APRALAN™ (Apramycin Sulfate) SOLUBLE POWDER
Environmental Impact Analysis Report

Applicant: Elanco Products Company
A Division of Eli Lilly and Company
740 South Alabama Street
Indianapolis, Indiana 46285

SUMMARY

The applicant has filed a New Animal Drug Application to provide for the use of apramycin sulfate as an additive to swine drinking water for the treatment of porcine colibacillosis. Apramycin sulfate is a water-soluble antibiotic of the aminocyclitol group. It is to be marketed in the form of a water soluble powder for incorporation into the drinking water of young swine.

The following environmental information is included as prescribed by 21 CFR 25.1(j) (0).:

I. Describe the Proposed Action

A. Elanco Products Company has filed a New Animal Drug Application for APRALAN (apramycin sulfate) SOLUBLE POWDER to be used for the treatment of porcine colibacillosis by addition to the drinking water of weanling swine.

APRALAN SOLUBLE POWDER will be recommended for use at a concentration which will provide 0.75 g apramycin activity/gallon of drinking water with label directions that treated pigs should consume enough medicated drinking water to receive 25 mg apramycin

activity per kg of body weight daily. The treatment should be continued for seven days.

B. The environments affected if this action is taken are the apramycin fermentation and packaging sites and the farms where swine would be treated.

Fermentation and product recovery of apramycin will be performed by Eli Lilly and Company subsidiary, Eli Lilly Industries, Inc., Carolina, Puerto Rico.

Blending and filling will be at Eli Lilly and Company, Omaha Laboratories, Omaha, Nebraska or Clinton Laboratories, Clinton, Indiana or Tippecance Laboratories, Lafayette, Indiana.

II. Discuss the Probable Impact of the Proposed Action on the Environment Including Primary and Secondary Consequences

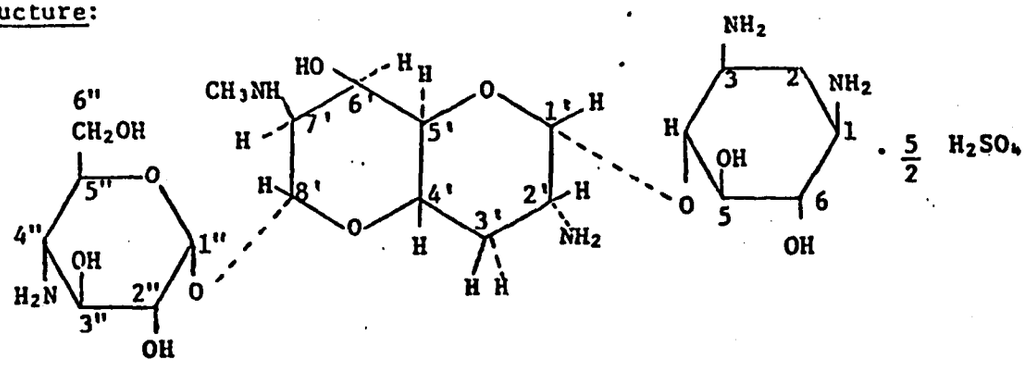
Describe Probable Adverse and Beneficial Effects:

2.(a) Physical Chemical Data

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-dipyranos-1-yl]-2-deoxy-sulfuric acid salt

Formula: $C_{21}H_{41}N_5O_{11}.\frac{5}{2}\ H_2SO_4$

Structure:



Molecular Weight: 784.8

Water Solubility: Larger than 300 g/L

pKa Values: The pKa values were determined by aqueous titration (1) and by using ^{15}N and ^{13}C nuclear magnetic resonance spectroscopy (2).

Aqueous titration resulted in pKa values of: 5.4; 6.2; 7.2; 7.8 and 8.5.

^{15}N and ^{13}C nuclear magnetic resonance spectroscopy resulted in pKa values of:

6.4 - 6.6 (N-4"), 6.6 - 6.8 (N-3), 7.3 - 7.4 (N-7'), 7.6 - 7.7 (N-2'), 8.1 (N-1).

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

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

Vapor Pressure: Apramycin and apramycin sulfate are non-volatile solids.

Octanol-Water Partition Coefficient: The n-octanol-to-water partition coefficient (K) of apramycin was determined to have a value of 5×10^{-4} at a pH of 13. It is estimated that the partition coefficient of apramycin sulfate is at least as small as that of apramycin.

Ion Exchange Capability: Apramycin in its protonated or partially protonated form binds strongly to weak or strong cation exchange resins. The clay fraction of soil as well as the organic fraction of soil are known to carry negative charges and are believed to interact with apramycin in a similar manner.

(1) S. O'Conner et al, J. Org. Chem., 41, 2087 (1976)
(2) J. W. Paschal and D. E. Dorman, Org. Magn. Reson., 11, 632 (1978).

B. Environmental Introduction

Apramycin will be introduced into the environment primarily in the feces from pigs treated with apramycin. Urine will contain very small amounts of apramycin. The fecal and urinary radioactivity in pigs treated with ^{14}C labeled apramycin was predominantly unchanged apramycin, indicating little metabolism of the compound.

1. Expected Concentration in Soil

Colibacillosis is a diarrheal disease of weanling pigs. Calculations based on sow numbers and pigs farrowed/sow reported in the USDA Meat and Livestock Report, 1979, estimate an annual pig crop of 90 million. Assuming that 85 percent of all pigs (76.5 million) will contract a diarrheal disease following weaning (Billy E. Hooper, DVM, MS, Ph.D., Associate Dean for Academic Affairs, Purdue School of Veterinary Medicine, BVM Seminar, Feb. 23, 1978) and that all infected pigs are treated with APRALAN SOLUBLE POWDER for seven days (25 mg/kg X 10 kg pig X 7 days = 1750 mg apramycin per pig), the maximum amount of drug administered in the United States would be 133,875 kg annually.

Apramycin excreted by treated pigs will be added to the land surface as semi-solid or liquid waste spread onto the soil following storage in collection pits.

Approximately 70 percent of all weanling pigs are raised in nurseries using semi-solid or liquid waste disposal. (Large and Medium Volume Hog Producers, V. James Rhodes, Calvin Stemme, and Glenn Grimes, University of Missouri, Columbia Agriculture Experiment Station, February, 1979.) An example of a mode and rate of introduction of apramycin into the environment is as follows:

Assumptions:

a. Farrow to finish operation selling 20,000 hogs/year and producing 19,984 tons of manure/year[1] (semi-solid or liquid waste).

b. 100 percent of weanling pigs treated with 25 mg apramycin/kg/day in the drinking water for seven days (assume a pig weight of 10 kg).

c. 25 mg apramycin/kg/day x 10 kg x 7 days = 1750 mg = average drug consumed/pig over seven-day period.

d. 75 percent of dose is excreted in the urine and feces (estimated from ^{14}C excretion study).

e. 1 acre, 6" depth = 2×10^6 lb. soil

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[1]Personal Communication, A. L. Sutton, Ph.D., Department of Animal Science, Purdue University, West Lafayette, Indiana.

f. Application rate 40 T manure/A

1750 mg consumed x 0.75 = 1300 mg excreted/pig

1300 mg excreted/pig x 20,000 pigs/yr. = 2.6×10^7 mg excreted/yr.

$$\frac{2.6 \times 10^7 \text{ mg excreted/yr.}}{19{,}984 \text{ T manure/yr.}} = 1300 \text{ mg apramycin/T manure}$$

Apply 40 T manure/A

1300 mg apramycin/T x 40 T manure/A = 52,000 mg apramycin/A

A = 2×10^6 lb. = 907,185 kg[1]

$$\frac{52{,}000 \text{ mg apramycin/A}}{907{,}185 \text{ kg/A}} = 0.057 \text{ ppm apramycin}$$

Thus, the soil concentration of apramycin resulting from the treatment of 20,000 pigs over a one year period with APRALAN SOLUBLE POWDER would be 0.057 ppm.

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[1]The Nature and Properties of Soils, T. Lyon, H. Buchanan, W. Broody. MacMillan Company, 5th Ed., 1952, p. 60.

2. Expected Concentration in Manure in a Swine Waste Facility

Feces, from pigs treated with APRALAN SOLUBLE POWDER, entering a swine waste facility would result in a gradual increase in apramycin concentration in the waste. Using the same data as used in the calculation of apramycin soil concentration and assuming a cleaning of the waste facility once a year, the highest concentration of apramycin in manure attained would be:

$$\frac{2.6 \times 10^7 \text{ mg apramycin excreted/yr.}}{19{,}984 \text{ T manure/yr.}} = 1300 \text{ mg apramycin/T manure}$$

$$\frac{1300 \text{ mg apramycin/T}}{909 \text{ kg manure}} = 1.43 \text{ ppm}$$

No dilution of the manure with water would be a "worst case" assumption.

C. Environmental Fate

1. Accumulation in Soil

The soil persistence study with apramycin (NADA-106-964, pp. 72-85) indicated a soil half-life for apramycin of over one year. Therefore, it is projected that apramycin will accumulate in soils which receive manure from pigs fed apramycin over a period of several years.

Under worst possible conditions (a degradation rate of only 15 percent per year for apramycin in soil) and assuming 0.1 ppm apramycin added to the soil per year, the accumulation of apramycin in soil has been estimated graphically to infinity in Figure 1. The formula used for the calculation and actual apramycin values at specific times have been included. The highest possible accumulation of apramycin is 0.6667 ppm.

Considering the physical-chemical properties of apramycin, little, if any, environmental transformation would be expected when apramycin is added to soil. The pKa values indicated that apramycin will adhere strongly to soils. Pinck, et al.[1], evaluated the affinity of various

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[1]Pinck, L. A., Holton, W. F., and F. E. Allison, 1961. Soil Sci. 91: 22-28
Pinck, L. A., Soulides, D. A., and F. E. Allison, 1961. Soil Sci. 91: 94-99.

antibiotics to soils and concluded that the aminoglycosides were strongly bound to soils and were microbiologically inactive. Apramycin would react similarily. Strong chemical procedures must be utilized to extract apramycin from soil for assay.

The soil leaching study with apramycin (NADA 106-964, pp. 136-149) in which apramycin failed to leach through soil, also suggests strong adherence of apramycin to soil.

It can be concluded from these data that the ultimate fate of apramycin from usage in pigs will be the attachment to soil particles in a relatively immobile form. Under worst case conditions, accumulation of low levels of soil bound apramycin will occur followed by a steady state.

2. Expected Concentration in Streams or Ponds Due to Runoff

There are two major means whereby apramycin may conceivably be present in runoff streams or ponds. The first is the leaching of apramycin from soil by runoff water. The second is the washing of apramycin soil sediments by runoff water.

As discussed in section C. 1. above, the highest possible accumulative concentration of apramycin in soil would be

0.6667 ppm. Based upon the results of laboratory leaching studies, runoff streams and ponds would be expected to contain much less than 0.6667 ppm of dissolved apramycin.

Apramycin would, conceivably, be washed into ponds while bound to soil particles. However, the physical-chemical properties of apramycin, i.e., five amino groups with pKa values (aqueous titration) ranging from 5.4 to 8.5, eleven ether or hydroxyl groups, and a n-octanol-water partition coefficient of 5×10^{-4} all support the conclusion that apramycin would be energetically bound to charged soil particles. As Hamelink[1] has discussed, under these conditions apramycin would not be expected to be bioavailable to aquatic organisms in a pond.

3. Expected Concentration in Plants

Any accumulation of apramycin in plant material would be derived from the uptake by the roots of plants growing in soil containing apramycin. Based on the degree to which apramycin is bound to soil, plants would be expected to contain, if any, much less than 0.6667 ppm of apramycin, the highest possible accumulative concentration in soil.

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[1]Hamelink, J., Biotransformation and Fate of Chemicals In The Environment, A. W. Maki, K. L. Dickson, and J. Cairns, Jr., Eds. American Society of Microbiology, Wash. D.C., 1980, pp. 56-62.

4. Expected Bioaccumulation in Fish

The n-octanol-water partition coefficient (P) for apramycin is 5×10^{-4}. Neely, Branson, and Blau[1] developed a regression equation for projected steady-state residue concentrations in trout muscle versus calculated n-octanol-water partition coefficients for a variety of synthetic compounds:

$$\log (\text{bioconcentration factor, BCF}) = 0.542 (\log P) + 0.124$$

Using this equation the predicted BCF for apramycin is 0.026.

The solubility of apramycin is at least 300 g/L. The regression equation for BCF for fish versus water solubility as described by Kenaga and Goring as cited in Lambert[2]:

$$\log BCF = 2.791 - 0.564 \log S$$

Using this equation the predicted BCF for apramycin is 0.50, where S is expressed in units of mg/L.

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[1]Neely, W. B., Branson, D. R., and Blau, G. E., Environmental Science and Technology, Vol. 8, No. 13, Dec. 1974, pp. 1113-1115.
[2]Lambert, S. M., Agric. Food Chem. 16 340-343 (1968).

D. Environmental Effects and Assessment

1. Environmental Effects - Wildlife Mammals

Apramycin has also been studied extensively in toxicological studies to determine effects upon animals and humans. Toxicity studies involving mice, rats, rabbits, guinea pigs, chickens, cats and dogs were conducted to establish the safe level of apramycin residue and to ensure human safety in the manufacture, control, distribution and use of apramycin. The effects of large, single oral doses of apramycin (520-5200 mg activity/kg-depending on species) were studied in mice, rats, dogs, rabbits, guinea pigs, chickens, calves and pigs. In all cases, the maximum dose given was limited not by the toxicity of the compound, but by the volume of material that could be administered. In most experiments, there were no signs of toxicity other than transient diarrhea and cachexia. No significant irritation, sensitivity or systemic toxicity was seen in tests of dermal, ocular and inhalation toxicity conducted in rabbits, guinea pigs and rats. Apramycin was found to be non-mutagenic in three mutagenic screens utilizing current, accepted methodologies, sufficient dose ranges and both positive and negative controls. Apramycin produced no teratological effects in rats and rabbits at doses of 1000 mg apramycin activity/kg and 32 mg

activity/kg, respectively. Daily oral administration of 100 mg apramycin activity/kg to dogs for one year and 1000 ppm apramycin activity to rats for six months produced no discernible toxic effects.

2. Environmental Assessment - Wildlife Mammals

Exposure of wildlife mammals to apramycin would be through the ingestion of plant materials, prey, or by drinking runoff water contaminated with apramycin. As described in section D. 1., apramycin has a very low order of acute and chronic toxicity to a wide variety of mammalian species.

As described in section C., expected concentrations of apramycin in plant material or runoff streams and ponds is expected to be extremely low. Since apramycin has an extremely low predicted bioconcentration factor, it would not biomagnify in mammalian predator animals.

Therefore apramycin would not be expected to adversely affect wildlife mammals.

3. Environmental Effects - Avian Species

5-Day dietary studies in bobwhite quail and mallard ducks (NADA 106-964, submission March 13, 1980, pg. 204-229 and

230-255) concluded that there were no apparent toxic effects in ducks or quail that consumed diets containing as much as 0.500% w/w apramycin. Based on food consumption and body weight data for the two studies, these dietary concentrations equate to average doses of 1838 mg of apramycin/kg of body weight/day for mallards and 1050 mg of apramycin/kg of body weight/day for quail.

4. Environmental Assessment - Avian Species

Wildlife avian species could conceivably be exposed to apramycin by ingesting plant material containing apramycin residues, or by drinking stream or pond water containing apramycin. Based on the expected apramycin concentration in soil, plant materials or runoff water, apramycin should not have an adverse effect on avian species.

5. Environmental Effects - Fish

Rainbow trout and bluegills were exposed to an aqueous solution of apramycin for 96 hours (Apramycin Premix NADA, pg. 1528-1533 and 1534-1541). All fish survived and no changes were observed in their behavior or appearance. The no observed effect level for these studies were 300 ppm of apramycin, the highest level tested.

6. Environmental Assessment - Fish

As discussed above in section C., the expected concentration of apramycin in runoff streams or ponds would be less than the maximum soil concentrations of 0.6667 ppm.

Based on the above data, apramycin should not have an acute adverse effect on fish. If one uses an application factor of 0.1 to relate acute effects to estimate a Maximum Allowable Toxicant Concentration (MATC) for chronic effects[1],

$$MATC = (LC_{50}) \text{ (Application Factor)} = (>300 \text{ ppm}) \times (0.1) = > 30 \text{ ppm}$$

Thus apramycin would not be expected to have a chronic adverse effect on fish. This is supported by the low toxicity of apramycin in chronic and reproduction studies in mammalian species, and the low predicted bioconcentration for apramycin.

[1]U.S. EPA's draft guidance document entitled "Use of Effluent Toxicity Testing in the Second Round of NPDES Permit Issuance" (January 1981).

7. Environmental Effects - Aquatic Invertebrates

Daphnids were exposed to aqueous solutions of apramycin for 24 and 48 hours (Apramycin Premix NADA, pg. 1517-1527). Immobilization and activity were the responses observed. The no observed effect level at 24 and 48 hours for this study was 44.4 and 28.4 ppm of apramycin activity, respectively. The 24-hour EC_{50} (based on immobility) was 105.1 ppm of apramycin activity with 95% confidence limits of 90.6 to 121.9 ppm (dose-response line slope of 6.524); the 48-hour EC_{50} was 101.6 ppm of apramycin activity with 95% confidence limits of 88.3 to 116.8 ppm (dose-response line slope of 6.483). The dose-response data were linear in a chi square evaluation ($P \leq 0.05$).

8. Environmental Assessment - Aquatic Invertebrates

As discussed above in section C. the expected concentration of apramycin in runoff streams or ponds would be less than the maximum soil concentration of 0.6667 ppm.

Based on the above data, apramycin would not be expected to have an acute adverse effect on aquatic invertebrates.

9. Environmental Effects - Annelids

Earthworms were exposed to 100 ppm soil-incorporated apramycin for 14 days (Apramycin NADA, pg. 1510-1516). Apramycin caused no mortalities, no changes in live body weight and no changes in appearance of the animals.

10. Environmental Assessment - Annelids

As discussed in section B. the expected maximum soil concentration of apramycin is calculated to be 0.6667 ppm. Based on a 14-day no observed effect level of 100 ppm of apramycin for worms, apramycin would not be expected to adversely affect annelids.

11. Environmental Effects - Plants

Apramycin when tested in vitro against Rhizobium japonium, was active at levels of 0.1 to 10 mcg/ml. Nevertheless, under in vivo conditions, apramycin at levels as high as 160 ppm in the soil did not affect rhizobium activity in the roots of soybeans (NADA 106-964, pp. 100-106).

In addition to these studies, an apramycin phytotoxicity study (NADA 126-050, pp. 1504-09) evaluated the effect of

feces obtained from pigs fed apramycin at a level of 100 g/ton and applied to the soil at levels as high as 22.4 metric ton per hectare. There were no grossly detected effects.

12. Environmental Assessment - Plants

Based on the lack of effects observed when plants were exposed to high soil levels of apramycin, and the maximum soil concentration of 0.6667 ppm of apramycin from use of the product, apramycin would not be expected to cause adverse effects to plants.

13. Environmental Effects - Microorganisms

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 (NADA 106-964, pp. 107-135).

Apramycin was tested against a group of nitrogen fixing organisms to determine its effect on atmospheric nitrogen reduction and growth in broth culture. The organisms included an alga, Anabaena flos aquae, a free living bacterial heterotroph, Azotobacter chroococcum and several species of symbiotic nitrogen fixing rhizobia. All are gram negative organisms and showed different degrees of sensitivity to the antibiotic. Growth of A. flos aquae, A. chroococcum, and the pea symbiont Rhizobium leguminosarum was inhibited by apramycin concentrations of 0.1 ppm. Four strains of R. japonicum, the soybean symbiont, were inhibited by apramycin concentrations between 0.1 and 10 ppm.

The ability of the free living organisms to fix atmospheric nitrogen was determined by acetylene reduction and test results paralleled growth. Rhizobium cultures do not reduce atmospheric nitrogen outside the legume root nodule and tests were not performed in the in vitro test system (REF: NADA 106-964, Submission March 18, 1980, pg. 82-99).

14. Environmental Assessment - Microorganisms

Apramycin did not cause detectable effects in an aerated sewage system at 102.4 ppm. This level is 71 times the expected concentration of apramycin in manure (1.43 ppm).

Apramycin would not be expected to affect swine waste treatment systems.

Apramycin in broth culture does have inhibiting activity on nitrogen fixing organisms as described herein. However, as discussed in section C. apramycin, like other aminoglycosides would be expected to be strongly bound to soils and would therefore be microbiologically inactive. Strong chemical procedures must be utilized to extract apramycin from soil for assay. Based on these data, apramycin would not be expected to adversely affect soil microorganisms.

In conclusion, there are no known adverse effects upon the environment, animals, or man which will result from the use of APRALAN SOLUBLE POWDER.

a. Describe measures taken to avoid or mitigate potential adverse environmental effects.

As discussed above, there are no anticipated adverse environmental effects. Therefore, no steps to mitigate such effects are planned.

b. Analyze the environmental impact of the manufacturing processes of the article.

1) Identification of pollutants expected to be emitted: The manufacturing processes for apramycin are designed to have minimal environmental impact. Apramycin is produced by standard fermentation techniques, separated by adsorption and eluted, concentrated, converted to the sulfate, dried and blended. Waste streams are concentrated and segregated at the source and are either dried for landfilling or treated in the activated sludge waste treatment plant.

Air Emissions

The manufacturing of apramycin does not produce air emissions, which are considered potentially dangerous. Air emissions include steam generation, fermentor vents and exhausts. Since fuel oil is the energy source, sulfur dioxide, hydrocarbons, etc., are at low concentrations. Apramycin itself does not volatilize.

Water Wastes

Water wastes from the fermentation process are treated in a biological waste treatment system.

Solid Wastes

Solid wastes including trash containers, paper other solid combustible materials are delivered contract hauler to a landfill for burial.

2) Applicable Federal, State and Local Emission Requirements

Water effluent limits are established by the U. Environmental Protection Agency, with reviews b Environmental Quality Board, Puerto Rico, using unpublished pharmaceutical guidelines for treat efficiency. NPDES Permit No. PR 0021423, March 1977, has been issued to Eli Lilly Industries,

Air emission limits are established by the U.S. Environmental Protection Agency and by the Environmental Quality Board, Puerto Rico. Construction permits for 2 boilers, a vent cond and ammonia scrubber have been issued to Eli Li Industries, Inc. under reference 475-0110-II-0. Operating permits are forthcoming.

3) Certification

We hereby certify that the described emissions are in compliance with the cited requirements.

III. Discuss the probable adverse effects that cannot be avoided

Under the worst possible case (farm producing 20,000 pigs/year, all treated with APRALAN SOLUBLE POWDER, manure spread on some acreage for > 20 years), accumulation of 0.6667 ppm of apramycin would be achieved. Considering the chemical-physical characteristics of apramycin, concentrations of this magnitude should not produce adverse effects.

Any manufacturing process must unavoidably make some minimal contribution of by-products, organic and inorganic, to the environment. The precautionary measures outlined above will keep these to an absolute minimum.

Further, it is readily apparent if the production of apramycin sulfate is compared with the scope of the entire fermentation industry, e.g., medicinals, chemicals and alcohol, the adverse contribution of the proposed action to the total environmental burden would be nil.

IV. Evaluate alternatives to the proposed action

There is no known alternative product possessing all of the desirable characteristics of APRALAN SOLUBLE POWDER for the use described above. There are no feasible alternatives to the raw materials used in the manufacture of apramycin sulfate which results in a reduced environmental burden.

V. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity

Short-term effects upon the environment are negligible as discussed in Sections "II" and "III". There is no cumulative adverse effect upon the environment since potential materials are added and dispersed at a low controlled rate as described in Section 2. Because of these factors, there will be no long-term detrimental effect upon the productivity of the environment.

Considerable overall benefits will accrue from the use of apramycin sulfate in exchange for possible minimal local effects due to use and manufacture of the product. The therapeutic use of apramycin sulfate will decrease losses due to morbidity and mortality caused by colibacillosis and result in better weight gain and feed efficiency. The more efficient use of feed supplied to the pigs will thus make more pork protein available as a source of human food.

Improving the quality and efficiency of swine management means that more pounds of meat for human consumption will be produced per ton of feed and unit of energy. In the long run, food will be provided for a larger number of people without increasing the environmental burden resulting from the production of feed and energy.

VI. Describe any irreversible and irretrievable commitment of resources which would be involved in the proposed action should it be implemented

A portion of the raw materials used in the manufacture of apramycin sulfate will be ultimately discharged into the ecosphere as indicated in Sections "II" and "III". The organic portion of the by-products will be bio-degraded and ultimately returned to the natural carbon and nitrogen cycles. Due to the economics of the processes involved, such chemical entities are irretrievable and, therefore, the original commitment of resources may be regarded as irreversible.

VII. Discuss the objections raised by other agencies, organizations, or individuals which are known to the applicant

The applicant is unaware of any such objections. Numerous other products for the medication of swine feeds and water have been used in the United States and elsewhere for many years without apparent significant adverse effects upon the environment.

VIII. If proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement for 30 days from the filing of a final environmental impact statement, explain why

The information presented herein obviates the requirements for an environmental impact statement since the proposed action will result in no significant or cumulative adverse effect upon the environment.

IX. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risks to the environment

Implementation of the proposed action will provide a valuable improvement to the techniques of swine husbandry with the foreseeable benefits outlined in Sections "II" and "V". A further

foreseeable benefit will be an increase in the supply of pork. An additional benefit is provided by the more efficient utilization of natural resources such as feed and energy in the production of pork for human consumption. There is only minimal potential risk due to the introduction of apramycin sulfate into the environment through the swine excreta or from the emission of by-products during manufacture. Irretrievable depletion of natural resources due to the manufacture of apramycin sulfate is negligible in practical terms.

The benefit to the public of the proposed action greatly outweighs any potential present or future risk to the environment.

August 12, 1981

Date

Carl S Pruitt.

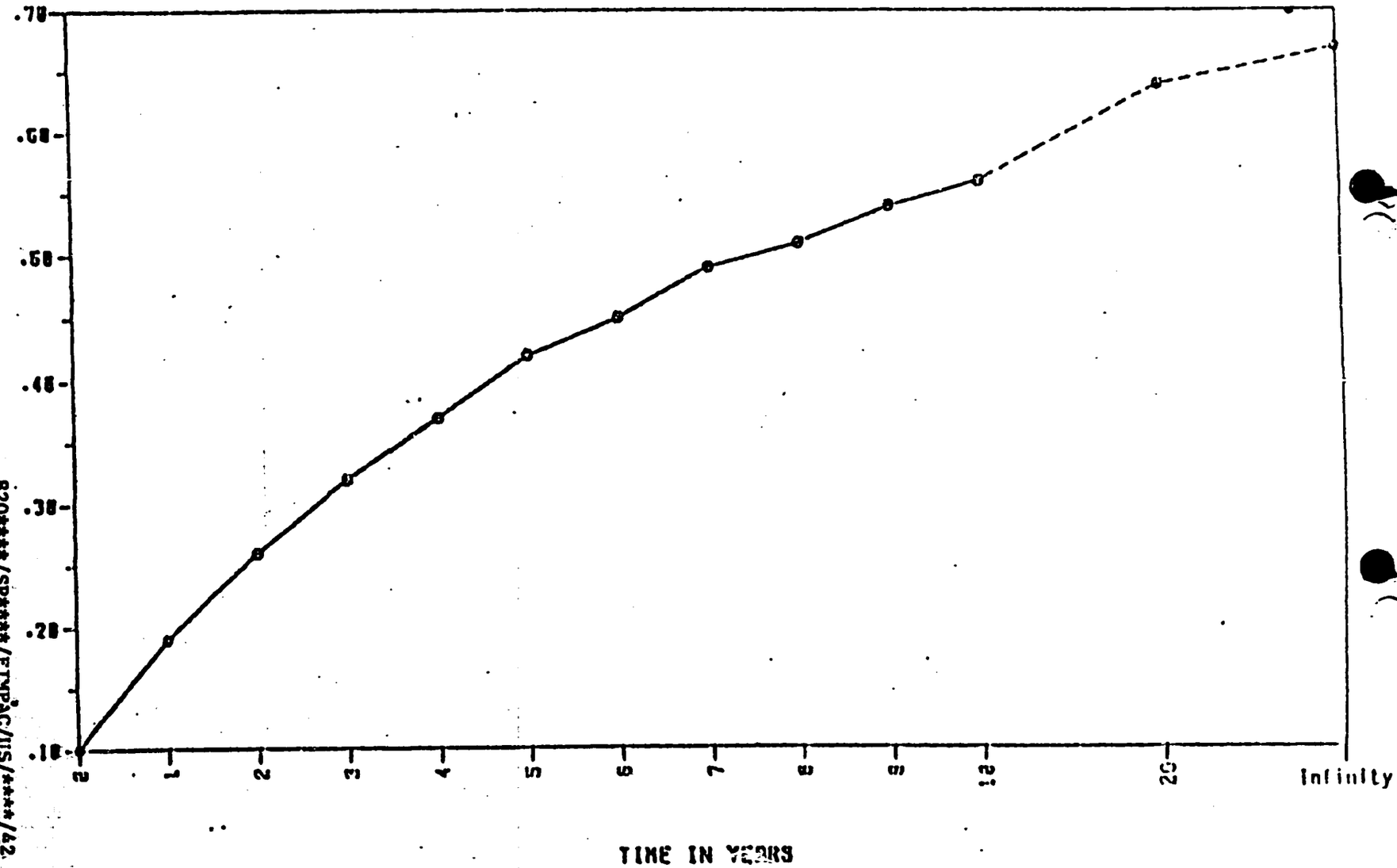
Signature

C. S. Pruitt, D.V.M.

820****/SP****/EIMPAC/US/****/41

FIGURE 1

ACCUMULATION OF APRAMYCIN IN THE SOIL. ASSUMING WORST CASE (15%) DEGRADATION/YEAR*



*With the addition of 0.1 ppm per year

Attached is a table of the accumulation of apramycin in the soil over time. Assuming an initial concentration of 0.1 ppm, a yearly degradation of 15% of the amount of apramycin at the beginning of each year, and a once-a-year addition of 0.1 ppm of apramycin, the compound would accumulate in the soil up to a theoretical maximum of 0.6667 ppm. The concentration at the beginning of each year for the first ten years, after twenty years, and the maximum are given in the table and plotted in the accompanying figure. The concentration at any point in time can be computed from the following equation:

$$A_{t_n} = A_o \frac{1-e^{-nkD}}{1-e^{-kD}} e^{-kt}$$

where A_{t_n} = concentration at time t after n doses of the compound (i.e. n years)

A_o = initial concentration

k = rate constant associated with 15% degradation (0.1625)

D = dose interval (i.e. D = 1 year)

n = number of doses (years)

t = time (ranges from 0 to D)

Fundamentals of Clinical Pharmacokinetics - John G. Wagner
Drug Intelligence Publications, Inc., Hamilton, Illinois 6234l, First Edition 19
Hamilton Press, Inc., Hamilton, Illinois 6234l

Years of Accumulation	Concentration in the Soil after Addition of 0.1 ppm of Apramycin
0	.1
1	.1850
2	.2573
3	.3187
4	.3709
5	.4152
6	.4529
7	.4850
8	.5123
9	.5357
10	.5554
20	.6446
	.6667

SUMMARY OF ENVIRONMENTAL FATE AND EFFECTS TESTS

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

A ^{14}C apramycin leaching study was conducted using two different soil textures. Each column was leached with a total of 48 ml deionized water which was equivalent to 60 cm of rainfall. The data indicated that apramycin did not leach in sandy loam or silt loam soil textures.

(REF: NADA 106-964, Submission March 18, 1980, Pg. 136-149)

^{14}C Apramycin Greenhouse Soil Decline Study

The decline of ^{14}C apramycin in soil was studied. Soil was fortified with ^{14}C apramycin (sulfate salt) at a nominal level of 10 mg apramycin base per kilogram of soil. The fortified soil was maintained in the greenhouse and samples were assayed periodically for total radioactivity, extractable radioactivity, and apramycin. After 52 weeks, the total radioactivity was 83% of the initial value and the extractable radioactivity was 53% of the initial value. Most of the extractable radioactivity was ^{14}C apramycin.

A more vigorous extraction of the spent 52-week soil sample released approximately 18% more of the total radioactivity, of which approximately one-third was ^{14}C apramycin. The radioactivity in the 52-week sample was approximately two-thirds ^{14}C apramycin. The remaining one-third was nearly equally divided between extractable degradation products and tightly bound radioactivity (not extractable with hot alkali). Therefore, there was a decline of ^{14}C apramycin in soil during the 52-week observation period.

(REF: NADA 106-964, Submission March 18, 1980, pg. 1-10)

72-85

The Susceptibility of Selected Species of Nitrogen Fixing Organisms to Apramycin

Apramycin was tested against a group of nitrogen fixing organisms to determine its effect on atmospheric nitrogen reduction and growth in broth culture. The organisms included an alga, Anabaena flos aquae, a free living bacterial heterotroph, Azotobacter chroococcum and several species of symbiotic nitrogen fixing rhizobia. All are gram negative organisms and showed different degrees of sensitivity to the antibiotic. Growth of A. flos aquae, A. chroococcum, and the pea symbiont R. leguminosarum was inhibited by apramycin concentrations of 0.1 ppm. Four strains of R. japonicum, the soybean symbiont, were inhibited by apramycin concentrations between 1 and 10 ppm but not by 0.1 ppm.

0.01-1

The ability of the free living organisms to fix atmospheric nitrogen was determined by acetylene reduction and test results paralleled growth. Rhizobium cultures do not reduce atmospheric nitrogen outside the legume root nodule and tests were not performed in the in vitro test system.

(REF: NADA 106-964, Submission March 18, 1980, pg. 82-99)

86-99

9/11/81 - [illegible]

Apramycin incorporated to depths of 4 and 9 inches at rates of 480 lbs/A (160 ppm) and below caused no statistically significant inhibition of nodulation or acetylene reduction (nitrogenase) activity. The data from the high apramycin rate in the 9 inch incorporation study indicated a 24.8% reduction in mean nodule weight. However, a wide range of plant to plant variation in nodule development and size is expected. In this case one of the replicates from the high treatment group produced 23% more nodules than the control average. The second highest rate, 120 lb/A (40 ppm) also showed a decrease from controls which was statistically insignificant. The coefficient of variation was above 40% in 5 of 6 treatments and above 60% in half the treatments.

Nodules collected from plants treated with 480 lb/A (160 ppm) apramycin were compared with nodules from controls for nitrogenase activity. Duplicate samples from each replicate were assayed with the single most deviant value from each group dropped from the statistical analysis. The average values from control and treated plants were not significantly different. The coefficient of variation was 15% for apramycin treatments and 23% for controls.

Analysis by Duncan's multiple range test for ordered means showed no difference with either parameter for any treatment. The results are summarized in the following table.

Summary Table

	Effect on Nodules			Nitrogenase Activity	
Apramycin Rate	Gm Nod. Wt/ 8 Plants		%C*	m acetylene reduced/ gram nodules/hr	
160 ppm (480 lb/A)	3.25	A	-24.80	8.33	A
40 ppm (120 lb/A)	3.49	A	-19.17		
10 ppm (30 lb/A)	4.62	A	+6.96		
2.5 ppm (7.5 lb/A)	4.45	A	+3.07		
0.63 ppm (1.88 lb/A)	4.23	A	-2.08		
Control	4.32	A	0.00	8.36	A

Letters following a mean indicate significance based on a Duncan's Multiple Range Test at the 5% level.

* %C reflects the percentage change between treated and control plants.

(REF: NADA 106-964, Submission March 18, 1980, pg. 100-106)

The Effect of Apramycin on Microorganisms in Aerated Sewage

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. Apramycin was not detrimental to the digestive process.

(REF: NADA 106-964, pg. 107-135)

Acute Oral Toxicity - Bobwhite Quail

Adult bobwhite quail (Colinus virginianus) were given single oral doses of apramycin in two separate studies. Doses of 0, 700, 1000, 1400, and 2000 mg apramycin activity/kg were given in study 7039-78. Signs of toxicity included diarrhea, ataxia, lethargy, weakness, and ruffled feathers. The 14-day LD_{50} for male and female birds combined was 1669 $\pm$ 568 mg/kg. Doses of 0, 250, and 500 mg apramycin activity/kg were given in study 7004-79. There were no observed abnormalities or mortalities.

(REF: NADA 106-964, Submission March 18, 1980, pg. 256-279)

5-Day Dietary Study - Bobwhite Quail

Bobwhite quail (Colinus virginianus), 11 days old, were fed diets containing 0.0, 0.0625, 0.125, 0.250, or 0.500% w/w apramycin activity. The treated diets were fed for 5 days followed by 3 days of basal diet. Although control birds consumed slightly more food during the 5-day and the 3-day phases of the test, body weight gain in treated birds for the same 5- or 3-day period was equal to or greater than that of the controls. There were no overt signs of toxicity or mortality in any test group. There were no apparent toxic effects in bobwhite quail that consumed diets containing as much as 0.500% w/w apramycin activity during the 8-day test.

(REF: NADA 106-964, Submission March 18, 1980, pg. 230-255)

5-Day Dietary Study - Mallard Ducks

Mallard ducks (Anas platyrhynchos), 10 days old, were fed diets containing 0.0, 0.125, 0.250, or 0.500% w/w apramycin activity. The treated diets were fed for 5 days followed by 3 days of basal diet. A decrease in food consumption and a statistically significant ($p \leq 0.05$) reduction in body weight gain were observed during the basal diet phase in the group that had received the 0.500% treated diet. There were no overt signs of toxicity or mortality at any of the dietary levels studied. There were no apparent toxic effects in mallard ducks that consumed diets containing as much as 0.500% w/w apramycin activity during the 8 day test.

(REF: NADA 106-964, Submission March 18, 1980, pg, 204-229)

TABULAR SUMMARY

Test	Concentration (ppm) at Which No Mortalities or Gross Effects were Seen	Apramycin Safety Factor
Greenhouse phytotoxicity	50 - 60	71.4
Rhizobium japorium, in vitro	1 - 10	1.4
Soybean nodulation, in vivo	<150	228.6
Earthworms (Lumbricus terrestris)	> 100 (14 days)	142.9
Daphnia magna	28.4 (48 hrs.)	40.6
Rainbow trout (Salmo gairdneri)	Static test, > 300 (96 hrs.)	428.6
Bluegill (Lepomis machrochirus)	Static test, > 300 (96 hrs.)	428.6
Bobwhite quail (Colinus virginianus)	Acute, > 500	714.3
Bobwhite quail (Colinus virginianus)	Dietary (.500% w/w), 5 days	714.3
Mallard duck (Anas platyrhynchos)	Dietary (.500% w/w), 5 days	714.3

Considering the tremendous safety factors built into the apramycin soil concentration factor (.7 ppm) and the significant safety factors in these tests even with the use of .7 ppm, these data demonstrate that apramycin will not adversely affect the environment.

OVER-ALL SUMMARY: In view of the very low octanal-water partitioning 5 coefficient*, the strong soil binding characteristic of the drug and the safety studies noted above, there is no reason to believe that apramycin will adversely affect the environment.

*NADA 106-964, Submission May 18, 1981

Environmental Impact Data Sheet

1. Date: November 6, 1981

2. Applicant/Petitioner: Elanco Products Company

3. Address: 740 South Alabama Street
Indianapolis, IN 46285

4. Description of proposed action:

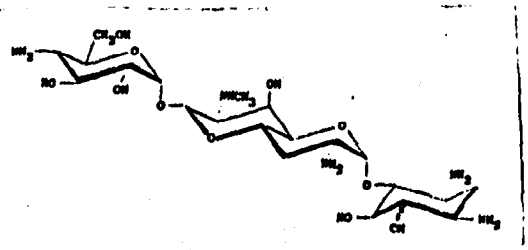
NADA 106-964 requests FDA approval to provide for use of apramycin sulfate (Apralan®) as an additive to swine drinking water for the treatment of porcine colibacillosis (weanling pig scours) caused by susceptible strains of E. coli. Treatment lasts for 7 days. Treated pigs should consume enough medicated water to receive 25 mg of apramycin per kilogram of body weight per day (11.4 mg/lb/day). There is a 28 day withdrawal period before treated animals could be slaughtered for food use.

5. Chemical identity:

Trade name - Apralan® Soluble Powder

Chemical name - apramycin sulfate; 4-O-[(8R)-2-Amino-8-O-(4-amino-4-deoxy- α-D-glucopyranosyl)-2,3,7-trideoxy-7-(methyl-amino)-D-glycero--D-allo-octadialdo-1,5:8,4-dysyranos-1-yl]-2-deoxy-D-streptamine (Merck, 1976).

Structural diagram -



Merck (1976)

Formula - $C_{21}H_{41}N_5O_{11} \cdot \frac{5}{2} H_2SO_4$ (apramycin sulfate)

Source - Fermentation product of Streptomyces tenebrarius

Mol. wt. - 784.8 (sulfate salt), 539.60 (apramycin base)

6. Introduction into the environment

Manufacture - Produced by standard fermentation techniques separated by adsorption and eluted, concentrated, converted to sulfate, dried and blended. Waste streams segregated at source and dried for landfilling or treated with activated sludge. Facility located in Puerto Rico.

Air emissions - steam generation, fermentor vents and exhausts. Fuel oil burned as energy source. Permits for construction of facilities (2 boilers, vent condenser and ammonia scrubber) issued by EPA and Environmental Quality Board, Puerto Rico.

Water emissions - treatment of fermentation wastes by activated sludge waste treatment plant, NPDES permit No. PR 0021423.

Solid wastes - trash containers, paper, and solid combustible material are landfilled.

Use in weanling pigs

- # animals potentially treated - 90 million per year (1979 est.)
- Maximum annual use of product, assuming 85% of pig crop is treated for scours - 133,875 kg
- Measured conc. of apramycin in fresh feces from swine fed ration containing 110 ppm apramycin was 50-60 ppm. NADA 126-050, p. 1506.
- Est. avg. conc. in swine wastes during treatment (7 days) + withdrawal period (28 days).

  Assume: - all residues are excreted during 35 day period
  - 75% of dose administered is excreted as apramycin or bioactive metabolite = 1300 mg/pig
  - avg. volume waste/pig-day = 0.1 cu foot (liquids and solids) Animal Waste Management (1971)

  Calculation: $\frac{1300 \text{ mg Apramcyin}}{0.1 \text{ cu ft/day} \times 35 \text{ days} \times 28.32 \text{ liters/cu ft.}} =$

  $\frac{*13.1 \text{ mg apramycin}}{\text{liter (ppm)}}$

*Bureau of Veterinary Medicine calculation.

- Est. conc. apramycin in swine wastes based on yearly production of 20,000 pig facility = 1.43 ppm
- Est. conc. of apramycin in agricultural soil amended with wastes from swine: 0.057 ppm, one application of 40T manure wet weight/acre.

7. Fate in the environment:

Melting point - decomposes before melting

Ultraviolet and visible absorption spectra - End absorption observed below 250 nm. No visible or UV maxima.

Vapor pressure - low; non-volatile solid

Water solubility - greater than 300 g/L

pKa values - from aqueous titration: pH 5.4, 6.2, 7.2, 7.8, and 8.5; from nuclear magnetic resonance spectroscopy: pH 6.4-6.6, 6.6-6.8, 7.3-7.4, 7.6-7.7, and 8.1.

Octanol/water partitioning coefficient - at pH 5,7,9, and 13 all coefficients are considerably less than 10 (0.00022-0.0011) (NADA 106-964, Aug. 13, 1981 submission, Dorulla, July 1981).

Degradation/inactivation - mechanism probably biological

- Rate in soil (greenhouse conditions) - 15% loss per year = half-life ($T_{\frac{1}{2}}$) - 3 1/3 years. Steady state soil conc. when $T_{\frac{1}{2}}$ = 3 1/3 years is 0.6667 ppm reached in 20+ years, assuming one application of manure per year (EIAR calc.) Possibly inactive when bound to soil particles. (NADA 106-964, Expt. ABC-0015, pp. 72-85.)

- Rate with sewage organisms (Static BOD bottles) see Table 1 below based on data from NADA 106-964, pp. 107-135, Table X.

Table 1. Half-lives of apramycin calculated from loss of activity in BOD bottles innoculated with sewage organisms.

Apramycin conc. (C_o) (g/ml = ppm)	Residual apramycin after 14 days (C_R) (g/ml)	% lost	%/day	Half-life ($T_{\frac{1}{2}}$) (days)
0	0			
0.1	0	--	--	--
0.2	0	--	--	--
0.4	0	--	--	--
0.8	0.18	77.5	5.54	9.0
1.6	0.58	63.8	4.56	11.0
3.2	1.33	58.4	4.17	12.0
6.4	2.96	53.8	3.84	13.0
12.8	5.64	55.9	3.99	12.5
25.6	12.3	52.0	3.71	13.5
51.2	27.3	46.7	3.34	15.0
102.4	83.8	18.2	1.3	38.5

calc.

$\%\ \text{lost} = \frac{C_o - C_R}{C_o} \times 100$. Assuming losses are at a constant linear rate,

$$\%\ \text{lost/day} = \frac{\%\ \text{lost}}{14\ \text{days}} \quad \text{and} \quad T_{\frac{1}{2}} = \frac{50\%}{\%\ \text{lost/day}} = \text{days}$$

* * * * *

Adsorption/desorption in soils - relatively immobile in soils (column leaching test, NADA 106-964, pp. 136-140 and 9/11/81 amendment p. 24)

Bioaccumulation - based on octanol/water partitioning, bioaccumulation expected to be very low. Bioconcentration factor (BCF) in fish muscle estimated to be 0.26 based on Neely, Branan, and Blau (1974) equation (EIAR calc.).

8. Effects in environment:

Microorganisms

Nitrogen-Fixing Organisms
Minimal Inhibitory Concentration Range
In Vitro Test

Organism	MIC Range ppm#
Azotobacter chroococcum	.01 - 0.1 (free-living soil bacterium)
Anabaena flos-aquae	.01 - 0.1 (blue-green alga)
Rhizobium japonicum 31 1B 110	0.1 - 1.0 (symbiotic, root nodule bacterium)
R. japonicum 31 1B 142	0.1 - 1.0 " " "
R. japonicum 31 ATCC 10311	0.1 - 1.0 " " "
R. leguminosarum	0.1 - 1.0 " " "
Soybean isolate	1.0 - 10 " " "

72 hours' incubation

(NADA 106-964, pp. 86-99 I-BSD-79-03,-05, and -10 as modified by 9/11/81 submission, p. 22A)

No statistically significant effect on soybean nodulation was found when apramycin was incorporated into soil at 160 ppm. (NADA 106-964, pp. 100-106).

Table 2. Antibacterial activity of apramycin

Organism	No. of strains	MIC (μg/ml) Broth				Gradient plate					
		4	8	16	32	1	2	4	8	16	32
Escherichia coli	18			17	1			4	5	8	1
Salmonella spp.	24	2	8	7	7	2		10	5	6	1
Shigella sonnei	10			6	4			1	5	4	
Vibrio cholerae	4				4					4	

(Walton, 1978)

Table 3. Comparative activity of apramycin, neomycin and streptomycin

Organism	No. of strains	Antibiotic	MIC (μg/ml) <1	1	2	4	8	16	32	64	128	256	512	> 512
Escherichia coli	149	Apramycin			7	59	74	7	1		1			
		Neomycin	2	59	60	9				2	2	4	8	3
		Streptomycin				23	26	4	9	15	24	22	19	7
Klebsiella aerogenes	2	Apramycin			1	1								
		Neomycin	1		1									
		Streptomycin			1	1								
Pasteurella spp.	4	Apramycin				1	1	1	1					
		Neomycin	1	1	1	1								
		Streptomycin			1	1		2						
Proteus spp.	3	Apramycin				1	2							
		Neomycin		1	1							1		
		Streptomycin				1	1	1						
Pseudomonas spp.	4	Apramycin					3	1						
		Neomycin			2	1		1						
		Streptomycin						2	1			1		
Salmonella spp.	121	Apramycin	1	4	22	47	42	5						
		Neomycin	23	46	37	11	3							1
		Streptomycin	1	3	8	4	3	45	32	5	12	6	1	1
Staphylococcus spp.	29	Apramycin		1	8	12	8							
		Neomycin	19	7	2	1								
		Streptomycin		1	5	8	8	1		3	1	1	1	

(Ryden and Moore, 1977)

Apramycin at gradually increasing concentrations of 0.1-102.4 ppm over a 26 day period had no detectable effects on the functioning of sewage digesting microorganisms in a 30 day laboratory activated sludge test. Apramycin at concentrations of 51.2 ppm or greater depressed oxygen utilization of sewage sludge organisms in static BOD bottle tests. (NADA 106-964 Expts. AAC-79-06 and T93-230 pp. 107-135) and 9/11/81 submission, pp. 22-23, see also Table 1 above).

Other Organisms

Test Organism	Test Description	Endpoint	Apramycin conc. causing endpoint	Ref.
Invertebrates				
1. *Lumbricus terrestris* (earthworms)	14 day acute toxicity	mortality	no mortality at highest level tested (100 ppm in soil)	NADA 106-964 pp. 45-51 Aug. 13, 1981 submission
2. *Daphnia magna* (water flea)	48 hours, static test	50% immobilization (EC50)	101.6 ± 7.3 ppm in water	NADA 106-964 pp. 52-62 Aug. 13, 1981 submission
		0% immobilization (EC0)	28.4 ppm in water	
Fish				
3. *Salmo gairdneri* (rainbow trout)	96 hours, static test	mortality	no mortality at highest dose tested, 300 ppm	NADA 106-964 pp. 63-68 Aug. 13, 1981 submission
4. *Lepomis machrochirus*	96 hours, static test	mortality	no mortality at highest dose tested, 300 ppm	NADA 106-964 pp. 69-76 Aug. 13, 1981 submission
Birds				
5. *Colinus virginianus* (bobwhite quail)				
a. adults	acute oral	mortality following single dose	LD50 = 1669 ± 568 mg/kg, no mortality or effects on weight gain observed at 500 mg/kg	NADA 106-964 pp. 256
b. 11 days old	5-day dietary	mortality weight gain	no mortality or weight gain effects observed at conc's. up to 0.5% w/w in feeds	NADA 106-964 pp. 230-255
6. *Anas platyrhynchos* (mallard duck)	5-day dietary	mortality weight gain	no mortality or weight gain effects observed at conc's. up to 0.5% w/w in feeds	NADA 106-964 pp. 204-229

Test Organisms	Test Description	Endpoint	Apramycin conc. causing endpoint	Ref.
7. Chicken	acute oral	mortality	LD0>520 mg/kg	NADA 106-964
Mammals				
8. Mouse	acute oral	mortality	LD0>5200 mg/kg	NADA 106-964
9. Rat	acute oral	mortality	LD0>4,160 mg/kg	NADA 106-964
10. Rabbit	acute oral	mortality	LD0>832 mg/kg	NADA 106-964
11. Guinea pig	acute oral	mortality	LD50>1,250 mg/kg	NADA 106-964
12. Dog	acute oral	mortality	LD50>520 mg/kg	NADA 106-964
13. Rat	90 day dietary subacute toxicity	effects on body gain, food utilization histological changes in tissues	no effects at highest doses (520 ppm A activity in feed and 0.52% A activity in water)	NADA 106-964
14. Rat	6-month dietary subacute	effects on mortality, growth, food consumption water consumption, blood chemistry	transient blood chemistry effects at conc. 1000-5000 ppm No effects at 6 months at 5000 ppm	NADA 106-964
15. Dog	90 day oral subacute toxicity	effects on body weight, behavior and appearances, hematology, urinalysis and histology of tissues	No effects at highest dose (13 mg/kg body wt/day) administered	NADA 106-964
16. Dog	6-month oral subacute toxicity	effects on body weight hearing, blood chemistry, tissues	body weight and blood chemistry effects at 100 mg/kg body wt/day no effect 50 mg/kg body wt/day	NADA 106-964

Test Organisms	Test Description	Endpoint	Apramycin conc. causing endpoint	Ref.
17. Rat	Teratogenicity	fetal defects, fetal toxicity, maternal toxicity	No effects at highest doses tested (1000 mg/kg body wt/day)	NADA 106-964
18. Rabbit	Teratogenicity	fetal defects, fetal toxicity, maternal toxicity	Dose related maternal toxicity (2 mg-32 mg apramycin/day). Increased resorption and decreased fetal weight with increased dose.	NADA 106-964
Plants				
19. a. Medicago sativa (alfalfa) b. Festuca elatior (fescue) c. Cucumis sativus (cucumber) d. Oryza sativa (rice) e. Capsicum annuum (pepper) f. Gossypium hirsutum (cotton) g. Lycopersicon esculentum (tomatoes) h. Zea mays (corn)	21-day phytotoxicity to 14 species of plants from manure from medicated animals, 2 ppm apramycin	Stunting, mortality	no apramycin related effects observed at about 2 ppm soil conc.	NADA 106-964 pp. 77-83 August 13, 1981 submission and addendum Sept. 11, 1981 to NADA 106-964

Test Organisms	Test Description	Endpoint	Apramycin conc. causing endpoint	Ref.
i. Beta vulgaris (sugar beet)				
j. Hordeum vulgare (barley)				
k. Glycine max (soybean)				
l. Triticum aestivum (wheat)				
m. Sorghum vulgare (sorghum)				
n. Avena sativa (oats)				
20. L5178Y TK^{+1}- mouse lymphona cells	in vitro forward mutation assay with and without rat liver enzyme activation	cytotoxicity—	minimal cytotoxicity at conc. up to 1000 µg/ml	NADA 106-964 pp. 150-157
		mutagenicity	non-mutagenic at conc. up to 1000 µg/ml	
Salmonella typhimurium TA 100, TA 1538, TA 1535, TA 98, TA 1537	Modified Ames back mutation assay with and without rat liver enzyme activation	mutagenicity	non-mutagenic at all conc's tested (3-300 g/plate)	NADA 106-964 pp. 158-187
Adult rat hepatocytes	induction of DNA repair synthesis as screen for mutagens and carcinogens	induction of DNA repairs	cytotoxic at 1000 nmoles/ml but inactive in inducing DNA repair	NADA 106-964 pp 188-203

References

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N-OCTANOL-TO-WATER PARTITION COEFFICIENT OF APRAMYCIN AT pH 5.0, 7.0 AND 9.0

The apparent n-octanol-to-water partition coefficient (K) of apramycin was determined to have a range of 0.00022 to 0.0011. The mean apparent partition coefficient of 0.0005 represents an average of eight determinations at pH's of 5.0, 7.0, and 9.0 and a ten-fold concentration difference at pH 7.0.

Compounds, as in the case of apramycin, whose partition coefficients are <100 should not bioaccumulate in the environment.

Static bioassays were conducted to determine the toxicity of apramycin to bluegills (Lepomis machrochirus). The fish were exposed to 100 and 300 ppm activity for 96 hours. There were no signs of toxicity and no mortalities. The no-effect level was > 300 ppm apramycin activity. (REF: NADA 106-964, pg. 69-76, Aug. 13, 1981, submission.)

Greenhouse Phytotoxicity

There was no apramycin-related phytotoxicity to plants grown in soil treated with feces obtained from swine which were fed apramycin at a level of 110 ppm in the diet. Standard greenhouse phytotoxicity tests were conducted using 14 plant species grown in soil treated with 11.2 and 22.4 metric tons of feces per hectare (5 and 10 tons/acre). Feces from control (untreated) swine and apramycin-treated swine were tested. No treatment-related phytotoxicity was noted in any plant species. (REF: NADA 106-964, p. 77-83, Aug. 13, 1981, submission)

SUMMARIES OF EIAR STUDIES

Earthworm Toxicity

Earthworms (Lumbricus terrestris) were exposed to 10 to 100 ppm soil-incorporated apramycin activity for 14 days. There were no signs of toxicity and all animals survived. The no-effect level was > 100 ppm apramycin activity. (REF: NADA 106-964, pg. 45-51, Aug. 13, 1981, submission)

Daphnia 48-Hour Static Test

Toxicity tests were conducted to determine the acute effects of apramycin on first instar Daphnia magna. Daphnia were exposed to 0.0, 17.8, 28.4, 44.4, 71.0 and 106.5 ppm apramycin activity for 48 hours. The criterion for effect was immobilization expressed as EC_{50} (the concentration of test material estimated to be effective in producing immobility in 50% of the test animals at the specified time). The no-effect level at 24 and 48 hours was 44.4 and 28.4 ppm apramycin activity, respectively. The 24-hour $EC_{50} \pm$ SE was 105.1 $\pm$ 8 ppm apramycin activity. The 48-hour $EC_{50} \pm$ SE was 101.6 $\pm$ 7.3 ppm apramycin activity. (REF: NADA 106-964 pg. 52-62, Aug. 13, 1981, submission)

Fish 96-hour Static Tests

Static bioassays were conducted to determine the toxicity of apramycin to rainbow trout (Salmo gairdneri). The fish were exposed to 50, 100 and 300 ppm apramycin activity for 96 hours. There were no signs of toxicity nor were there any mortalities. The no-effect level was > 300 ppm apramycin activity. (REF: NADA 106-964, pg. 63-68, Aug. 13, 1981, submission)