by MS-FAB of isolates from HPLC chromatographic separations. Although the method of applying ^{14}C-laidlomycin propionate to the soil samples was constant, the distribution into different metabolites as the samples aged was not always consistent among replicates.

The distribution of radioactivity in extracts from CA soils is presented in Figure 1, where the average values for the components of replicates are plotted for increasing incubation times. In general, ^{14}C-laidlomycin sodium (loss of C-26 propionyl group) is the major product initially formed in CA soil. On average, the level of laidlomycin sodium decreases from day 21 to study end at day 120. By day 120, from 37% to 97% of the extractable radioactivity appears in the form of more polar components. Variable, small amounts of C-3 despropionyl-^{14}C-laidlomycin are formed between days 56 and 120.

The distribution of radioactivity in extracts from MS soils is presented in Figure 2, where the average values for the components of replicates are plotted for increasing incubation times. In general, the formation of very polar ^{14}C-metabolites is about as extensive as the formation of ^{14}C-laidlomycin sodium. On average, 41% of the recovered extractable radioactivity was in the form of polar metabolites at test termination on day 179. Variable, small amounts of C-3 despropionyl-^{14}C-laidlomycin are formed between days 120 and 179.

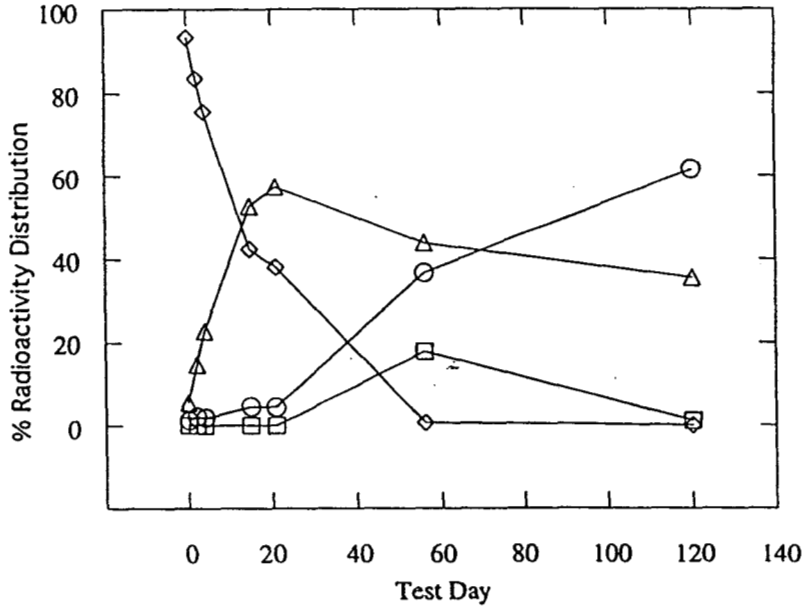
The distribution of radioactivity in extracts from TX soils is presented in Figure 3, where the average of values for the components of replicates are plotted for increasing incubation times. In general, the ^{14}C-laidlomycin sodium is the major compound initially formed, although the amount decreases from day 56 to day 120. The increase in the level of ^{14}C-polar metabolites during this period indicates that many biotransformation processes are operative after day 56. On average, 51% of extracted radioactivity was in the form of polar metabolites at test

termination on day 120. No significant amounts of C-3 despropionyl-^{14}C-laidlomycin are formed in TX soil.

Reference experiments in each of the three soil types were performed with radioactive ^{14}C-glucose, resulting in ^{14}C-CO_2 evolution in the range of 38.3% to 43.4% of the applied material. It was concluded that the soils contained a metabolically active microbial population.

Figure 1

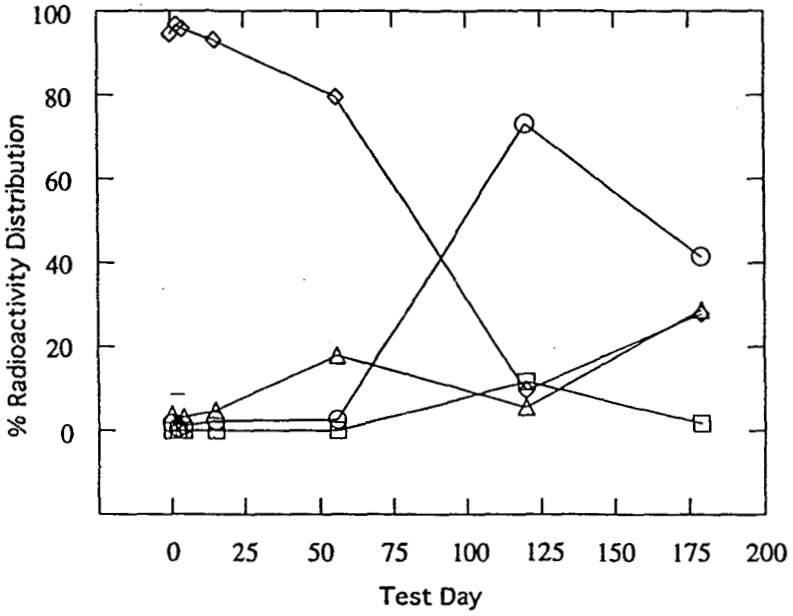
Radioactivity Distribution of Laidlomycin Propionate And Its Metabolites in Extracts of California Soil As Determined By HPLC-RAM



Diamonds: Laidlomycin Propionate
Up Triangles: Laidlomycin Sodium
Circles: Polar Metabolites
Squares: C-3-despropionyl

Figure 2

Radioactivity Distribution of Laidlomycin Propionate And Its Metabolites in Extracts of Mississippi Soil As Determined By HPLC-RAM



Diamonds: Laidlomycin Propionate

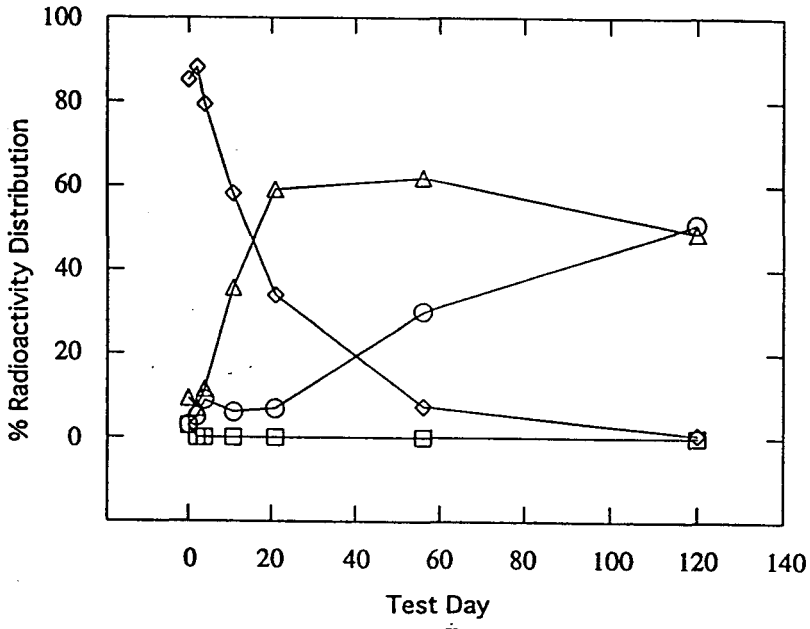
Up Triangles: Laidlomycin Sodium

Circles: Polar Metabolites

Squares: C-3-despropionyl

Figure 3

Radioactivity Distribution of Laidlomycin Propionate And Its Metabolites in Extracts of Texas Soil As Determined By HPLC-RAM



Diamonds: Laidlomycin Propionate
Up Triangles: Laidlomycin Sodium
Circles: Polar Metabolites
Squares: C-3-despropionyl

Appendix K: Report Summary

Title: Mammalian Toxicity Study Summaries
Nonclinical Toxicology Summary of Laidlomycin Propionate (RS-11988-047), Syntex Report #RS-11988 ATv 4847

Acute Studies with Laidlomycin Propionate (Potassium)

Acute (single dose), subchronic, and chronic toxicity and reproduction studies were conducted in laboratory animals. A subchronic tolerance study and a chronic safety study were conducted in cattle. In all studies, laidlomycin propionate potassium was administered orally. In addition, four short-term *in vitro* genotoxicity tests were conducted. The results of these studies are summarized below.

Acute Mouse Study: Seven groups, each composed of 6 males and 6 female mice, were administered a single dose by gavage 0, 5, 10, 20, 50, 100, or 200 mg/kg of laidlomycin propionate. The minimum lethal dose (LD_0) was estimated to be 50 to 100 mg/kg and the acute lethal median dose (LD_{50}) at 24 hours was calculated to be 133 mg/kg. Clinical changes and mortality due to laidlomycin propionate were present at 100 and 200 mg/kg. Based on gross pathological changes, cause of death was considered due to enteropathy.

Acute Rat Study: Seven groups, each composed of 6 male and 6 female rats, were administered a single dose by gavage 0, 2, 4, 10, 20, 40, or 100 mg/kg of laidlomycin propionate. The LD_0 was estimated to be 20 to 40 mg/kg and the LD_{50} after 48 hours was calculated to be 63 mg/kg. Clinical changes due to laidlomycin propionate were present at doses of 20 mg/kg or greater. Based on gross pathological changes, cause of death was considered due to enteropathy and/or gastroenteropathy.

Acute Dog Study: Two groups, each composed of 1 male and 1 female beagle dog, were administered by gavage a single dose of 20 or 60 mg/kg of laidlomycin propionate. The LD_0 was estimated to be between 20 and 60 mg/kg. Clinical signs due to laidlomycin propionate were present at 20 and 60 mg/kg. No drug-related deaths occurred at 20 mg/kg (although 1 dog died of malintubation). The male that received 60 mg/kg was killed in a moribund condition 3 days postdosing. Pathological examination revealed congestion and hemorrhage and/or edema in multiple organ systems.

Subchronic and Chronic Studies with Laidlomycin Propionate (Potassium)

Three-Month Rat Study: Five groups, each consisting of 20 male and 20 female rats, received by gavage daily doses of 0 (vehicle), 1, 2, 4 or 8 mg laidlomycin propionate/kg body weight for three months. No evidence of drug-related effects on clinical conditions, ophthalmology examination results, clinical pathology (hematology, serum chemistry and urinalysis), or gross or microscopic pathology examination results were observed. High-dose males exhibited decreased food intakes and rate of weight gain. The no-observed-effect level (NOEL) was 4 mg/kg/day for males and 8 mg/kg/day for females.

Three-Month Dog Study: Five groups, each consisting of four male and four female dogs, received by gavage twice daily doses of 0 (vehicle), 0.25, 0.50, 1.0 or 2.0 mg laidlomycin propionate/kg body weight for three months (91 days). Total doses were 0, 0.5, 1.0, 2.0 or 4.0 mg/kg/day. Evidence of toxicity, observed at the highest dose level, 4 mg/kg/day, was manifested by peripheral neuropathy and poor clinical condition. The NOEL was 2 mg/kg/day.

One-Year Dog Study: Four groups, each consisting of six male and six female beagle dogs, received by gavage twice daily doses of 0 (vehicle), 0.375, 0.75 or 1.5 mg laidlomycin propionate/kg body weight. Total doses were 0, 0.75, 1.5 or 3.0 mg/kg/day. Doses of 1.5 and 3.0 mg/kg/day produced dose-related increased serum triglyceride levels, skeletal fiber vacuolation and clinical and pathological changes consistent with peripheral neuropathy. These effects are typically observed for this class of compounds, i.e. ionophore. The NOEL was 0.75 mg/kg/day.

Reproductive Toxicity of Laidlomycin Propionate (Potassium)

Rat Teratology Study: Four groups, each consisting of 25 female rats with evidence of mating, received by gavage daily doses of 0 (vehicle), 1, 2 or 4 mg laidlomycin propionate/kg body weight from day 7 through 16 of gestation. No drug-related changes were present in the clinical condition of the dams. No evidence of teratogenicity was found. A dose of 4 mg/kg/day did produce an approximate 10% decrease in body weight gain. The NOEL was 2 mg/kg/day.

Rabbit Teratology Study: Four groups, each consisting of 20 artificially inseminated female rabbits, received by gavage daily doses of 0 (vehicle), 1, 2 or 4 mg laidlomycin propionate/kg body weight from day 7 through 19 of gestation. No drug-related changes were present in the clinical condition of the dams. No evidence of teratogenicity was found. A dose of 4 mg/kg/day did produce an approximate 10% decrease in body weight gain. The NOEL was 2 mg/kg/day.

Rat Two-Generation Reproduction Study: Four groups, each consisting of 30 males, received vehicle or laidlomycin propionate in the diet beginning ten weeks prior to cohabitation with treated females and continuing until F1 offspring were weaned. Four groups, each consisting of 30 females, received vehicle or laidlomycin propionate in their diet beginning 2 weeks prior to cohabitation with treated males until F1 offspring were weaned. Concentrations of laidlomycin propionate were 0, 50, 100 or 300 ppm. After all F1 pups were weaned, 30 male and 30 female pups per group were randomly selected as P2 breeders. P2 breeders received the same diet as their parents. At approximately 13 weeks of age each P2 male was cohabitated with 1 P2 female from the same dose group for 1 week. The P2 breeders remained on their test or placebo diets until day 21-23 postpartum.

P1 animals received an average dose of 0, 4.0, 7.4 or 24.7 mg laidlomycin propionate/kg body weight/day. P2 animals received an average dose of 0, 5.4, 11.3 or 37.4 mg/kg/day. Treatment with laidlomycin propionate did not affect reproduction and fertility parameters in either generation. The NOEL for reproductive effects was 300 ppm which provided averaged doses of approximately 25 and 37 mg/kg/day of laidlomycin for the first and second generations, respectively.

Genotoxicity

Ames Assay: Doses ranging from 4.88 to 312.5 µg laidlomycin propionate/plate were administered in the presence and absence of S9 metabolic activation. No mutagenic activity to the *Salmonella typhimurium* was observed.

Yeast Mitotic Recombination Assay: Doses ranging from 1.0 to 10,000 µg laidlomycin/plate were administered in the presence and absence of S9 metabolic activation. No treatment related gene conversions in the yeast strain D4 were observed.

Chinese Hamster Ovary Cell/HGPRT Mutation Assay: Doses ranging from 10 to 250 µg laidlomycin propionate/mL were administered in the presence and absence of S9 metabolic activation. No treatment related mutations in Chinese hamster ovary cells were induced.

Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay: Doses ranging from 0.51 to 253 µg laidlomycin propionate/mL were incubated with primary rat hepatocytes from an adult male Fischer 344 rat. Neither genetic activity nor DNA damage were observed.

Target Animal Studies

Bovine Tolerance Study: Two steers and two heifers received seven daily doses of 1500 mg laidlomycin propionate by capsule (mean dose = 5.55 mg/kg/day). One steer and 1 heifer received vehicle (soybean millrun) using the same regimen. Drug related effects consisted of anorexia, watery diarrhea, rumen atony, abnormal eating behavior, dullness and/or listlessness, bradycardia, myocardial fiber degeneration and death (1/4) due to cardiopathy.

Bovine Safety Study: Four groups, each consisting of seven mixed beef-breed steers (141 kg mean initial body weight) and heifers (137 kg mean initial body weight) were fed 0, 10, 30 or 50 g laidlomycin propionate/ton finished feed for 265 days. These doses represented 1X, 3X and 5X the maximum intended field dose. Three replicate groups of each sex were fed control and 10 g/ton treated diets and two replicate pens of each sex were fed diets containing 30 or 50 g/ton laidlomycin propionate. No statistically significant differences in feed efficiency, hematologic or clinical chemistry parameters, nor gross or microscopic pathology were observed. Increased incidence of intermittent loose feces were present in heifers fed 50 g laidlomycin propionate/ton.

Special Toxicity Studies

Rabbit Dermal Irritation Study with Laidlomycin Propionate: Laidlomycin propionate powder was applied (with occlusion) to one intact and one abraded skin site (0.5 g/site) on six female rabbits for 24 hours. Each site was then scored for erythema/edema at 1, 2, 3, 7, 14 and 21 days following removal of the patch. Slight erythema with no evidence of edema was observed. Evidence of recovery was seen 14 days post-dosing. Laidlomycin propionate is considered a slight, dermal irritant.

Rabbit Dermal Irritation Study with Laidlomycin: Laidlomycin powder was applied (with occlusion) to one intact and one abraded skin site (0.5 g/site) on six male rabbits for 24 hours. Each site was then scored for erythema/edema at 1, 2, and 3 days following removal of the patch. No erythema/edema was present in any animals up to 3 days post-dosing. The solid form of laidlomycin is not considered a dermal irritant.

Rabbit Ocular Irritation Study with Laidlomycin Propionate: Laidlomycin propionate powder was instilled into the right conjunctival sac (0.1 g) of three female rabbits. Both eyes were examined prior to dosing and at 1 hour, 1, 2, 3 and 7 days after treatment. The treated eyes were washed with 0.9% saline following day 1 observations. The animals exhibited severe corneal opacities, chemosis, ocular discharge and hypopyon (pus in the anterior chamber). One rabbit died 2 days post-dosing and one died 6 days post-dosing. The eye damage in the remaining rabbit was determined to be irreversible and the study terminated 7 days post-dosing. The unformulated laidlomycin propionate is considered a severe ocular irritant.

Rabbit Ocular Irritation Study with Laidlomycin: Laidlomycin powder was instilled into the right conjunctival sac (0.1 g) of three male rabbits. Both eyes were examined prior to dosing and at 1 hour, 1, 2, 3 days after treatment. The animals exhibited slight injection of the iris, conjunctival redness, chemosis and ocular discharge up to 24 hours post-dosing. All eyes were normal by 48 hours post-dosing. Laidlomycin is considered a slight, reversible ocular irritant. The release of propionic acid in the rabbit eye is considered the cause of the ocular irritation from solid, unformulated laidlomycin propionate. The decreased eye irritancy of laidlomycin supports this conclusion.

Appendix L: MSDS Sheets for Laidlomycin Sodium, Laidlomycin Propionate Potassium, and Laidlomycin Propionate Potassium Premix 11%

Material Safety Data Sheet

SYNTEX

Date: 29 September 1992
Supersedes: 31 July 1991

SECTION 1. COMPANY AND MATERIAL IDENTIFICATION

Supplier of Data: Syntex (U.S.A.) Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

In emergency, call: Environmental Health & Safety, (415) 855-5050

MATERIAL IDENTIFICATION

Common Name: **Laidlomycin sodium salt**

SECTION 2. PRODUCT COMPOSITION

Chemical Name: 9-Hydroxy-beta-(1-oxopropoxy)-2-[5-methyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-[hydroxymethyl]-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-alpha,gamma,2,8-tetramethyl-1,6-dioxaspiro-[4,5]decane-7-butyric acid sodium salt.

Chemical Formula: $C_{37}H_{61}O_{12}Na$

CAS #: 61489-98-3

Synonyms: RS-37728-037

Typical Percent Composition: ~95%

SECTION 3. HEALTH HAZARDS

WARNING STATEMENT

WARNING: Slight eye irritant. Overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin contact and eye contact. Material intended for veterinary pharmaceutical manufacturing use only.

SECTION 3. HEALTH HAZARDS (CONT'D)

Routes of Absorption

Inhalation, Skin and Eye Contact, Accidental Ingestion.

NOTE: Potential eye and skin irritation hazards data for laidlomycin sodium salt is based on animal studies using laidlomycin.

Eye

Animal studies indicate that laidlomycin causes slight eye irritation.

Skin

Results from a rabbit skin irritation study indicate that laidlomycin is not a skin irritant.

NOTE: The following potential health hazards data for laidlomycin sodium salt is based on animal studies using laidlomycin propionate.

Systemic

Acute: Laidlomycin propionate is considered very toxic if ingested. The oral LD_{50} (dose that kills 50% of the test animals) in rats is 63 mg/kg body weight. Signs of toxicity in these animals included labored breathing, paleness, salivation and collapse. Death was caused by gastrointestinal effects. Similar results were observed in dogs following acute oral administration of 60 mg/kg body weight.

Chronic: Three month oral toxicity studies in beagle dogs and rats resulted in salivation, lack of coordination, tremors and peripheral nerve effects in the beagles and decreased food consumption and weight gain in the rats.

A 12 month oral toxicity study in beagle dogs resulted in salivation, labored breathing, abnormal gait and decreased or absent peripheral reflexes at doses of 1.5 and 3.0 mg/kg/day.

Reproductive and Developmental Toxicity

Laidlomycin propionate was not shown to be teratogenic (or causing birth defects) when administered to rats and rabbits at doses up to and including 4 mg/kg/day. Treatment with laidlomycin propionate did not affect reproduction or fertility in a two generation rat reproductive study.

Carcinogenicity and Mutagenicity

Laidlomycin propionate is not listed by NTP, IARC or OSHA as a carcinogen and has not been tested for carcinogenicity potential in animals. No genotoxic activity was demonstrated in gene mutation, chromosomal or DNA damage studies of laidlomycin propionate.

SECTION 3. HEALTH HAZARDS (CONT'D)

Medical Conditions Aggravated by Exposure

Not known or reported.

Occupational Exposure Limit

No level has been established by OSHA, NIOSH or ACGIH.

Syntex has established an Occupational Exposure Limit (OEL) based on currently available information to assess the potential adverse health effects of laidlomycin, which is also applicable to the sodium salt. The OEL for laidlomycin is **0.05 mg/m^3** of air. The OEL is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be repeatedly exposed day after day without harmful effects.

SECTION 4. FIRST AID MEASURES

Eye Contact

Immediately flush eyes thoroughly with water for at least 15 minutes and contact medical personnel and supervisor.

Skin Contact

Immediately wash thoroughly with soap and water for 15 minutes. If an irritation develops, contact medical personnel and supervisor.

Inhalation

Immediately move to fresh air and contact medical personnel and supervisor.

Ingestion

Give moderate amount (8-12 oz.) of water and immediately contact medical personnel and supervisor.

SECTION 5. FIRE PROTECTION

Flash Point

Not applicable - laidlomycin sodium salt is a solid.

Explosability

This material is very sensitive to electrical ignition sources. The minimum explosive concentration is 0.045 oz/ft^3 (similar to corn starch). The minimum ignition energy is 0.045 joules.

SECTION 5. FIRE PROTECTION (CONT'D)

Extinguishing Media

Water, multipurpose dry chemical or halon-fire extinguisher.

Special Fire Fighting Procedures

Wear full protective clothing and NIOSH/MSHA-approved, positive pressure, self-contained breathing apparatus. Thoroughly wash all equipment after use.

SECTION 6. SPILL AND RELEASE MEASURES

If material is released or spilled, cordon off spill area. For small spills of powders, do not attempt to sweep up dry materials; use water or an appropriate solvent to dilute and clean up. Soak up material with paper towels and wash spill area thoroughly with soap and water. For larger spills, wet down spilled material with water or an appropriate solvent. Scoop into suitable containers appropriate for either recovery or disposal to avoid exposure and to comply with applicable waste disposal regulations. Alternatively, use an industrial vacuum cleaner equipped with a high efficiency particulate filter. Remove filter and dispose of it in a manner to avoid exposure and to comply with applicable waste disposal regulations. Take proper precautions to minimize exposure by using appropriate personal protective equipment.

SECTION 7. HANDLING AND STORAGE

Avoid contact with skin, eyes or clothing. Use adequate ventilation to minimize dust generation. Wash thoroughly after handling. Store in a cool, well-ventilated area.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

Eye Protection

Wear safety glasses with side shields, chemical splash goggles, and/or full face shield to prevent contact with eyes. The choice of protection should be based on the job activity and potential for exposure to the eyes and face.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION (CONT'D)

Respiratory Protection

Where applicable, this material should be handled in closed processes or containers. If it is properly handled in a glove box, laboratory hood, or with effective local exhaust ventilation, respiratory protection may not be needed. For exposures up to 10 times the OEL, a / mask air-purifying respirator with NIOSH/MSHA approval for dusts and mists is the minimum respiratory protection recommended. A full face piece air-purifying respirator with NIOSH/MSHA approval for dust and mists is recommended for exposures greater than 10 times but less than 50 times the OEL. A "hood or helmet-type" powered air purifying respirator or a supplied-air respirator may be required if exposures exceed 50 times the OEL.

Skin Protection

Rubber (latex) gloves are recommended to minimize potential for skin contact when handling the active in dry form or in aqueous solutions. When the compound is dissolved in organic solvent, wear gloves that provide protection against that solvent. Wear lab coat or other protective overgarment. The choice of skin protection should be based on the job activity and potential for exposure to the skin.

Engineering Controls

When practicable, the material should be handled in enclosed or contained processes, a properly operating laboratory hood or with other effective local exhaust ventilation.

Other

Wash hands, face and other potentially exposed areas immediately after working with this material (especially before eating, drinking, or smoking). All protective equipment should be thoroughly cleaned as well.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES

Boiling Point:	Not applicable - solid
Melting Point:	247-278°C
Molecular Weight:	720.86
Solubility:	Partially soluble in water; soluble in most organic solvents.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES (CONT'D)

Vapor Pressure:	Nil
Specific Gravity:	No data
pH (of 1% aqueous solution):	No data
Percent Volatile:	Nil
Vapor Density:	Not applicable - solid
Evaporation:	Nil
Appearance, Color, Odor:	White crystalline powder, Odor not determined

SECTION 10. STABILITY AND REACTIVITY

Stability
Stable.

Incompatibility
Strong oxidizing agents (e.g., peroxides, permanganates, nitric acid, etc.) may produce violent reactions.

Hazardous Decomposition Products
Information not available.

Hazardous Polymerization

Will not occur.

SECTION 11. TOXICOLOGICAL INFORMATION

See Section 3. Health Hazards.

SECTION 12. ENVIRONMENTAL INFORMATION

Persistence and Degradability:

Respirometry testing indicates that laidlomycin containing wastewater would not readily biodegrade in a wastewater treatment plant.

Aquatic Toxicity:

Bluegill sunfish 96 hour LC_{50} = 9.5 mg/L
Rainbow trout 96 hour LC_{50} = 2.7 mg/L
These LC_{50} values (lethal concentration to kill 50% of the test species) are considered to be toxic to fish by most criteria.

SECTION 13. WASTE DISPOSAL METHODS

All wastes containing the compound should be specially contained, properly labeled, and stored separately from other facility waste discharges. Dispose of any waste residues according to prescribed Federal, state, and local guidelines, e.g., appropriately permitted chemical waste incinerator. Rinsewaters resulting from spill cleanups should be discharged in an environmentally safe manner, e.g., appropriately permitted municipal or on-site wastewater treatment facility.

Disposal methods should be used which in addition to preventing environmental contamination, also prevent human exposure to the waste residues. For example, ensure that there will not be any chance for humans to be exposed to the undiluted/active waste residues.

SECTION 14. TRANSPORTATION INFORMATION

Hazard Class

Not regulated

UL Number

Not assigned

SECTION 15. LABELING/REGULATORY INFORMATION

Bulk containers of Laidlomycin sodium salt should have affixed the following warning label (in addition to the product identity label):

WARNING: Slight eye irritant. Overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin and eye contact. Material intended for veterinary pharmaceutical manufacturing use only. Read and understand the Material Safety Data Sheet for additional information before using this product.

****

The above information is based on data available to us and is believed to be correct. Since the information may be applied under conditions beyond our control and with which we may be unfamiliar, we do not assume any responsibility for the results of its use and all persons receiving it shall make their own determination of the effects, properties, and protections which pertain to their particular conditions.

No representation, warranty, or guarantee, express or implied (including a warranty of fitness or merchantability for a particular purpose), is made with respect to the material, the accuracy of this information, the results to be obtained from the use thereof, or the hazards connected with the use of the material. Caution should be used in the handling and use of the material because it is a biologically active compound.

Material Safety Data Sheet

SYNTEX

Date: 30 September 1992
Supersedes: 31 July 1991

SECTION 1. COMPANY AND MATERIAL IDENTIFICATION

Supplier of Data: Syntex (U.S.A.) Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

In emergency, call: Environmental Health & Safety, (415) 855-5050

MATERIAL IDENTIFICATION

Common Name: **Laidlomycin propionate potassium salt**

SECTION 2. PRODUCT COMPOSITION

Chemical Name: 9-Hydroxy-beta-(1-oxopropoxy)-2-[5-methyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-[(1-oxopropoxy)methyl]-3,5-dimethyl-2H-pyran-2-yl]-2-furyl]-2-furyl]-alpha,gamma,2,8-tetramethyl-1,6-dioxaspiro-[4,5]decane-7-butyric acid potassium salt.

Chemical Formula: $C_{40}H_{65}O_{13}K$

CAS #: 84799-02-0

Synonyms: RS-11988-047

Typical Percent Composition: ~95%

SECTION 3. HEALTH HAZARDS

WARNING STATEMENT

DANGER: Severe eye irritant. Direct contact may cause eye injury and skin irritation. Overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin contact and eye contact. Material intended for veterinary pharmaceutical manufacturing use only.

Routes of Absorption

Inhalation, Skin and Eye Contact, Accidental Ingestion.

NOTE: The following potential health hazards data for laidlomycin propionate potassium salt is based on animal studies using laidlomycin propionate.

Eye

Animal studies indicate that laidlomycin propionate is a severe eye irritant and may cause irreversible corneal damage.

Skin

Results from a rabbit dermal irritation study indicate that laidlomycin propionate may cause slight skin irritation.

Systemic

Acute: Laidlomycin propionate is considered very toxic if ingested. The oral LD_{50} (dose that kills 50% of the test animals) in rats is 63 mg/kg body weight. Signs of toxicity in these animals included labored breathing, paleness, salivation and collapse. Death was caused by gastrointestinal effects. Similar results were observed in dogs following acute oral administration of 60 mg/kg body weight.

Chronic: Three month oral toxicity studies in beagle dogs and rats resulted in salivation, lack of coordination, tremors and peripheral nerve effects in the beagles and decreased food consumption and weight gain in the rats.

A 12 month oral toxicity study in beagle dogs resulted in salivation, labored breathing, abnormal gait and decreased or absent peripheral reflexes at doses of 1.5 and 3.0 mg/kg/day.

Reproductive and Developmental Toxicity

Laidlomycin propionate was not shown to be teratogenic (or causing birth defects) when administered to rats and rabbits at doses up to and including 4 mg/kg/day. Treatment with laidlomycin propionate did not affect reproduction or fertility in a two generation rat reproductive study.

Carcinogenicity

Laidlomycin propionate is not listed by NTP, IARC or OSHA as a carcinogen and has not been tested for its carcinogenic potential in animals.

Mutagenicity

No genotoxic activity was demonstrated in gene mutation, chromosomal or DNA damage studies of laidlomycin propionate.

SECTION 3. HEALTH HAZARDS (CONT'D)

Medical Conditions Aggravated by Exposure

Not known or reported.

Occupational Exposure Limit

No level has been established by OSHA, NIOSH or ACGIH.

Syntex has established an Occupational Exposure Limit (OEL) based on currently available information to assess the potential adverse health effects of laidlomycin and laidlomycin propionate, which is applicable to the potassium salt. The OEL for laidlomycin and laidlomycin propionate is **0.05 mg/m^3** of air. The OEL is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be repeatedly exposed day after day without harmful effects.

SECTION 4. FIRST AID MEASURES

Eye Contact

Immediately flush eyes thoroughly with water for at least 15 minutes and contact medical personnel and supervisor.

Skin Contact

Immediately wash thoroughly with soap and water for 15 minutes. If an irritation develops, contact medical personnel and supervisor.

Inhalation

Immediately move to fresh air and contact medical personnel and supervisor.

Ingestion

Give moderate amount (8-12 oz.) of water and immediately contact medical personnel and supervisor.

SECTION 5. FIRE PROTECTION

Flash Point

Not applicable - laidlomycin propionate potassium salt is a solid.

Explosability

This material is very sensitive to electrical ignition sources. The minimum explosive concentration is 0.045 oz/ft^3 (similar to corn starch). The minimum ignition energy is 0.045 joules.

SECTION 5. FIRE PROTECTION (CONT'D)

Extinguishing Media

Water, multipurpose dry chemical or halon-fire extinguisher.

Special Fire Fighting Procedures

Wear full protective clothing and NIOSH/MSHA-approved, positive pressure, self-contained breathing apparatus. Thoroughly wash all equipment after use.

SECTION 6. SPILL AND RELEASE MEASURES

If material is released or spilled, cordon off spill area. For small spills of powders, do not attempt to sweep up dry materials; use water or an appropriate solvent to dilute and clean up. Soak up material with paper towels and wash spill area thoroughly with soap and water. For larger spills, wet down spilled material with water or an appropriate solvent. Scoop into suitable containers appropriate for either recovery or disposal to avoid exposure and to comply with applicable waste disposal regulations. Alternatively, use an industrial vacuum cleaner equipped with a high efficiency particulate filter. Remove filter and dispose of it in a manner to avoid exposure and to comply with applicable waste disposal regulations. Take proper precautions to minimize exposure by using appropriate personal protective equipment.

SECTION 7. HANDLING AND STORAGE

Avoid contact with skin, eyes or clothing. Use adequate ventilation to minimize dust generation. Wash thoroughly after handling. Store in a cool, well-ventilated area.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

Eye Protection

Wear safety glasses with side shields, chemical splash goggles, and/or full face shield to prevent contact with eyes. The choice of protection should be based on the job activity and potential for exposure to the eyes and face.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION (CONT'D)

Respiratory Protection

Where applicable, this material should be handled in closed processes or containers. If it is properly handled in a glove box, laboratory hood, or with effective local exhaust ventilation, respiratory protection may not be needed. For exposures up to 10 times the OEL, a / mask air-purifying respirator with NIOSH/MSHA approval for dusts and mists is the minimum respiratory protection recommended. A full face piece air-purifying respirator with NIOSH/MSHA approval for dust and mists is recommended for exposures greater than 10 times but less than 50 times the OEL. A "hood or helmet-type" powered air purifying respirator or a supplied-air respirator may be required if exposures exceed 50 times the OEL.

Skin Protection

Rubber (latex) gloves are recommended to minimize potential for skin contact when handling the active in dry form or in aqueous solutions. When the compound is dissolved in organic solvent, wear gloves that provide protection against that solvent. Wear lab coat or other protective overgarment. The choice of skin protection should be based on the job activity and potential for exposure to the skin.

Engineering Controls

When practicable, the material should be handled in enclosed or contained processes, a properly operating laboratory hood or with other effective local exhaust ventilation.

Other

Wash hands, face and other potentially exposed areas immediately after working with this material (especially before eating, drinking, or smoking). All protective equipment should be thoroughly cleaned as well.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES

Boiling Point:	Not applicable - solid
Melting Point:	200°C
Molecular Weight:	793.04
Solubility:	0.5 mg/ml water; very soluble in tetrahydrofuran, methanol, methylene chloride
Vapor Pressure:	Nil
Specific Gravity:	Not available

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES (CONT'D)

pH (of 1% aqueous solution):	Not available
Percent Volatile:	Nil
Vapor Density:	Not applicable - solid
Evaporation:	Nil
Appearance, Color, Odor:	Straw-colored powder; odor not determined

SECTION 10. STABILITY AND REACTIVITY

Stability
Stable.

Incompatibility
Strong oxidizing agents (e.g., peroxides, permanganates, nitric acid, etc.) may produce violent reactions.

Hazardous Decomposition Products
Information not available

Hazardous Polymerization
Will not occur.

SECTION 11. TOXICOLOGICAL INFORMATION

See Section 3. Health Hazards

SECTION 12. ENVIRONMENTAL INFORMATION

Persistence and Degradability:
Respirometry testing indicates that laidlomycin containing wastewater would not readily biodegrade in a wastewater treatment plant.

Aquatic Toxicity:

Bluegill sunfish 96 hour LC_{50} = 9.5 mg/L
Rainbow trout 96 hour LC_{50} = 2.7 mg/L
These LC_{50} values (lethal concentration to kill 50% of the test species) are considered to be toxic to fish by most criteria.

SECTION 13. WASTE DISPOSAL METHODS

All wastes containing the compound should be specially contained, properly labeled, and stored separately from other facility waste discharges. Dispose of any waste residues according to prescribed Federal, state, and local guidelines, e.g., appropriately permitted chemical waste incinerator. Rinsewaters resulting from spill cleanups should be discharged in an environmentally safe manner, e.g., appropriately permitted municipal or on-site wastewater treatment facility.

Disposal methods should be used which in addition to preventing environmental contamination, also prevent human exposure to the waste residues. For example, ensure that there will not be any chance for humans to be exposed to the undiluted/active waste residues. Water may be used to dilute the waste residues.

SECTION 14. TRANSPORTATION INFORMATION

Hazard Class
Not regulated

UL Number
Not assigned

SECTION 15. LABELING/REGULATORY INFORMATION

Bulk containers of Laidlomycin propionate potassium salt or its formulations should have affixed the following warning label (in addition to the product identity label):

DANGER: Severe eye irritant. Direct contact may cause eye injury and skin irritation. Overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin and eye contact. Material intended for veterinary pharmaceutical manufacturing use only. Read and understand the Material Safety Data Sheet for additional information before using this product.

SECTION 15. LABELING/REGULATORY INFORMATION (CONT'D)

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The above information is based on data available to us and is believed to be correct. Since the information may be applied under conditions beyond our control and with which we may be unfamiliar, we do not assume any responsibility for the results of its use and all persons receiving it shall make their own determination of the effects, properties, and protections which pertain to their particular conditions.

No representation, warranty, or guarantee, express or implied (including a warranty of fitness or merchantability for a particular purpose), is made with respect to the material, the accuracy of this information, the results to be obtained from the use thereof, or the hazards connected with the use of the material. Caution should be used in the handling and use of the material because it is a biologically active compound.

A6M0588

Material Safety Data Sheet

SYNTEX

Date: September 30, 1992
Supersedes: 4 December 1991

SETION 1. COMPANY AND MATERIAL IDENTIFICATION

Supplier of Data: Syntex (U.S.A.) Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

In emergency, call: Environmental Health & Safety, (415) 855-5050

MATERIAL IDENTIFICATION

Common Name: **Laidlomycin propionate potassium premix 11%**

SECTION 2. PRODUCT COMPOSITION

Components	CAS #	Formula	Percent
Non-hazardous materials (carbohydrates and fiber)			54%
Laidlomycin propionate potassium salt	84799-02-0	$C_{40}H_{65}O_{13}K$	11%
Calcium hydroxide	1305-62-0	$Ca(OH)_2$	30%
Sodium hydroxide	1310-73-2	NaOH	5%

NOTE: This Material Safety Data Sheet (MSDS) is for use during the handling of premix during feed mill or similar operations, and for the transport of bulk packages or containers of this product. Please consult the MSDS for the active ingredient (laidlomycin propionate potassium salt) for precautions to be used when handling premix or its ingredients during premix manufacture.

SECTION 3. HEALTH HAZARDS

WARNING STATEMENT

DANGER: Direct contact be irritating and corrosive to the eye and irritating to the skin. Repeated overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin contact and eye contact. Material intended for veterinary pharmaceutical use only.

SECTION 3. HEALTH HAZARDS (CONT'D)

Routes of Absorption

Inhalation, Skin and Eye Contact, Accidental Ingestion.

Eye

Animal studies indicate that laidlomycin propionate potassium premix is a severe eye irritant and corrosive agent and may cause irreversible corneal damage.

Skin

Laidlomycin propionate potassium premix contains highly corrosive agents that may cause skin irritation and may be irritating or corrosive to abraded skin.

Systemic

NOTE: Some of the data described in the remaining parts of Section 3 are based on toxicity studies on laidlomycin propionate. These studies are applicable to this veterinary product formulation.

Acute: Because of the caustic (corrosive) nature of sodium hydroxide and calcium hydroxide, laidlomycin propionate potassium premix may cause severe esophageal and gastrointestinal injury if accidentally ingested.

Laidlomycin propionate is considered very toxic if accidentally ingested. The oral LD_{50} (dose that kills 50% of the test animals) in rats is 63 mg/kg body weight. Signs of toxicity in these animals included labored breathing, paleness, salivation and collapse. Death was caused by gastrointestinal effects.

Chronic: Three month oral toxicity studies of laidlomycin propionate in beagle dogs and rats resulted in salivation, lack of coordination, tremors and peripheral nerve effects in the beagles and decreased food consumption and weight gain in the rats.

A 12 month oral toxicity study of laidlomycin propionate in beagle dogs resulted in salivation, labored breathing, abnormal gait and decreased or absent peripheral reflexes at doses of 1.5 and 3.0 mg/kg/day.

Reproductive and Developmental Toxicity

Treatment with laidlomycin propionate did not affect reproduction or fertility in a two generation rat reproductive study. Laidlomycin propionate was not shown to be teratogenic when administered to rats and rabbits at doses up to and including 4 mg/kg/day, i.e., it did not cause birth defects in laboratory animals. Sodium hydroxide and calcium hydroxide have not shown reproductive or development toxicity.

Carcinogenicity & Mutagenicity

None of the components of laidlomycin propionate potassium premix are listed by NTP, IARC or OSHA as carcinogenic. The components of laidlomycin propionate potassium salt premix are not considered to be mutagenic.

SECTION 3. HEALTH HAZARDS (CONT'D)

Medical Conditions Aggravated by Exposure

Not known or expected.

Occupational Exposure Limit

No level has been established by OSHA, NIOSH or ACGIH for laidlomycin propionate potassium premix. Syntex has established an Occupational Exposure Limit (OEL) based on currently available information to assess the potential adverse health effects of laidlomycin propionate (which is applicable to exposure to the potassium salt, the active ingredient in this mixture). The OEL for laidlomycin propionate is **0.05 mg/m^3** of air as a time-weighted average for a normal 8-hour workday and a 40-hour work week.

OSHA, NOISH, and ACGIH have established Permissible Exposure Limits (PELs), Recommended Exposure Limits (RELs) and Threshold Limit Values (TLVs), respectively, for sodium hydroxide and calcium hydroxide. The ceiling exposure limit (the maximum exposure of sodium hydroxide for a period of fifteen minutes) is **2 mg/m^3** of air.

The PEL, REL, and TLV for calcium hydroxide is **5 mg/m^3** of air. The PEL, REL, and TLV are defined as the time-weighted average concentration for a normal 8-hour workday and a 40-hour work week.

SECTION 4. FIRST AID MEASURES

Eye Contact

Immediately flush eyes thoroughly with water for at least 15 minutes and contact medical personnel and supervisor.

Note to Physician: *If material contacts eyes, consultation with an ophthalmologist is recommended.*

Skin Contact

Immediately wash thoroughly with soap and water for 15 minutes and contact medical personnel and supervisor.

Inhalation

Immediately move to fresh air and contact medical personnel and supervisor.

Ingestion

Give moderate amount (8-12 oz.) of water and immediately contact medical personnel and supervisor.

SECTION 5. FIRE PROTECTION

Flash Point

Not applicable - laidlomycin propionate potassium salt is a solid.

Explosability

As a finely divided solid, laidlomycin propionate potassium premix should be handled as a potentially combustible powder.

Extinguishing Media

Water, multipurpose dry chemical or halon-fire extinguisher.

Special Fire Fighting Procedures

Wear full protective clothing and NIOSH/MSHA-approved, positive pressure, self-contained breathing apparatus. Thoroughly wash all equipment after use.

SECTION 6. SPILL AND RELEASE MEASURES

If material is released or spilled, cordon off spill area. For small spills of powders, do not attempt to sweep up dry materials; use water or an appropriate solvent to dilute and clean up. Soak up material with paper towels and wash spill area thoroughly with soap and water. For larger spills, wet down spilled material with water or an appropriate solvent. Scoop into suitable containers appropriate for either recovery or disposal to avoid exposure and to comply with applicable waste disposal regulations. Alternatively, use an industrial vacuum cleaner equipped with a high efficiency particulate filter. Remove filter and dispose of it in a manner to avoid exposure and to comply with applicable waste disposal regulations. Take proper precautions to minimize exposure by using appropriate personal protective equipment.

SECTION 7. HANDLING AND STORAGE

Avoid contact with skin, eyes or clothing. Use adequate ventilation to minimize dust generation. Wash thoroughly after handling. Store in a cool, well-ventilated area.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

Eye Protection

It is recommended that safety glasses with side shields or chemical splash goggles be worn to prevent contact with eyes. Base the choice of protection on the job activity and potential for exposure to the eyes and face.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION (CONT'D)

Respiratory Protection

Air monitoring studies have shown that if general feed mill dust is adequately controlled, pre-mix dust levels are well within the Syntex OEL of 50 mcg/m^3 (laidlomycin) and that additional respiratory protection should not be required. While the potential for exposure to laidlomycin exposure in feed mill operations appears to be low, this product should be handled in well ventilated processes where feasible to minimize potential for exposure..

Skin Protection

Rubber (latex) gloves are recommended to minimize potential for skin contact when handling the active in dry form or in aqueous solutions. The choice of skin protection should be based on the job activity and potential for exposure to the skin.

Other

Wash hands, face and other potentially exposed areas immediately after working with this material (especially before eating, drinking, or smoking). All protective equipment should be thoroughly cleaned as well.

SECTION 9. PHYSICAL/CHEMICAL PROPERTIES

Boiling Point:	Not applicable - solid
Melting Point:	Degrades at ~160°C
Solubility:	Partially water soluble
Vapor Pressure:	Nil
Specific Gravity:	0.4 - 0.6
pH (of 1% aqueous solution):	~pH 11
Percent Volatile:	Nil
Vapor Density:	Nil
Evaporation:	Not applicable - solid
Appearance, Color, Odor:	A brown free-flowing granular powder with a characteristic calcic (alkaline or limepit) odor.

SECTION 10. STABILITY AND REACTIVITY

Stability
Stable

Incompatibility
Strong oxidizing agents (e.g., peroxides, permanganates, nitric acid, etc.) maleic anhydride, nitroethane, nitromethane, nitroparaffins, and nitropropane, may produce violent reactions

Hazardous Decomposition Products
Information not available

Hazardous Polymerization
Will not occur

SECTION 11. TOXICOLOGICAL INFORMATION

– See Section 3. Health Hazards

SECTION 12. ENVIRONMENTAL INFORMATION

Persistence and Degradability:
Respirometry testing indicates that laidlomycin containing wastewater would not readily biodegrade in a wastewater treatment plant.

Aquatic Toxicity:
Bluegill sunfish 96 hour LC_{50} = 9.5 mg/L
Rainbow trout 96 hour LC_{50} = 2.7 mg/L
These LC_{50} values (lethal concentration to kill 50% of the test species) are considered to be toxic to fish by most criteria.

SECTION 13. WASTE DISPOSAL METHODS

All wastes containing the compound should be specially contained, properly labeled, and stored separately from other facility waste discharges. Dispose of any waste residues according to prescribed Federal, state, and local guidelines, e.g., appropriately permitted chemical landfill or appropriately permitted chemical waste incinerator. Rinsewaters resulting from spill cleanups should be discharged to appropriately permitted municipal or on-site wastewater treatment facility.

Disposal methods should be used which, in addition to preventing environmental contamination, also prevent human exposure to the waste residues. For example, if liquid wastes are sewered, ensure that there will not be any chance for humans to be exposed to the undiluted/active waste residues. Water may be used to dilute the waste residues.

SECTION 14. TRANSPORTATION INFORMATION

Hazard Class
Not regulated

UN Number
Not assigned

SECTION 15. LABELING/REGULATORY INFORMATION

Bulk containers of laidlomycin propionate potassium premix or its formulations should have affixed the following warning label (in addition to the product identity label):

DANGER: Direct contact may be irritating and corrosive to the eye and irritating to the skin. Repeated overexposure may cause nervous system effects (tremors or affecting reflexes). Avoid ingestion, inhalation, skin and eye contact. Material intended for veterinary pharmaceutical manufacturing use only.

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The above information is based on data available to us and is believed to be correct. Since the information may be applied under conditions beyond our control and with which we may be unfamiliar, we do not assume any responsibility for the results of its use and all persons receiving it shall make their own determination of the effects, properties, and protections which pertain to their particular conditions.

No representation, warranty, or guarantee, express or implied (including a warranty of fitness or merchantability for a particular purpose), is made with respect to the material, the accuracy of this information, the results to be obtained from the use thereof, or the hazards connected with the use of the material. Caution should be used in the handling and use of the material because it is a biologically active compound.

Appendix M: Report Summary

Title: Acute Toxicity of Laidlomycin Sodium to *Daphnia magna*.

Investigator: Blasberg, J.W., Hicks, S.L., Friesen, K.C., Syntex Report #RS-11988 ATv 5757

Preliminary tests with laidlomycin sodium indicated an immobility effect at the 100 mg/L nominal test level and no adverse affects at or below the 10 mg/L test level. These nominal values corresponded to the measured values of 55 mg/L and 8.3 mg/L, respectively.

Nominal laidlomycin sodium concentrations for the definitive study were 55, 31, 17, 9.5 and 5.3 mg/L and a dilution water control. In the basic test design, five *Daphnia magna* were added to each of four replicate test beakers per test concentration and to the dilution water control. Water from the five exposure levels and the dilution water control was analyzed at 0 and 48 hours for laidlomycin sodium. The mean measured values were 33, 20, 10, 5.5 and 3.0 mg/L. Water quality parameters of temperature, dissolved oxygen, pH, hardness and alkalinity were measured at 0, 24, 48 hours and reported to be within acceptable limits.

The results of the 48-hour static acute *Daphnia magna* toxicity study are summarized. Based on the absence of immobility and other abnormal affects after 48-hours, the no-observed effect level was reported to be 5.5 mg/L and the dose-response slope was reported to be 4.2.

EC_{50} (mg/L)[a]	
24-hour	48-hour
>33	21
(na)	(17-26)

a 95% confidence interval in parentheses

Appendix N: Report Summary

Title: Acute Toxicity of Laidlomycin Sodium to *Hyalella azteca.*

Investigator: England, D., ABC Laboratories, Inc., Syntex Report #RS-37728 CH 0271

A range finding test at nominal concentrations of 10, 100, and 1000 mg/L indicated no mortality at 10 or 100 mg/L in 96 hours. A definitive static acute toxicity bioassay was conducted at nominal laidlomycin sodium concentrations ranging from 17 to 270 mg/L with a loading factor of one Hyalella per 100 mL of solution. Actual measured concentrations at test termination were 15, 25, 36, 52, and 54 mg/L, owing to a lower than expected solubility in the dilution water used in this study. Abnormal effects, including mortality, lethargy, and/or test organisms tending to the bottom of the chamber were observed in the 36, 52, and 54 mg/L test levels at test termination. However, there was insufficient mortality to calculate an LC_{50}, or a dose-response curve over the concentration range employed. The lowest-observed effect level, based on measured concentrations at test termination, was 36 mg/L, and the no-observed effect level was 25 mg/L.

Appendix O: Report Summary

Title: (Laidlomycin Sodium) - Acute Toxicity to Bluegill sunfish (*Lepomis macrochirus*) Under Flow-through Conditions.

Investigator: Machado, M., ABC Laboratories, Inc., Syntex Report #RS-11988 ATv 5759

A preliminary flow-through study was conducted in which bluegill sunfish were exposed to nominal concentrations of laidlomycin sodium ranging from 50 - 3.9 mg/L. At test termination, 96 hours, 100% and 50% mortality was reported among fish exposed to 50 and 30 mg/L, respectively. Sub-lethal effects were observed among two of the surviving fish exposed to the 30 mg/L treatment level. No mortality or sublethal effects were reported among fish exposed to the remaining concentrations, (18, 11, 6.5, and 3.9 mg/L). Based on these data, nominal concentrations of 50, 30, 18, 11, 6.5 and 3.9 mg/L were selected for the definitive study.

In the definitive study, pH and dissolved oxygen concentration of the exposure solutions remained within acceptable ranges for the survival of bluegill sunfish. Daily temperature range of 21-22^{O}C was maintained throughout the exposure period. Twenty organisms (ten per replicate) were exposed in duplicate test aquaria to mean measured test concentrations of 19, 9.5, 5.9, 3.5, 2.1, and 1.2 mg/L and a dilution water control. Biological observations were made and recorded at test initiation and every 24 hours thereafter until the test was terminated. Following 96 hour of exposure, 100 and 50% mortality were reported in the two highest concentrations, 19 and 9.5 mg/L, respectively. Mortality ranging from 0 - 5% was reported among fish exposed to the remaining concentrations tested. Sub-lethal effects were reported among surviving fish in the 9.5 mg/L treatment level. The concentration of laidlomycin was determined in the treatment and control aquaria by a spectrophotometric procedure to judge whether sufficient quantities of test material were being delivered and maintained. No mortalities were reported among the control groups. LC_{50} values and the corresponding confidence intervals are reported in the following table. The no-observed effect concentration was reported to be 5.9 mg/L.

LC_{50}, (mg A.I./L)[a,b]			
24-hour[c]	48-hour[d]	72-hour[d]	96-hour[d]
11	10	9.9	9.5
(9.3-13)	(5.9-19)	(5.9-19)	(5.9-19)

a Based on mean measured concentrations
b Corresponding 95% confidence interval in parentheses
c LC_{50} value calculated by probit analysis
d LC_{50} value estimated by non-linear interpolation; 95% confidence interval calculated by binomial probability

Appendix P: Report Summary

Title: (Laidlomycin Sodium) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-through Conditions.

Investigator: Machado, M., Springborn Laboratories, Inc., Syntex Report #RS-11988 ATv 5758

A preliminary flow-through study was conducted at nominal concentration of laidlomycin sodium ranging from 20 - 1.6 mg/L. At test termination (96 hours) 100% mortality was reported among fish exposed to the two highest treatment levels, 20 and 12 mg/L. No mortality was reported among fish exposed to the remaining concentrations tested (7.2, 4.3, 2.6, and 1.6 mg/L), although sublethal effects were reported among all surviving fish exposed to 7.2 mg/L treatment level. Based on these data, nominal concentrations of 20, 12, 4.3, 2.6 and 1.6 mg/L were selected for the definitive study.

In the definitive test, the pH and dissolved oxygen concentration of the exposure solutions remained within acceptable ranges for the survival of rainbow trout. Twenty organisms, (ten per replicate) were exposed in duplicate test aquaria to each of six concentrations of laidlomycin sodium and a dilution water control. During the test, the mean measured test concentrations were reported to be 11, 6.7, 3.8, 2.2, 1.2 and 0.95 mg/L. Biological observations were made and recorded at test initiation and every 24 hours thereafter until test termination. Following 96 hours exposure, 100, 100, and 75% mortality was reported in the three highest concentrations tested, 11, 6.7 and 3.8 mg/L, respectively. Mortality ranging from 5-25% was reported among fish exposed to the remaining concentrations tested, 2.2-0.95 mg/L. Sub-lethal effects were reported among surviving fish in the 2.2 and 1.2 mg/L treatment levels. The concentration of laidlomycin sodium was determined in the treatment and control aquaria by spectrophotometric procedure to judge whether sufficient quantities of test material were being delivered and maintained. Based on these data, it was reported that the effects observed during this study were concentration dependent. No mortalities were reported among the control groups. LC_{50} values and the corresponding confidence intervals are reported in the following table. The no-observed effect concentration was reported to be 1.2 mg/L.

LC_{50}, (mg A.I./L)[a,b]			
24-hour[c]	48-hour[c]	72-hour[c]	96-hour[c]
5.7	3.9	3.1	2.7
(4.9-6.7)	(3.3-4.5)	(2.7-3.6)	(2.3-3.2)

a Based on mean measured concentrations
b Corresponding 95% confidence interval is in parentheses
c LC_{50} calculated by probit analysis

Appendix Q: Report Summary

Title: RS-78585-037: Acute Toxicity to the Water Flea, *Daphnia magna*, Under Static Test Conditions

Investigator: Cindy McElwee, Toxicon Environmental Sciences , Syntex Report #RS-78585 CH 0272

The toxicity of C-3 despropionyl laidlomycin sodium (RS-78585-037) to *Daphnia magna* was determined in a well controlled study at nominal test concentrations ranging from 39 to 500 mg active ingredient (ai) per L. Actual test concentrations ranges from 95 to 105% of nominals. Test temperature during the 48 hour exposure ranged from 19.9 - 20.5^{O}C. Freshwater with a hardness, alkalinity, and conductivity ranged from 72-80, 26-54 mg/L as $CaCO_3$, and 513-623 µmhos was used for dilution water. Dimethylformamide, up to a maximum of 0.5 mL per liter of dilution water, was used as solvent for C-3 despropionyl laidlomycin sodium. However, undissolved test chemical was present in solutions prepared at 300 and 500 mg ai/L.

The 48-hour EC_{50} was 239 mg/L (based on measured concentrations), with a 95 percent confidence range of 202-283 mg/L. The no-observed effect concentration was 109 mg/L based on the lack of mortality and sublethal effects at this test concentration.

Appendix R: Report Summary

Title: Procedure for Determining the Effects of Laidlomycin Sodium on Seed Germination and Root Elongation

Investigator: E. Feutz, D. Schwab, ABC Laboratories, Inc., Syntex Report #RS-11988 CH 0270

A seed germination and root elongation study was conducted to determine the toxicity of laidlomycin sodium to six species of terrestrial plants: Cabbage (*Brassica oleracea*), corn (*Zea mays*), cucumber (*Cucumis sativus*), pinto bean (*Phaseolus vulgaris*), perennial ryegrass (*Lolium perenne*), and wheat (*Triticum aestivum*). The preliminary test concentrations of laidlomycin sodium were l, 10, 100, and 300 ppm, as well as deionized water control. A vehicle blank treatment (5% methanol) was also used.

The definitive concentrations were then used to determine the effect/no-effect range. The experimental procedure used was identical to that used in the preliminary test. The definitive laidlomycin sodium concentrations for cabbage, pinto bean, and ryegrass were 1.5, 2.7, 4.9, and 8.8 ppm, and for cucumber and wheat, 15, 27, 49, and 88 ppm. The definitive test concentrations for corn were 8.0, 14, 26, 47, and 84 ppm.

The results of the germination tests indicated that exposure to laidlomycin sodium up to 300 ppm did not significantly affect the germination of corn and cucumber. The no-observed effect concentration (NOEC) for corn and cucumber was 300 ppm. The NOEC for the germination of cabbage and pinto bean was 10 ppm. Perennial ryegrass and wheat had NOEC levels of 4.9 and 27 ppm, respectively.

Analysis of the root length data indicated that laidlomycin sodium inhibited growth in all species tested. The level of inhibition was species specific and varied from the preliminaruy and definitive tests. The NOEC for radicle lengths were determined from the results from the preliminary and definitive tests. The NOEC for cabbage, corn, cucumber, pinto bean, perennial ryegrass and wheat radicle lengths were 1.0, 14, 10, 1.5, 2.7, and 10 ppm, respectively. The following summary table gives the results of the preliminary and definitive seed germination and root elongation tests with laidlomycin sodium.

Summary Table

Results From the Seed Germination and Root Elongation Tests

with Laidlomycin Sodium

	No Observed Effect Concentration, ppm	
Species	Germination	Root Elongation
Cabbage	10[a]	1.0[a]
Corn	300[a]	14
Cucumber	300[a]	10[a]
Pinto Bean	10[a]	1.5
Perennial Ryegrass	4.9	2.7
Wheat	27	10[a]

(a) Based on results from preliminary test

Appendix S: Report Summary

Title: Laidlomycin Sodium-Determination of Effects on Seedling Growth of Six Plant Species

Investigator: J. Hoberg, Springborn Laboratories, Inc., Syntex Report #RS-11988 CH 0269

Preliminary range finding tests at 100, 10, 1.0 and 0.1 mg/L laidlomycin sodium per liter were conducted with corn, cucumber, perennial ryegrass, soybean, wheat, and tomato as test species. Based on the results, definitive tests were conducted at the following nominal concentrations of test material:

Corn	4.0,	2.0,	1.0,	0.50,	0.25,	0.13 mg/L	
Cucumber		2.0,	1.0,	0.50,	0.25,	0.13 mg/L	
Ryegrass			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Soybean			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Wheat			1.0,	0.50,	0.25,	0.13,	0.063 mg/L
Tomato				0.50,	0.25,	0.13,	0.063, 0.031 mg/L

Chemical analyses were performed to establish the concentration of laidlomycin sodium in the nutrient solutions. From the statistical effects observed, a lowest-observed effect concentration and the no-observed effect concentration were determined for each species as shown in the following Table.

Plants were grown under regulated environmental conditions of temperature, light intensity, relative humidity, and photoperiod. Carbon dioxide concentrations, measured daily, were maintained by a continuous input of air drawn from the outside laboratory. Nutrient solution containing essential minerals in water and dosed with laidlomycin sodium was provided daily by sub-irrigation. Shoot lengths were recorded on test days 1, 3, 5, 7, 14, and 21. At the conclusion of the test, measurements of dry shoot and root weights were recorded.

Summary Table

Species	No-observed Effect Level, mg/L			Mean Control Weights, g	
	Shoot Length	Shoot Wt.	Root Wt.	Shoot	Root
Corn	0.96	0.12	0.12	0.462	0.161
Cucumber	0.70	0.70	0.70	0.628	0.179
Ryegrass*	0.21	0.21	0.11	0.062	0.024
Soybean	0.21	0.056	0.70	1.069	0.145
Wheat	0.36	0.36	0.70	0.0951	0.0520
Tomato	0.36	0.36	0.022	0.554	0.101

* *Lolium perenne*

Appendix T: Report Summary

Title: Laidlomycin Sodium - Determination of Effects on Seedling Growth of Seven Plant Species in a Natural Field Soil

Investigator: J. Hoberg, Springborn Laboratories, Inc., Syntex Report #RS-37728 CH 0276

A preliminary study was conducted in which cucumbers were grown in sand and bottom watered with nutrient solution containing a nominal concentration of 1.0 mg/L. Based on the initial amount of laidlomycin sodium added to sand, the nominal concentration in sand was 0.17 mg/kg. Analysis of the sand after 21 days of exposure indicated that the concentration of test compound in top one-half of the pot sand increased substantially during the test. The initial mean measured concentration in pots sacrificed at the start of the study was 0.10 mg/kg (range of 0.91 to 0.12 mg/kg), but at the end of 21 days, the concentration in the top one-half of the pots had increased to 1.26 mg/kg (range of 1.20-1.35 mg/kg). The increase in concentration is expected from the rate at which nutrient solution (containing test compound) is drawn up into the pot as a result of evaporation and plant transpiration. A second preliminary study in which perennial ryegrass seedlings were grown in a CA sandy loam soil treated with nutrient solutions containing from 1 to 100 mg/L of laidlomycin sodium indicated that the no-observed effect levels were from 20 to 450 times the levels observed when seedlings were grown in sand.

The phytotoxicity of laidlomycin sodium incorporated into blended California sandy loam soil (pH 7.8-8.2, CEC 5.3 - 12.4 meq/g, and 1.4% OM) was evaluated in a well controlled study in using corn, cucumber, annual ryegrass, perennial ryegrass, soybeans, tomato, and wheat seedlings. Laidlomycin sodium was dissolved in acetone solvent, and added to the soil to achieve concentrations of 0.2 to 50 mg/kg. After planting, pots were maintained at field moisture capacity by sub-irrigation with nutrient solution.

A solvent control, soil treated with acetone used to apply laidlomycin sodium, untreated soil, and an untreated sand control was used for each plant species. Sub-irrigation with nutrient solution was used to maintain pots at field moisture capacity.

Chemical analyses were performed to establish the concentration of laidlomycin sodium in each of the stock solutions used to treat the soil samples. From the statistical effects observed, lowest-observed effect concentrations (LOEC) were determined for the shoot length, shoot weight, and root weight for each species as shown in Summary Table I. Comparison of the shoot and root weights for control plants grown in soil and in sand indicate that corn, tomato and annual ryegrass appear to be stressed when grown in sand.

The no–observed effect concentrations (NOEC) were also determined for the shoot length, shoot weight, and root weight of each plant species. Based on the LOEC and NOEC values for the most sensitive parameter(s), an overall LOEC and NOEC value for each plant species was obtained as shown in Summary Table II.

Summary Table I: LOEC Values for Plant Species Grown in a Natural Field Soil

Species	Lowest-observed Nominal Effect Level, mg/kg†			Mean Control Weights, g			
	Shoot Length	Shoot Wt.	Root Wt.	Shoot		Root	
				Sand	Soil*	Sand	Soil*
Corn	13	3.3	13	0.353	0.613	0.139	0.133
Cucumber	6.5	6.5	6.5	0.981	0.933	0.166	0.170
Ryegrass*	6.5	3.3	6.5	0.062[a]	0.090[b]	0.024[a]	0.021[b]
Wheat	6.5	6.5	25	0.091	0.108	0.041	0.035
Tomato	13	13	13	0.244	0.297	0.063	0.035
Ryegrass***	3.3	3.3	3.3	0.035	0.074	0.021	0.018
Soybean	6.5	3.3	3.3	0.539	0.604	0.078	0.090

† Based on nominal starting concentrations.
* Solvent control soil
** *Lolium perenne*
*** *Lolium multiflorum*
(a) Previous seedling growth study, SLI #11734.0291.6105.620
(b) Preliminary study for growth in CA soil, report dated 7 Oct 91, using same seeds and growing conditions as Study SLI #1174.0291.6105.620

Summary Table II. LOEC and NOEC Values for Plant Species Grown in a Natural Field Soil

Species	Concentration Laidlomycin Sodium, mg/kg			
	LOEC[a]		NOEC	
	Nominal[b]	Measured[c]	Nominal[b]	Measured[c]
Corn	3.3[d]	2.57	1.6[h]	1.25
Cucumber	6.5[e]	5.07	< 6.5	< 5.07
Annual Ryegrass*	3.3[e]	2.57	1.6	1.25
Perrenial Ryegrass**	3.3[f]	2.57	1.6	1.25
Soybean	3.3[g]	2.57	1.6	1.25
Tomato	13[d]	10.14	6.5	5.07
Wheat	6.5[d]	5.07	3.3	2.57

a LOEC and NOEC values were based on the most sensitive parameter(s).
b Based on nominal concentration of laidlomycin sodium.
c Based on an average 78% recovery of laidlomycin sodium in all test levels. See Section 8.D.(2).
d Based on statistically significant effects on shoot length and shoot weight.
e Based on statistically significant effects on shoot length, shoot weight, and root weight.
f Based on statistically significant effects on shoot weight.
g Based on statistically significant effects on shoot weight and root weight.
h The two–tailed Dunnett's Test, as required by the study protocol, indicates that the 25, 13, and 3.3 mg A.I./kg treatment levels are significantly different from the solvent control. Consequently, a conservative NOEC of 1.6 mg A.I./kg has been selected.
* *Lolium multiflorum*
** *Lolium perenne*

Appendix U: Summary of Environmental Fate and Effect Tests for Laidlomycin, Laidlomycin Salts, Laidlomycin Propionate Potassium, and C-3 Despropionyl Laidlomycin Sodium

Laidlomycin Free acid, Laidlomycin Sodium, and Laidlomycin Potassium	
Property or Test	**Experimental Value**
Molecular Weight, Sodium salt	720.86
Molecular Formula, Sodium salt	$C_{37}H_{61}O_{12}Na$
Dissociation Constant, Free Acid	5.5
Aqueous Solubility, Sodium salt	145 mg/L
Aqueous Solubility, Potassium salt	314 mg/L
Aqueous Solubility, Free acid	0.30 mg/L
K_{sp}, Sodium salt	1.17 x 10-7 $(mol/L)^2$
K_{sp} , Potassium Salt	5.21 x 10-7 $(mol/L)^2$
K_{ow} , Sodium salt	1.88 x 10^4 (pH 9)
Vapor Pressure, Sodium salt	3.20 x 10^{-7} torr (270°C); 3 x 10^{-12} torr (20°C, est.)
K_{oc} , Sodium salt	8.0x10^2, CA sandy loam; 1.3x10^4, MS clay loam; 3.6x10^2, MO silt loam; 1.4x10^3, TX silt loam
Hydrolysis (free acid, Na, and K salts)	No decomposition in pH range 5–9 (41 days)
Mineralization in Soils (65 day period), Sodium salt	0.1% mineralized, MS silty clay; 4.9%, TX silty clay loam; 1.0%, CA sandy loam
Biodegradation of Laidlomycin Sodium in Cattle Feedlot Waste	47.8%–66.3% biotransformed† (56 days); 93%–94% biotransformed† (98 days)
Microbial Growth Inhibition of Laidlomycin Sodium Expressed as Minimum Inhibitory Concentration (MIC)	>1000 mg/L, *Aspergillus niger*†; >1000 mg/L, *Trichoderma viride*†; 0.4 mg/L, *Clostridium perfringens* † (Gram positive); 0.4 mg/L, *Bacillus subtilis*† (Gram positive); 40 mg/L, *Nostoc*†
Acute (48 hour) Toxicity of Laidlomycin Sodium to *Daphnia magna*	EC_{50}: 21 mg/L* NOEL: 5.5 mg/L*

Laidlomycin Free acid, Laidlomycin Sodium, and Laidlomycin Potassium, Cont.	
Property or Test	**Experimental Value**
Acute (96 hour) Toxicity of Laidlomycin Sodium to *Hyalella azteca*	LC_{50}: Could not be calculated NOEL: 25 mg/L*
Acute (96 hour) Toxicity of Laidlomycin Sodium to Bluegill Sunfish	LC_{50}: 9.5 mg/L* NOEL: 5.9 mg/L*
Acute (96 hour) Toxicity of Laidlomycin Sodium to Rainbow Trout	LC_{50}: 2.7 mg/L* NOEL: 1.2 mg/L*
Laidlomycin Sodium NOEC Levels for Seed Germination	10 mg/L, cabbage; 300 mg/L, corn; 300 mg/L, cucumber; 10 mg/L, pinto bean; 4.9 mg/L, perennial ryegrass; 27 mg/L, wheat
Laidlomycin Sodium NOEC Levels for Root Elongation	1.0 mg/L, cabbage; 14 mg/L, corn; 10 mg/L, cucumber; 1.5 mg/L, pinto bean; 2.7 mg/L, perennial ryegrass; 10 mg/L, wheat
Laidlomycin Sodium NOEC Levels for Seedling Growth in Sand	0.12 mg/L, corn*; 0.70 mg/L, cucumber*; 0.11 mg/L, perennial ryegrass*; 0.056 mg/L, soybean*; 0.022 mg/L, tomato*; 0.36 mg/L, wheat*
Laidlomycin Sodium NOEC Levels for Seedling Growth in a Natural Field Soil	1.6 mg/kg, corn†; <6.5 mg/kg, cucumber†; 1.6 mg/kg, perennial ryegrass†; 1.6 mg/kg, annual ryegrass†; 1.6 mg/kg, soybean†; 6.5 mg/kg, tomato†; 3.3 mg/kg, wheat†

* Values based on measured concentrations.
† Values based on nominal concentrations.

Laidlomycin Propionate Potassium	
Property or Test	**Experimental Value**
Molecular Weight	793.04
Molecular Formula	$C_{40}H_{65}O_{13}K$
Biodegradation of Laidlomycin Propionate in Cattle Feedlot Waste	$T_{1/2}$ for biodegradation: 18 days; 92.3%-99.3% biotransformed (84 days)†
Biodegradation of Laidlomycin Propionate in Soils Amended with Cattle Feedlot Waste	0.3–1.3% mineralized (120 to 179 days); 87.5%-100% biotransformed† (120 to 179 days)

* Values based on measured concentrations.
† Values based on nominal concentrations.

C-3 Despropionyl Laidlomycin Sodium	
Property or Test	**Experimental Value**
Molecular Weight	664.80
Molecular Formula	$C_{34}H_{57}O_{11}Na$
Acute (48 hour) Toxicity to *Daphnia magna*	EC_{50}: 239 mg/L* NOEL: 109 mg/L*

* Values based on measured concentrations.
† Values based on nominal concentrations.