ENVIRONMENTAL ASSESSMENT

FOR

APRALAN® PREMIX FOR SWINE

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

July 1985

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1. DATE — July 1985

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

3. ADDRESS — Lilly Corporate Center
Indianapolis, Indiana 46285

4. DESCRIPTION OF THE PROPOSED ACTION

New Animal Drug approval has been requested for APRALAN PREMIX to be used in the feed of swine for the treatment of porcine colibacillosis. About 165 ppm (150 grams per ton of feed) of apramycin activity as the sulfate salt would be used for a maximum of 14 days in the feed of young swine. Daily intake of apramycin activity would be about 13 mg per kilogram body weight for the 14-day period.

Use of APRALAN® SOLUBLE POWDER in the drinking water of swine has already been approved by the Food and Drug Administration for the treatment of porcine colibacillosis. The active ingredient in APRALAN SOLUBLE POWDER is also apramycin sulfate. Daily intake of apramycin activity in water by swine is about 25 mg per kilogram body weight for a seven-day period. New Animal Drug approval for apramycin premix would then allow swine to ingest APRALAN PREMIX in their diet at about one-half the daily intake rate of APRALAN SOLUBLE POWDER. APRALAN PREMIX would be administered twice as long as APRALAN SOLUBLE POWDER. The total estimated intake of apramycin activity as APRALAN PREMIX or as APRALAN SOLUBLE POWDER would be about the same.

APRALAN® PREMIX (apramycin sulfate, Elanco)
APRALAN® SOLUBLE POWDER (apramycin sulfate, Elanco)

Approval of the proposed action would authorize the fermentation plants of Eli Lilly and Company at Carolina, Puerto Rico, at Indianapolis, Indiana, at Lafayette, Indiana, and at Liverpool, England, to manufacture apramycin sulfate for sale in the United States as APRALAN PREMIX. Formulation and packaging of APRALAN PREMIX may be performed in Eli Lilly and Company facilities at Omaha, Nebraska.

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

a) The environment adjacent to the manufacturing plants.

b) Swine farms where residues may be found in animal waste.

c) Agricultural lands where waste products from swine are used as fertilizer.

d) Aquatic systems where runoff may collect from sites receiving waste products from swine.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES

A. PRODUCT DESCRIPTION

APRALAN PREMIX will be incorporated into the feed of swine. Apramycin sulfate is the active ingredient of APRALAN PREMIX. APRALAN PREMIX will contain 165 g of apramycin activity per kilogram of premix. APRALAN PREMIX will also contain approved carriers such as soybean mill run or ground rice hulls.

1) Apramycin

Apramycin is produced by a fermentation process with the actinomycete Streptomyces tenebrarius. Apramycin is extracted from the fermentation medium and produced as apramycin sulfate at a purity of at least 85%. A microbiological assay is used to determine apramycin activity as equivalents of apramycin base, without the sulfuric acid salt group.

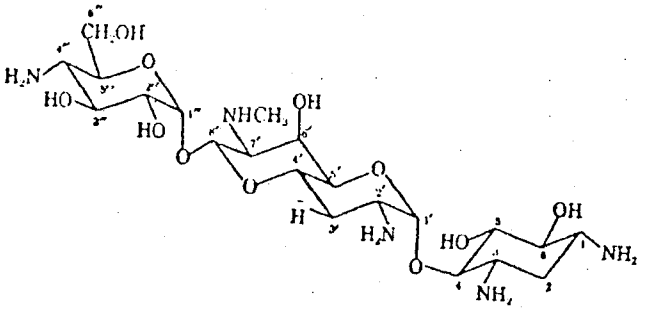
Chemical Name: D-Streptamine,4-O-[(8R)-2-amino-8-O-(4-amino-4-deoxy-α-D-glucopyranosyl)-2,3,7-trideoxy-7-(methylamino)-D-glycero-α-D allo-octodialdo-1,5:8,4-dipyranosyl-yl]-2-deoxy-sulfuric acid salt.

CAS Registry Number: 65710-07-8

Molecular Formula: $C_{21}H_{41}N_5O_{11} \cdot 5/2\ H_2SO_4$

Molecular Weight: 784.8

Structure of Apramycin Base:



Melting Point: Apramycin sulfate and amorphous apramycin decompose before melting. A sharp melting point of 245-247°C has been reported for apramycin monohydrate (1).

Solubility in Water: Greater than 300 g/L

pKa Values: Aqueous titration provided pKa values of 5.4, 6.2, 7.2, 7.8 and 8.5 (1).

n-Octanol/Water Partition Coefficient: The n-octanol/water partition coefficients for apramycin at pH 5.0, 7.0 and 9.0 ranged from 0.00022 to 0.0011 (Appendix A).

Vapor Pressure: Non-volatile solid based on molecular weight and melting point. Thermogravimetric analysis of apramycin indicated weight loss beginning at 25°C resulting in a 3.6% loss at 106°C. Another loss begins at 221°C and continues through decomposition.

Ultraviolet and Visible Absorption Spectra: Apramycin does not contain any specific ultraviolet or visible absorption maxima. End absorption is observed below 250 nm.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCES FROM THE MANUFACTURING SITE

The manufacturing process for apramycin sulfate, the active ingredient in APRALAN PREMIX, in conjunction with the corresponding pollution control practices at each of the plant sites, is designed to have minimal environmental impact. Apramycin sulfate is produced by a fermentation process and unit operations such as sorption and elution by ion exchange, concentration, drying, grinding, screening and blending. APRALAN PREMIX is manufactured using operations such as formulating and packaging.

The only releases of apramycin activity from manufacturing operations may be in spent fermentation broth, spent ion exchange regenerants, or in dilute washwaters. These apramycin-containing process wastewaters from the fermentation plants in Puerto Rico or Indiana would be treated by microbiological degradation, by land application, by concentration and pyrolysis, or by concentration, with or without drying, and landfilling. The discharge of such process wastewaters from the production site in England will be performed in accordance with the pertaining environmental control laws of the United Kingdom, the Control of Pollution Act (1974) as implemented by national and local authorities.

Any one of the Eli Lilly and Company fermentation plants, which are located at Carolina, Puerto Rico, at Indianapolis, Indiana, or at Lafayette, Indiana, may become the principal site for producing apramycin sulfate in the United States. Formulating and packaging of APRALAN PREMIX may be performed in Eli Lilly and Company facilities at Omaha,

Nebraska. The plant in Carolina, Puerto Rico, already produces apramycin sulfate for APRALAN SOLUBLE POWDER and the Omaha plant blends and packages that product. Alternatively, the Eli Lilly plant at Liverpool, England, may become the major site for producing apramycin sulfate and may produce APRALAN PREMIX as well as APRALAN SOLUBLE POWDER.

The highest estimated concentration of apramycin in surface waters would occur during an acute low-flow period for the surface water body which receives wastewaters originating from the sorption of apramycin from its fermentation broth or from the subsequent purification operations. The plant in Carolina, Puerto Rico, discharges its microbiologically treated wastewaters to the Rio Grande de Loiza about nine miles from the Atlantic Ocean. The low flow in this river is lower than flows in the rivers to which the other Lilly fermentation plants, referred above, discharge their wastewaters, whether directly or indirectly through publicly-owned treatment plants. The lowest measured flow in the Rio Grande de Loiza just upstream of the Carolina plant is 11.7 ft^3/sec. (U.S. Geological Survey). It may be assumed that wastewaters at this plant might carry as much as 50 kg of apramycin each day to its treatment facilities until September, 1986, when this plant should to be connected to the publicly-owned Carolina regional wastewater facility that is now under construction. Although apramycin does not inhibit the ability of aerated sewage sludge to treat biodegradable substances when compared to negative controls (Appendix N), there is no information to indicate the rate at which apramycin itself would biodegrade in any of the microbiological treatment plants which may receive wastewaters from the manufacture of apramycin. Since the apramycin removal efficiency of

microbiological treatment is not known, it may be assumed that, in the worst case, apramycin would not be removed by such treatment. Under such an assumption, if as much as 50 kg of apramycin were discharged each day to the Rio Grande de Loiza and mixed with the river water at its lowest measured flow, the highest estimated concentration of apramycin in the river would be about 1.75 mg/L.

Calculation:

$$\frac{50 \text{ kg apramycin/day} \times 1{,}000{,}000 \text{ mg/kg}}{11.7 \text{ ft}^3\text{/sec} \times 28.32 \text{ liters/ft}^3 \times 86{,}400 \text{ sec/day}} = 1.75 \text{ mg apramycin/liter}$$

The highest estimated concentration of apramycin during such an acute low-flow period in the Rio Grande de Loiza is substantially below concentrations which have been found to have no acute effect on aquatic organisms. A nominal apramycin concentration of 300 mg/L did not affect the behavior or survival of either bluegill (Appendix K) or rainbow trout (Appendix L). A nominal apramycin concentration of 28.4 mg/L did not affect the behavior or survival of Daphnia magna (Appendix M). These values are 171 and 16 times higher, respectively, than the highest estimated concentration of apramycin in the Rio Grande de Loiza at acute low flow.

The average estimated concentration of apramycin in a river which receives apramycin-containing wastewaters daily for a period of time from product isolation and purification operations at one of the above referenced fermentation plants can be based on average flow in that river. A conservative estimate of the average flow in the Rio Grande de Loiza, based on data for a point upstream of the Carolina plant, is 232 ft^3/sec (U.S. Geological Survey and Puerto Rico Aqueduct and Sewer Authority). This is lower than the average flows in the surface waters to which the

other Lilly fermentation plants, referred to above, discharge their wastewaters, whether directly or indirectly through publicly-owned treatment plants. Again, based on a worst case assumption, if as much as 50 kg apramycin/day were continuously discharged to the Rio Grande de Loiza and mixed with the river water at its average flow for a period of time, the average estimated concentration of apramycin in the Rio Grande de Loiza would be about 0.088 mg/L.

Calculation:

$$\frac{50 \text{ kg apramycin/day} \times 1{,}000{,}000 \text{ mg/kg}}{232 \text{ ft}^3\text{/sec} \times 28.32 \text{ liters/ft}^3 \times 86{,}400 \text{ sec/day}} = 0.088 \text{ mg apramycin/liter}$$

The average estimated concentration of apramycin, based on estimated average flow in the Rio Grande de Loiza, is substantially below concentrations which can be calculated to have no chronic effects on aquatic organisms. An application factor of 100 can be used with median effect concentrations from acute studies to extrapolate the concentrations which have no observed effects on the test organisms during chronic exposure. The calculated chronic no-observed-effect concentration (NOEC) for Daphnia magna is 1.02 mg/L. Median lethal concentrations were not calculated for the studies with bluegill and rainbow trout because no mortalities were found. When the application factor is applied to the highest nominal concentration tested in these acute studies with fish, the calculated chronic NOEC values for these fish have to be at least 3.0 mg/L. These calculated NOEC values are 11.6 and 34.1 times higher than the average estimated concentration of apramycin in the Rio Grande de Loiza at its conservatively estimated average flow. After connecting the Carolina fermentation products plant in Puerto Rico to the publicly-owned Carolina regional wastewater facility, the discharge of wastewaters will receive infinite dilution in the Atlantic Ocean.

The manufacturing site at Indianapolis, Indiana may discharge wastewaters from the manufacture of apramycin into a river with a higher flow than that of the Rio Grande de Loiza. The Lilly fermentation plant in Indianapolis discharges its process wastewaters to the publicly-owned Indianapolis Belmont Street sewage treatment facility, which in turn discharges into the White River. The three-day-average low flow for the White River that would occur once in ten years is 46 ft^3/sec. (U.S. Geological Survey). Increased manufacturing requirements at the time any manufacturing of apramycin is done in Indianapolis may result in as much as 91 kg of apramycin being discharged each day to that publicly-owned treatment works and to the river, again assuming as a worst case that apramycin would not be removed by such treatment. In this worst case, after the discharge from the treatment works mixes with the river at low flow, the highest estimated concentration of apramycin in the White River would be about 0.808 mg/L. The average flow for the White River from 50 years of data is 1,388 ft^3/sec (U.S. Geological Survey). If as much as 91 kg of apramycin were continuously discharged into the river each day, the average estimated concentration of apramycin in the White River would be 0.0268 mg/L. Apramycin concentrations which resulted in no mortalities or behavioral abnormalities in acute studies with Daphnia magna (28.4 mg/L) and rainbow trout and bluegill (300 mg/L) are 31.1 and 371 times higher, respectively, than the highest estimated concentration of apramycin in the White River at acute low flow. The previously calculated NOEC values for these organisms, 1.02 mg/L for Daphnia magna and 3.0 mg/L for rainbow trout and bluegills, are 38.1 and 112 times higher, respectively, than the average estimated concentration of apramycin in the White River.

The manufacturing facility at Lafayette, Indiana discharges its microbiologically-treated wastewaters into the Wabash River. The three-day-average low flow of the Wabash River that would occur once in ten years near the manufacturing facility discharge site is 558 ft^3/sec (U.S. Geological Survey). As much as 100 kg of apramycin could be discharged each day from the manufacturing operation to the treatment facility and then to the river, assuming no reduction of apramycin during treatment. In this case, after the effluent from this plant mixes with the river at low flow, the highest estimated concentration of apramycin in the Wabash River would be about 0.0732 mg/L. The average estimated concentration of apramycin in the Wabash River is based on its average flow over a fifty-six year period of 6,383 ft^3/sec (U.S. Geological Survey). If as much as 100 kg of apramycin were discharged daily to the river, the average estimated concentration of apramycin in the Wabash River would be about 0.0064 mg/L. Apramycin concentrations which resulted in no mortalities or behavioral abnormalities in acute studies with Daphnia magna (28.4 mg/L) and rainbow trout and bluegill (300 mg/L) are 388 and 4098 times higher, respectively, than the highest estimated concentration of apramycin in the Wabash River. The previously calculated NOEC values for these organisms, 1.02 mg/L for Daphnia magna and 3.0 mg/L for rainbow trout and bluegill, are 159 and 469 times higher, respectively, than the average estimated concentration of apramycin in the Wabash River.

The highest estimated and the average estimated concentrations of apramycin that might be found in a river which may receive treated or untreated wastewaters from the manufacture of apramycin appear to be acutely and chronically safe to aquatic organisms. Even untested aquatic

organisms, which could be somewhat more sensitive to apramycin than those organisms tested, should be safe.

Residual biodegradable fermentation nutrients from the manufacture of other fermentation products at each of the plant sites are discharged to receiving waters at rates significantly below permitted limitations. Since apramycin will not be the only fermentation-based product manufactured at any of the above-referenced plant sites, it will account for only a small portion of the permitted discharge of residual nutrients expressed as biological oxygen demand (BOD). At the plant in Puerto Rico, spent fermentation broth and certain wash waters, both of which are believed to contain negligible apramycin, are transported by a contract hauler to the publicly-owned Puerto Nuevo regional wastewater facility in order to comply with water-quality-based BOD limitations for the plant's wastewater discharge to the Rio Grande de Loiza.

Essentially no other wastewater pollutants or liquid, solid or gaseous pollutants from the manufacture of apramycin will be allowed to enter the environment. Therefore, the manufacture of apramycin will have a minimal effect on the environment at any of these plant sites.

Limitations for atmospheric pollutant emissions and wastewater pollutant discharges, and disposal practices for other liquid and solid wastes applicable to the Puerto Rico and the Indiana plant sites, are defined by regulations administered by the U.S. Environmental Protection Agency (EPA) and, as appropriate, either by Puerto Rico's Environmental Quality Board (EQB) or by Indiana's Air Pollution Control Board (APCB), Stream Pollution Control Board (SPCB), or Environmental Management Board (EMB).

The following list shows the operating permits issued by EQB and by APCB for those manufacturing and emission control facilities which would produce apramycin at the Carolina, Puerto Rico, and Lafayette, Indiana, plants, respectively:

Location	Permit Identification No.	Issued	Expiration
Carolina	PFE-16-0383-0157-I-II-0	June 28, 1984	June 28, 1986
Lafayette	79-01-86-0264	Mar. 22, 1982	Jan. 1, 1986
Lafayette	79-01-86-0277	Mar. 22, 1982	Jan. 1, 1986

The EPA and SPCB have issued the following NPDES permits for the discharge of wastewaters from Carolina and Lafayette, respectively:

Location	NPDES Permit No.	Issued	Expiration
Carolina	PR 0021423	September 28, 1984	October 31, 1989*
Lafayette	IN 0002861	March 10, 1982	Dec. 31, 1982**

(*This permit was issued with interim limitations under an administrative order to provide time for complying with certain water-quality-based limitations, which should occur by September, 1986, by connecting this plant's discharge to the publicly-owned Carolina regional wastewater facility.)

(**A renewal permit is pending on the basis of timely submission of an application, and under authority of State and Federal statutes tne above permit is being extended administratively until a renewal permit is issued.)

On July 25, 1983, the Indianapolis Department of Public Works issued a permit, No. 283004, for the discharge of wastewaters from the Kentucky

Avenue fermentation facility of Eli Lilly and Company to the municipal sewer system for treatment. This permit expires on July 31, 1986. Emissions of non-hazardous particulate matter to the atmosphere from apramycin manufacturing operations at this facility would be too low to require a permit; such permits are not issued for emissions of particulate matter less than de minimis rates of 5 lbs/hour or 25 lbs/daily.

Limitations for atmospheric pollutant emissions from Nebraska plant sites are defined by regulations promulgated by the EPA and by Nebraska's Environmental Control Council. Under authority granted by Nebraska to the city of Omaha, the latter's Air Quality Control Division has issued certificates of approval limiting particulate matter emissions to the atmosphere from existing facilities which would be used to formulate and package APRALAN PREMIX at Omaha Laboratories of Eli Lilly and Company. These certificates are as follows:

Certificate No.	Issued	Expiration
2440/CR23545	March 3, 1971	Not Stated
25331/CR95881	January 27, 1982	Not Stated

The Nebraska Department of Environmental Control has provided letter authorization with no stated expiration for Omaha Laboratories of Eli Lilly and Company to dispose of packaging materials and animal health products, such as antibiotics in feed premixes, by landfilling. No other environmental permits are required for formulating and packaging APRALAN PREMIX at Omaha Laboratories.

No hazardous wastes and essentially no solid wastes will be generated in the manufacture of apramycin. Packaging materials, nonrecyclable tailings and floor sweepings from the manufacture of apramycin at the

Puerto Rico or the Indiana plants would be incinerated with industrial and domestic trash from other sources or would be landfilled. Manufacture of apramycin in the production facility at Liverpool, England, and disposal of wastes from apramycin processing performed there, will comply with all the pertaining environmental control laws of the United Kingdom.

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

B. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

The United States Department of Agriculture statistics show that there are about 85 million pigs produced in the United States annually. Most of this production is centered in the states of Iowa, Illinois, Indiana, Ohio, Minnesota, Missouri, South Dakota, Nebraska, North Carolina and Georgia. About 85% of all these pigs (72.3 million) will contract a diarrheal disease following weaning. APRALAN PREMIX can be mixed in the diet of these young swine for up to 14 days for the treatment of porcine colibacillosis. A pig from 28 to 42 days old can eat 21.7 pounds of treated feed in 14 days. Treated feed would contain 75 mg of apramycin activity per pound. A pig could then ingest, at most, 1628 mg of apramycin activity. If all of the swine that contracted diarrheal disease annually in the United States were treated with APRALAN PREMIX, as much as 0.118×10^6 kg of apramycin activity would be used (1.628 g per pig $\times$ 72.3×10^6 pigs). This is equivalent to the use of 0.715×10^6 kg of APRALAN PREMIX in a year (165 g activity/kg premix). A

more realistic market penetration of 24% could result, at most, in an annual use of about 0.028×10^6 kg of apramycin activity and 0.172×10^6 kg of APRALAN PREMIX.

Swine farms will be the primary site from which apramycin could be introduced into the environment. APRALAN PREMIX will be mixed into feed at the swine farm and apramycin may be introduced into the environment via the waste products from swine.

Most of the apramycin ingested by swine is excreted as parent compound in the feces and less than 10% appears in the urine (Appendices B and C). There is very little metabolism of apramycin following oral dosing. For the purpose of calculating the concentration of apramycin in swine waste, it can be assumed that, at most, all apramycin fed to swine passes unchanged into the waste. The highest possible concentration of apramycin activity in swine waste can be calculated given that a young swine could ingest, at most, 1628 mg of apramycin activity and that a farrow to finish operation selling 20,000 pigs/year produces 19,984 tons of manure/year (semi-solid or liquid waste). The highest possible concentration of apramycin activity in swine waste in a farrow to finish operation would then be 1.79 mg activity/kg waste ((20,000 pigs/year x 1628 mg/pig) $\div$ 18.17×10^6 kg manure). The primary manner by which apramycin activity could be introduced into the environment is by use of swine manure as fertilizer on cropland.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

The use of large amounts of swine waste as fertilizer for crops could result in measurable amounts of apramycin in soil. Because of its large molecular weight and relatively high melting point, and because thermogravimetric analysis showed little weight loss of apramycin until thermal decomposition, apramycin is considered to be a non-volatile solid. Measurable concentrations of free apramycin activity will not, therefore, occur in the atmosphere. It may be possible, however, to find apramycin activity in soil to which it has been applied, and in adjacent aquatic systems.

A. POTENTIAL CONCENTRATION OF APRAMYCIN ACTIVITY IN SOIL

The highest expected initial activity level of apramycin in soil can be estimated. The highest expected activity level of apramycin in swine feces used to fertilize cropland is 1.79 mg/kg. A reasonable estimate of the application rate of swine manure as fertilizer is 10 tons/A (22.4×10^3 kg/ha). It is standard practice to incorporate manure into the top six inches of soil to avoid loss of nutrients in runoff. A six-inch deep soil layer in one hectare weighs about 2.25×10^6 kg. The highest initial activity level of apramycin in soil is, therefore, about 0.018 ppm ((1.79 mg/kg x 22.4×10^3 kg/ha) ÷ 2.25×10^6 kg of soil/ha).

Apramycin activity may accumulate in soil which receives yearly applications of swine waste. Apramycin does not leach (Appendix D) and strong chemical procedures are required to remove apramycin residue from soil (Appendices E, F and G). Total activity of apramycin declines slowly in soil. Approximately two-thirds (Appendix E) and 75% (Appendix F) of the apramycin activity initially applied to soils in the

greenhouse could be extracted from the soils after one year. A study of the decline of apramycin in field soil indicated that about 75% of the initially applied apramycin activity could be extracted from the soil after two years (Appendix G). Using the slowest decline rate found for apramycin activity in soil (about 25% in two years), the expected apramycin activity would be, at most, 87% of the initial level one year after application. If apramycin is reapplied in swine waste to cropland annually, apramycin activity would accumulate to some asymptotic value.

The highest expected asymptotic concentration of apramycin activity in field soil, which has received repeated applications of swine waste containing apramycin, can be calculated using a yearly decline rate of 13% and the highest expected level for the annual addition of apramycin activity to field soil, 0.018 mg/kg. A differential equation, which describes the change in the concentration of apramycin in soil, can be written to account for the yearly loss and addition of apramycin in soil.

(1) $$\frac{dC}{dt} = -rC + I$$

where:

C = Concentration of apramycin activity in soil (mg activity/kg soil)
r = Decline rate constant for apramycin activity (yr^{-1})
I = Input rate of apramycin activity (mg activity/kg soil/year)
t = Time (years)

Solution of this differential equation yields the following equation:

$$(2) \quad C = C_0 e^{-rt} + \frac{I}{r}\left[1-e^{-rt}\right]$$

where:

C = Concentration of apramycin activity in the soil at time t (mg activity/kg soil)
C_0 = Initial concentration of apramycin activity in the soil (mg activity/kg soil) when swine waste with apramycin is first applied to the soil
r = Decline rate constant for apramycin activity (yr^{-1})
I = Input rate of apramycin activity (mg activity/kg soil/year)
t = Time (years)

The asymptotic concentration of apramycin in soil can be calculated by setting t (years) to infinity. This simplifies Equation 2 to:

$$C = \frac{I}{r}$$

Using the previously described values for I (0.018 mg activity/kg soil/year) and r (0.13 per year), the highest expected asymptotic concentration of apramycin activity in cropland can be calculated to be 138.5 µg activity/kg soil.

B. POTENTIAL CONCENTRATION OF APRAMYCIN ACTIVITY IN AQUATIC SYSTEMS

1) Surface Water

It is possible that runoff water from heavy rainfall could carry some apramycin activity into surface waters. Even though apramycin can easily be dissolved in water, it cannot be easily leached from soil particles with water (Appendix D). Strong chemical procedures must be utilized to extract apramycin from soil. This information and physical-chemical properties, such as five pKa values ranging from 5.4 to 8.5, 11 ether or hydroxyl groups, and a n-octanol/water partition coefficient from 0.00022 to 0.0011, all support the conclusion that apramycin is very

tightly bound to charged soil particles. An estimate of 0.5% has been made for the loss of soil-incorporated pesticides from application sites during a season into runoff water (2,3). However, if a large runoff event occurs soon after application, as much as three times this amount may be lost (2,3). Apramycin lost in runoff would probably be tightly bound to soil particles.

Based on the slow decline of apramycin activity in soil (Section 7A), it is possible that the decline of apramycin activity in water would also be slow. Given that apramycin could be leached from soil particles washed into surface water and assuming that there is no degradation of apramycin in water, the highest possible asymptotic concentration of apramycin in surface water can be calculated using a 40-acre (16.2 hectares) watershed with a 2.5-acre pond (average depth, 2.5 ft) as the surface water receiving runoff (4). Yearly runoff can be presumed to equal at least an average of 0.5 acre-in/acre/yr (4), although more runoff might be needed to carry enough soil particles to actually discharge 1.5% of the apramycin in the soil into the pond. The pond would contain 7.71×10^6 liters (6.25 acre-ft x 43, 560 ft^3/acre-ft x 28.32 liters/ft^3). An average runoff of 0.5 acre-in/acre/yr would fill the pond in four years ((6.75 acre-ft x 12 acre-in/acre-ft) ÷ (40 acres x 0.5 acre-in/acre/yr)). The 16.2-hectare watershed would contain, at most, 5.05 kg of apramycin activity when the highest expected asymptotic apramycin concentration was reached in soil ((138.5 µg activity/kg soil x 2.25×10^6 kg soil/ha x 16.2 ha) ÷ 10^9 µg/kg). The losses of apramycin activity from the watershed over a year could be as high as 76 g (5.05 kg x 1.5%). Given no degradation of apramycin in water, the concentration of apramycin in the pond would eventually reach an asymptote at the

average concentration of apramycin in runoff water. The highest possible asymptotic concentration of apramycin in surface water can then be estimated to be 0.040 ppm ((76 g apramycin activity/yr) ÷ (40 acres x 0.5 acre-in/acre/yr x 1.028 x 10^5 liters/acre-in). Even if it were possible for all of the apramycin activity in this watershed (5.05 kg) to be instantaneously dispersed in the pond, the level of apramycin activity would only be 0.655 ppm (5.05 kg ÷ 7.71 x 10^6 L).

2) Occurrence of Apramycin Activity in Groundwater

Apramycin does not leach from soil when applied to soil with or without swine feces. A laboratory leaching study shows that ^{14}C-apramycin adsorbs tightly to sandy loam and silt loam soil and cannot be leached with water (Appendix D). When apramycin was incorporated with swine feces in the surface soil layer (0-8 cm) in the field, only 7.2% of the radioactivity moved to the next soil layer (8-16 cm) after two years (Appendix G).

Given the immobility of apramycin demonstrated in the laboratory and in the field and given the strong chemical procedures needed to extract apramycin from soil, it is very unlikely that any apramycin activity would be found in groundwater.

C. FATE OF APRAMYCIN IN TERRESTRIAL AND AQUATIC ORGANISMS

Apramycin would not bioconcentrate in terrestrial or aquatic organisms. Apramycin adsorbs tightly to soil so very little would be available to plants, animals or aquatic organisms. The high water solubility (>300 g/L), very low n-octanol/water partition coefficient and relatively large molecular size of apramycin indicates that it would not be easily absorbed by animals that may ingest soil or by plants. Most of the apramycin activity fed to swine is excreted in the feces (Appendices B and C). Any apramycin that may run off via soil particles into receiving water would not bioconcentrate in fish. Neely, Branson and Blau (5) developed a regression equation for projected steady-state residue concentrations in trout muscle versus measured n-octanol/water partition coefficients for a variety of synthetic compounds:

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

Using this equation, and the experimentally derived log K_{ow} value of -2.66 (Appendix A), the predicted BCF is less than 0. Based on the predicted BCF value, fish would not be expected to bioconcentrate apramycin activity. Even though the decline rate of apramycin in soil is slow, terrestrial and aquatic organisms would be exposed to very low concentrations of apramycin and apramycin would not be expected to bioconcentrate.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY TESTS

An in-depth testing program has been completed with various laboratory animal species to determine the toxicological properties of apramycin. Complete reports of all of these studies have been submitted to support the proposed action. Studies which are critical to determine the safety of apramycin to the public and the environment are briefly described below. A more complete summary of mammalian toxicology information can be found in a Freedom of Information (FOI) Summary for APRALAN PREMIX (6).

Acute Studies

Oral LD_0 for ICR Mice: > 5.2 g apramycin activity/kg body weight (>10 g apramycin sulfate/kg body weight) for both males and females.

Oral LD_0 for Wistar Rats: > 4.16 g apramycin activity/kg body weight (>8 g apramycin sulfate/kg body weight) for both males and females.

Hazard Evaluation Studies

Guinea Pig Dermal Sensitization: No sensitization with a 40% aqueous solution of apramycin.

Dermal Irritation in Rabbits: No signs of toxicity with 1040 mg apramycin activity/kg body weight (2000 mg apramycin sulfate/kg). Slight erythema was recorded when the occlusive dressings were removed. The erythema cleared in five days.

Ocular Irritation in Rabbits: Only slight irritation was found in rabbit eyes treated with 36 mg of apramycin sulfate. The slight conjunctival redness cleared in all eyes by 96 hours, even though the eyes were not rinsed.

Inhalation by Wistar Rats: No adverse effects were observed in rats exposed for one hour to an atmospheric concentration of 211 ± 14 mg apramycin activity/m^3 of air. The appearance and behavior of all rats were normal throughout the 14-day observation period.

Chronic, Reproduction and Teratology Studies

One-Year Beagle Dog Study: Dogs were given daily oral doses of up to 100 mg apramycin activity/kg body weight/day for one year. There were no significant signs of toxicity. Analysis of body weight gain, hematology, clinical chemistry, organ weight and histopathology data did not reveal any effects which could be attributed to administration of apramycin.

Two-Year Rat Study: Significant toxicity and carcinogenicity were not found in rats fed diets containing 10,000 mg apramycin activity/kg diet. This concentration provided time weighted average daily doses of 488 and 610 mg apramycin activity/kg rat for males and females, respectively.

Rat Multigeneration Reproduction and Teratology Study: Apramycin was administered to four successive generations in their diet. No significant reproductive or teratogenic effects were found at a dietary concentration of 10,000 mg apramycin activity/kg diet, the highest level tested. This was equivalent to an average daily dose of 785 mg activity/kg body weight/day.

Rabbit Teratology Study: Apramycin was administered orally by gavage to rabbits in daily doses of 2, 8, and 32 mg/kg on gestation days 6 through 18. A low incidence of fetal defects was observed in this study. These findings occurred in fetuses from the control as well as the apramycin-treatment groups. In the apramycin groups, the

fetuses with deviations frequently were small and were from females with marked signs of toxicity. It was concluded that apramycin was not teratogenic to the rabbit at doses which produced maternal toxicity.

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

1) Production of Apramycin and Manufacture of APRALAN PREMIX

Apramycin and APRALAN PREMIX will be produced in only two plants and engineering controls and personal hygiene precautions are effective in minimizing exposure. Considering the extent of these measures, the magnitude of the acute LD_{50} values with apramycin, and the fact that in laboratory animals apramycin is not a teratogen, carcinogen, or a reproductive toxin, it is concluded that workers producing apramycin and APRALAN PREMIX would not be adversely affected by the proposed action.

2) Human Exposure to Apramycin Via the Food Supply

Exposure of humans to large amounts of apramycin via the food supply is unlikely. As discussed in preceding sections (7B1 and 7B2), it is improbable that measureable amounts of apramycin activity would occur in drinking water from groundwater or surface water sources. Details of exposure of humans to any apramycin residues in meat are listed in a Freedom of Information (FOI) Summary (6). Adverse effects are not expected for humans exposed to any small residues of apramycin. Based on this information, it is concluded that the proposed action would not adversely affect human health through the food supply.

C. EFFECTS OF APRAMYCIN ON NONTARGET ORGANISMS

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

Avian Species

Bobwhite Quail 14-day Acute Oral Toxicity Study (Appendix H): Adult bobwhite quail (Colinus virginianus) were given single oral doses of 0, 250, and 500 mg apramycin activity/kg and 0, 700, 1000, 1400, and 2000 mg apramycin activity/kg in two 14-day studies. At doses of 250 and 500 mg apramycin activity/kg there were no substantial changes in mean body weights or food consumption and no behavioral effects or mortalities were found. Effects from treatment were found at doses of 700, 1000, 1400, and 2000 mg/kg. These effects included diarrhea, ataxia, lethargy, ruffled feathers, and death. One, five, six, and four cumulative mortalities occurred at doses of 700, 1000, 1400 and 2000 mg apramycin activity/kg, respectively. No deaths occurred after day three. All survivors displayed normal behavior by day eight. Male birds initially lost weight at doses of 1400 and 2000 mg activity/kg and females showed substantial loses in mean body weight at doses of 1000, 1400, and 2000 mg activity/kg. An equal number of males and females died from single oral doses of apramycin, so the mortalities were combined to calculate a single dose-response curve. The 14-day LD_{50}, the 95% confidence interval for the 14-day LD_{50}, and the slope of the dose

response curve for adult bobwhite were 1669 mg apramycin activity/kg, 857 to 3253 mg apramycin activity/kg, and 1.8, respectively.

Bobwhite Quail 5-day Dietary Study (Appendix I): Bobwhite quail (Colinus virginianus), 11-days old, were fed diets for five days that contained nominal apramycin activity levels of 0.0, 625, 1250, 2500, and 5000 mg/kg of diet. These birds were observed for another three days while being fed an untreated diet. Although control birds consumed slightly more diet than the treated birds, body weight gains for treated and control birds were not statistically different. No mortalities or any overt signs of toxicity, such as ataxia or lethargy, were seen in control or in any treatment group. There were no apparent toxic effects in bobwhite quail that consumed diets containing as much as 5000 mg apramycin activity/kg (assayed as 5000 mg apramycin activity/kg). Based on food consumption and body weight data, this dietary concentration provided an average dose of 1050 mg apramycin activity/kg of body weight/day for the bobwhite quail.

Mallard Duck 5-day Dietary Study (Appendix J): Mallard ducks (Anas platyrhynchos), 10-days old, were fed diets for five days that contained nominal apramycin activity levels of 0.0, 1250, 2500, and 5000 mg/kg of diet. These birds were observed for another three days while being fed an untreated diet. A decrease in food consumption and a significant reduction in body weight gain were observed when birds that received the diet treated with 5000 mg activity/kg were fed an untreated diet for three days. No other

differences in body weight gain or food consumption were found. No mortalities or any overt signs of toxicity, such as ataxia or lethargy, were seen in control or in any treatment group. There were no apparent toxic effects in mallard ducks that consumed diets containing as much as 5000 mg of nominal apramycin activity/kg (assayed as 4700 mg apramycin activity/kg). Based on food consumption and body weight data, mallards consumed an average dose of 1838 mg apramycin activity/kg of body weight/day.

Aquatic Species

96-Hour Bluegill Toxicity Study (Appendix K): A static toxicity test was conducted to determine the acute effects of apramycin on the bluegill (Lepomis macrochirus). Juvenile bluegill were exposed for 96 hours to nominal apramycin activity levels of 0, 100, and 300 mg/L. Analyzed activity levels averaged 116 and 331 mg/L. No mortalities and no behavioral abnormalities were found in the control or in the treatment groups.

96-Hour Rainbow Trout Toxicity Study (Appendix L): A static toxicity test was conducted to determine the acute effects of apramycin on the rainbow trout (Salmo gairdneri). Juvenile trout were exposed to nominal apramycin activity levels of 50, 100, and 300 mg/L. No mortalities and no behavioral abnormalities were found in the control or in the treatment groups.

48-Hour Daphnia Toxicity Study (Appendix M): First-instar Daphnia magna ≦ 18 hours of age were exposed for 48 hours to nominal apramycin levels of 0.0, 17.8, 28.4, 44.4, 71.0 and 106.5 mg apramycin activity/L. No immobile daphnids and no hypoactive or prostrate daphnids were found at apramycin activity levels ≦ 28.4 mg/L. At 48 hours, seven daphnids were hypoactive and 24 were normal at an apramycin level of 44.4 mg/L. At an apramycin level of 71.0 mg/L, 24 daphnids were hypoactive, two were prostrate and six were immobile. At the highest apramycin level, 11 daphnids were hypoactive, three were prostrate, and 16 were immobile. The 48-hour EC_{50} (based on immobility) was 101.6 mg apramycin activity/L, with 95% confidence limits of 88.3 to 116.8 mg activity/L and a slope of 6.483 for the concentration-response line.

Sewage Microorganisms (Appendix N): Apramycin had little effect on sewage-digesting organisms as determined by standard methods for examining waste water. Using a laboratory scale, semicontinuous aerated sewage system, apramycin was tested at an initial concentration of 0.1 ppm and gradually increased to 102.4 ppm. In 26 days, changes that occurred in the biochemical oxygen demand, bacterial populations, pH and solid's content of treated systems also occurred in the negative controls.

Terrestrial Species

Earthworm 14-day Toxicity Study (Appendix O): Earthworms (Lumbricus terrestris) were exposed to nominal soil concentrations of 0.0, 10, and 100 mg apramycin activity/kg soil for 14 days. Mortalities, changes in appearance, and reductions in body weight gain were not found in control or in the treatment groups. There were no apparent signs of toxicity at the two concentrations of apramycin activity that were tested.

Greenhouse Phytotoxicity Study of Manure from Apramycin-treated Swine (Appendix P): Raw feces from untreated swine and from swine treated with apramycin were mixed with greenhouse potting soil to test the phytotoxicity of apramycin. Feces from the apramycin-treated swine contained about 50 to 60 mg apramycin activity/kg waste. Feces from control and treated animals were mixed in soil at levels equivalent to manure application rates of 11.2 and 22.4 metric tons of fresh feces per hectare. Nominal apramycin activity levels in the soil would then be at least 0.249 and 0.498 ppm. Twenty-one days after seeds of alfalfa, fescue, cucumber, rice, pepper, cotton, tomatoes, corn, sugar beets, barley, soybeans, wheat, sorghum, and oats were planted, germination, plant stunting, and phytotoxicity were assessed. At the highest manuring level with feces from both control and treated swine, some stunting was noted for peppers. No other stunting or crop injury was noted for plants exposed to feces from control or treated swine. There was no apramycin-related phytotoxicity in the species tested.

Nitrogen-fixing Microorganisms (Appendix Q): Apramycin was tested against a group of nitrogen-fixing organisms to determine its effects on atmospheric nitrogen reduction and/or growth in broth culture. The bluegreen alga, Anabaena flos aquae was inhibited by concentrations of 0.1 and 1.0 mg apramycin/L, yet culture growth and nitrogen fixation were uninhibited by an apramycin concentration of 0.01 mg/L. An apramycin concentration of 0.1 mg/L appeared to inhibit growth of the free-living heterotroph Azotobacter chroococcum, while a concentration of 0.01 mg/L inhibited growth in one experiment and did not inhibit total growth in another replicated experiment. Rhizobia leguminosarum was also inhibited by an apramycin concentration of 0.1 ppm. The growth of three strains and a field isolate of Rhizobia japonicum, a symbiotic nitrogen-fixing organism, was inhibited by an apramycin concentration of 1 and 10 ppm, but not by a concentration of 0.1 ppm. An apramycin concentration of 0.01 ppm can be considered the concentration which should result in no reduction in total culture growth for all of the species tested.

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

1) Potential Adverse Effects on Aquatic Organisms

Based on the slow decline of apramycin activity in soil (minimum of 13% activity loss/year), it is possible that any decline of apramycin activity in water would also be slow. In fact, the highest possible asymptotic concentration of apramycin activity in water was estimated based on no degradation of apramycin in water (Section 7B1). This highest possible concentration was also calculated using the assumption that all apramycin could be leached from soil particles washed into surface water, an unlikely event given that strong chemical procedures are required to remove apramycin from soil. Assuming that apramycin might not degrade in water and that it could be leached into water, aquatic organisms may be chronically exposed to low concentrations of apramycin.

The highest possible asymptotic concentration of apramycin activity was calculated to be 0.040 mg/L for a pond. No mortalities and no behavioral abnormalities were found for bluegill or rainbow trout during 96-hour exposures to concentrations of apramycin as high as 331 and 300 mg activity/L, respectively. The 48-hour EC_{50} for daphnids was 101.6 mg apramycin activity/L, based on immobility. No behavioral abnormalities and no immobile daphnids were found at apramycin activity levels $\leqq$ 28.4 mg/L. The concentrations of apramycin which resulted in no acute mortalities nor any behavioral abnormalities in bluegill, rainbow trout and daphnids are 8275, 7500, and 710 times greater, respectively, than the highest possible asymptotic concentration of apramycin in pond water. Even sewage microorganisms are unaffected by an apramycin concen-

tration (102.4 ppm) that is 2560 times larger than the highest possible asymptotic concentration of apramycin in surface water.

The highest possible asymptotic concentration of apramycin in surface water is substantially below concentrations which can be calculated to have no chronic effects on aquatic organisms. An application factor of 100 can be used with median effect concentrations from acute studies to extrapolate the concentrations which have no observed effects on the test organisms during chronic exposure. Median lethal concentrations were not found for rainbow trout or for bluegill, so the application factor will be used with the concentrations which resulted in no acute mortalities nor behavioral abnormalities for these organisms. Calculated chronic no-observed-effect concentrations for bluegill, rainbow trout, and daphnids are 3.31 mg/L (331 mg/L ÷ 100), 3.0 mg/L (300 mg/L ÷ 100), and 1.02 (101.6 mg/L ÷ 100), respectively. These concentrations are between 26 and 83 times higher than the highest possible asymptotic concentration of apramycin in surface water receiving runoff from soil fertilized with swine manure.

The highest possible asymptotic concentration of apramycin in surface water appears to be acutely and chronically safe to aquatic organisms. Even untested aquatic organisms, which might be somewhat more sensitive to apramycin than those organisms tested, should be safe.

2) Potential Adverse Effects on Avian Species

Apramycin is not expected to bioaccumulate in plants (Section 7C) and could occur in only very low concentrations in surface water (Section 7B1). The highest concentration of apramycin in any material will be in swine feed. The recommended concentration of apramycin activity in swine

feed is 165 ppm. No compound-related mortality, reduction in body weight gain, reduction in food consumption, change in appearance, or change in behavior occurred for mallard ducks or bobwhite quail fed diets containing 4700 and 5000 ppm of apramycin activity, respectively. These dietary levels are 28 to 30 times higher than the concentration of apramycin activity in swine feed. Even if wild birds were allowed to forage swine feed for several days, an impact on bird populations would not be expected.

3) Potential Adverse Effects on Terrestrial Species

The highest expected asymptotic concentrations of apramycin in soil (138.5 µg/kg soil) and surface water (40 µg/L) would not be expected to cause toxic effects in mammals, earthworms, or fourteen species of crops. At these concentrations, apramycin is not acutely toxic to mammals, apramycin would not cause dermal sensitization, or dermal or ocular irritation in mammals, and apramycin is not a teratogen, carcinogen or a reproductive toxin in mammals (Section 8A). No mortalities, changes in appearance, or reductions in body weight gain would be found for earthworms exposed to 138.5 µg apramycin/kg soil, or to soil concentrations 722 times higher (Section 8C). No treatment-related effects on germination, stunting, or phytotoxicity were found for alfalfa, fescue, cucumber, rice, pepper, cotton, tomatoes, corn, sugar beets, barley, soybeans, wheat, sorghum, and oats when exposed to concentrations of apramycin in soil (0.249 and 0.498 ppm) that were as much as 3.6 times higher than the highest expected asymptotic concentration of apramycin in cropland (Section 8C).

Apramycin does inhibit growth of nitrogen-fixing organisms when it is freely available in broth culture. Apramycin is however, strongly

adsorbed to soil and strong chemical procedures must be utilized to extract the compound from soil (Section 7B). Because adsorbed apramycin would not be available to microbiological organisms, the compound would not be expected to adversely affect populations of nitrogen-fixing microorganisms in soil.

9. USE OF RESOURCES AND ENERGY

Manufacturing APRALAN PREMIX will require an amount of energy similar to that used to produce and package any conventional fermentation product for animals. Disposal of waste washwater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources. Manufacture of APRALAN PREMIX will occur at facilities already designed for production of fermentation materials. Unusual levels of noise, odors, construction, or other disruptions should not be required in the manufacture of this product.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or on the environment. Therefore, no mitigation measures are necessary.

11. ALTERNATIVES TO THE PROPOSED ACTION

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

12. LIST OF PREPARERS

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

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13. CERTIFICATION

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

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

July 29, 1985
Date

14. REFERENCES

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APPENDIX A: Report Summary

Title: N-Octanol-to-Water Partition Coefficient of Apramycin at pH 5.0, 7.0, and 9.0.

Study Number: I-EAD-7901

Date: July 1981

Test Article: ^{14}C Labeled apramycin crystalline (2.70 μCi/mg)

Names and Addresses of Investigators: G. K. Dorulla, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test System: N-Octanol-to-water partition

Summary of Experimental Design:

The design of this study was based on protocols described in the Federal Register, Vol. 45, No. 227, Nov. 21, 1980, pp. 77350-77352 which provides for the use of standardized buffers at pH 5.0, 7.0, and 9.0. ^{14}C apramycin was quantified radiochemically from aqueous and n-octanol experimental samples after partitioning.

Summary of Results:

The partition coefficient (K) is defined as:

$$K = \frac{\text{Conc. of apramycin in n-octanol at equilibrium}}{\text{Conc. of apramycin in water at equilibrium}} = \frac{Cnoct}{Caq}$$

The experimental data and the determined partition coefficient for apramycin is shown in the following table:

		At equilibrium		
No.	pH	Cnoct (mcg/ml)	Caq (mcg/ml)	Cnoct/Caq
1	5.0	0.416	931	0.00045
2	5.0	0.427	914	0.00047
3	7.0	0.326	938	0.00035
4	7.0	0.356	910	0.00039
5	7.0	0.108	98	0.0011
6	7.0	0.103	95	0.0011
7	9.0	0.197	911	0.00022
8	9.0	0.200	908	0.00022

The partition coefficient (K) of apramycin was determined to have a range of 0.00022 to 0.0011 and a mean of 0.0005. These results indicate that apramycin should not bioaccumulate in the environment.

APPENDIX B: Report Summary

Title: ^{14}C Apramycin Tissue Residue Levels in Swine at Zero Time and Seven Days Following Oral Dosing.

Study Number: ABC-0024

Study Dates: March 1, 1979 to November 2, 1979

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

Test Article: ^{14}C Apramycin (sulfate salt)

Test System: Swine

Summary of Experimental Design:

Six pigs weighing approximately 8 kg each were dosed orally with ^{14}C apramycin (25 mg/kg) daily for five days. One female and two males were sacrificed at a practical zero withdrawal (four hours after the last dose) and a similar group was sacrificed at seven days' withdrawal. Edible tissues were assayed for total radioactivity and selected samples were assayed for apramycin.

Summary of Results:

The total radioactivity levels expressed as net parts per million (ppm) are given in the following table:

	Zero Withdrawal			7-Day Withdrawal		
	Female	Male	Male	Male	Male	Female
Muscle	0.059	0.215	0.107	0.039	0.055	0.037
Liver	0.202	4.011	1.939	0.099	0.190	0.078
Kidney	1.388	70.990	9.735	0.106	0.524	0.165
Fat	0.126	0.303	0.132	0.094	0.123	0.114

Virtually all of the total kidney radioactivity at zero withdrawal was ^{14}C apramycin. Approximately 80% of the 0.524 ppm seven-day withdrawal kidney sample was ^{14}C apramycin. The remaining two kidney samples were too low in total residue for quantitative evaluation of the ^{14}C apramycin level. Approximately 85% of the total radioactivity in the livers of the two male pigs at zero withdrawal was ^{14}C apramycin. Liver from the female pig contained a lower total residue (0.202 ppm) and ^{14}C apramycin accounted for at least half of the total. None of the other tissues contained sufficient radioactivity for apramycin determination. These data support the conclusion that the apramycin level in the kidney is a reliable index to apramycin-derived residues in apramycin-fed swine.

Balance-excretion data were derived for the female in the seven-day withdrawal group. Recovery of radioactivity was 86.93% of the administered dose with 81.61% in the feces and 5.32% in the urine.

APPENDIX C: Report Summary

Title: Excretion and Tissue Distribution of ^{14}C Apramycin in Swine Following Oral Dosing.

Study Number: ABC-0013

Study Dates: April 15, 1978 to December 15, 1978

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

Test Article: ^{14}C Apramycin (sulfate salt)

Test System: Swine

Summary of Experimental Design:

Three pigs, one female (gilt) and two males (barrows), weighing approximately 10 kg each were dosed orally with ^{14}C apramycin (25 mg/kg) daily for five days. Fourteen days after the last dose, the pigs were killed. Tissues, urine, and feces were assayed for total radioactivity by liquid scintillation counting and selected samples were assayed for apramycin by fractionation of radioactivity and semiquantitative thin-layer bioautography.

Summary of Results:

The radioactivity was excreted primarily in the feces of the three pigs (72 to 91%). Urine contained less than 10% (1.5 to 9.7%). Total recovery of the dose was 82 to 92%. The urinary and fecal radioactivity was predominantly (75% or more) unchanged ^{14}C apramycin indicating that there was very little metabolism of the compound.

Kidney contained the highest level of radioactivity (equivalent to 0.173, 0.050, and 0.291 ppm ^{14}C apramycin for the gilt and two barrows, respectively). Muscle contained less than 0.05 ppm, and fat and liver contained 0.150 ppm or less for all three pigs. Approximately two-thirds of the kidney radioactivity was ^{14}C apramycin. Therefore, a determination of apramycin in kidney is a reliable index for monitoring total apramycin-derived residues in apramycin-fed swine.

APPENDIX D: Report Summary

Title: Leaching of ^{14}C Apramycin Through Soil in Laboratory Studies.

Date: December 1978

Test Article: ^{14}C labeled apramycin crystalline (1.04 uCi/mg)

Name and Address of Investigators: G. K. Buchanan, W. L. Sullivan, and A. Loh, Lilly Research Laboratories, Division of Eli Lilly and Company, Box 708, Greenfield, Indiana 46140

Test System: Laboratory soil leaching

Summary of Experimental Design:

The design for this soil column leaching study was based on protocols described in Guidelines for Registering Pesticides in the U.S., Vol. 40, No. 123, June 25, 1975, pp. 26884-26886. Apramycin was applied to the surface of the 30 cm soil columns at a rate equivalent to 16 kg/ha and leached with the equivalent of 60 cm of rainfall. Two soil types, silt loam and sandy loam, were leached over an eight day period in triplicate. The leachate was collected in six increments and radiochemically assayed for apramycin. At the end of the experiment the soil column was divided into six segments and assayed for apramycin.

Summary of Results:

The results of the laboratory leaching study are summarized in Table I and standard recovery data is listed in Table II.

Soil pH was 7.7 for sandy loam and 7.0 for the silt loam. Both columns required eight days to leach. All of the apramycin recovered was found in the top 5 cm of the soil columns tested and no detectable amount of apramycin was found in any of the lower soil segments or in any of the leachate fractions. This experiment indicates that apramycin does not have the potential to leach into groundwater.

APPENDIX D (continued)

TABLE I
Apramycin Soil Leaching Date

Sample(cm)	Percent of Initial Apramycin Added to Soil Columns[1]					
	Sandy Loam Columns			Silt Loam Columns		
Soil	A	B	C	A	B	C
0-5	84.3	83.3	91.5	116	111	115
5-10	ND[2]	ND	ND	ND	ND	ND
10-15	"	"	"	"	"	"
15-20	"	"	"	"	"	"
20-25	"	"	"	"	"	"
25-30	"	"	"	"	"	"
Leachate						
0-10	ND[3]	ND	ND	ND	ND	ND
10-20	"	"	"	"	"	"
20-30	"	"	"	"	"	"
30-40	"	"	"	"	"	"
40-50	"	"	"	"	"	"
50-60	"	"	"	"	"	"
Total Percent	84.3	83.3	91.5	116	111	115

[1] Corrected for standard recovery
[2] None detected above 0.15 mcg per soil segment
[3] None detected above 0.08 mcg per leachate increment

TABLE II
Apramycin Standard Recovery Data

Matrix	Mean Percent Recovery	S.D.
Sandy Loam Soil	108.6[1]	5.31
Silt Loam Soil	80.3[1]	2.36
Sandy Loam Leachate	92.7[2]	1.84
Silt Loam Leachate	89.6[1]	3.33

[1] Mean of 3 samples
[2] Mean of 2 samples

APPENDIX E: Report Summary

Title: ^{14}C Apramycin Greenhouse Soil Decline Study.

Study Number: ABC-0015

Study Dates: August 18, 1978 to November 1, 1979

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

Test Article: ^{14}C Apramycin (sulfate salt)

Test system: Soil flats maintained in the greenhouse.

Summary of Experimental Design:

^{14}C Apramycin as the sulfate salt was incorporated into nonsterile greenhouse potting soil at a nominal concentration of 10 ppm (apramycin base equivalents). The potting soil was a mixture of equal parts sand and Brookston loam. Flats containing apramycin-treated and untreated soil were prepared and maintained in a greenhouse which was not rigidly environmentally controlled. The flats were watered from the bottom as needed to maintain soil moisture. At intervals up to one year after initiation, replicated samples were taken for assay of radioactivity and parent apramycin.

Summary of Results:

Results are summarized in Table 1. Total radioactivity concentrations did not change appreciably during the study. There was a decline in extractable radioactivity with time and a proportional increase of tightly bound radioactivity, ie. radioactivity not extractable with 0.5 N NaOH. Semiquantitative bioautography indicated that most of the extractable radioactivity was parent ^{14}C apramycin.

A more vigorous extraction of the 52-week soil sample with hot alkali after the standard initial extraction released approximately 18% more of the total radioactivity, of which approximately one-third was parent apramycin. Therefore, after one year approximately two-thirds of the radioactivity in the soil was recovered as parent apramycin and the remainder was degraded and/or tightly bound to soil particles.

APPENDIX E (continued)

TABLE 1

Radioactivity and Apramycin Levels in Soil Fortified with ^{14}C Apramycin at a Nominal Level of 10 PPM

Sampling Time (Weeks)	Radioactivity ppm		Apramycin (ppm)[b]
	Total	Extractable[a]	
0	12.9	11.0	10
1	11.8	10.0	9
2	10.9	10.1	9
3	11.1	9.8	9
8	10.8	7.5	-[c]
12	10.6	9.0	9
20	11.0	7.0	7-8
28	11.6	6.5	5-6
52	10.7	5.8	5-6

[a] Extractable with 0.5 N NaOH.

[b] Estimated by semiquantitative bioautography.

[c] The calculated value of 15 ppm appears to be high due to a low response from the recovery sample in that set.

APPENDIX F: Report Summary

Title: ^{14}C Apramycin Greenhouse Soil Decline Study.

Study Number: ABC-0029

Study Dates: June 13, 1979 to August 1, 1980

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

Test Article: ^{14}C Apramycin (sulfate salt)

Test System: Soil flats maintained in the greenhouse.

Summary of Experimental Design:

^{14}C Apramycin as the sulfate salt was incorporated into nonsterile field soil (Brookston loam from Greenfield, Indiana) at a nominal concentration of 15 ppm. The soil was also fortified with feces from nonmedicated swine equivalent to an application rate of 11.2 metric tons per hectare (5 U.S. tons per acre). Control soil containing swine feces but no ^{14}C apramycin was also prepared. Duplicate flats of ^{14}C apramycin treated soil and control soil were prepared and maintained under nominal greenhouse growing conditions. Temperatures ranged from approximately 27° to 43°C with a range of 20 to 40% relative humidity. The flats were watered from the bottom as needed to maintain soil moisture. At intervals up to one year after initiation, replicated samples were taken for assay of radioactivity and parent apramycin.

Summary of Results:

Data from assay of total and extractable radioactivity are presented in Table 1. After 12 months, the total radioactivity was 90% of the initial value and the extractable radioactivity was 87% of the initial extractable value (approximately 75% of the initial total radioactivity). Most of the extractable radioactivity was parent ^{14}C apramycin. Therefore, after a 12-month observation period, approximately three-fourths of the total radioactivity in the soil appeared to be parent apramycin.

TABLE 1
Total and Extractable Radioactivity in
Manured Soil Fortified with 15 ppm ^{14}C Apramycin

Sampling Time (months)	Radioactivity Concentration (ppm)	
	Total	Extractable
0	17.6	15.3
1	16.6	14.3
2	17.3	13.7
6	16.1	11.4
12	15.9	13.3

APPENDIX G: Report Summary

Title: ^{14}C Apramycin Field Soil Decline Study.

Study Number: ABC-0030

Study Dates: June 13, 1979 to August 1, 1981

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

Test Article: ^{14}C Apramycin (sulfate salt)

Test System: Field soil plot

Summary of Experimental Design:

^{14}C Apramycin as the sulfate salt equivalent to approximately 10 ppm apramycin base activity was incorporated into the top 8-cm soil layer in a field plot at Greenfield, Indiana. The soil was also fortified with feces from nonmedicated swine at an application rate of 11.2 metric tons per hectare (5 U.S. tons/acre). The soil plot consisting of Brookston loam was subjected to conditions of natural weathering for a period of two years. Samples were assayed periodically for total radioactivity, extractable radioactivity (extractable with 1 N ammonium hydroxide), and apramycin concentrations.

Summary of Results:

Total radioactivity in the soil and relative quantities at two sampling depths are shown in Table 1. There was only a small loss of radioactivity over two years (89% remained) and there was very little movement of radioactivity from the treated soil layer (0-8 cm) into the layer below (8-16 cm). After 24 months, 80% of the radioactivity in the 0-8 cm layer sample was extractable with 1 N ammonium hydroxide, and most of this radioactivity was parent ^{14}C apramycin. Therefore the apramycin remaining after 24 months was estimated to be approximately three-fourths of that applied.

The apramycin application rate in this study was one to two orders of magnitude higher than that expected under actual use conditions.

APPENDIX G (continued)

TABLE 1

Total Radioactivity in Field Soil Treated with ^{14}C Apramycin and Relative Quantities of Radioactivity at Two Sampling Depths

Sampling Period (months)	Total Radioactivity dpm/cm^2	% of Initial Radioactivity in 2 soil layers	
		0-8 cm	8-16 cm
0	76.5×10^3	97.5	2.5
2	75.1×10^3	92.8	5.4
12	69.6×10^3	82.4	8.6
24	68.4×10^3	82.2	7.2

APPENDIX H: Report Summary

Title: The Toxicity of Compound 47657 (apramycin) to Bobwhite Quail in an Acute Oral Study.

Study Numbers: 7039-78 and 7004-79

Study Dates: November 13 to November 27, 1978 and February 13 to February 27, 1979.

Names and Addresses of Investigators: R. E. Karnak, C. C. Kehr and J. L. Hamelink. Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Test Article: Apramycin

Lot Number: X-24144, contains 71.4% apramycin activity

Species: Adult bobwhite quail (Colinus virginianus)

Number of Animals: 5/sex/dose

Levels of Dosing: Doses of 0.0, 700, 1000, 1400, and 2000 mg apramycin activity/kg bird were administered in aqueous solution for Study 7039-78. Doses of 0.0, 250 and 500 mg apramycin activity/kg bird were administered in aqueous solution for Study 7004-79.

Length of Observation: 14 days for both studies.

Route: Single oral gavage.

Parameters Studied: Food consumption, body weight, overt signs of toxicity (diarrhea, ataxia, lethargy, ruffled feathers) and mortality.

Summary of Results:

Study 7039-78: Substantial decreases in mean body weight were found for females given doses of 1000, 1400, and 2000 mg activity/kg body weight. Males treated with 1400 and 2000 mg activity/kg body weight showed slight decreases in mean body weight up to day 3, then steadily increasing body weights for the rest of the test. No dose-related pattern in food consumption was found. Effects from treatment were found at doses of 700, 1000, 1400, and 2000 mg/kg. These effects included diarrhea, ataxia, lethargy, ruffled feathers, and death. One, five, six, and four cumulative mortalities occurred at doses of 700, 1000, 1400 and 2000 mg apramycin activity/kg, respectively. No deaths occurred after day three. All survivors displayed normal behavior by day eight. An equal number of males and females died from single oral doses of apramycin, so the mortalities were combined to calculate a single dose-response curve. The 14-day LD_{50}, the 95% confidence interval for the 14-day LD_{50}, and the slope of the dose response curve for adult bobwhite were 1669 mg apramycin activity/kg, 857 to 3253 mg apramycin activity/kg, and 1.8, respectively.

APPENDIX H (continued)

Study 7004-79: At doses of 250 and 500 mg apramycin activity/kg there were no substantial changes in mean body weights or food consumption and no behavioral effects or mortalities were found.

APPENDIX I: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Bobwhite Quail in a 5-Day Dietary Study.

Study Number: A011-79

Study Dates: May 17 to May 25, 1979

Names and Addresses of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboaratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, IN 46140

Test Article: Apramycin

Lot Number: X-31385, contains 78.7% apramycin activity

Species: Bobwhite quail (Colinus virginianus), 11 days old

Number of Animals: 10/treatment

Levels of Exposure: 0.0, 0.0625, 0.125, 0.250 or 0.500% (w/w nominal) apramycin activity

Route: Dietary

Length of Exposure: Treated diet, 5 days; basal diet, 3 days

Parameters Studied: Food consumption, body weight gain, overt signs of toxicity (ie., ataxia, lethargy, and ruffled feathers), and mortality.

Summary of Results:

Although control birds consumed slightly more diet than the treated birds, body weight gains for treated and control birds were not statistically different. No mortalities or any overt signs of toxicity, such as ataxia or lethargy, were seen in control or in any treatment group. There were no apparent toxic effects in bobwhite quail that consumed diets containing as much as 5000 mg apramycin activity/kg (assayed as 5000 mg apramycin activity/kg). Based on food consumption and body weight data, this dietary concentration provided an average dose of 1050 mg apramycin activity/kg of body weight/day for the bobwhite quail.

APPENDIX J: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Mallard Ducks in a 5-Day Dietary Study.

Study Dates: December 13 to December 21, 1978

Study Number: 7043-78

Names and Addresses of Investigators: R. E. Karnak, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, IN 46140

Test Article: Apramycin

Lot Number: X-30881, contains 83.2% apramycin activity

Species: Mallard ducks (Anas platyrhynchos), 10 days old

Number of Animals: 10/treatment

Levels of Exposure: 0.0, 0.125, 0.250, or 0.500% (w/w nominal) apramycin activity

Route: Dietary

Length of Exposure: Treated diet, 5 days; basal diet, 3 days

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

Summary of Results:

A decrease in food consumption and a significant reduction in body weight gain were observed when birds that received the diet treated with 5000 mg apramycin activity/kg were fed an untreated diet for three days. No other differences in body weight gain or food consumption were found. No mortalities or any overt signs of toxicity, such as ataxia or lethargy, were seen in control or in any treatment group. There were no apparent toxic effects in mallard ducks that consumed diets containing as much as 5000 mg apramycin activity/kg (assayed as 4700 mg apramycin activity/kg). Based on food consumption and body weight data, mallards consumed an average dose of 1838 mg apramycin activity/kg of body weight/day.

APPENDIX K: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Bluegills in a 96-Hour Static Test.

Study Dates: November 27 to December 1, 1978

Study Number: 2142-78

Names and Addresses of Investigators: E. A. Yinger, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Test Article: Apramycin

Lot Number: X-24144, contains 71.4% apramycin activity

Species: Bluegill (Lepomis macrochirus)

Experimental Design:

Groups of ten juvenile bluegill (mean weight and length: 0.282 g and 27.8 mm, respectively) were exposed to test solutions with nominal apramycin concentrations of 0, 100 and 300 mg/L for 96 hrs (average assayed apramycin activity levels of 0, 116, and 331 mg/L). Jars with 15 L of test or control solution were used to contain each group of ten fish. Three replicates of ten fish were used for the highest concentration tested. Dissolved oxygen concentration, pH and temperature were recorded daily for each jar. Total hardness, total alkalinity, and conductivity were recorded once for the dilution water. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior, disoriented and/or labored respiration, and prostration) and mortalities were noted for fish in each jar on a daily basis. Test and control solutions were aerated during the last 24 hours of the study.

Results:

The water quality characteristics were as follows: pH, 7.7 to 8.4; dissolved oxygen, 47.5% to 98.6% saturation; temperature 22.5 to 24°C; total hardness, 239.4 mg/L as $CaCO_3$; total alkalinity, 285 mg/L as $CaCO_3$; and conductivity, 510 µmhos/cm. No mortalities and no behavioral abnormalities were found in the control or in the treatment groups.

APPENDIX L: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Rainbow Trout in a 96-hour Static-Test.

Study Dates: May 31 to June 4, 1978

Study Number: 2073-78

Names and Addresses of Investigators: E. A. Yinger, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Test Article: Apramycin

Lot Number: X-24144, contains 71.4% apramycin activity

Species: Rainbow Trout (Salmo gairdneri)

Experiment Design:

Groups of ten juvenile rainbow trout (mean weight and length: 0.315 g and 33.3 mm, respectively) were exposed to test solutions with nominal apramycin activity levels of 0, 50, 100, and 300 mg/L for 96 hours. Jars with 15 L of test or control solution were used to contain each group of ten fish. Three replicates of ten fish were used for the highest concentrations tested. Dissolved oxygen concentration, pH and temperature were recorded daily for each jar. Total hardness, total alkalinity, and conductivity were recorded once for the dilution water. Behavioral signs of toxicity (hypoactivity, minimal swimming behavior, disoriented and/or labored respiration, and prostration) and mortalities were noted for fish in each jar on a daily basis.

Results:

The water quality characteristics were as follows: pH, 8.2 to 8.7; dissolved oxygen, 67.6% to 97.2% saturation; temperature 12 to 12.5°C; total hardness, 239 mg/L as $CaCO_3$; total alkalinity, 310 mg/L as $CaCO_3$; and conductivity, 450 µmhos/cm. No mortalities and no behavioral abnormalities were found in the control or in the treatment groups.

APPENDIX M: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Daphnia magna in a 48-Hour Static Test.

Study Dates: November 28 to November 30, 1978

Study Number: 5058-78

Names and Addresses of Investigators: E. A. Yinger, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Test Article: Apramycin

Lot Number: X-24144, contains 71.4% apramycin activity

Species: Daphnia magna

Experimental Design:

Groups of 29 to 31 Daphnia, ≤ 18 hours old, were exposed to nominal apramycin levels of 0.0, 17.8, 28.4, 44.4, 71.0, and 106.5 mg activity/L for 48 hours. Each of three beakers with 200 ml of solution were used to contain 9 to 11 Daphnia, for each treatment or control solution. Test solutions were maintained at 18°C and had the following water quality characteristics: dissolved oxygen, at least 83% of saturation; pH, 8.2; total hardness, 240 mg/L as $CaCO_3$; total alkalinity, 290 mg/L as $CaCO_3$; and conductivity, 450 µmhos/cm. Daphnia were assessed for hypoactivity, prostration, and immobility.

Results:

No immobile daphnids and no hypoactive or prostrate daphnids were found at apramycin activity levels ≤ 28.4 mg/L. At 48 hours, seven daphnids were hypoactive and 24 were normal at an apramycin level of 44.4 mg/L. At an apramycin level of 71.0 mg/L, 24 daphnids were hypoactive, two were prostrate and six were immobile. At the highest apramycin level, 11 daphnids were hypoactive, three were prostrate, and 16 were immobile. The 48-hour EC_{50} (based on immobility) was 101.6 mg apramycin activity/L, with 95% confidence limits of 88.3 to 116.8 mg activity/L and a slope of 6.483 for the concentration-response line.

APPENDIX N: Report Summary

Title: The Effect of Apramycin on Microorganisms in Aerated Sewage

Study Number: S-AAC-79-06 and T93-230

Study Dates: S-AAC-79-06 - February, 1979 to October, 1979
T93-230 - January, 1979 to October, 1979

Name and Address of Investigator: R. M. Kline, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Test Article: Apramycin sulfate containing ^{14}C apramycin base

Test System: Laboratory aeration chambers and standard laboratory methods of analysis

Summary of Experimental Design:

Inoculum from a sewage plant's aerated lagoon was treated with apramycin. Treatments were made daily with increasing concentrations of apramycin up to a maximum of 102.4 mcg/ml. Total volume in the aeration vessels was held constant by removing a portion of the supernate before replacing it with a nutrient solution. The vessels were aerated with laboratory air at 3 ft^3/hr.

The effect of the compound on sewage microorganisms was determined by measuring biochemical oxygen demand (BOD-5 day), viable cell counts, pH, and solids content (dry weight).

Apramycin content during the experiment was determined by microbiological assay and radiochemical analyses.

Dissolved oxygen consumption using an acclimated inoculum, was determined in closed test systems with apramycin concentrations from 0.1 to 102.4 mcg/ml. The test extended for 14 days.

Summary of Results:

Using the standard methods for measuring biological activity in sewage systems, the effect of apramycin was minimal even at high concentrations.

BOD-5 day analyses on apramycin treated systems performed in the same manner as negative controls. High BOD values were observed immediately after feeding and reduced to low levels following incubation. Very low "0-time" values were obtained in latter stages of the experiment, but the microbial populations remained viable as determined by adding glucose-glutamic acid to the test containers.

At day 26 in Experiment T93-230 when the apramycin concentration was measured to be 78.6 ± 2.0 mcg/L the BODs at "0-time" and 4 days post treatment are shown in the following table.

APPENDIX N (continued)

BOD-5 day (mg/L)

Day	Post Treatment Time(Days)	Treated Systems	Negative Controls
26	0	2.3±1.4	1.5±.75
	4	0	.98±.88

Results of a separate experiment (study 79-06) were as follows:

BOD-5 day (mg/L)

Day	Post Treatment Time(Days)	Treated Systems	Negative Controls
23	0	131±32	334±11.6
	3	7.8±.8	3.75±.5

Apramycin assay results at 23 days were 79.7±3.9 mcg/ml.

Viable cell counts at the end of both experiments are listed in the following table.

Viable Cell Counts ($x10^5$ cells/ml)

Day	Post Treatment Time(Days)	Treated Systems	Negative Controls
Exp. T93-230			
26	0	62.0±11	23.9±5.7
	4	18.4±5.3	2.7±.15
Exp. 79-06			
26	0	18.1±3.1	13.6±5.0
	4	17.0±6.6	199±12.8*

*Only one tank was sampled.

There were also no significant differences between the pH values of controls and treated systems as shown below.

pH

	79-06		T93-230	
	Control	Treated	Control	Treated
Initial	7.8	7.85	7.40	7.45
Terminal	6.1	6.25	8.30	8.55

Dissolved oxygen utilization by apramycin acclimated sewage organisms was not affected until concentrations of 6.4 mcg/ml were reached. A range of concentrations from 0.1 to 102.4 mcg/ml was tested. These results reflect the low inoculum level and the broad spectrum of the antibiotic.

APPENDIX O: Report Summary

Title: The Toxicity of Compound 47657 (Apramycin) to Earthworms in a 14-Day Soil Incorporated Study.

Study Dates: July 14 to July 28, 1978

Study Number: 6015-78

Names and Addressed of Investigators: E. A. Yinger, C. C. Kehr, and J. L. Hamelink, Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Test Article: Apramycin

Lot Number: X-24144, contains 71.4% apramycin activity

Species: Lumbricus terrestris

Average Initial Weight: 3.9 g

Number of Animals: 10 animals/treatment,
15 animals in untreated control,
15 animals in solvent control

Levels of Exposure: 0.0 (control and solvent control),
10, and 100 ppm (nominal)

Length of Exposure: 14 days

Route: Incorporated into test media (rabbit feces, water, and non-sterile potting soil).

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

Experimental Design:

Test media was placed in 2 L cylindrical glass jars. Three jars were used for control and for the solvent control (0.02% Tween 80 was used as a wetting agent) and two jars were used at each of the treatment levels. Five worms were placed into each jar at the beginning of each study. The study was conducted at 12.5°C. Worms were weighed and their appearance was noted on test days 0, 3, 7, and 14.

Results:

Mortalities, changes in appearance, and reductions in body weight gain were not found in control, solvent control, or in the treatment groups. There were no apparent signs of toxicity at the two concentrations of apramycin activity that were tested.

APPENDIX P: Report Summary

Title: Greenhouse Phytotoxicity Study of Manure from Apramycin-Treated Swine.

Study Number: ABC-0011

Study Dates: March 1, 1978 to May 1, 1978

Name and Address of Investigators: L. L. Zornes, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140.

Test Article: Manure from apramycin-treated swine

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

Summary of Experimental Design:

Feces from swine which had been fed 110 ppm ^{14}C apramycin (as the sulfate salt) served as the test article and feces from swine fed a control ration served as the feces control. The test article contained 71% moisture and approximately 54 ppm apramycin on a wet basis or approximately 186 ppm on a dry weight basis. Air-dried feces were incorporated into greenhouse potting soil at rates equivalent to 11.2 and 22.4 metric tons of wet feces per hectare (5 and 10 U.S. tons per acre). Greenhouse flats were prepared at these rates for both the treated and control feces, as well as control soil to which no feces were added.

A standard greenhouse phytotoxicity test was conducted in which 14 mono- and dicotyledonous plants were grown from seed in the treated and untreated soils. The plant species were alfalfa (Medicago sativa), fescue (Festuca elatior), cucumber (Cucumis sativus), rice (Oryza sativa), cotton (Gossypium hirsutum), tomato (Lycopersicon esculentum), pepper (Capsicum annuum), corn (Zea mays), sugar beet (Beta vulgaris), barley (Hordeum vulgare), soybean (Glycine max), wheat (Triticum aestivum), grain sorghum (Sorghum bicolor), and oats (Avena sativa). Plants were rated for phytotoxic injury (0 = no injury, to 10 = complete kill) and injury, described as chlorosis, burning, stunting, or reduced germination, was noted 21 days after planting.

Summary of Results:

All ratings were zero (no injury) compared to the untreated soil control with the exception of peppers. Peppers in the 22.4 mt/ha flats for both control feces and treated feces had ratings of 2S (slight stunting injury). The fact that this stunting occurred in both the control feces and treated feces flats demonstrates that it was not due to apramycin treatment. Therefore, there was no apramycin-related phytotoxicity due to growing these 14 plant species in soil manured with feces from apramycin-treated swine.

APPENDIX Q: Report Summary

Title: The Susceptibility of Selected Species of Nitrogen Fixing Organisms to Apramycin

Study Number: I-BSD-79-03, I-BSD-79-05, I-BSD-79-10

Study Dates: March, 1979 - September, 1979

Name and Address of Investigator: R. M. Kline, Lilly Research Laboratories, Division of Eli Lilly and Company, P. O. Box 708, Greenfield, Indiana 46140

Test Article: Crystalline apramycin

Test System: Pure culture techniques under laboratory conditions using standard laboratory procedures.

Summary of Experimental Design:

The effect of apramycin on growth of the following organisms was determined:

Azotobacter chroococcum	A.T.C.C. 9043
Anabaena flos-aquae	U.TEX. 1444
Rhizobium japonicum (1)	3I 1b 110
Rhizobium japonicum (2)	3I 1b 142
Rhizobium japonicum (3)	A.T.C.C. 10311
Rhizobium japonicum (4)	Field Isolate
R. leguminosarum	128C53

All were studied in pure culture and the blue-green algae was axenic.

Nitrogen fixation, as determined by acetylene reduction was determined using cultures of A. flos-aquae and Azotobacter chroococcum.

APPENDIX Q (continued)

Summary of Results:

Growth of the gram-negative organisms showed different degrees of sensitivity to apramycin as shown in the following table:

Organism	Concentration Showing Growth Reduction
A. flos-aquae	0.1 mcg/ml
A. chroococcum	0.1 mcg/ml
R. japonicum (1)	1.0 mcg/ml
R. japonicum (2)	1.0 mcg/ml
R. japonicum (3)	10.0 mcg/ml
R. japonicum (4)	10.0 mcg/ml
R. leguminosarum	0.1 mcg/ml

All readings were made at 72 hours posttreatment.

Nitrogenase activity as measured by the conversion of acetylene to ethylene was variable but apramycin was effective in reducing nitrogen fixation by A. chroococcum at 0.01 ppm in vitro. There was also a reduction in acetylene reduction by A. flos-aquae at 1 ppm but after 96 hours the response appeared equal to nontreated controls.