ENVIRONMENTAL ASSESSMENT
for
Virginiamycin in Turkeys
NADA 91-467

1. **DATE:** March 3, 1988

2. **NAME OF APPLICANT:** SmithKline Beckman Animal Health Products

3. **ADDRESS OF APPLICANT:** SmithKline Animal Health Products
1600 Paoli Pike
West Chester, PA 19380

4. **DESCRIPTION OF THE PROPOSED ACTION:**

SmithKline Animal Health Products is requesting approval for a supplemental New Animal Drug Application to NADA 91-467 for use of virginiamycin in turkeys. This animal drug is currently approved for the following indications: for treatment and control of swine dysentery, increased rate of weight gain and improved feed efficiency in swine and broiler chickens and prevention of necrotic enteritis caused by *Clostridium perfringens* susceptible to virginiamycin in broiler chickens. This supplemental application would add the intended use for increased rate of weight gain and improved feed efficiency in turkeys at a dose level of 10 to 20 g/ton using previously approved Stafac® 500, 50, 20 and 10 type A medicated articles. No change is proposed for the manufacturing, formulation, production methods or dosage forms.

Virginiamycin will continue to be produced in Genval, Belgium, by Recherche et Industrie Therapeutiques, S.A., a wholly owned subsidiary of SmithKline Beckman Corporation. Manufacturing of premixes will continue at our facility in Omaha, Nebraska.

Since virginiamycin is a growth enhancer proposed for use in turkey feed, the geographic area of predominant usage will naturally coincide with the area of greatest meat-type turkey production. The following table lists the relative number of turkeys raised by each state as compared to the rest of the country.

TURKEYS: PRODUCTION, 1986[1]

STATE	NUMBER RAISED [a] (1,000 head)
ARK	16,500
CALIF	21,900
CONN	40
GA	2,426
ILL	347
IND	9,370
IOWA	7,000
KANS	104
MD-DEL	125
MASS	145
MICH	2,700
MINN	34,200
MO	13,500
NEBR	1,437
NH	26
NJ	100
NY	343
NC	39,100
N DAK	1,030
OHIO	3,100
OREG	1,510
PA	7,800
SC	3,900
S DAK	1,968
UTAH	3,390
VA	14,307
W VA	2,220
WIS	6,128
OTH STS [b]	12,500
U.S.	207,216

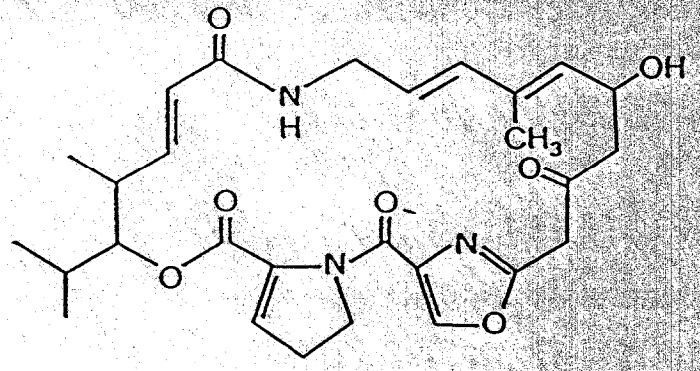
a. BASED ON TURKEYS PLACED SEP 1, 1985 THROUGH AUG 31, 1986. EXCLUDES YOUNG TURKEYS LOST.
b. COLO, OKLA, AND TEX COMBINED TO AVOID DISCLOSING INDIVIDUAL OPERATIONS.

The type of environment present at and adjacent to turkey production facilities can include open fields, streams, ponds and woodland usually in a rural area. Approximately 91% of the turkeys are raised commercially in buildings which vary in construction according to the climate.[1,2] The remainder, approximately 9%, are raised on range.

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

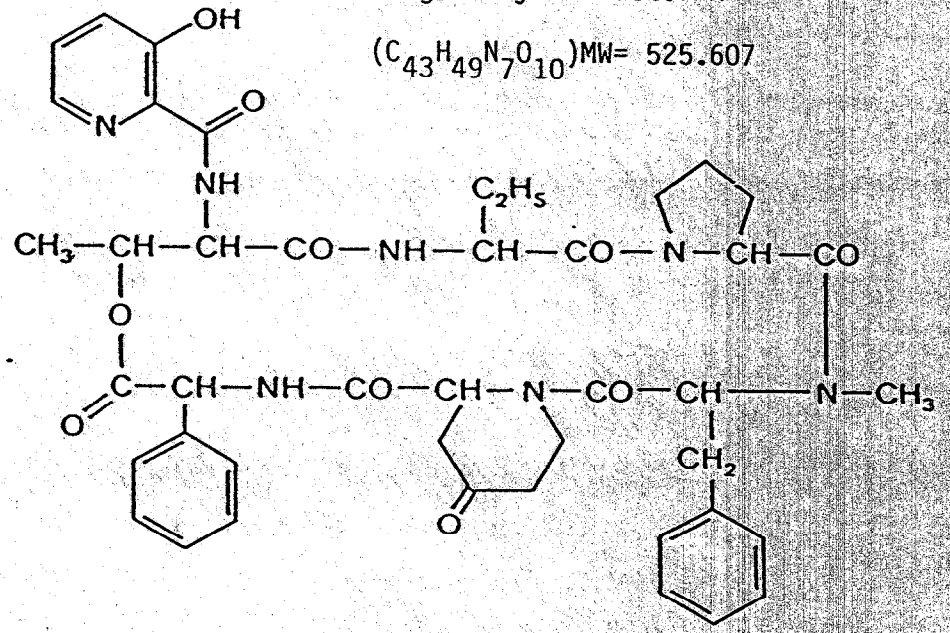
Virginiamycin (CAS-11006-76-1) is a composite antibiotic produced by *Streptomyces virginiae*[3]. It contains a mixture of two principal antibiotic components, virginiamycin Factor M and virginiamycin Factor S which act synergistically against a wide range of gram positive organisms. Factor M is present in the largest concentration and is mainly active against *Staphylococcus aureus*, while Factor S is mainly active against *Bacillus subtilis*.

The chemical structures of the two factors comprising virginiamycin follow with pertinent physical and chemical information.



virginiamycin Factor M

$(C_{43}H_{49}N_7O_{10})$MW= 525.607



virginiamycin Factor S

$(C_{28}H_{35}N_3O_7)$MW= 823.91

Virginiamycin is an amorphous, white powder which is sparingly soluble in water and dilute acid. It dissolves in aqueous alkali above pH 9.5 with rapid inactivation. Virginiamycin is soluble in methanol, ethanol, acetone, ethyl acetate, chloroform and benzene.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

Introduction of virginiamycin into the environment can occur from three sources, (A.) the virginiamycin production facility, (B.) the virginiamycin premix production facility and (C.) the sites of intended use in turkeys. A description of each source follows with calculations of the total virginiamycin entering the environment.

A. DESCRIPTION OF VIRGINIAMYCIN PRODUCTION FACILITY AND CALCULATION OF ENVIRONMENTAL EXPOSURE.

Virginiamycin is produced at SmithKline RIT, S.A., Que de l'Institut 89, B-1330, Rixensart, Belgium. General Environmental Control criteria are evaluated as part of a local governmental "General Plant Operating Permit", issued by the Province after consultation with the Ministry of Employment and with the (Walloon) Ministry of Environment and also the local community. The permit is effective for 30 years. The review covers noise level, odor, water discharge and toxic emissions (if applicable).

In addition, specific permits are issued covering the following:

Sewer discharge permit: National Ministry of Health (1976)
Surface discharge (River) Permit: National Ministry of Health (1976)
Well Pumping Permit: Walloon Region; Ministry of Environment

Specific Environment Control practices utilized at the plant are as follows:

1) <u>Hazardous Liquid Waste Stream</u>

Spent solvent streams generated in the process are recovered and recycled for process reuse. Residue recovery still bottoms are sent to the spent broth storage tank for processing.

The organic stream is a closed system with no direct discharge to the environment.

2) Liquid Waste Stream

Spent Broth

Non-volatile residuals from recovery are combined and classified as spent broth. The broth is sent to a by-product recovery unit, for production of an animal feed nutrient. The processing involves the following steps:

temperature control
concentration
drying to a powder

A waste stream from the concentration step which contains no virginiamycin is discharged to the municipal sewer. As a result of approval of virginiamycin for turkey production in the United States, approximately 45 additional tons of spent broth per year will be processed for animal feed use containing approximately 25 ppm of virginiamycin, based on an estimated market share.

Excess Spent Broth

Dependent on animal feed nutrient sales, excess spent broth is disposed of in one of two methods.

Off-site contract incineration. Approximately 30 additional tons per year of spent broth will be disposed of by incineration in compliance with our "General Plant Operating Permit".

Discharge to a local municipal waste treatment system for biological treatment (under permit). Approximately 18 additional tons per year of spent broth will be discharged into the municipal waste treatment system in compliance with our "General Plant Operating Permit".

Other Waste Streams

Waste streams are generated from other manufacturing processes:

The scrubber blowdown containing no virginiamycin is discharged with non-contact cooling water to the Lasne River by permit of the National Ministry of Health (1976).

The other wastes are blended with the Antibiotic Plant discharges and monitored for flow volume, pH, biological oxygen demand, chemical oxygen demand and detergents, during discharge to the municipal sewer.

These discharges are controlled by agreement with IWB (Intercommuale du Brabant Walloon) municipal Sewer Plant and are in compliance with our "General Plant Operating Permit."

3) Air Emissions

Vapors

Air emissions from the animal feed nutrient dryers are scrubbed in a packed column, with blowdown from the system discharged to the river as it contains no active virginiamycin. These vents are not regulated by any formal government regulation, but incorporate a "state-of-the-art" treatment system.

Solvent storage tanks are equipped with flame arrestors.

Dust

No significant amount of dust escapes into the environment since dust from the fermentation process is disposed of in the scrubber blowdown waste discharge.

All dust from conveying dry feed systems is vented through a bag filter and the collected solids are reprocessed.

4) Dry Solid Wastes

All solid waste materials such as trash, bags and used process drums are disposed of by a contractor in compliance with local and national laws.

5) Calculation of environmental exposure

Approximately 45 additional tons of spent broth will be processed to an animal feed nutrient which contains residual amounts of virginiamycin at a concentration of 25 ppm. This amounts to a total of 1.0 kg of virginiamycin in these 45 tons. Approximately 30 additional tons of spent broth per year will be disposed of by incineration. Approximately 18 additional tons of spent broth, which amounts to approximately 400 g of virginiamycin, will be discharged to a waste treatment system under permit.

Additional detailed proprietary manufacturing information is provided in a confidential appendix only for the purpose of the Agency's review of this document.

B. DESCRIPTION OF VIRGINIAMYCIN PREMIX PRODUCTION FACILITY AND CALCULATION OF ENVIRONMENT EXPOSURE.

'Stafac' Medicated Premixes are manufactured at our facility located at 4444 South 76th Street, Omaha, Nebraska 68127. Environmental control practices followed at the plant are as follows:

1) Liquid Waste Stream

Waste liquids are generated from the manufacturing of 'Stafac' medicated premixes as a result of washing the empty production equipment after use and small quantities from the analytical laboratory. This liquid waste water contains very small amounts of virginiamycin and inert carrier. This waste is regulated by the City of Omaha Municipal Code Chapter 31. Treatment of this waste by the City of Omaha Waste Water System, NPDES Permit no. NE0036358, is regulated by the Nebraska Department of Environmental Control under Title 128, Chapter 2, Titles 118, 119 and 127 as subject to regulations under Section 307b of the Clean Air Act, 40 CFR Part 439. This liquid waste requires no pretreatment and is in compliance with the above referenced laws and regulations.

2) Air Emissions

Air emissions from the production of 'Stafac' Medicated Premixes that escape our production system consist of dust which contains virginiamycin and the inert carrier. Only negligible amounts of this dust escape outside the plant. The dust is contained inside the plant by keeping the manufacturing system closed as much as possible and using the central dust collector (MAC Model 72AV25, bag filter, 25,000 CFM) to extract dust that escapes the system. The dust collected by the system is deposited in a central container and is disposed of at the City of Omaha County landfill by the Tecrep Company. This system meets the requirements of the Nebraska Department of Environmental Control, Title 129, Rules and Regulations Governing Air Pollution in Nebraska and OSHA Safety and Health Standards (29 CFR 1910) Subpart 2, Section 1910.000 (Air Emissions). Air emissions associated with the production of 'Stafac' medicated premixes contain no hazardous materials regulated by the State of Nebraska; therefore, the state does not require a permit.

3) Dry Solid Waste

Dry solid waste is disposed of at the City of Omaha County landfill by Browning-Ferris Industries Waste Systems. This municipal landfill is regulated by the Nebraska Department of Environmental Control, Title 128, Rules and Regulations Governing Hazardous Waste in Nebraska, Code Section 4, 10, 12 and 14 per CFR 40, Part 261 and Title 132, Rules and Regulations pertaining to Solid Waste Management. The dry solid waste consists of flush material used to clean equipment, floor sweepings, dust from the dust collector (approximately 600 lbs/year) and outdated and returned goods (approximately 500 lbs/year) which contains approximately 57 pounds of virginiamycin activity. This waste contains virginiamycin and inert carrier and is disposed of in accordance with the above referenced laws and regulations.

4) Employee Protection

Material Safety Data Sheets are available for employees who work in the production area, Appendix I. In addition, employees in the production and packaging areas wear protective clothing and dust respirator as needed, to assure compliance with OSHA standards, CFR 29, Part 1900 to 1910 and OSHA's Hazard Communication, CFR 29, Part 1910. Employee training and industrial hygiene programs are routine plant operations.

5) Calculation of Environmental Exposure

The amount of virginiamycin contained in liquid waste is negligible. Approximately 600 lbs/year of dust and approximately 500 lbs/year of returned goods disposed of at the City of Omaha landfill amount to a maximum of approximately 22 kg of virginiamycin waste at this facility on a yearly basis.

C. CALCULATION OF ENVIRONMENTAL EXPOSURE DUE TO USE IN TURKEYS.

In turkeys, as in other poultry (chickens), since the ureters empty directly into a cloaca, within which the urine mixes with the solid waste, the entire amount of virginiamycin excreted is contained in these droppings. Therefore, the stability of virginiamycin in these poultry droppings is the major determining factor of environmental impact.

Environmental fate studies were conducted with chicken excreta and reported previously in NADA 91-467, February 1, 1979, to support the approval, March 27, 1981, for use of virginiamycin in broiler chickens. This information is discussed in section 7 of this assessment to support the environmental considerations for use of virginiamycin in turkeys, since the maximum use level is the same for both species and any effect on the environment would be similar.

The following information will be used to calculate the quantity and concentration of virginiamycin that could possibly enter the environment assuming all commercially raised turkeys receive virginiamycin for their entire life at the maximum approved level of use.

250,000,000	=	conservative estimate of total turkeys raised commercially[1].
20 g/ton	=	virginiamycin anticipated maximum approved use level.
37.37 kg.	=	feed consumption for entire life of a male turkey[4]
17.18 kg.	=	feed consumption for entire life of a female turkey[4]
27.275 kg. (60 lbs.)	=	Average feed consumption per turkey life since approximately equal numbers of each sex are raised commercially.
27.275 kg. (60 lbs.)	=	Average fecal output per turkey life on a wet basis which equals average feed consumption per turkey life.[5]
12 lbs.	=	Average fecal output per turkey life on a dry matter basis equals 20% of wet basis.[5]

Calculation of total virginiamycin exposure from use in turkeys assuming exposure to all marketed turkeys:

Total virginiamycin consumed in feed:

$$\frac{250{,}000{,}000 \text{ turkeys}}{} \left| \frac{20\text{g}}{\text{ton}} \right| \frac{\text{ton}}{2000\text{lbs}} \left| \frac{60\text{lbs. feed}}{} \right| \frac{\text{kg}}{1000\text{g}} = 150{,}000 \text{ kg virginiamycin}$$

The amount of virginiamycin excreted cannot be more than the amount consumed; therefore, in the absence of actual virginiamycin fecal concentration data, the amount of virginiamycin in feces on a wet basis weekly will be assumed as equivalent to feed intake on a weekly basis for fate calculations in Section 7.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT.

The probable environmental impact of using virginiamycin in turkeys is negligible. The use of virginiamycin in turkey feed should have no significant impact on the environment in terms of its accumulation and uptake into the flora. In order for a compound ingested by animals, such as turkeys, to be a significant factor in pollution, that compound must find its way into the environment in significant amounts, and break down very slowly or not at all.

Degradation of virginiamycin in fecal droppings

As mentioned in Section 6, page 8, the environmental exposure from use of virginiamycin in turkeys as in chickens, is through fecal droppings and the stability of virginiamycin in the environment. Since the maximum use level and residue profile for both species are similar, the environmental studies performed with chickens are being used to support this environmental assessment for turkeys.

To determine this stability, chicken droppings were fortified to a level of 30 ppm of virginiamycin, and maintained at room temperature (18-22°C). After seven days, more than 80% of the virginiamycin had degraded and by the 14th day more than 94% degradation had occurred, Appendix II. Similar results were obtained when the droppings were identically fortified but maintained outdoors at ambient temperatures (18-76°F), in order to better simulate practical circumstances. After seven and 14 days, more than 78% and 94%, respectively, of the virginiamycin had degraded.

To further support this data, chicken litter (a combination of droppings and straw from the pens) was also fortified to a level of 30 ppm of virginiamycin and maintained at room temperature (18-22°C). Samples tested three and seven days later revealed that more than 69% and 83%, respectively, of the antibiotic had degraded, Appendix II.

The fortification level of 30 ppm represents an approximately two-fold multiple of the actual mean concentrations found in feces of swine maintained for 34 days on a diet containing virginiamycin (95.7 g/ton of feed).[6] Since the highest anticipated use level in chicken feed and turkey feed is 20 g/ton, actual fecal concentrations of virginiamycin should be even lower than those observed in swine and the fortification level of 30 ppm is therefore greatly exaggerated. This fortification level was used in the degradation studies for two reasons: 1) clearly, the results would more than adequately describe the maximum concentration of drug ever expected to be present in the (poultry) environment and the rate of degradation (% over time) could be easily applied to lesser concentrations; 2) the higher fortification level greatly facilitated microbiological assay of the drug in chicken excreta and allowed development of a more complete degradation profile. Even at this magnified concentration of drug, degradation occurs rapidly in chicken feces and litter, thereby minimizing an environmental hazard from excreted virginiamycin.

Degradation of virginiamycin in soil

When virginiamycin (30 PPM) was added to control soil containing swine fecal material, no antibiotic was detected after 84 days, with approximately 80% degradation occurring after nine days. No difference could be detected in the rates of degradation between the soil-feces matrix versus feces alone, Appendix III.

Degradation of virginiamycin in water

Stability experiments on the degradation rate of virginiamycin in water, at variable temperature and pH, demonstrated that after 48 hours, less than 50% of the antibiotic content remained. The data also show that significant degradation occurs in unbuffered water, and that the rate is accelerated as temperature increases, therefore minimizing the possibility of water contamination by leaching, Appendix IV.

Octanol/water partitioning of virginiamycin

An octanol/water partitioning study was performed in order to evaluate the potential for virginiamycin absorption in animals and plants. Results of the study suggest that virginiamycin is highly lipid soluble, since 100% of the antibiotic was detected in the octanol layer. Based on this, one would expect the antibiotic to be orally absorbed in animals. However, when virginiamycin was fed to chickens for 5 days at a rate of 20 g/ton, no significant blood levels could be detected, indicating poor absorption in spite of high lipid solubility. Similar results would be expected for turkeys due to a similar tissue residue profile. Low tissue residues were also observed in laboratory animals used in comparative metabolism studies as well as in swine, further supporting this poor absorption. This suggests that factors other than polarity are involved. These factors are probably related to molecular cross-section or size. Because of its high molecular weight and size, virginiamycin cannot easily penetrate the sites for absorption, even though it exhibits high lipid solubility, Appendix V.

Prediction of environmental concentrations of and exposure to virginiamycin entering the environment.

a. Air

Virginiamycin is not volatile, therefore the only exposure to air would be in the form of dust at production sites. However, this exposure is minimal and would not be expected to have any affect on the environment as discussed in Section 6, pages 6 and 7.

b. Freshwater, estuarine and marine ecosystems

Any exposure of virginiamycin to water would occur from settlement of dust from production sites on to ground with subsequent possible runoff or from the spreading of turkey manure on fields with subsequent possible runoff into waterways. Additionally, turkeys raised on open range, approximately 9% of total, would provide direct exposure of fresh manure to the environment which could potentially runoff into waterways.

The practice of applying livestock manure to fertilize agricultural soil, necessitates an assessment of the maximum concentration of excreted virginiamycin in the soil.

The maximum encountered fecal concentration, 33 ppm, was obtained from a pig receiving 95.7 gm of virginiamycin per ton of feed. This was reported in the environmental impact analysis report for approval of virginiamycin use in swine (NADA submission March 15, 1978, approval September 19, 1978). An immediate application of that excreta at the maximum rate of 5 tons/acre (dry weight) assuming no degradation of drug was estimated to produce a 0.165 ppm concentration of virginiamycin. However, the drug is readily biodegradable, and turkey feed contains only 10-20 g/ton virginiamycin; consequently, these application levels are not likely to occur.

If the total population of turkeys receives virginiamycin at the highest use level, 20g/ton, the total virginiamycin used is 150,000 kg as calculated in Section 6. Since 91% of turkeys are raised in confinement and 9% on range, the use of virginiamycin would be as follows:

Confinement	136,500 kg
Range	13,500 kg

A typical turkey farm raises 50,000 turkeys annually.[2] A turkey barn is usually 40 x 500 feet and holds either 7,000 toms or 10,000 hens. During the summer 7,000-10,000 turkeys are grown on a range.

Assuming that all of the virginiamycin consumed is eliminated in turkey manure, which is an overestimation, the concentration in wet manure can be calculated using the feed consumption data in the following table.

Feed Consumption of Large-Type Turkeys[4]

Age (weeks)	Feed Consumption per Week (kg) M	Feed Consumption per Week (kg) F	Cumulative Feed Consumption (kg) M	Cumulative Feed Consumption (kg) F
1	0.10	0.10	0.10	0.10
2	0.20	0.17	0.30	0.27
3	0.45	0.39	0.75	0.66
4	0.61	0.46	1.36	1.12
5	0.70	0.60	2.06	1.72
6	0.86	0.76	2.92	2.48
7	1.08	0.89	4.00	3.37
8	1.30	1.04	5.30	4.41
9	1.51	1.18	6.81	5.59
10	1.78	1.34	8.59	6.93
11	1.99	1.47	10.58	8.40
12	2.25	1.59	12.83	9.99
13	2.51	1.70	15.34	11.69
14	2.66	1.75	18.00	13.44
15	2.89	1.82	20.89	15.26
16	3.05	1.92	23.94	17.18
17	3.13	2.03	27.03	19.21
18	3.27	2.07	30.34	21.28
19	3.43	2.15	33.77	23.43
20	3.60	2.23	37.37	25.66
21	3.71	--	41.08	--
22	3.82	--	44.90	--
23	3.94	--	48.84	--
24	4.05	--	52.89	--

Since virginiamycin degrades more than 94% within 14 days in poultry feces and the amount of excreta increases as the turkeys grow larger, the last two weeks (19, 20 for toms and 15, 16 for hens) residual virginiamycin activity is calculated as an estimated virginiamycin concentration in the total excreta without consideration of degradation during the last week.

The amount of excrement on a wet basis per week is equal to the amount of feed intake per week[5]. Hence, the concentration of virginiamycin in turkey manure is as follows:

Turkeys raised in confinement

		Feed Consumed (kg)	Feed Consumed (lbs)	Virginiamycin (g)
toms	week 19	3.43	7.56	0.0756
	week 20	3.60	7.93	0.0793
hens	week 15	1.82	4.01	0.0401
	week 16	1.92	4.23	0.0423

				Degradation		Virginiamycin Remaining/Tom
toms	week 19	0.0756 g	x	0.223	=	0.0169 g
	week 20	0.0793 g	x	1.000	=	0.0793 g
						0.0962 g

				Degradation		Virginiamycin Remaining/Hen
hens	week 15	0.0401 g	x	0.223	=	0.0089 g
	week 16	0.0423 g	x	1.000	=	0.0423 g
						0.0512 g

Estimated concentration of virginiamycin in manure at end of production cycle:

Toms $\frac{0.096\ g \mid 1000\ mg \mid}{\qquad\quad g \mid 37.37\ kg\ total\ manure}$ = 2.57 ppm/tom (~3 ppm)

Hens $\frac{0.051\ g \mid 1000\ mg \mid}{\qquad\quad g \mid 17.18\ kg\ total\ manure}$ = 2.97 ppm/hen (~3 ppm)

Using the same approach for estimating soil concentration as in the environmental impact analysis report for swine, Appendix VI, the virginiamycin soil concentration resulting from spreading turkey manure is estimated as follows:

Maximum feasible level of manure spread per acre = 5 tons (dry weight/acre = 4545.45 kg/acre
Incorporation into top six inches of soil = 9.09×10^5kg soil/acre
Estimated concentration of virginiamycin in turkey manure = 3 ppm (mg/kg) wet basis.
Estimated concentration of virginiamycin in turkey manure = 15 ppm (mg/kg) dry basis, (3 ppm x 100/20) since 80% of wet manure is moisture.

$$\frac{15 \text{ mg}}{\text{kg}} \mid \frac{4545.45 \text{ kg}}{} = 68{,}182 \text{ mg applied per acre}$$

$$\frac{68{,}182 \text{ mg}}{909{,}000 \text{ kg}} = 0.075 \text{ mg/kg or 75 ppb virginiamycin concentration in soil}$$

The typical turkey facility raising 50,000 birds annually would accumulate approximately 1,500 tons of manure on a wet basis or 300 tons on a dry basis. If virginiamycin was fed at 20 g/ton to all turkeys raised at this facility approximately

$$\frac{50{,}000 \text{ Turkeys} \mid 27.275 \text{ kg excreta}}{\text{turkey}} \mid \frac{3 \text{ mg virginiamycin}}{\text{kg excreta}} \mid \frac{\text{kg}}{10^6 \text{mg}} = 4.09 \text{ kg virginiamycin}$$

4 kg of virginiamycin would reside in the manure at an approximate concentration of 3 ppm (wet basis) after one production cycle. A typical turkey facility will clean out manure on a yearly basis resulting in one-half of the virginiamycin concentration (1.5 ppm) calculated above for toms (2 cycles/year) and one-third the virginiamycin concentration (1 ppm) calculated above for hens (3 cycle/year). These figures would translate to maximum anticipated concentrations of 37.5 ppb and 25 ppb, respectively, when the manure is spread on fields resulting in soil concentrations significantly lower than 75 ppb.

Turkeys raised on range

Typically 7,000 toms or 10,000 hens are raised on 3 acres of a 20,000 acre range at any one time, and rotated every 1-2 weeks to a clean 3 acre range to minimize the concentration of manure and preserve the pasture.[7] The largest concentration of virginiamycin will be excreted during the last two weeks of the May to October growing season as in the calculation above for turkeys raised in confinement. The estimated concentration of virginiamycin in the soil resulting from turkeys raised on range using the longer two week rotation period follows:

Toms $\frac{0.0962 \text{ g virginiamycin}}{\text{Tom}} \left| \frac{7{,}000 \text{ toms}}{3 \text{ acre}} \right| \frac{\text{acre}}{9.09 \times 10^5 \text{ kg}} \left| \frac{1{,}000 \text{ mg}}{\text{g}} \right. = 0.247 \text{ ppm}$

Hens $\frac{0.0512 \text{ g virginiamycin}}{\text{Hen}} \left| \frac{10{,}000 \text{ Hens}}{3 \text{ acre}} \right| \frac{\text{acre}}{9.09 \times 10^5 \text{ kg}} \left| \frac{1{,}000 \text{ mg}}{\text{kg}} \right. = 0.188 \text{ ppm}$

Therefore, the maximum estimated concentration of virginiamycin will be approximately 250 ppb in soil without considering further degradation from the natural elements (water, soil, etc.).

Considering the low solubility of virginiamycin in water, and that virginiamycin degrades rapidly in feces, soil (57% in 2 days, 89% in two weeks) and water (50% in 2 days), any runoff of virginiamycin into waterways would be negligible from turkeys raised either in confinement or on range.

The level of virginiamycin in the animal feed nutrient by-product being 25 ppm would result in negligible environmental exposure since rapid degradation would occur during normal use or disposal.

c. Terrestrial ecosystems

As discussed above in (b.) the concentration of virginiamycin exposed to the terrestrial ecosystems would be negligible. Since the product quickly degrades in the droppings, soil and water, there can be no opportunity for accumulation in the environment, thereby eliminating the possibility for build-up to an inhibitory concentration against soil microbes or cause any other environmental concern. Any uptake by plants would be negligible for similar reasons and results from phytotoxicity studies in plants discussed in Section 8, page 18, demonstrates that virginiamycin in fecal material has no significant effect on plants. Due to the low absorption of virginiamycin by the target species (turkeys as well as swine and chickens) as evident from tissue residue studies, it's reasonable to expect that the same low absorption will occur with other animals if they happen to ingest the antibiotic[8]. Virginiamycin would be unlikely to reach groundwater since virginiamycin degrades to less than 50% within 48 hours when exposed to water.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES.

Virginiamycin is classified as a narrow spectrum antibiotic primarily active against gram-positive bacteria and not used in human medicine in this hemisphere. Virginiamycin has met the human and animal safety criteria for antibiotics in animal feeds and its use does not constitute a risk or a human health hazard.

Virginiamycin is non-toxic. No toxic effect attributable to virginiamycin could be demonstrated in any of the following acute and chronic toxicity studies performed on a variety of animals including mice, rats, dogs and turkeys.

Target animal safety in turkeys - Turkeys were fed 0, 10, 100 and 200 g of virginiamycin per ton of feed from one day of age until 16 weeks and 20 weeks for hens and toms, respectively, without any adverse effect.

Acute toxicity in mice - Results from an oral acute toxicity study in mice reflected a low order of toxicity; the lethal dose in 50% of the animals tested (LD_{50}) was greater than 1500 mg/kg.

Subacute toxicity in rats - Virginiamycin was administered by intubation daily for three months to 15 rats of each sex per group, at levels of 5.0, 22.5 or 100 mg/kg. Ophthalmoscopic examinations, hematology, blood chemistries, urinalyses and histopathology were performed on appropriate animals with no drug-related effects noted.

Subacute toxicity study in dogs - Virginiamycin was administered daily at levels of 5.0, 22.5 or 100 mg/kg via gelatin capsules for three months. Following ophthalmoscopic examinations, hematologic studies, blood chemistry, urinalyses and histopathology at terminal necropsy performed on appropriate animals, no effect related to virginiamycin administration was observed.

Teratology in rats - Ninety-six presumed pregnant CD® Sprague-Dawley rats assigned 24 to each of four treatment groups received 0, 25, 75 or 200 mg virginiamycin/kg daily from 6-15 days of gestation. No mortality occurred. At postmortem examination no treatment-related effects were noted in the uteri of low and mid-dose groups. There was no effect of any kind on any of the fetal parameters. The no-effect level was considered to be 75 mg/kg.

Teratology in mice - One hundred presumed pregnant Crl: CD-1 (ICR) BR mice were assigned 25 each into four treatment groups. The mice received either 0, 25, 160 or 1000 mg of virginiamycin/kg/day on days 6 through 15 of pregnancy. There were no deaths during the study and no adverse clinical signs. No postmortem lesions were found. The few skeletal and soft tissue abnormalities which were noted were attributed to expected genetic variants. An increased incidence of dilation of the renal pelvis in the high-dose group was considered a reversible development delay, probably related to reduced maternal gain when treatment was stopped. Statistical analyses of all parameters which provided suitable data for analysis indicated no significant differences among the four (including control) dosage groups. The no-effect level was identified as 1000 mg/kg.

Subchronic toxicity in beagle dogs - Six beagle dogs of each sex in each of four treatment groups were administered virginiamycin 0, 25, 200 and 750 mg/kg/day in gelatin capsule once daily for six months. Emesis, probably related to the large volume of compound given, was noted sporadically in the high-dose group. No significant difference in hematologic parameters were noted, including the bone marrow smears in a hematology study.

Urinalysis revealed no treatment-related effects. Proliferated bile duct epithelium was noted in some livers of the high dose (750 mg/kg) dogs and only adjacent to the gall bladder. This finding was not made in the low (25 mg/kg) or mid-dose (200 mg/kg) groups. The 25 mg/kg dose level was considered the no-effect level.

Two generation reproduction study in rats - Virginiamycin was administered via the diet at dose levels of 0, 25, 65 or 300 mg/kg/day for ten weeks prior to mating and throughout the mating, gestation, lactation and rest periods of two litter intervals. The F_0 generation was composed of 100 male and 100 female CD® Sprague-Dawley derived rats. The F_2 generations were produced from 100 males and 100 females from the F_{1b} generation.

Necropsies were performed on all animals on test and tissues from selected animals were taken for histological examination. Body weights and food consumption were determined. Survivorship, mating or pregnancy indices were recorded for adults; litter data, pup viability and pup survival were recorded for the pups. Histology was not performed on the tissues because the relevant tissues were to be examined from animals on a concurrent carcinogenicity study.

Reduced weights were observed in the high-dose F_0 adult males and periodically in the high-dose F_0 adult females. Reduced pup weights (F_{1a}) were also reported on days 14 and 21 of lactation. Reductions in weights of adult females and pups were not noted when the dose of virginiamycin was reduced from 300 to 100 mg/kg/day. Increased food consumption was noted for treated males but not for treated females.

No treatment-related effects were recorded on survival, mating or pregnancy indices for adult or litter data, viability or survival for pups. The no-effect level was established as 100 mg/kg.

Chronic toxicity (carcinogenicity) study in rats - Five hundred sixty Sprague-Dawley CD® rats, equal numbers both sexes were used. Seventy rats of each sex were allotted to each of four treatment groups.

Doses of 0, 25, and 50 mg of virginiamycin/kg/day were administered continuously in the feed to both males and females. The high dose was 250 mg/kg/day for males and 300 mg/kg/day for females. The test drug was administered for two years. Various observations and tests were made/performed at predetermined intervals during the treatment period.

Reduced body weights, increased food consumption and minor alterations in some clinical laboratory parameters in both sexes at the highest dose were observed. Increased parathyroid hyperplasia was related to nephropathy in individual animals and was not related to treatment. There was a reduction in the incidence of biliary hyperplasia in treated animals, but this is of neither favorable nor unfavorable significance. There were no findings of toxicologic or oncogenic significance at any dose level. The no effect level was considered to be 50 mg/kg/day.

Chronic toxicity (carcinogenicity) study in mice - Virginiamycin was administered orally, via dietary admixture, to 420 CD® - 1 mice (70/sex/group) 7 days a week for a period of approximately 24 months at dose levels of 0, 25, 75 and 1000 mg/kg/day.

Physical observations, body weight and food consumption data were evaluated on all animals pretest and at selected intervals during the treatment period. Hematology and clinical chemistry evaluations were performed at 6 and 12 months and at study termination.

Physical observations of the mice throughout the study revealed no treatment-related effects. Scabs and tears in the ears were noted in control and treated animals and were, therefore, considered unrelated to treatments.

Mean food consumption values were elevated at a number of the measuring periods. The most consistent increases occurred in the mid- and high-dose males and because, to some extent, they exhibited a dose response, they were considered treatment-related.

Hematology and clinical chemistry conducted at 6, 12 and 24 months revealed no patterns which could be attributed to the treatments.

The mean kidney to body weight ratio of the high dose females was elevated compared to controls. This was not evident in the males. Since histopathology of the kidneys was negative, this finding is probably of no pathological significance. There were no other organ/body weight ratio alterations.

Gross pathology revealed a number of lesions. However, they were either sporadic or observed with equal frequency in both treated and control animals.

Microscopic examination of tissues indicated there was an increase in lymphoma rate among the treated mice. However, tumors of the lymphoid system are often observed in control animals at variable rates. The incidence of all primary tumors of the lymphoid system in the treatment groups was comparable to control animals. Moreover, there was no significant increase in tumor incidence in other organs. The 1000 mg/kg dose level was established as the no-effect level.

In a phytotoxicity study, litter from chickens consuming virginiamycin medicated feed (20 g/ton) was applied to loam soil in greenhouse flats at a rate of 4-10 tons per acre. These flats, and others containing untreated loam or application of litter from non-medicated chickens (120 total flats) were planted with wheat, barley, fescue, peppers, tomatoes or corn. At termination of the study, no adverse effect resulting from virginiamycin application was noted. No abnormalities were noted in the organic content and texture of the litter collected from medicated animals, Appendix VII.

In other environmental studies:

1) Housefly toxicity study

Litter from poultry fed virginiamycin medicated feed (20 g/ton) was used as growth media for eggs collected from adult houseflies. Appropriate control manure and CSMA standard fly larval media comprised the control treatments. Eggs collected from adult houseflies reared on the media, were in all cases viable; no adverse effects were noted on the eggs or larval development, Appendix VIII.

2) Earthworm toxicity study

Medicated poultry litter [identical to that used in the housefly toxicity study], or non-medicated litter was applied to soil (containing a controlled number of red earthworms) at a rate of two and one-half to ten tons/acre. No significant adverse effect was seen upon the general condition of the worms, nor upon the number of eggs and young collected from medicated soil as compared to controls, Appendix IX.

3) Fish toxicity studies

Rainbow trout and bluegill sunfish were exposed to virginiamycin treated water for periods of 24, 48 or 96 hours. Toxicity was evaluated in terms of the concentration of drug which produced 50% mortality (LC_{50}). The test showed that extremely high concentrations of virginiamycin (more than 225 ppm), were required to produce 50% mortality in either type of fish, Appendix X.

Effects at the ecosystem level

a. Air

As discussed in Section 7, virginiamycin would not present any adverse effect on the environment since it is non-volatile. Additionally, any dust from production facilities is minimal and would only result in exposure to the soil or water where it is rapidly degraded and found not to affect any environmental circumstances tested.

b. Fresh water, estuarine, and marine ecosystems

Virginiamycin concentration in the water ecosystem would be negligible as calculated in Section 7. It is rapidly degraded in water and found to be non-toxic to fish, target animals, laboratory test animals, houseflies, earthworms or various crops exposed to it in poultry or swine manure. Therefore, no adverse effect on the environment would be expected.

c. Terrestrial ecosystems

The major exposure of virginiamycin to the terrestrial ecosystem is through turkey manure spread on soil. The concentration of virginiamycin estimated to be spread in manure is negligible due to the degradation of virginiamycin in manure, 94% within 14 days. The amount exposed as calculated in Section 7, would be adequately degraded in water or soil with no adverse effects to the animals, plants and soil microbes tested.

Listed below are a number of microbes indigenous to soil, and M.I.C. of virginiamycin. Considering the data regarding possible soil concentration and degradation, it is inconceivable that soil levels of virginiamycin would ever approach the M.I.C.s listed below.

In Vitro Minimal Inhibitory Concentrations (M.I.C.)

ORGANISM	M.I.C. OF VIRGINIAMYCIN µg/ml(ppm)[9]
Mycoplana bullata ATCC 4279	*20*
Mycoplana dimorpha ATCC 4279	*100*
Hydrogenomonas sp.	*100*
Citrobacter sp. 1	*100*
Citrobacter sp. 2	*1000*
Flavobacterium sp.	*1000*
Klebsiella sp.	*1000*
Thiobacillus thiooxydans 504 DSM	*10*
Cythophaga johnsonae 425 DSM	*10*
Rhodopseudomonas sp.	*1000*
Hyphomicrobium sp.	*100*
Rhodopseudomonas sphaeroides 158 DSM	*100*
Nitrobacter sp.	*1000*

Virginiamycin may be categorized primarily as a gram positive antibiotic. Based on its ***in vitro*** spectrum of activity, it exhibits neither antifungal nor gram negative activity. This immediately eliminates any impact on those organisms, the gram negatives, which play a major role in the decomposition of organic matter in the soil and nutrient recycling of the soil. For example, the major soil reactions are all accomplished by gram negative organisms such as ***Nitrosomonas***, ***Nitrobacter***, and ***Thiobacillus***.

Other gram negative organisms are important in the processes of nonsymbiotic and symbiotic nitrogen fixation. The genus ***Azotabacter*** is responsible for non-symbiotic nitrogen fixation, while that of ***Rhizobium*** is responsible for nodulation and symbiotic nitrogen fixation. By virtue of the gram negative origin for all of these soil reactions, virginiamycin residues should have no impact because, a) it is exclusively a gram positive antibiotic, and b) only very low concentrations can be expected in the soil under approved conditions of use.

Some gram positive anaerobes, such as the ***Clostridia***, play some role in the fixation of nitrogen. These organisms are more acid tolerant than aerobes and perhaps for that reason are more widespread. Under suitable conditions, they too can fix some nitrogen.

Regarding the gram positive anaerobes, the minimal inhibitory concentration (M.I.C.) of virginiamycin against ***Clostridium welchii***, is 0.5 µg/ml or approximately two to six times greater than the above mentioned, exaggerated, maximum estimated soil concentrations.

Another important factor to be considered regarding the effect of virginiamycin residues on soil microorganisms is the impact on soil yeast and fungi. Again, a review of the ***in vitro*** spectrum leads one to conclude an absence of significant impact, based on no activity against the yeast ***Candida albicans***, and fungus ***Trichophyton mentagrophytes*** 8410.

Since the product quickly degrades in the droppings, there can be no opportunity for accumulation in the environment, thereby eliminating the possibility for build-up to an inhibitory concentration against similar anaerobes.

Rapid inactivation of virginiamycin in soil and water preclude the bioaccumulation of virginiamycin from turkey or other animal excreta. The extensive compilation of laboratory animal and target animal safety data as well as the lack of effects in toxicity studies of environmental species indicate that adverse effects on the environment would not be expected.

Virginiamycin has a combination of features which distinguish it from many antibacterial agents. It exhibits the feature of bacteriopause; i.e., bacteria which come into contact with virginiamycin for a short time lose their ability to multiply for a considerable time after withdrawal of the product. It is bactericidal, acting primarily on gram-positive organisms, through its ability to inhibit protein synthesis. Although its mode of action is not completely understood, evidence supports the theory that virginiamycin binds to an acceptor site on the ribosomal subunit thus interfering with peptide chain formation. This binding is irreversible and probably accounts for the bactericidal nature of its activity.

Total antibiotic activity of virginiamycin depends on synergistic interaction between its two component factors (M & S). Each factor has a different spectrum of activity. For example, Factor M is active against both Micrococci (Staphylococci and Streptococci), but the combination of the two factors is far stronger in activity. Against *Corynebacterium xerosis*, Factor M alone has a Minimum Inhibitory Concentration (M.I.C.) of 0.2 µg/ml, while the M.I.C. of virginiamycin against *C. xerosis* is 0.03 µg/ml. The activity of Factor M is undoubtedly potentiated by the presence of Factor S, although Factor S alone has little or no activity against *C. xerosis*. Thus, the activity of the two factors together is nearly seven-fold that of either separately.

Plasmid-mediated cross-resistance between virginiamycin and other streptogramin and peptolide antibiotics has been demonstrated *in vitro* with strains of *Staphylococcus aureus* and *Streptococcus faecalis*[10]. The strains were first made resistant to virginiamycin by repeated subculture in the presence of increasingly higher concentration of the antibiotic, utilizing standard *in vitro* techniques.

Studies show that this cross-resistance to erythromycin (and other macrolides) is unidirectional. That is to say, strains made resistant to virginiamycin are also resistant to erythromycin, but strains made resistant to erythromycin are not generally resistant to virginiamycin[11,12]. Erythromycin-resistant gram-positive bacteria were found in the feces of virginiamycin-treated dogs[13]. The enterococci disappeared upon discontinuation of virginiamycin and were replaced by the normal phage type sensitive to macrolides. Evidence of resistance is sparse and no similar data has been found.

In chickens, artificially infected with *Salmonella typhimurium* and treated with virginiamycin (25 g/ton of feed) the persistence, incidence, or susceptibility of the excreted *Salmonella* remained unchanged. Whereas the resistance profile of the *E. coli* in the feces of the same chickens showed, for the most part, only temporary variations in relation to a few of the 12 antibiotics tested. These studies demonstrate that virginiamycin has no affect on incidence of *Salmonella* or *E. coli* in poultry[14].

Implications from the public health standpoint are minimal for a number of reasons, listed below:

- Virginiamycin is not administered to humans in this hemisphere.
- After many years of use in the U.S. and in Europe, few resistant bacterial strains resistant to virginiamycin, have been isolated either from farmers, in feed mixing facilities, or in hospitals, thereby indicating the lack of spread of resistant virginiamycin organisms in an environment, where the antibiotic has been under extensive use for eight and seventeen years, respectively.
- Among antibiotics, a great number (including erythromycin) are active against gram-positive bacteria. Therefore, should an unlikely increase in erythromycin resistant microbes materialize, the abundant availability of alternative agents would minimize any resulting impact.
- Virginiamycin has already met the Human and Animal Health Safety Criteria for Antibiotics in Animal Feeds.

The following table lists the M.I.C. of virginiamycin against a variety of bacterial organisms.

In vitro Minimal Inhibitory Concentrations (M.I.C.) in μg/ml[3]

Organism	M.I.C. of virginiamycin
Staphylococcus aureus	*0.2*
Sarcina lutea	*0.03*
Streptococcus pneumoniae	*0.07*
Streptococcus faecalis	*15*
Corynebacterium xerosis	*0.03*
Hemophilus pertussis	*0.4*
Neisseria meningitidis	*0.1*
Clostridium welchii	*0.5*
Bacillus subtilis	*0.04*
Lactobacillus acidophilus	*0.5*
Escherichia coli	*>100*
Proteus mirabilis	*>100*
Pasteurella pestis	*3*
Shigella flexneri	*>100*
Brucella abortus	*75*
Mycobacterium tuberculosis	*1*
Candida albicans	*>100*
Trichomonas vaginalis	*>100*
Mycoplasma gallisepticum	*0.05*
Leptospirae	*0.002*
Trichophyton mentagrophytes 8410	*>100*
Treponema hyodysenteriae	*0.65*

There are no known adverse environmental effects. Potential pollutants resulting from the manufacturing process are contained within the manufacturing facility and are disposed of in compliance with Provincial, Federal, State and local regulations. The compound is excreted in very low concentrations as the intact drug even after administration at the highest recommended use level for prolonged periods. Virginiamycin is non-toxic, rapidly degraded in feces and soil and only sparingly soluble in water; thus the possibility of water contamination by leaching or other entry into the food chain as a contaminant is practically non-existent. Therefore, no further measures are necessary to mitigate or avoid potential adverse environmental impacts since no potential adverse effects are apparent.

9. USE OF RESOURCES AND ENERGY:

Minimal natural resources and energy are required to transport, use and dispose of waste resulting from the production of virginiamycin or from its use as a feed additive. Solvents are recycled and waste broth is converted to an animal feed additive to recover nutrient resources used in its production. The transportation to a landfill of any excess fermentation waste is minimized and disposal is performed in accordance with national, state and local requirements. No additional resources are required to remove waste containing virginiamycin from use in turkeys since manure is removed from a turkey production facility regardless of additive use. Energy resources (gas, oil, electricity, etc.) are non-recoverable resources and are considered normal requirements of manufacturing/ production.

Virginiamycin has no known effects on endangered or threatened species or on property listed in or eligible for listing in The National Register of Historic Places.

Since virginiamycin is produced by a bacterial fermentation process, the expenditure of manufacturing resources is minimal, and the solvents used are approximately 99% recovered and recycled. Hence, no significant commitment of irretrievable resources will result from the production of virginiamycin.

10. MITIGATION MEASURES

Material Safety Data Sheets are available for employees who work in the production area. In addition, employees in the production and packaging areas wear protective clothing and dust respirators as needed, to assure compliance with OSHA standards as discussed in Section 6. No other mitigation measures are necessary since virginiamycin is non-toxic and does not pose any known harm to the environment.

11. ALTERNATIVE TO THE PROPOSED ACTION:

No potential environmental impacts are apparent for the use of virginiamycin in turkeys as well as in currently approved species (swine and chickens). The only specific alternative to the proposed actions would be refusal to approve the New Animal Drug Application. This would, however, deny the producer the benefits which could be realized by use of virginiamycin in terms of the economic gain afforded by increased weight gain and improved feed efficiency in turkeys; such action would hardly seem justifiable in view of the lack of toxicity, the absence of human health hazard, and the negligible impact on the environment associated with the use of virginiamycin.

Other factors which distinguish virginiamycin from many if not all antibiotics currently approved for poultry are:

- It is a composite antibiotic and consequently less likely to induce bacterial resistance than single-entity products.

- No withdrawal period is required because it is poorly absorbed from the digestive tract of domestic animals.

- It is not, in this hemisphere, used in human therapeutics; however, it has met the Human and Animal Health Safety Criteria for Antibiotics in Animal Feeds and is currently dispensed as a swine and broiler chicken growth enhancer.

- It is non-toxic, excreted in very low concentrations and rapidly degraded.

These factors illustrate the numerous advantages virginiamycin offers over some presently available products.

In recent years, there have been significant changes in the agricultural sector of the American economy. Growing populations--both here and abroad--have increased the demand for the entire range of grain and meat food products. Large scale production to meet this rising need has become a highly technical and more efficient process. Among the numerous tools employed toward this end are a vast array of animal health products. By employing antibacterial agents to control disease and stimulate rate of growth, a more efficient utilization of feedstuffs has been realized. The result has been to increase the abundance of food by enriching the supply of food-animal products with the high quality protein value essential for good nutrition and health at prices within the grasp of the consumer.

Controlled clinical studies have demonstrated the potential benefits virginiamycin could offer the turkey producer in terms of increased growth rate as well as feed efficiency resulting in lower unit production costs. In the marketplace, these benefits could be translated into increased availability of poultry at a lower cost to the consuming public, in return for negligible changes in the environment.

12. LIST OF PREPARERS

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Twenty years of Environmental Engineering experience in the consulting and engineering industry.

13. **CERTIFICATION**

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the firm responsible for preparation of this environmental assessment.

Duane E. Thurman

Duane E. Thurman, Ph.D.
General Manager, U.S. and Canada

3-3-88

Date

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Appendix I

SmithKline Beckman
CORPORATION

MATERIAL SAFETY DATA SHEET

NFPA Designation

NO.

SK&F NO.: 7988

CAS REGISTRY NUMBER: 21411-53-0 and 23152-29-6

CHEMICAL NAME: Virginiamycin

STRUCTURE:

Factor M_1 - $C_{28}H_{35}N_3O_7$

Factor S - $C_{43}H_{49}N_7O_{10}$

I. PRODUCT IDENTIFICATION		
MANUFACTURER'S NAME	SmithKline Animal Health Products	Phone 215-251-7424 Emer. Phone 215-251-7410
ADDRESS	1600 Paoli Pike, West Chester, PA 19380 and Rue de l' institut B1330, Rixensart, BELGIUM	
TRADE NAME	'Stafac' Eskalin	
SYNONYMS	Virginiamycin	
STOCK ITEM OR INTERNAL #		

II. HAZARDOUS INGREDIENTS		
MATERIAL OR COMPONENT	%	HAZARD DATA

III. PHYSICAL DATA		
BOILING POINT 760 MM HG		MELTING POINT
SPECIFIC GRAVITY (H_2O = 1)		VAPOR PRESSURE
VAPOR DENSITY (AIR = 1)		SOLUBILITY IN H_2O, % BY WT. 600 mcg/l at pH 6
% VOLATILES BY VOL.		EVAPORATION RATE
APPEARANCE AND ODOR	Brown powder with characteristic odor, very bitter caste, non-hydroscopic	MOLECULAR WEIGHT

NO.

IV. FIRE AND EXPLOSION DATA

FLASH POINT (TEST METHOD)		AUTOIGNITION TEMPERATURE	
FLAMMABLE LIMITS IN AIR, % BY VOL.	LOWER		UPPER
EXTINGUISHING MEDIA	CO_2, foam or dry powder extinguishers		
SPECIAL FIRE FIGHTING PROCEDURES			
UNUSUAL FIRE AND EXPLOSION HAZARD			

V. HEALTH HAZARD INFORMATION

HEALTH HAZARD DATA: Oral LD 50 in mice - 1.5 gm/kg

INHALATION

SKIN: Mild to moderately severe contact dermatitis, probably related to sensitization, has been described.

EYE

INGESTION

GENERAL

EMERGENCY AND FIRST AID PROCEDURES

EYES: Use eye wash fountain, flush eyes for at least 15 minutes.

SKIN: Wash well with soap and water.

INHALATION

INGESTION

SPECIAL NOTES: Toxic effects attributable to virginiamycin were not demonstrated in chronic toxicity studies in rats, (5 to 100 mg/kg), dogs (5 to 100 mg/kg) or swine, (100 to 500 mg/kg).

NO. ______

VI. REACTIVITY DATA

CONDITIONS CONTRIBUTING TO INSTABILITY

Avoid excessive heat

INCOMPATIBILITY

Alkalies, oxidants

HAZARDOUS DECOMPOSITION PRODUCTS

Unknown

CONDITIONS CONTRIBUTING TO HAZARDOUS POLYMERIZATION

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

Sweep up spills and place in sealed containers

NEUTRALIZING CHEMICALS

1% caustic or hypochlorite followed by water.

WASTE DISPOSAL METHOD

Landfill

VIII. STORAGE

SPECIAL INSTRUCTIONS FOR STORAGE

IX. SPECIAL PROTECTION INFORMATION

VENTILATION REQUIREMENTS

Normal ventilation

SPECIFIC PERSONAL PROTECTIVE EQUIPMENT

MINIMUM RESPIRATORY (SPECIFY IN DETAIL) 3M Brand Dust Respirator 8710 or equivalent for nuisance dust (not surgical mask).

EYE Safety glasses for lab quantities, goggles for manufacturing quantities.

GLOVES Impervious glove, cotton lined or with replaceable cotton liners

OTHER CLOTHING AND EQUIPMENT

Disposable overgarment, or one which can be laundered.
Launder after use.

PREPARED BY: [signature]

APPROVED BY: [signature] **DATE:** 12/1/83

SmithKline Beckman
CORPORATION

One Franklin Plaza, P.O. Box 7929, Philadelphia, PA 19101 - (215) 751-4661

Appendix II

STABILITY OF VIRGINIAMYCIN IN POULTRY EXCRETA AND LITTER

INTRODUCTION

Three fortification studies were conducted to determine the degradation rate of virginiamycin in poultry litter and excreta when stored at room temperature or under ambient conditions. Litter was employed in the first fortification study at room temperature to obtain virginiamycin stability data applicable to those open-housing facilities where poultry excreta becomes mixed with soil. The second study using virginiamycin fortified poultry excreta was also conducted at room temperature. In the third study, fortified poultry excreta was subjected to ambient temperature conditions in order to better duplicate temperatures and environmental factors encountered during actual use.

METHODS

A. POULTRY LITTER AT ROOM TEMPERATURE (STUDY 1)

Fresh poultry litter was collected from chickens housed at Truslow Farms, Chestertown, Maryland. The birds had been maintained on an unmedicated commercial diet. The litter was air-dried overnight, processed by a homoloid mill to make a powder and stored at 4°C until use.

Replicate 20 g samples of dry poultry litter were weighed into polypropylene bottles and 41.0 mls distilled water (2.05 mls water/gram of soil) was added to each bottle to achieve 70% field capacity*. The replicate samples were fortified with virginiamycin at a level of 30 ppm. Containers were stored loosely capped at room temperature (18-22°C) for the course of the 3 month stability experiment.

After the appropriate degradation period, triplicate samples were extracted, diluted and assayed microbiologically for virginiamycin using the disc method.

B. POULTRY EXCRETA AT ROOM TEMPERATURE (STUDY 2)

Fresh excreta were collected from chickens maintained on an unmedicated commercial diet and housed at Truslow Farms, Chestertown, Maryland. Upon arrival, excreta was stored at 4°C until use.

* Field Capacity (100%): The amount of water held in soil after the gravitational water has drained away.

Replicate 20 g samples of the pooled excreta were weighed into polypropylene bottles. The samples were fortified with virginiamycin at a level of 30 ppm. The fortified samples were stored loosely capped at room temperature (18-22°C).

After the appropriate degradation period, samples were extracted diluted and assayed microbiologically using the disc method.

C. POULTRY EXCRETA AT AMBIENT TEMPERATURE (STUDY 3)

This study employed the same poultry excreta obtained from Truslow Farms and used in the room temperature stability study.

Replicate 20 g samples of the pooled excreta were weighed into 50 ml capacity polycarbonate weighing jars and fortified with virginiamycin at a level of 30 ppm. Total weights of jar and fortified excreta were recorded.

The samples were kept outside during the day with lids removed and brought in at night, except during inclement weather, when samples were kept inside. Outside temperature readings were recorded for the length of the study, with a range in temperature of 18°F to 76°F. Due to weight loss in samples through evaporation, samples were reweighed every other day during the study and brought back to their initial weight with distilled water.

After the appropriate degradation period, triplicate samples were quantitatively transferred to polypropylene bottles and extracted then diluted and assayed microbiologically using the disc method.

RESULTS

The data from study #1 demonstrated that 69% degradation of virginiamycin occurred within three days and 83.2% within seven days. In study #2 80% of the virginiamycin degraded within seven days and 94.4% within fourteen days. Study #3 provided similar results with 78% of the virginiamycin degrading within seven days and 94.7% within fourteen days.

CONCLUSION

Virginiamycin when fortified into poultry excreta and poultry litter at a level of 30 ppm, is unstable and degrades rapidly at room temperature and under ambient conditions with approximately 69% degradation occuring within three days, 80% within seven days and 94% within fourteen days.

Appendix III

STABILITY OF VIRGINIAMYCIN IN SOIL MIXED WITH SWINE FECES AT ROOM TEMPERATURE

INTRODUCTION

The purpose of this study was to determine the stability of virginiamycin in a swine feces/soil mixture. This was performed to duplicate those conditions where swine are reared in outside lots.

METHODS

Soil mixed with swine feces was obtained from a commercial operation in which pigs are reared on outside lots. An analysis of the soil was performed by the United States Testing Company, Inc., located in Memphis, TN. Following are the results:

Parameter	Swine Dirt
pH	6.4
Organic Matter, %	3.8
Cation Exchange Capacity, meq/100g	16.9
1/3 Bar Moisture, %	17.0
Texture	silt loam
Sand %	23.2
Silt %	74.0
Clay %	2.8

The pig soil was air dried, large particles sifted out, and then ground to a fine powder. The soil was maintained at 70% field capacity. This was determined by taking duplicate 20 g dried soil samples and adding 100 ml of water to each. The amount of water remaining in the soil was measured by difference and then divided by 20. This was equal to 100% field capacity. When this figure was multiplied by 0.70 the 70% field capacity volume was obtained.

Duplicate 20 gram dried soil samples were weighed into 250 ml polypropylene bottles and brought to 70% field capacity (13.14 ml water/sample). Each sample was fortified with virginiamycin at 30 ppm. The bottles were stored loosely capped at room temperature for three months. Each of the samples was extracted and assayed microbiologically utilizing the disc method.

RESULTS

As in previous stability studies, the antibiotic is rapidly degraded, with 57% of the initial content degraded within 2 days, 80% within 9 days and 89% degraded within 14 days. The concentration of virginiamycin steadily declined, until none was detectable after 84 days.

CONCLUSION

A mixture of feces and soil obtained from a commercial swine operation (where pigs are reared in outside lots) was fortified with virginiamycin and stability studies performed over a three month period at room temperature. The results confirm that the drug is rapidly degraded, with 89% degraded after 14 days, and none detectable in 84 days. Comparisons from this experiment to room temperature stability studies performed on swine feces only, revealed no significant differences in the rates of degradation.

Appendix IV

STABILITY OF VIRGINIAMYCIN IN WATER

INTRODUCTION

The purpose of these experiments was to evaluate the degradation rate of virginiamycin in water in the presence of swine and without the presence of swine. Experiments were also conducted to determine the effect of pH and elevated temperature.

METHODS

Five experiments were performed to assess the stability in water. Four of the experiments were performed at room temperature and the fifth experiment was performed at conditions of room temperature and 37°C.

Experiment 1 and 2 were carried out in the presence of swine under actual field conditions. Pigs were housed in concrete floored pens at the Applebrook Research Center, SmithKline Corporation. Watering containers were made of galvanized metal with a 15 gallon capacity. They were equipped with a device to maintain a constant level in the drinking pan which is fed from the reservoir.

Virginiamycin was added to tap water in various concentrations and samples taken from the reservoir immediately after preparation and again 22 hours later from the drinking pan. The samples were assayed for virginiamycin using an Ehrlich colorimetric method.

Experiment 3 and 4 were conducted without the presence of swine, using the same conditions and procedures as Experiment 1.

The effect of pH and temperature on the degradation rate of virginiamycin in water was evaluated in Experiment 5. This was a laboratory experiment without the presence of swine. Synthetic hard water was prepared to produce a hardness of 123 mg/l (123 ppm) expressed as $CaCO_3$. The pH was adjusted using hydrochloric acid or sodium bicarbonate to give the final pH of 6, 7 and 8, respectively. Virginiamycin was added to the synthetic hard water at a concentration of 47 mg per Liter. The resultant solutions were stored in galvanized metal pails at room temperature and 37°C. Samples were taken immediately after preparation (initial) and 23 and 48 hours after storage and assayed as in Experiment 1.

RESULTS

Results of virginiamycin degradation experiments conducted in the presence of swine indicated that within 22 hours, at least 37 percent of the Virginiamycin had degraded. Without the presence of swine, an average of 25 percent of the virginiamycin had degraded at the end of 22 hours.

The fifth experiment demonstrated the effect of hard water and elevated temperature on the degradation rate of virginiamycin. At room temperature after 23 hours, 36 percent of the virginiamycin had degraded at all pH levels tested. While at 37°C 59 percent had degraded. After 48 hours these values had increased to 53 percent at room temperature and 69 percent at 37°C, respectively.

CONCLUSIONS

These experiments demonstrate the rapid degradation rate of virginiamycin in water and that elevated temperatures accelerate that rate. More than 35 percent of virginiamycin is degraded in water after 22 hours in the presence of swine. More than 50 percent is degraded after 48 hours at room temperature while at 37°C the degradation rate is accelerated.

Appendix V

A WATER/n-OCTANOL PARTITIONING STUDY ON VIRGINIAMYCIN

INTRODUCTION

The purpose of these studies, performed at Applebrook Research Center, was to determine the lipid/water partitioning of virginiamycin. The following partitioning coefficient data was obtained by shaking virginiamycin with two immiscible solvents and then analyzing the concentration in both phases.

METHODS

A water solution of virginiamycin was prepared and its concentration confirmed utilizing the disc microbiological assay procedure. Equal volumes (50 ml) of the virginiamycin water solution and n-octanol were placed in bottles. Duplicate samples were agitated on a horizontal shaker at room temperature and 37°C. After 17 hours, the bottles were removed from the shakers and the phases separated by centrifugation. An aliquot of the water and n-octanol phases was withdrawn for biological assay. Phases were then re-combined in the bottles and replaced onto the shakers at their appropriate temperature conditions. Partitioning systems were continuously agitated for 36 hours, with aliquots withdrawn for biological assay, as described, at 24 and 36 hours.

RESULTS

In this study the initial virginiamycin water solution assayed at 60 PPM. Water phase samples taken at 17, 24 and 36 hours assayed negative, thereby indicating that all the virginiamycin was concentrated in the n-octanol phase.

CONCLUSION

A partition experiment with virginiamycin in a n-octanol/water system was performed to assess the lipid solubility of virginiamycin and its subsequent potential for passive diffusion across membranes. The results of this experiment suggest that virginiamycin is lipid soluble and, therefore, may have potential for passive diffusion across membranes. However, this suggestion for extensive absorption is inconsistent with actual results obtained from animal studies.

V. Maximum Possible Concentrations of Virginiamycin In Agricultural Soils And Their Impact On Soil Microbial Flora

A. Estimates Based on Initial Concentrations - Independent of Stability Data

Table 2 provides individual and mean elimination data for virginiamycin in feces. Based on this data, when virginiamycin is administered to swine as a medicated feed at its highest approved level, one can expect an average fecal concentration of 16 ppm ± 1%. Based on this range, calculations were performed to determine the concentration of virginiamycin to be expected in agricultural soils when fecal material containing the residues is applied at the maximum feasible level (approx. 5 dry weight tons per acre) and incorporated into the top six inches of soil (approx. 9.09×10^5 kgs soil/acre). For the purposes of this calculation, no consideration was given to the stability of virginiamycin in feces, which, as indicated in our previous stability studies, is very low. For purposes of this estimation, the highest mean range of fecal concentrations was utilized, based on the data provided in Table 2. Obviously, utilization of the lowest mean range would result in no virginiamycin residues at all.

1. Sample Calculations

a. The addition of 5 dry weight tons/acre is equivalent to the addition of 4545.5 kg/acre.

5 tons = 10,000 lbs = 4545.45 kg

b. The highest mean range of virginiamycin concentrations expected in feces is 33 ppm.

33 ppm = 33 µg/g

33 µg/g X 1000 g = 33,000 µg of residue/kg of feces

c. The total residue applied is:

4545.5 kg (33,000 µg) = 150,001,500 µg applied per acre

d. The final concentration in the soil per acre is:

$\frac{150,001,500 \text{ µg}}{909,000 \text{ kg}}$ = 165 µg/kg or .165 ppm concentration in soil

Appendix VII

GREENHOUSE PHYTOTOXICITY EVALUATIONS OF LITTER FROM VIRGINIAMYCIN TREATED BROILERS ON SEVEN CROPS

OBJECTIVE

The objectives of this project were to determine the effects on crop growth of litter from poultry that were fed Virginiamycin treated feed. The treated feed contained 20 grams per ton of Virginiamycin. The litter was incorporated into the covering soil to a depth of 2 ½ inches at 4 - 10 tons per acre.

METHODS & MATERIALS

During the fall of 1976, Smith Kline Animal Health collected litter from pens of broilers which were fed a basal ration and a medicated ration containing Virginiamycin at 20 grams per ton of feed.

The following were employed in this study:

1. Three separate drums of air-dried poultry medicated litter; approximately 40 kg each.
2. Three separate drums of air-dried poultry control litter; approximately 40 kg each.
3. Five jars of fresh medicated poultry manure.
4. Five jars of fresh control poultry manure.

One-third of each drum was ground in a Hobart food chopper for two minutes and returned to the same drum in a sealed plastic container. The sealed drums were stored at an average temperature of 60°F. Random samples of the ground air-dried manure were submitted for proximate analysis along with the fresh samples for moisture determinations.

Soil for the project was obtained from Wipperfurth & Endres, Waunakee, WI 53597. During 1976 wheat was grown on the soil. The last two previous years the soil was used for growing lima beans. A representative sample of the soil was sent to the state soil lab for analysis and type determination.

The soil was sifted through a 0.5 cm mesh screen and put in 2.25 square foot flats in the greenhouse. The litter application rates for the seven crops were based on the recommendations as presented by the following publications:

University of Maryland Fact Sheet 39
Poultry Manure is Valuable Fertilizer
V.A. Bondel, C.S. Shaffner and H.A. Hunter
Depts. of Agronomy, Poultry an Agronomy
Revised, May 1966

University of Georgia Leaflet 206
Poultry Waster - Georgia's 30 Million Dollar Forgotten Crop
Harry D. Muller, Extension Poultry Scientist
November, 1974

The dosage rate for 1 ton per acre is 46.7 grams per flat. All dosages were calculated on the basis of moisture determinations made directly prior to the start of the experiment.

For both control and medicated poultry manure an application rate of four tons per acre was used for barley, fescue, wheat, green beans and peppers; five tons per acre was used for cucumbers and 10 tons per acre for corn.

The samples which were previously ground were weighed in amounts appropriate for the respective flats. Two and one-half inches of the covering soil of each flat was placed in a five gallon container and mixed with the sample for four minutes with a Hobart blender. The flats were tagged with a marker as the mixes were completed.

The treated and untreated flats were placed on the greenhouse bench and seeded. A planting form was used which contained 20 holes equidistant from the flat sides and from each other. The crop, variety, number of seeds per flat and planting depth were as follows:

Crop	Variety	Seeds per Flat	Planting Depth (cm)
Corn	Wis. 900	20	2.54
Cucumber	Improved Chicago Pickling	20	1.27
Green Bean	Green Podded Bush	20	2.54
Pepper	California Wonder 357	20	1.27
Wheat	Timwin	40	2.54
Barley	Dickson	40	2.54
Fescue	Pennlawn	100	1.27

Immediately after planting, each flat was watered with 2 liters using a sprinkler head to evenly distribute the moisture. Equal moisture per flat was added daily as required.

RESULTS AND DISCUSSION

Barley - At 23 days after planting the total stand count in each plot was recorded. At the same time the average heights of ten plants in the poultry medicated and poultry control plots and twenty plants in the untreated plots were recorded. In each plot the readings were taken for the first two plants in row one, plants two, three and four in rows two and three and the last two plants in row four. In those instances where no plants or one plant was present it was so noted.

Wheat - At 22 days after planting the total stand count in each plot for the wheat was recorded. At the same time the average heights of two plants per ten locations per plot were recorded. In each plot the same readings were taken as for barley.

Fescue - At 33 days a stand count of plants for each of five rows in a plot were recorded and totaled. All plants from each plot were cut 3.7 centimeters from the soil surface and the weight for each plot was recorded. At 53 days all plants from each plot were cut 2.54 centimeters from the soil surface and the weight for each plot was recorded.

Corn - At 22 days after planting the total stand count for corn was recorded. The height of the plants per plot were recorded accordingly: 0 - 15, 15 - 30, and 30+ centimeters. Those plants with wilting of the new growth were recorded for each plot.

Green Beans - At 22 days after planting the total stand count for green beans was recorded. In addition those plants with primary leaves at least five centimeters wide and eight centimeters long or longer were recorded as well as all those seedlings which were smaller.

The weights of all larger bean plants per plot were recorded and the average weight of those plants with leaves at least 5 centimeters wide and 8 centimeters long noted.

Cucumber -

At 34 days after planting the total stand count for cucumbers were recorded. In addition the height of the plants per plot were recorded accordingly: 0 - 15, 15 - 30, and 30 + centimeters.

All cucumber plants 15 cm or larger were cut at the soil level and the weight for each plot was recorded. The roots were removed for observation.

The degree of plant injury was noted and the number of leaves with necrotic lesions was recorded.

Pepper and Tomato -

At 19 days after the pepper had been seeded 10 (6 - 8 centimeters) stokesdale tomato seedlings were transplanted in each flat.

At 56 and 42 days after transplanting, the number, size and phytotoxic effects were observed and recorded for the pepper and tomato seedlings, respectively.

CONCLUSION

Litter from poultry fed with Virginiamycin treated feed (20 grams per ton) had no effect on the growth of wheat, pepper, tomato, barley and fescue when applied to loam soil at 4 tons per acre and no effect on corn at 10 tons per acre.

The growth of beans and cucumbers were somewhat inhibited. The number of large bean plants was about 15% less than the controls at 4 tons per acre and the number of large cucumber plants about 20% less at 5 tons per acre. However, no phytotoxic symptoms were observed on any plants and all roots were normal in all treatments.

Appendix VIII

EVALUATION OF THE POTENTIAL ADVERSE ACTIVITY OF VIRGINIAMYCIN RESIDUES CONTAINED IN BROILER LITTER TO HOUSEFLY EGGS AND LARVAE

OBJECTIVE

The purpose of this project was to determine whether manure from poultry fed virginiamycin treated feed had any effect on the development of housefly eggs and larvae.

METHODS & MATERIALS

Larval Media

All media were prepared the day prior to housefly egg collections.

Poultry manure

Equal quantities of manure were taken from each drum which had previously been ground and thoroughly mixed. 2500 gram quantities of air-dried manure specimens for each test series were reconstituted to a fresh litter basis.

CSMA

This is a standard media used as a reference comparison. A 2500 gram quantity of CSMA Standard Fly Larval Medium was mixed with 8 liters of a deionized water suspension containing 80 ml of nondistatic diamalt and 45 grams of active dry yeast. The medium was mixed thoroughly and equal quantities were transferred to five battery jars (16 centimeter diameter by 19 centimeters deep) and covered with a cloth.

Eggs

The morning following media preparations, eggs were collected from the food dishes containing mature F58W strain houseflies. Two hundred viable eggs were counted onto lined filter paper. The eggs were washed into a 1 cm wide by 2.54 cm deep trench in the center of the media. The eggs were then covered with the media and the jar openings were covered with a cloth.

Pupae

Since mature larvae migrate to the surface to pupate, a two-inch layer of vermiculite was placed on each jar of medium three days after seeding. Six days after seeding the mixture of vermiculite and pupae was poured on a tray and then screened to recover the pupae. All recovered pupae were counted tabulated and combined for each test series.

Pupae(Con't.)

They were placed in a 30 by 30 centimeter screened cage, fitted with a sleeve opening and the adult emergence observed. Eggs (0.1 ml) from the emerging adults were collected and seeded into CSMA media and the number of pupae and emerging adults were recorded.

RESULTS AND CONCLUSION

Litter from poultry fed with virginiamycin treated feed (50 grams per ton) had no adverse effects on housefly eggs and larvae development. In all instances, eggs collected from adult houseflies reared on the various manure treatments were viable.

Appendix IX

EVALUATION OF THE POTENTIAL ADVERSE ACTIVITY OF VIRGINIAMYCIN RESIDUES CONTAINED IN BROILER LITTER TO EARTHWORMS

OBJECTIVE

The purpose of this project was to determine whether manure from poultry fed virginiamycin treated feed had any effect on the general condition of earthworms and their reproductive activity.

METHODS & MATERIALS

The soil and litter for the project were the same as previously described in Appendix VIII.

Manure Samples

Moisture determinations were made on composites of the air dried ground manures and fresh manures.

The following chart shows the amount of air dried manure which was mixed with each quart of air dried soil.

Tons of Fresh Manure per Acre	Grams of Fresh Manure per Test*	Equivalent Grams of Air Dried Manure per Test*
Broiler Medicated		
2½	4.4	1.9
4	7.1	3.1
10	17.7	7.6
Broiler Control		
2½	4.4	1.8
4	7.1	2.9
10	17.7	7.2

*Test containers are one quart jars.

The 5 quarts of soil and manure for each rate were mixed in a V-shell blender for 5 minutes. The mixture was divided into 5 equal parts.

One hundred twenty five milliliters of tap water was added to each treatment. Fifty red worms were added to each container and the soil was covered with a damp cheesecloth. The test containers were held at 62°F. Similar moisture levels were maintained by keeping the surface and cheesecloth damp as required.

The condition of the containers and worms was observed at 3 and 7 days. After 10 days exposure the containers were emptied and the number of worms and their condition were recorded. After returning the worms to their respective containers, 6 grams of food (CSMA fly larval media) was added to the surface before replacing the damp cheesecloth.

After 25 days exposure the containers were emptied and the numbers of worms were recorded. Observations of eggs and young were recorded. After returning the worms to their respective containers, 6 grams of CSMA fly larval media was added to the surface and 15 ml of water was added to the cheesecloth on the surface of each container.

After 35 days the experiment was terminated. Containers were emptied and the number of adults, egg and young worms were recorded.

RESULTS AND CONCLUSIONS

Total earthworm recovery data was used to compare the broiler medicated versus the broiler control treatments. The total numbers of adult earthworms were essentially constant throughout all experiments. The individual replicates showed a wide range in the number of earthworms eggs and young, but the total number for each experiment were similar.

Litter from poultry fed with virginiamycin treated feed (20 grams per ton) had no adverse effects on the general condition of earthworms and only slight differences on the number of eggs and young.

APPENDIX X

TROUT AND BLUEGILL SUNFISH FISH TOXICITY STUDY WITH VIRGINIAMYCIN

OBJECTIVE

To determine the toxicity of virginiamycin in trout and bluegills.

METHOD

The protocol for bioassay techniques was in accordance with the Environmental Protection Agency Fish-Pesticide Acute Toxicity Test Guideline. Statistical analyses followed the procedure of Lithfield, J.T., Jr. and Wilcoxon, F. entitled A simplified method of evaluating dose-effect experiments, J. Pharm. and Exp. Therap. 96:99-113 (may and August) 1949.

RESULTS

Rainbow Trout

24 hours: LC_{50} = 430 ppm
48 hours: LC_{50} = Between 225 ppm and 338 ppm
96 hours: LC_{50} = Between 225 ppm and 338 ppm

Bluegill Sunfish

24 hours: LC_{50} = 252 ppm
48 hours: LC_{50} = 240 ppm
96 hours: LC_{50} = Between 225 ppm and 338 ppm

CONCLUSION

Trout and bluegill sunfish were found to have a high tolerance for virginiamycin.