ENVIRONMENTAL IMPACT ANALYSIS REPORT

Date: February 1, 1979

Name of Applicant: SmithKline Animal Health Products
Division of SmithKline Corporation

Address: 1500 Spring Garden Street
Philadelphia, PA 19101

Environmental Information:

1. PROPOSED ACTION

SmithKline Animal Health Products holds approved New Animal Drug Applications (91-467 and 91-513), which demonstrate safe and effective use of virginiamycin premixes for manufacture of swine feeds. The following conditions are proposed for the use of these premixes in poultry feed:

1) that a level of five grams of virginiamycin per ton of feed be administered for improved feed efficiency;

2) that levels of five to 20 grams of virginiamycin per ton of feed be administered for increased weight gain.

a) Purpose of the proposed action:

By this proposed action of permitting the addition of five to 20 grams of virginiamycin per ton in poultry feeds, SmithKline Animal Health Products hopes to give farmers a means of increasing rate of gain and improving feed efficiency in growing broiler chickens.

b) Environment to be affected:

Since virginiamycin is a growth enhancer, proposed for use in poultry feed, the geographic area of predominant usage will naturally coincide with the area of greatest meat-type poultry production; i.e., the Southern states. The following table lists the relative distribution of broiler chicken populations in these states as compared to the rest of the country.

U.S. BROILER Production* --- 1976 (in Thousands)

State	# Broilers	%
Arkansas	540,428	17.0
Georgia	451,531	13.9
Alabama	430,225	12.9
North Carolina	315,589	10.4
Mississippi	257,442	7.2
Maryland	199,008	6.2
Texas	190,703	5.9
Total in the above States	2,384,926	73.5
Rest of U.S.	895,196	26.5
Total U.S.	3,280,122	100.0

Source: USDA, Statistical Reporting Service
Agricultural Statistics, 1977 p.408

2. PROBABLE IMPACT ON THE ENVIRONMENT

a) The probable impact of the above proposed action is negligible. The use of virginiamycin in poultry 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 chickens, 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.

In 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.

To determine this stability, poultry droppings were fortified to a level of 30 ppm of virginiamycin, and maintained at room temperature (18-22° C). After three days, more than 79% of the virginiamycin had degraded and by the 14th day more than 94% degradation had occurred.[1] Similar results were obtained when the droppings were identically fortified but maintained outdoors at ambient temperatures (8-24° C), in order to better simulate practical circumstances. After seven and 14 days, more than 77% and 94%, respectively, of the virginiamycin had degraded.

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*Commercial broiler production including production of other meat-type breeds, excludes States producing less than 500,000 birds. Estimates of commercial broilers are for the Dec. 1, 1975 through Nov. 30, 1976 marketing year.

[1] Appendix III

To further support this data, poultry 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 68% and 83% respectively, of the antibiotic had degraded.[2]

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).[3] Since the highest anticipated use level in chicken 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) can 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.

The practice of applying livestock manure to fertilize agricultural soil, necessitates an assessment of:

- The maximum concentration of excreted virginiamycin in the soil
- The potential phytotoxic effects from the excreted virginiamycin.

The maximum encountered fecal concentration, 33 ppm, was obtained from a pig receiving 95.7 gm of virginiacmycin per ton of feed. An immediate application of that excreta at the rate of 5 tons/acre (assuming no degradation of drug) would produce a 0.165 ppm concentration of virginiamycin.[4] However, the drug is readily biodegradable, and poultry feed contains only 5-20 g/ton; consequently, these application levels are not likely to occur. Moreover, the concentration of virginiamycin expected in the soil would be well below that required to exhibit an inhibitory effect on soil flora.

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[2] Appendix III
[3] Approved NADA 91-513 (Analytical Methods for Residues)
[4] Kraeer, P., Presented at 5th World International Pig Veterinary Society Congress, Zagreb, Yugoslavia. 1978.

Listed below are a number of microbes indigenous to soil and the 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 µ/ml[5]
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
Rhodospeudomonas sphaeroides 158 DSM	100
Nitrobacter sp.	1000

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

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.

Stability experiments on the degradation rate of virginiamycin in water, at variable temperature and pH, demonstrated that after 72 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.[6]

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[5] Van Dijck, P. and H. Van de Voorde. Applied and Environmental Microbiology, 31:1, 332-356, 1976.

[6] Appendix IV

An octanol/water partitioning study was performed in order to evaluate the potential for virginiamycin absorption into 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 offered, no significant blood levels could be detected, indicating poor absorption in spite of high lipid solubility. 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. The impact of this finding on the environment is minimal, since virginiamycin is rapidly degraded in the feces and therefore unavailable for absorption.[7] 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 applications 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.[8]

In other environmental studies:

1) <u>Housefly toxicity study</u>

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.

2) <u>Earthworm toxicity study</u>

Medicated poultry litter [identical to that used in (1)], 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.

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[7] Appendix V
[8] Appendix II

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 (LD_{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.

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.[9]

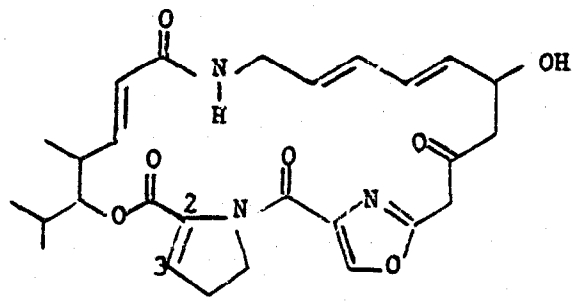
Virginiamycin has been shown to be a suitable alternative growth promotant for poultry and swine in the United Kingdom after restrictions were placed on the use of penicillin, tetracyclines, sulfonamides and nitrofurans, following the recommendations of the Swann Committee. Virginiamycin has also met the criteria of the European Economic Community for inclusion in Annex I of the list of antibacterial substances that may be used continuously at sub-therapeutic levels in the feed of swine, poultry, and calves, for improvement in rate of weight gain and feed efficiency.

Virginiamycin has a combination of features which distinguishes 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) both of which are produced by the same Streptomyces.

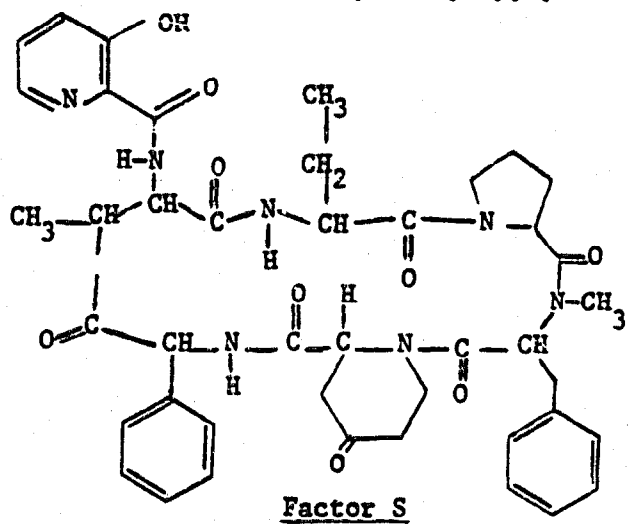
[9] Approved NADAs 91-467 and 91-513.

The M factor has a macrocyclic lactone structure,



Factor M

whereas the S factor is a cyclic polypeptide.



Factor 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 antibictics 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 concentrations 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] Recently, erythromycin-resistant gram-positive bacteria were found in the feces of virginiamycin-treated dogs.[13] However, the evidence 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.[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 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 years.

SmithKline Animal Health Products
a SmithKline company

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[10] DeSomer, P. and Van Dijck, P. J., Antibiot Chemother 5: 632-639, 1955.
[11] Jones, W. F., Nichols, R. L. and Finland, M., Proc Soc Exp Biol Med 93: 388-393, 1956.
[12] Kienholz, M. and Krigar, G., Arzneim. Forsch 16: 1104-1105, 1966.
[13] Silver, P., Leming, B. and Cohen, E., In Current Chemotherapy, Bol. I, W. Siegenthaler and R. Luthey eds. American Society for Microbiology, Wash., DC 1978.
[14] Section 8.ii, Appendix A, of this submission.

- 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[15]

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

[15] VanDijck, P.J. Chemotherapy 14:322-32, 1969.

b) Virginiamycin is extremely non-toxic. No toxic effect attributable to virginiamycin could be demonstrated in any of a number of acute and chronic toxicity studies performed on a variety of animals including mice, rats, dogs, swine and chickens.

The oral LD_{50} of virginiamycin in mice was greater than 1500 mg/kg; higher doses were precluded by the extreme viscosity of the resultant suspension. Three-month chronic oral toxicity studies of virginiamycin were conducted in rats and beagle dogs at dose levels of 5, 22.5 or 100 mg/kg/day. All animals grew well and showed no signs of local systemic toxicity. Numerous biochemical tests performed during the studies were normal in all respects, as were microscopic examinations of tissues from the animals at the conclusion of the experiments.

c) Virginiamycin, the active ingredient in the products which are the subject of the proposed actions, is manufactured in Genval, Belgium, by Recherche et Industrie Therapeutiques, S.A., a wholly owned subsidiary of SmithKline Corporation. It is produced by a fermentation process in which wastes are minimized as much as possible. Solvents are 99%, or more, recovered and recycled. Disposal of the waste water conforms with provincial and local requirements.

With respect to manufacturing operations performed in this country, i.e. blending of the lesser concentrated premixes ('Stafac' 22 and 'Stafac' 110) from the primary premix ('Stafac' 500), our manufacturing facilities comply with all local and state regulations for waste water and air filtration systems.

SmithKline Animal Health Products hereby certifies that, during the course of the above mentioned manufacturing operations, effluent emissions into the environment will be within the limits set forth by Federal, State or local regulations.

3. PROBABLE UNAVOIDABLE ADVERSE ENVIRONMENTAL EFFECTS

As stated above, the probable impact of the proposed action is beneficial. There are no known adverse environmental effects. Potential pollutants resulting from the manufacturing process are 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.

4. ALTERNATIVES TO THE PROPOSED ACTION

The only specific alternative to the proposed actions would be refusal to approve the New Animal Drug Application. This would, however, deny the farmer 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 poultry; 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.

There are several antibiotics used in poultry which have one or more of the same claims presently approved for virginiamycin. However, it may be noted that many of these products contain tetracyclines and/or penicillin. The subcommittee on low-level antibiotics in animal feed of the National Advisory Food and Drug Committee has recommended that use of penicillin and tetracyclines be discontinued for growth promotion and feed efficiency for species where there are satisfactory substitutes available. Virginiamycin has been shown to be a viable alternative to penicillin and the various tetracyclines, for improving weight gain and feed efficiency in poultry.

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

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

5. SHORT-TERM USE OF THE ENVIRONMENT VS LONG-TERM PRODUCTIVITY

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.

6. IRREVERSIBLE OR IRRETRIEVABLE COMMITMENTS OF RESOURCES

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

7. OBJECTIONS TO THE PROPOSED ACTION

No known objections have been raised by other agencies, organizations or individuals.

8. The information presented in this Environmental Impact Analysis Report demonstrates that the proposed action will not adversely affect the quality of the human environment within the meaning of the National Environmental Policy Act. Therefore, an Environmental Impact Statement is not required.

9. BENEFIT TO THE PUBLIC VS POTENTIAL RISK

Controlled clinical studies have demonstrated the potential benefits virginiamycin could offer the chicken farmer 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.

001365

Appendix I

WARF INSTITUTE, INC.
MADISON, WISCONSIN

001366

Greenhouse Phytotoxicity Evaluations of Litter from Virginiamycin Treated Broilers on Seven Crops

For:

Smith Kline Animal Health Products
Applebrook Research Center
1600 Paoli Pike
West Chester, PA 19380

By:

WARF Institute, Inc.
P. O. Box 7545
Madison, WI 53707

Study Director: G. E. Schmolesky
Head, Pesticide Evaluation Department

WARF Institute No. 6121161 - 1199 II
6121226 - 1228 II

WARF INSTITUTE, INC.
MADISON, WISCONSIN

001367

SUMMARY

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.

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 1/2 inches at 4 - 10 tons per acre.

METHODS & MATERIALS

Litter - 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 received from Smith Kline on December 7, 1976:

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.

Date Received	WARF Institute No.	Sample Designation (Air-Dried)	
12-7-76	6121168	Drum No. 3, Poultry Medicated	40.9 kg
12-7-76	6121169	Drum No. 3, Poultry Medicated	44.6 kg
12-7-76	6121170	Drum No. 3, Poultry Medicated	40.2 kg
12-7-76	6121171	Drum No. 4, Poultry Control	38.4 kg
12-7-76	6121172	Drum No. 4, Poultry Control	41.6 kg
12-7-76	6121173	Drum No. 4, Poultry Control	42.6 kg
		Jar No. (Fresh Sample)	
12-7-76	6121175	2 Control Poultry	10-26-76
12-7-76	6121177	4 Medicated Poultry	10-26-76
12-7-76	6121179	6 Control Poultry	10-28-76
12-7-76	6121181	8 Medicated Poultry	10-28-76
12-7-76	6121183	10 Control Poultry	11-1-76
12-7-76	6121185	12 Medicated Poultry	11-1-76
12-7-76	6121187	14 Control Poultry	11-3-76
12-7-76	6121189	16 Medicated Poultry	11-3-76
12-7-76	6121191	18 Control Poultry	11-5-76
12-7-76	6121193	20 Medicated Poultry	11-5-76

Sample Preparation

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 3 North, average temperature 60°F.

Date of Sample Grinding	WARF Institute No.	Sample Designation
12-15-76	6121168	Drum No. 3, Poultry Medicated 40.9 kg
12-16-76	6121169	Drum No. 3, Poultry Medicated 44.6 kg
12-16-76	6121170	Drum No. 3, Poultry Medicated 40.2 kg
12-15-76	6121171	Drum No. 4, Poultry Control 38.4 kg
12-15-76	6121172	Drum No. 4, Poultry Control 41.6 kg
12-15-76	6121173	Drum No. 4, Poultry Control 42.6 kg

Moisture Determinations

Random samples of the ground air-dried manure were submitted to the proximate lab of WARF Institute along with the fresh samples for moisture determinations.

WARF Institute No.	Sample Designation	Percent Moisture
6121168	Drum No. 3, Poultry Medicated	10.5
6121169	Drum No. 3, Poultry Medicated	26.1
6121170	Drum No. 3, Poultry Medicated	10.6
	Average	15.7
6121171	Drum No. 4, Poultry Control	11.8
6121172	Drum No. 4, Poultry Control	10.2
6121173	Drum No. 4, Poultry Control	9.8
	Average	10.6
6121185	Drum No. 12, Poultry Medicated	62.3
6121189	Drum No. 16, Poultry Medicated	65.5
6121193	Drum No. 20, Poultry Medicated	58.8
6121177	Drum No. 4, Poultry Medicated	64.1
6121181	Drum No. 8, Poultry Medicated	63.0
	Average	62.7
6121175	Drum No. 2, Poultry Control	70.4
6121179	Drum No. 6, Poultry Control	61.4
6121183	Drum No. 10, Poultry Control	54.2
6121187	Drum No. 14, Poultry Control	66.4
6121191	Drum No. 18, Poultry Control	63.9
	Average	63.3

WARF INSTITUTE, INC.
MADISON, WISCONSIN

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Soil Source and Analysis

Soil for the project was obtained from Wipperfurth & Endres, Waunakee, WI 53597. During 1976 wheat was grown on the soil by farmer, D. Hoffman. 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 and physical analysis of the soil used in the experiment are attached in the following (2) reports.

COOPERATIVE EXTENSION PROGRAMS
UWEX University of Wisconsin–Extension
University of Wisconsin–Madison

Soil & Plant Analysis Laboratory, 8996 South Park Street, Madison, Wisconsin 53715; 608-262-4364

DEPARTMENT OF SOIL SCIENCE

February 16, 1977
Acct. 900
Lab No. 01177

MEMO

TO: G. Schmolesky
WARF Institute, Inc.
PO Box 7545
Madison, WI 53707

FROM: Soil/Plant Analysis Lab

SUBJECT: Results of physical analyses on 1 soil sample submitted December 22, 1976.

Sample ID	Sand	Silt	Clay
	----------	%	----------
1	14	63	23

If you have any questions concerning these analyses, please feel free to contact us.

/sf
Encl.

COOPERATIVE EXTENSION PROGRAMS
University of Wisconsin–Extension
University of Wisconsin–Madison

Soil & Plant Analysis Laboratory, 806 South Park Street, Madison, Wisconsin 53715; 608-262-4364

DEPARTMENT OF SOIL SCIENCE

00372

December 16, 1976
Acct. 900
Lab No. OH0535

MEMO

TO: G. Schmelesky
WARF Institute, Inc.
3301 Kinsman Blvd.
Madison, WI 53707

FROM: Soil/Plant Analysis Lab

SUBJECT: Results of analyses on 1 soil sample.

Sample ID	pH	SMP	O.M.	P	K	SS*
			T/A	----lbs/A----		
1	5.7	5.7	57	350	270	10

**SS = soluble salts in mhos x 10^{-5}/cm

The physical analysis was missed on this sample and the soil was inadvertently discarded before the error was noticed. We will be happy to run the physical analysis if you care to resubmit another soil sample. We are sorry if this error has caused you any inconvenience.

If you have any questions concerning these analyses, please feel free to contact us.

/sf

Litter Application, Treatment Rates and Planting

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 and Agronomy
Revised, May 1966

University of Georgia Leaflet 206
Poultry Waste - 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 given below were calculated on the basis of moisture determinations made directly prior to the start of the experiment.

Poultry Medicated

For barley and fescue:

4 tons per acre is 186.8 grams wet manure or
84.1 grams of air dried manure per flat

For wheat, green beans and peppers:

4 tons per acre is 186.8 grams wet manure or
89.8 grams of air dried manure per flat

For cucumbers:

5 tons per acre is 233.6 grams wet manure or
112 grams of air dried manure per flat

For corn:

10 tons per acre is 467.2 grams wet manure or
224 grams of air dried manure per flat

Poultry Control

For barley and fescue:

4 tons per acre is 186.8 grams wet manure or
76.6 grams of air dried manure per flat

For wheat, green beans and peppers:

4 tons per acre is 186.9 grams wet manure or
78.2 grams of air dried manure per flat

For cucumbers:

5 tons per acre is 233.6 grams wet manure or
97.7 grams of air dried manure per flat

For corn:

10 tons per acre is 467.2 grams wet manure or
195.4 grams air dried manure per flat

The samples which were previously ground were weighed in the above amounts. 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 with the following designations:

PM	Poultry Medicated	No. 1 through 5 (replicates)
PC	Poultry Control	No. 1 through 5 (replicates)
CK	No Manure	No. 1 through 5 (replicates)

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:

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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

The fescue seeds were planted in five rows (20 seeds per row) equidistant from the flat sides and from each other.

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 - The results are given in Table I

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.

Prior to discarding the plots, 35 mm pictures of replicate one for the barley medicated, control and untreated were taken.

Wheat - The results are given in Table II.

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 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 one plant was present it was so noted.

Prior to discarding the plots, 35 mm pictures for replicate three for wheat poultry medicated, poultry control and untreated plot were taken.

Fescue - The results are given in Table III.

At 33 days a stand count of plants for each of five rows in a plot were recorded and totaled.

35 mm pictures of replicate one of the poultry medicated, poultry control and untreated plot were taken.

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 at 2.54 centimeters from the soil surface and the weight for each plot was recorded.

Corn - The results are given in Table IV.

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.

35 mm pictures of replicate three of the poultry medicated, poultry control and untreated plot were taken.

Those plants with wilting of the new growth were recorded for each plot.

Green Beans - The results are given in Table V.

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.

35 mm pictures of replicate four for the poultry medicated, poultry control and untreated plots were taken.

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 -The results are given in Table VI.

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.

At 40 days 35 mm pictures of replicate four of the poultry medicated, poultry control and the untreated plot were taken. 001378

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 - The results are given in Table VII.

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

At 40 days after seeding 35 mm pictures of replicate three of the poultry medicated, poultry control and the untreated plot were taken.

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

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Table I

Barley (Dickson)

Treatment	Repl.	Average Height (cm) of 1 Seedling per 10 Locations per Plot (2 Seedlings in the Untreated) 23 Days After Planting										Average Height (cm)	Stand Count
Poultry Medicated 4 ton/acre	1	27	30	32	33	31	19	30	28	32	30	29.2	19
	2	-	25	18	-	31	10	31	34	23	31	25.4	18
	3	30	30	29	32	30	28	24	34	33	-	30.0	17
	4	29	28	26	27	26	30	28	-	32	31	27.4	16
	5	28	22	22	25	28	25	29	26	33	30	26.8	20
	Total											1,280	90
	Average											27.8	18
Poultry Control 4 ton/acre	1	21	27	29	31	31	32	25	33	28	-	28.5	18
	2	19	17	18	24	31	26	30	34	35	30	26.4	20
	3	29	30	29	27	28	30	28	31	31	24	28.7	19
	4	26	30	28	19	33	29	29	29	32	-	28.3	18
	5	-	18	27	27	25	31	14	22	28	31	24.8	18
	Total											1,286	93
	Average											27.3	18
Untreated	1	34	33*	34	33	34	30	30	32*	30	33	32.3	36
	2	26	29*	30*	30	26	25	26	30	32	30*	28.4	36
	3	29*	24	28	26	31	26	27	33	29	31	28.4	40
	4	34	33	32	33*	29	27	28	28	26	29	29.9	39
	5	32	30	30	31	33	32	30	29	33	30	31.0	40
	Total											1,500	191
	Average											30.0	38

*One Plant Only

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Table II

Wheat (Timwin)

Treatment	Repl.	Average Height (cm) of 2 Seedlings per 10 Locations per Plot 22 Days After Planting										Average Height (cm)	Stand Count
Poultry Medicated 4 ton/acre	1	25	24	18	16	22	19	19	20	25	25	21.3	37
	2	19	18	17	15	17	18	20	20	20*	22	18.7	38
	3	28	22	20*	25	21	2[illegible]	16	25	22	25	23.3	36
	4	29	22	27	19	19	22	20	19	24	26	22.7	40
	5	29	26	25	22	21	25	21*	21	23	24	23.7	37
Total												109.7	188
Average												21.9	37
Poultry Control 4 ton/acre	1	21	17	16	16	18*	20	21	15	24	24*	19.2	36
	2	21	20	14	14	17	23	22	20	22	21*	19.4	39
	3	26	24	23	19	19*	19	24	28	23	28	23.3	38
	4	20	21	20	23	23	19	22	20	18	20	20.6	40
	5	23	24	25	21	22	24	22	22	26	22	23.1	38
Total												105.6	191
Average												21.1	38
Untreated	1	18	16	18	18	16	20	22	20	25	26	19.9	39
	2	16	20	19	20	23	17	17	19	25	23	19.9	37
	3	18	21	26	17	27	26	20	28	24	25	23.2	40
	4	19	17	27	18	21	25	22	16	26	28	21.9	38
	5	24	26	23	25	22	25	26	17	28	26	24.2	37
Total												109.1	191
Average												21.8	38

*One Plant Only

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Table III

Fescue (Pennlawn)

Treatment	Repl.	Stand Count per Plot 5 Rows, 33 Days					Total Stand Count per Plot	33 Days Wt. of Plant Cut 3.7 cm from Soil Surface(gm)	53 Days Wt. of Plant Cut 2.54 cm from Soil Surface(gm)
Poultry Medicated ton/acre	1	16	13	15	16	16	76	0.90	3.8
	2	13	16	13	14	13	69	0.75	3.2
	3	18	13	17	15	17	80	1.15	4.5
	4	16	17	14	11	14	72	1.10	6.0
	5	17	16	16	17	14	80	1.25	4.5
Total							377	5.15	22.0
Average							75	1.0	4.4
Poultry Control 4 ton/acre	1	14	14	19	16	17	80	1.00	3.5
	2	17	17	17	16	14	81	1.20	5.0
	3	17	17	15	18	17	84	1.10	5.9
	4	12	15	16	17	16	76	1.25	6.2
	5	13	15	14	11	20	73	1.40	6.1
Total							394	5.95	26.7
Average							79	1.2	5.3
Untreated	1	15	16	16	19	16	82	1.10	5.2
	2	17	17	18	19	17	88	1.00	7.1
	3	17	18	15	15	16	81	1.30	6.6
	4	16	19	19	14	18	86	0.95	8.3
	5	16	17	18	18	20	89	0.80	5.6
Total							426	5.15	32.8
Average							85	1.0	6.6

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Table IV

Corn (Wis. 900)

Treatment	Repl.	Stand Count	22 Days Plants with Wilted New Growth	34 Days After Planting-Ht. of Plants in cm, Range Per Plot 0-5	15-30	30-45	45+	Plant Wt. in gm of All Plants 45+ cm	Average Wt. in gm per Plant	Plants with Wilted New Growth
Poultry	1	20	9	0	0	2	18	154.0	8.5	0
Medicated	2	19	2	0	2	1	15	119.2	7.9	1
10 ton/acre	3	20	4	0	0	0	20	197.3	9.8	1
	4	16	1	0	1	0	14	110.4	7.8	0
	5	18	0	1	2	4	11	89.1	8.1	0
	Total	93	16	1	5	7	80	670.0	-	2
	Average			1	5	8	86	134.0	8.4	-
Poultry	1	20	3	0	0	3	17	141.0	8.3	0
Control	2	19	4	0	1	3	15	123.4	8.2	0
10 ton/acre	3	20	5	0	1	2	17	149.4	8.8	1
	4	18	0	0	1	4	13	110.5	8.5	0
	5	19	0	2	3	1	13	107.1	8.2	0
	Total	96	12	2	6	13	76	631.4	-	1
	Average			2	6	14	79	126.3	8.3	-
Untreated	1	19	0	0	0	1	18	132.5	7.4	0
	2	18	0	1	2	0	15	126.7	8.4	0
	3	18	0	0	0	0	18	129.2	7.2	0
	4	19	0	0	1	5	13	74.0	5.7	0
	5	19	0	0	0	2	17	89.1	5.2	0
	Total	93	0	1	3	8	81	551.5	-	0
	Average			1	3	9	87	110.3	6.8	-

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Table V

Green Bean (Green Podded Bush)

22 Days After Planting

Treatment	Repl.	Plants with Primary Leaves 5 cm. wide and 8 cm. long or longer	Smaller Seedlings	Total Plants	Number of Bronzed Necrotic Leaves	Weight in Grams Larger Plants	Average Plant Weight in Grams
Poultry Medicated 4 to/acre	1	11	7	18	2 slight	36.1	3.3
	2	12	4	16	none	41.6	3.5
	3	14	3	17	5 slight	63.0	4.5
	4	12	6	18	2 slight	54.7	4.5
	5	11	2	13	none	34.9	3.2
	Total	60	22	82	9 slight	230.3	3.8
	Percent	73	27	-	-	-	-
Poultry Control 4 ton/acre	1	6	7	13	1 slight	22.3	3.7
	2	16	0	16	1 slight	58.0	3.6
	3	15	0	15	2 moderate	63.8	4.2
	4	14	3	17	2 moderate	53.1	3.8
	5	18	0	18	2 slight	59.0	3.3
	Total	69	10	79	4 slight	256.2	3.7
	Percent	87	13	-	4 moderate	-	-
Untreated	1	14	5	19	none	51.3	3.7
	2	14	1	15	2 slight	56.6	4.0
	3	12	4	16	4 slight	60.8	5.1
	4	17	1	18	3 slight	63.9	3.8
	5	18	0	19	none	71.8	4.0
	Total	75	11	86	9 slight	304.4	4.1
	Percent	86	14	-	-	-	-

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Table VI

Cucumber (Improved Chicago Pickling)

Treatment	Repl.	Stand Count	Height in cm 34 Days after Planting 0-15	15-30	30 +	Number of Plants 15 cm or larger	Total Plant Weight in Grams per Plot of Plants 15 cm or Larger	Average Weight in grams per Plant
Poultry	1	12	3	9	0	10	121.1	
Medicated	2	13	3	10	0	10	111.9	
5 ton/acre	3	12	3	9	0	10	140.7	
	4	12	8	4	0	7	57.0	
	5	16	11	5	0	7	63.2	
	Total	65	28	37	0	44	493.9	11.2
	Percent		33	57	0	-		
Poultry	1	12	2	9	1	11	163.8	
Control	2	16	2	11	3	14	147.8	
5 ton/acre	3	14	2	11	1	12	136.5	
	4	13	3	10	0	10	105.8	
	5	9	5	4	0	6	50.6	
	Total	64	14	45	5	53	604.5	11.4
	Percent		22	70	8	-		
Untreated	1	19	7	12	0	13	133.0	
	2	15	0	13	2	13	148.6	
	3	16	3	13	0	13	112.2	
	4	17	9	8	0	9	86.1	
	5	16	5	11	0	11	91.4	
	Total	83	24	57	2	59	571.3	9.6
	Percent		29	69	2	-		

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Table VII

Pepper (California Wonder 357) and Tomato Transplants (Stokesdale Hybrid)

Treatment	Repl.	Pepper - 56 Days Stand Count	Pepper - 56 Days Height less than 5 cm	Pepper - 56 Days Height greater than 5 cm	Tomato - 40 Days Stand Count	Tomato - 40 Days Height less than 20 cm	Tomato - 40 Days Height 20-30 cm
Poultry Medicated 5 ton/acre	1	9	2	7	10	0	10
	2	8	2	6	10	1	9
	3	13	5	8	10	0	10
	4	10	4	6	9	2	7
	5	9	5	4	10	3	7
	Total	49	18	31	49	6	43
	Percent		37	63		12	88
Poultry Control 5 ton/acre	1	6	6	0	10	0	10
	2	12	4	8	10	1	9
	3	13	6	7	9	1	8
	4	13	5	8	9	2	7
	5	11	4	7	10	3	7
	Total	55	25	30	48	7	41
	Percent		46	55		15	86
Untreated	1	11	4	7	9	1	8
	2	9	3	6	9	2	7
	3	11	2	9	9	1	8
	4	12	6	6	10	3	7
	5	11	7	4	10	2	8
	Total	54	22	32	47	9	38
	Percent		41	59		19	81

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Table VIII - Continued

Cucumber (Improved Chicago Pickling)

Treatment	Range in Height in Centimeters 34 Days After Planting 0 - 15	15 - 30	30+	Number of Plants 15 cm or larger at 40 Days	Average Weight in Grams per Plant
Poultry Medicated	28	37	0	44	11.2
Poultry Control	14	45	5	53	11.4
Untreated	24	57	2	59	9.7

Pepper and Tomato

(56 Days)

Treatment	Pepper Stand Count	Pepper less than 5 cm	Pepper greater than 5 cm	Tomato Stand Count	Tomato less than 20 cm	Tomato 20 - 30 cm
Poultry Medicated	49	18	31	49	6	43
Poultry Control	55	25	30	48	7	41
Untreated	54	22	32	47	9	38

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FORM 28-4A

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Table VIII - Continued

Fescue (Pennlawn)

Treatment	Range in Stand Count (Total)		Plant Weight (Grams) 33 Days Cut 3.7 cm from Surface	Plant Weight (Grams) 53 Days Cut 2.54 cm from Surface
Poultry Medicated	69 - 80	(377)	5.15	22.0
Poultry Control	73 - 84	(394)	5.95	26.7
Untreated	81 - 89	(426)	5.15	32.8

Bean (Green Podded Bush) 22 Days After Planting

Treatment	Large Plants	Small Plants	Total Plants	Necrotic Leaves	Range in Plant Weight in Grams (Large Plants)
Poultry Medicated	60	22	82	9 slight 4 slight	3.2 - 4.5
Poultry Control	69	10	79	4 moderate	3.3 - 4.2
Untreated	75	11	86	9 slight	3.7 - 5.1

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Table VIII - Summary

Corn (Wis. 900)

Treatment	Total Plants	22 Days Plants with Wilted New Growth	34 Days After Planting Range in Height cm 0-15	15-30	30-45	45+	Average Weight in grams per 45 cm+	Plants with Wilted New Growth
Poultry Medicated	93	16	1	5	7	80	8.4	2
Poultry Control	96	12	2	6	13	76	8.3	1
Untreated	93	0	1	3	8	81	6.8	0

Barley (Dickson) 23 Days After Planting

Treatment	Range in Stand Count	Total Plants	Average Height, Centimeters
Poultry Medicated	16 - 20	90	27.8
Poultry Control	18 - 20	93	27.3
Untreated	36 - 40	191	30.0

Wheat (Timwin) 22 Days After Planting

Treatment	Range in Stand Count	Total Plants	Average Height, Centimeters
Poultry Medicated	36 - 40	188	21.9
Poultry Control	36 - 40	191	21.1
Untreated	37 - 40	191	21.8

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FORM 26-4A

CONCLUSIONS

Beans

There were a total of 9 fewer large plants out of 69 in the poultry medicated compared to the poultry control.

The total stand counts compared favorably, and the average plant weight for the larger plants in the poultry medicated and poultry control plots were identical.

Some slight necrotic lesions were observed in many plots including the untreated plots with no manure. This likely was the result of over or under watering at a crucial period in the seedlings' growth.

Fescue

The stand counts and the cutting weights at 33 and 53 days were slightly less for poultry medicated than the poultry control but no phytotoxic or color differences were observed.

However, the stand count and final cutting weight were substantially higher for the untreated control plots.

Corn

The stand counts and range of plants for the poultry medicated and poultry control plots were comparable.

Both poultry medicated and poultry control plots had plants (30 cm or larger) with wilted new growth after 22 days. No injury occurred in the untreated plots.

The average weights per plant (45 cm or larger) were comparable for the poultry medicated and poultry control after 34 days.

The earlier plant injury symptoms had nearly disappeared. No differences in the roots were noted.

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Wheat & Barley

The stand counts and height of plants for the poultry medicated and poultry control plots were comparable. No phytotoxic effects were observed.

Cucumber

There were 9 fewer plants out of 53, 15 cm or larger, in the poultry medicated plots compared to the poultry control and 15 fewer plants compared to the untreated total of 59 plants.

No phytotoxic symptoms were observed on any plants and all roots were normal in the poultry medicated, poultry control and untreated plots.

Pepper & Tomato

The stand counts and height of plants for the poultry medicated and poultry control plots were comparable. No phytotoxic effects were observed.

Recommendations

It may be desirable to obtain additional data on cucumbers and beans as some inhibition of growth was noted.

Signed R. E. Schmalisky

By and For WARF Institute, Inc.

Date: May 12, 1977

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Addendum To:

Greenhouse Phytotoxicity Evaluations of Litter from

Virginiamycin Treated Broilers on Seven Crops

WARF Institute No. 6121161 - 1199 II
6121226 - 1228 II

The medicated poultry manure and control poultry manure treatments contained essentially the same number of bean and cucumber plants.

No phytotoxicity was observed which could be attributable to the treatments. Therefore, no further tests are necessary.

Signed

By and for WARF Institute, Inc.

Date: October 19, 1977

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001392

Evaluation of the Potential Adverse Activity of Virginiamycin Residues Contained in Pig Manure and Broiler Litter to Earthworms

For:

Smith Kline Animal Health Products
Applebrook Research Center
1600 Paoli Pike
West Chester, PA 19380

By:

WARF Institute, Inc.
P. O. Box 7545
Madison, WI 53707

Study Director: G. E. Schmolesky
Head, Pesticide Avaluation Dept.

WARF Institute No. 6121161 - 1199 IV
6121226 - 1228 IV

WARF INSTITUTE, INC.
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OBJECTIVE

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

The same manure and litter samples as referenced in the crop studies, WARF Institute 6121161 - 1199 I and II and 6121226 - 1228 I and II were used in the present studies.

SUMMARY

Manure from pigs fed with virginiamycin treated feed (50 grams per ton) and 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.

METHODS & MATERIALS

Soil Source and Analysis

The soil, manure and litter for the project were the same as previously described in WARF Institute No. 6121161 - 1199 I and 6121226 - 1228 I.

Manure Samples

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

	Percent Moisture	
	Fresh	Air Dried
Poultry Medicated	62.7	13.6
Poultry Control	63.3	10.2
Swine Medicated	70.1	22.1
Swine Control	69.5	18.7

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		
2 1/2	4.4	1.9
4	7.1	3.1
10	17.7	7.6
Broiler Control		
2 1/2	4.4	1.8
4	7.1	2.9
10	17.7	7.2
Swine Medicated		
10	17.7	6.8
20	35.4	13.6
Swine Control		
10	17.7	6.7
20	35.4	13.3

*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, eggs and young worms were recorded.

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RESULTS

Table I

Rate Ton/Acre	Replicate	Worms Active and Soil Condition 3 Days	Worms Active and Soil Condition 7 Days	Worms Recovered 10 Days	25 Days		
					Worms Recovered	Eggs Present	Young Worms Present
Broiler Medicated 2 1/2	1	OK	OK	50 Active	50	Yes	Yes
	2	OK	OK	50 Active	49	Yes	Yes
	3	OK	OK	50 Active	50	Yes	Yes
	4	OK	OK	48 Active	48	Yes	Yes
	5	OK	OK	49 Active	50	Yes	Yes
Broiler Control 2 1/2	1	OK	OK	49 Active	44	NO	NO
	2	OK	OK	49 Active	48	Yes	Yes
	3	OK	OK	47 Active	45	Yes	Yes
	4	OK	OK	50 Active	49	Yes	Yes
	5	OK	OK	50 Active	49	Yes	Yes
Broiler Medicated 4	1	OK	OK	49 Active	45	Yes	No
	2	OK	OK	49 Active	48	Yes	No
	3	OK	OK	50 Active	50	Yes	Yes
	4	OK	OK	50 Active	48	Yes	Yes
	5	OK	OK	50 Active	48	Yes	Yes
Broiler Control 4	1	OK	OK	46 Active	45	Yes	Yes
	2	OK	OK	50 Active	48	Yes	Yes
	3	OK	OK	50 Active	46	Yes	Yes
	4	OK	OK	50 Active	50	Yes	Yes
	5	OK	OK	48 Active	45	Yes	Yes
Broiler Medicated 10	1	OK	Surface Mold	43 Active	42	Yes	No
	2	OK	Surface Mold	50 Active	50	Yes	No
	3	OK	OK	50 Active	49	Yes	Yes
	4	OK	OK	50 Active	49	Yes	Yes
	5	OK	OK	47 Active	46	Yes	Yes

FORM 28-4A

RESULTS

Table I - Continued

Rate Ton/Acre	Replicate	Worms Active and Soil Condition 3 Days	Worms Active and Soil Condition 7 Days	Worms Recovered 10 Days	25 Days		
					Worms Recovered	Eggs Present	Young Worms Present
Broiler Control 10	1	Surface Mold	Surface Mold	47 Active	45	Yes	No
	2	OK	Surface Mold	50 Active	50	Yes	Yes
	3	OK	OK	50 Active	48	Yes	Yes
	4	OK	OK	50 Active	50	Yes	Yes
	5	OK	OK	50 Active	50	Yes	No
Swine Medicated 10	1	Surface Mold	Surface & Deep Mold	49 Active	46	Yes	No
	2	Surface Mold	Slight Deep Mold	50 Active	50	Yes	No
	3	OK	OK	50 Active	50	Yes	Yes
	4	Surface Mold	OK	50 Active	49	Yes	Yes
	5	OK	Surface Mold	50 Active	50	Yes	Yes
Swine Control 10	1	Surface Mold	Surface & Deep Mold	46 Active	42	Yes	No
	2	Surface Mold	No Surface but Deep Mold	49 Active	50	Yes	No
	3	OK	OK	50 Active	49	Yes	Yes
	4	OK	Slight Deep Mold	50 Active	50	Yes	Yes
	5	OK	Slight Deep Mold	49 Active	49	Yes	Yes
Swine Medicated 20	1	Surface & Deep Mold	Surface & Deep Mold	50 Active	47	Yes	No
	2	Surface & Deep Mold	Surface & Deep Mold	49 Active	49	Yes	No
	3	OK	Deep Mold	50 Active	49	Yes	Yes
	4	Surface & Deep Mold	Surface & Deep Mold	50 Active	49	Yes	Yes
	5	OK	OK	50 Active	49	Yes	Yes
Swine Control	1	Surface Mold	Surface & Deep Mold	48 Active	48	Yes	No
	2	Surface Mold	Slight Surface & Deep Mold	50 Active	49	Yes	No
	3	OK	Deep Mold	50 Active	50	Yes	Yes
	4	OK	OK	50 Active	50	Yes	Yes
	5	OK	Slight Surface Mold	50 Active	49	Yes	No

FORM 28-4A

RESULTS

Table I - Continued

Rate Ton/Acre	Replicate	Worms Active and Soil Condition 3 Days	Worms Active and Soil Condition 7 Days	Worms Recovered 10 Days	25 Days		
					Worms Recovered	Eggs Present	Young Worms Present
Untreated	1	OK	OK	50 Active	47	Yes	Yes
5.1 Grams	2	OK	OK	49 Active	50	Yes	No
CSMA Fly	3	OK	OK	49 Active	49	Yes	Yes
Larval Media	4	OK	OK	49 Active	49	Yes	Yes
	5	OK	OK	48 Active	50	Yes	No

001398

WARF INSTITUTE, INC.
MADISON, WISCONSIN

RESULTS

001399

Table II

Rate Ton/Acre	Replicate	Number Recovered - 35 Days Adults	Eggs	Young
Broiler Medicated 2 1/2	1	50	71	0
	2	45	64	2
	3	50	97	14
	4	47	42	19
	5	48	75	6
	Total	240	349	41
Broiler Control 2 1/2	1	44	23	0
	2	48	43	6
	3	45	71	7
	4	49	66	4
	5	49	50	7
	Total	235	253	24
Broiler Medicated 4	1	40	71	7
	2	47	31	2
	3	49	44	18
	4	48	58	20
	5	48	29	5
	Total	232	233	52
Broiler Control 4	1	42	32	2
	2	47	29	19
	3	48	29	9
	4	48	49	14
	5	44	19	7
	Total	229	158	51
Broiler Medicated 10	1	42	57	2
	2	50	22	2
	3	49	79	9
	4	49	55	20
	5	45	49	12
	Total	235	262	45

RESULTS

Table II - Continued

Rate Ton/Acre	Replicate	Number Recovered - 35 Days Adults	Eggs	Young
Broiler Control 10	1	45	58	5
	2	50	75	8
	3	48	109	14
	4	50	71	28
	5	49	46	11
	Total	242	269	66
Swine Medicated 10	1	45	49	0
	2	50	16	0
	3	50	53	8
	4	48	82	25
	5	49	28	18
	Total	242	228	51
Swine Control 10	1	42	45	0
	2	50	117	2
	3	49	61	24
	4	49	41	5
	5	49	51	15
	Total	239	315	46
Swine Medicated 20	1	47	105	0
	2	49	76	0
	3	48	89	25
	4	48	48	14
	5	49	41	17
	Total	241	359	56
Swine Control 20	1	48	48	0
	2	49	43	0
	3	50	97	15
	4	50	94	38
	5	49	105	27
	Total	246	387	80

RESULTS

Table II - Continued

Rate Ton/Acre	Replicate	Number Recovered - 35 Days		
		Adults	Eggs	Young
Untreated	1	46	75	11
5.1 Grams	2	49	42	10
CSMA fly	3	47	45	15
larval Media	4	48	54	4
	5	48	58	2
	Total	238	274	42

WARF INSTITUTE, INC.
MADISON, WISCONSIN

Summary of Results

Table III

Rate of Manure Ton/Acre	Total Earthworm Stages Which were Recovered from 5 Replicates After 35 Days		
	Adults	Eggs	Young
Broiler Medicated 2 1/2	240	349	41
Broiler Control 2 1/2	235	253	24
Broiler Medicated 4	232	233	52
Broiler Control 4	229	158	51
Broiler Medicated 10	235	262	45
Broiler Control 10	242	269	66
Swine Medicated 10	242	228	51
Swine Control 10	239	315	46
Swine Medicated 20	241	359	56
Swine Control 20	246	387	80
Untreated	238	274	42

DISCUSSION & CONCLUSIONS

The total numbers of adult earthworms were essentially constant throughout all experiments as shown in Table I. The individual replicates showed a wide range in the number of earthworm eggs and young as shown in Table II but the total numbers for each experiment were similar as shown in Table III.

The total earthworm recovery data in Table III was used to compare the broiler and swine medicated versus the broiler and swine control treatments.

Ton/Acre	Percentage Increase or Decrease of Recovered Earthworm Eggs and Young in Virginiamycin Treatment Compared to Controls
Broiler 2 1/2	+ 40
Broiler 4	+ 36
Broiler 10*	- 9
Swine 10*	- 23
Swine 20*	- 11

*Some of the treated and control treatments had mold present during the early portion of the experiment only.

Signed J. E. Schwenke

By and For WARF Institute, Inc.

Date: May 12, 1977

WARF INSTITUTE, INC.
MADISON, WISCONSIN

001404

Evaluation of the Potential Adverse Activity of Virginiamycin Residues Contained in Pig Manure and Broiler Litter to Housefly Eggs and Larvae

For:

Smith Kline Animal Health Products
Applebrook Research Center
1600 Paoli Pike
West Chester, PA 19380

By:

WARF Institute, Inc.
P. O. Box 7545
Madison, WI 53707

Study Director: G. E. Schmolesky
Head, Pesticide Evaluation Department

WARF Institute No. 6121161 - 1199 &
6121226 - 1228

WARF INSTITUTE, INC.
MADISON, WISCONSIN

SUMMARY

Manure from pigs fed with virginiamycin treated feed (20 grams per ton) and 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.

OBJECTIVE

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

The same manure and litter samples as referenced in the crop studies, WARF Institute No. 6121161 - 1199 I and II and 6121226 - 1228 I and II were used in the present studies.

METHODS & MATERIALS

Larval Media

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

I. Poultry and swine 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.

Sample	Grams solids per 100 gm Fresh	Grams solids per 100 gm Air Dry	ml of deionized water added to 2500 gm of air-dried manure
Swine Medicated	29.9	76.6	3,875
Swine Control	30.6	82.0	4,215
Poultry Medicated	37.3	84.3	3,160
Poultry Control	36.7	89.4	3,585

II. 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.

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.

These results are shown in Table I.

The experiments using swine medicated and swine control manure were repeated. The moisture contents of the previously ground samples were determined to be 23.7 and 20.4 percent respectively.

2500 gram quantities of air dried manure specimens for each test series were reconstituted to a fresh litter basis.

Sample	Grams solid per 100 gm Fresh	Air Dry	Deionized water per 2500 gm of air-dried manure
Swine medicated	29.9	76.3	3,878
Swine control	30.6	79.6	4,008

The results are shown in Table II. The experiment was again repeated and the results shown in Table III.

WARF INSTITUTE, INC.
MADISON, WISCONSIN

001408

Table I

Treatment	Repl.	No. of Pupae	Percent	Summary Range	Average	Pupae(1) from Eggs	Adults(1) Emerged
Swine Medicated	1	124	62				
	2	138	69				
	3	125	62				
	4	99	50				
	5	116	58				
	Total	602		50-69%	60%	592	574
Swine Control	1	190	95				
	2	186	93				
	3	200	100				
	4	195	98				
	5	188	94				
	Total	959		93-100%	96%	726	703
Poultry Medicated	1	173	86				
	2	137	69				
	3	161	81				
	4	140	70				
	5	157	78				
	Total	768		69-86%	77%	558	529
Poultry Control	1	136	68				
	2	158	79				
	3	166	83				
	4	155	78				
	5	157	78				
	Total	785		68-85%	79%	638	627
CSMA Media	1	194	97				
	2	169	84				
	3	162	81				
	4	199	100				
	5	184	92				
	Total	908		81-100%	91%	1140	1126

(1) 0.1 ml of eggs seeded in CSMA larval medium

001408

Table II

Treatment	Repl.	No. of Pupae	Percent	Summary Range	Summary Average	Summary Pupae(1) from Eggs	Summary Adults(1) Emerged
Swine Medicated	1	146	73				
	2	133	67				
	3	137	69				
	4	125	63				
	5	127	64				
	Total	668		63-73%	66%	884	851
Swine Control	1	108	54				
	2	132	66				
	3	190	95				
	4	64	32				
	5	102	51				
	Total	596		32-95%	59%	642	612
CSMA Media	1	136	68				
	2	150	75				
	3	138	69				
	4	166	83				
	5	153	77				
	Total	743		68-83%	74%	830	822

(1) 0.1 ml of eggs seeded in CSMA larval medium

Table III

Treatment	Repl.	No. of Pupae	Percent	Summary Range	Average	Pupae(1) from Eggs	Adults(1) Emerged
Swine Medicated	1	154	77				
	2	84	42				
	3	51	26				
	4	53	27				
	5	86	43				
	Total	428		27-77%	43%	838	812
Swine Control	1	74	37				
	2	36	18				
	3	134	67				
	4	97	49				
	5	145	73				
	Total	486		18-73%	49%	680	662
CSMA Media	1	176	88				
	2	176	88				
	3	120(2)	60				
	4	177	89				
	5	167	84				
	Total	816		60-89%	82	773	729

(1)0.1 ml of eggs seeded in CSMA larval medium
(2)Moldy Surface

Table IV

Summary

001411

Treatment	Total Pupae per 5 Repl.	Average, %	Pupae(1) From Eggs	Adults(1) Emerged
Experiment 1				
Swine Medicated	602	60	592	574
Swine Control	959	96	726	703
Poultry Medicated	768	77	558	529
Poultry Control	785	79	638	627
CSMA Media	908	91	1140	1126
Experiment 2				
Swine Medicated	668	66	884	851
Swine Control	596	59	642	612
CSMA Media	743	74	830	822
Experiment 3				
Swine Medicated	428	43	838	812
Swine Control	486	49	680	662
CSMA Media	816	82	773	729

(1)0.1 ml of eggs seeded in CSMA larval medium

DISCUSSION

Experiment 1 (Table I) showed a difference in pupae recovery for the swine medicated (60%) versus the swine control (96%) which did not occur for the poultry. Followup experiments 2 and 3 (Tables II and III) did not show these differences between the swine medicated and swine control treatments.

CONCLUSIONS

In all instances, eggs collected from adults reared through on the various manure treatments were viable. The eggs which were seeded onto standard CSMA larval media developed normally. Pupae and adult recovery were also normal.

Signed [signature]

By and For WARF Institute, Inc.

Date: May 12, 1977

WARF INSTITUTE, INC.

MADISON, WISCONSIN

8014119

REPORT

Analysis for Fish Toxicity: Trout, Bluegill

Description of Sample Virginiamycin, Feed Grade

Date Received 3/8/77 **Control Number** Lot # AFV/206/75

Submitted by Smith Kline Animal Health
West Chester, PA

Claimed Content

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

Method

Bioassay Techniques: Protocol was in accordance with the Fish-Pesticide Acute Toxicity Test Guideline, Environmental Protection Agency.

Statistical Analysis: Lithfield, J. T., Jr. and F. Wilcoxon. 1949 A simplified method of evaluating dose-effect experiments. J. Pharm. and Exp. Therap. 96:99-113. (May & August)

Remarks

Chi^2 analysis was run to obtain the "goodness of fit" of the linear line of the data.

The probit analysis work sheet is the present form being used by governmental departments within the U.S. Dept. of the Interior, Fish & Wildlife Service.

Signed John A. Bruemmer

by and for the WARF INSTITUTE, INC.

Date 5/12/77

WARF Institute No. 7031198

WARF INSTITUTE, INC.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

001414

Chemical Virginiamycin — Date Tested 4/12/77

Test Animal Rainbow Trout — Date Reported 5/12/77

Lot Number # AFV/206/75 — Temperature 55°F

Exposure Period 24 Hours — Water Quality Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
150	0/10	0	0	0	0
225	0/10	0 (0.3)	1.2	.09	0.005
338	2/10	20	19.0	1.0	0.000
507	7/10	70	70.0	0.0	0

Total animals = 40

K, No. of Doses = 4

Total Contribution to Chi 0.005

Chi^2 = contribution to Chi x $\frac{\text{total animals}}{K}$ = 0.050

Chi^2 (p=.05) for (K-2) 2 deg. of freedom = 5.99

LC_{84} = 575 ppm

LC_{50} = 430 ppm

LC_{16} = 330 ppm

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

S = 1.316

Confidence limits (.05) for LC_{50}

N' = 20

$fLC_{50} = S^{[2.77/\sqrt{N'}]}$ 1.316 0.6197

fLC_{50} = 1.183

LC_{50}/fLC_{50} = lower limit = 363 ppm

$LC_{50} \times fLC_{50}$ = upper limit = 509 ppm

Confidence limits (.05) for S:

R = ____ S = ____ A = ____

$fs = A^{[10\,(K-1)/K\sqrt{N'}]}$

fs =

S/fs = lower limit =

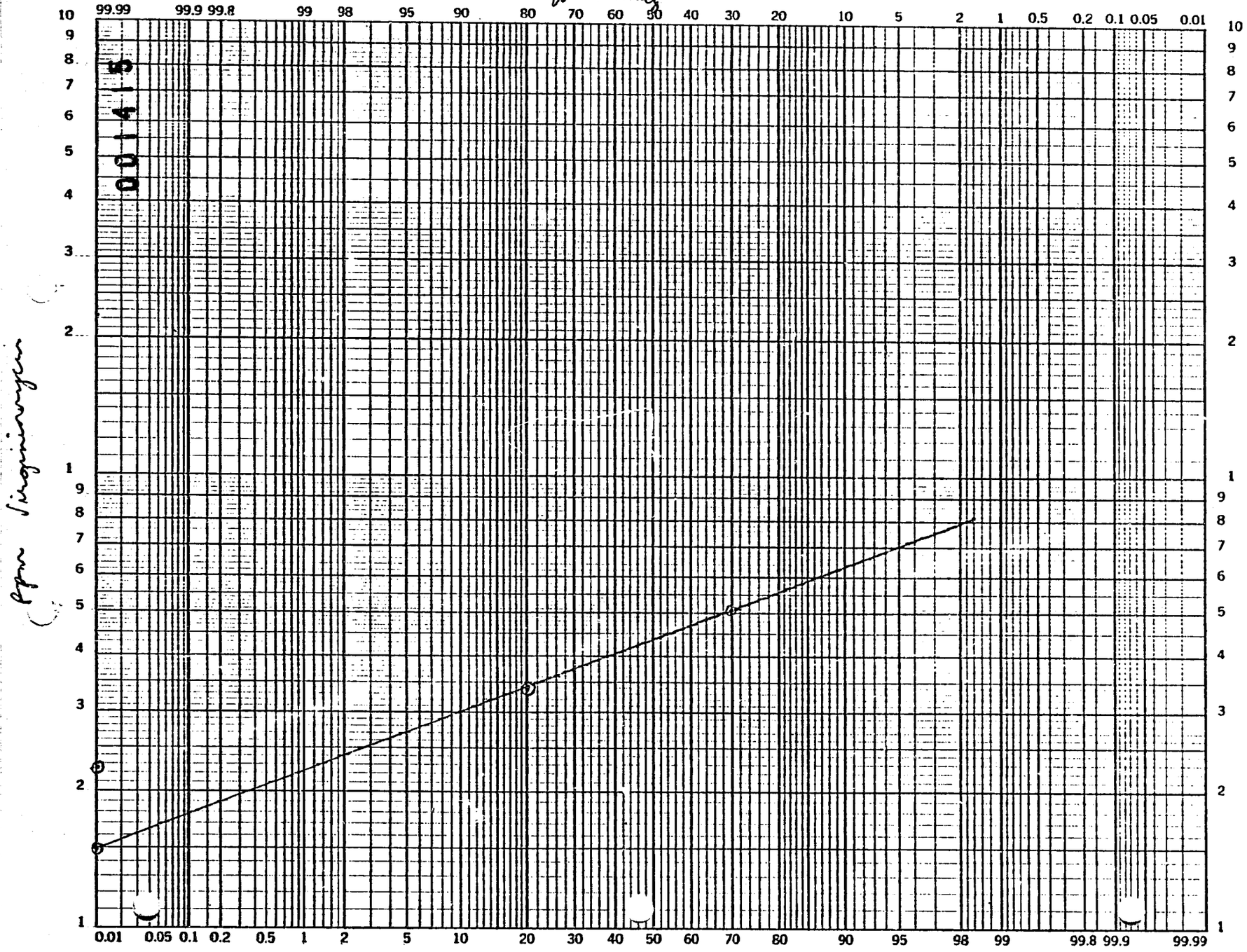
S x fs = upper limit =

Analysis By: John A. Brunmeier Date: 5/14/77

KEUFFEL & ESSER CO. MADE IN U.S.A.

46 8040

% mortality



ppm Virginiamycin

WARF INSTITUTE, I C.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

000416

Chemical: Virginiamycin

Date Tested: 5/12/77

Test Animal: Rainbow Trout

Date Reported: 5/12/77

Lot Number: #AFV/206/75

Temperature: 55°F

Exposure Period: 48 Hours

Water Quality: Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
100	0/10	0			
150	0/10	0			
225	2/10	20			
338	10/10	100			

Total animals = _____

K, No. of Doses = _____

LC_{84} = _____

LC_{50} = Between 225 & 338 ppm

LC_{16} = _____

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

S =

Confidence limits (.05) for LC_{50}

N' = _____

$fLC_{50} = S\ [2.77/\sqrt{N'}]$

fLC_{50} =

LC_{50}/fLC_{50} = lower limit =

$LC_{50} \times fLC_{50}$ = upper limit =

Total Contribution to Chi _____

Chi^2 = contribution to Chi $\times \frac{\text{total animals}}{K}$ = _____

Chi^2 (p=.05) for (K-2) ___ deg. of freedom = ___

Confidence limits (.05) for S:

R = _____ S = _____ A = _____

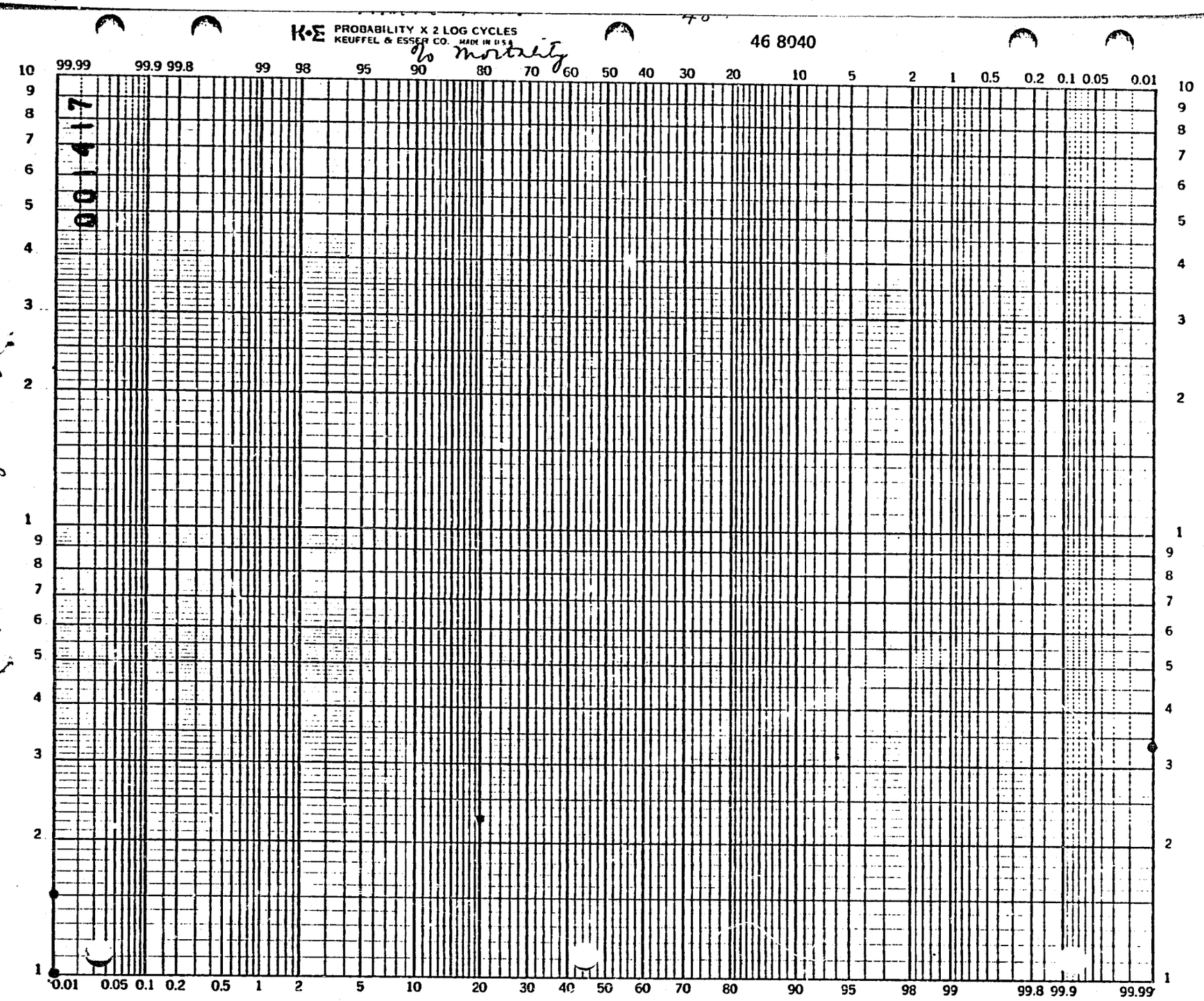
$fs = A\ [10\ (K-1)/K\sqrt{N'}]$

fs =

S/fs = lower limit =

S x fs = upper limit =

Analysis By: John A. Breismeister Date: 5/14/77


K•E PROBABILITY X 2 LOG CYCLES
KEUFFEL & ESSER CO. MADE IN U.S.A.
46 8040
% mortality
99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99
001417
ppm Virginiamycin

WARF INSTITUTE, INC.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

001418

Chemical Virginiamycin — Date Tested 4/12/77

Test Animal Rainbow Trout — Date Reported 5/12/77

Lot Number #AFV/206/75 — Temperature 55°F

Exposure Period 96 Hours — Water Quality Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
100	0/10	0			
150	2/10	20			
225	3/10	30			
338	10/10	100			

Total animals = _____

K, No. of Doses = _____

LC_{84} = _____

LC_{50} = Between 225 & 338 ppm

LC_{16} = _____

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

S =

Confidence limits (.05) for LC_{50}

N' = _____

$fLC_{50} = S\ [2.77/\sqrt{N'}]$

fLC_{50} =

LC_{50}/fLC_{50} = lower limit =

$LC_{50} \times fLC_{50}$ = upper limit =

Total Contribution to Chi _____

Chi^2 = contribution to Chi $\times \frac{\text{total animals}}{K}$ = _____

Chi^2 (p=.05) for (K-2) ____ deg. of freedom = ____

Confidence limits (.05) for S:

R = _____ S = _____ A = _____

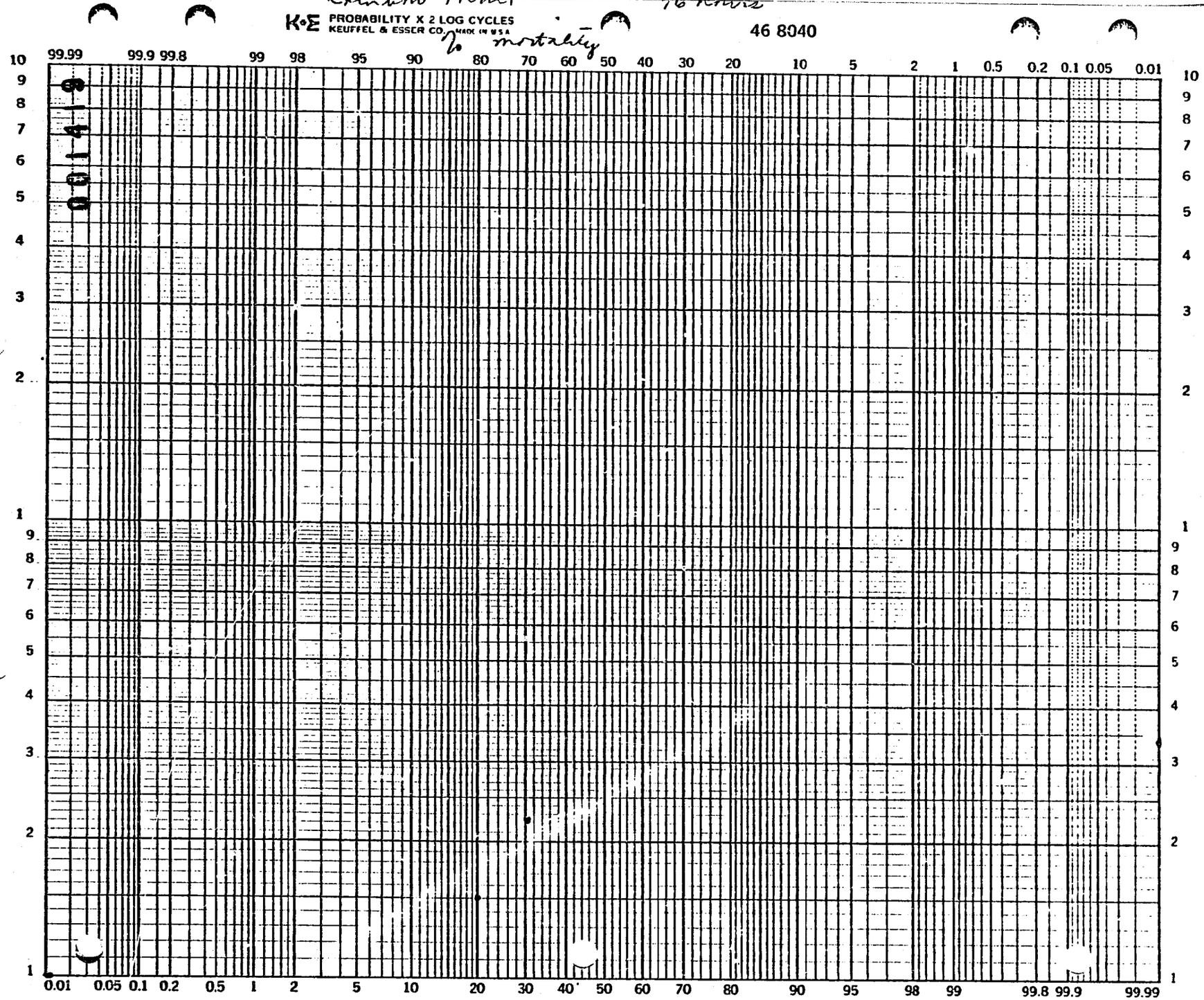
$fs = A\ [10\ (K-1)/K\sqrt{N'}]$

fs =

S/fs = lower limit =

S x fs = upper limit =

Analysis By: John A. Bowman — Date: 5/16/77


K•E PROBABILITY X 2 LOG CYCLES
KEUFFEL & ESSER CO. MADE IN U.S.A.
46 8040
% mortality
99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99

WARF INSTITUTE, INC.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

Chemical Virginiamycin — Date Tested 5/14/77

Test Animal Bluegill Sunfish — Date Reported 5/12/77

Lot Number #AFV/206/75 — Temperature 74°F

Exposure Period 24 Hours — Water Quality Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
100	0/10	0 (2.9)	0.9	2.0	0.045
150	1/10	10	9.0	1.0	0.00123
225	3/10	30	38.0	8.0	0.0255
338	8/10	80	78.0	2.0	0.0024

Total animals = 40

Total Contribution to Chi 0.07413

K, No. of Doses = 4

Chi^2 = contribution to Chi x $\frac{\text{total animals}}{K}$ = 0.7413

LC_{84} = 370 ppm

Chi^2 (p=.05) for (K-2) 2 deg. of freedom = 5.99

LC_{50} = 252 ppm

LC_{16} = 170 ppm

Confidence limits (.05) for S:

R = ____ S = ____ A = ____

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

$fs = A\,[10\,(K-1)/K\sqrt{N'}]$

fs =

S = 1.475

S/fs = lower limit =

S x fs = upper limit =

Confidence limits (.05) for LC_{50}

N' = 20 — 0.6197

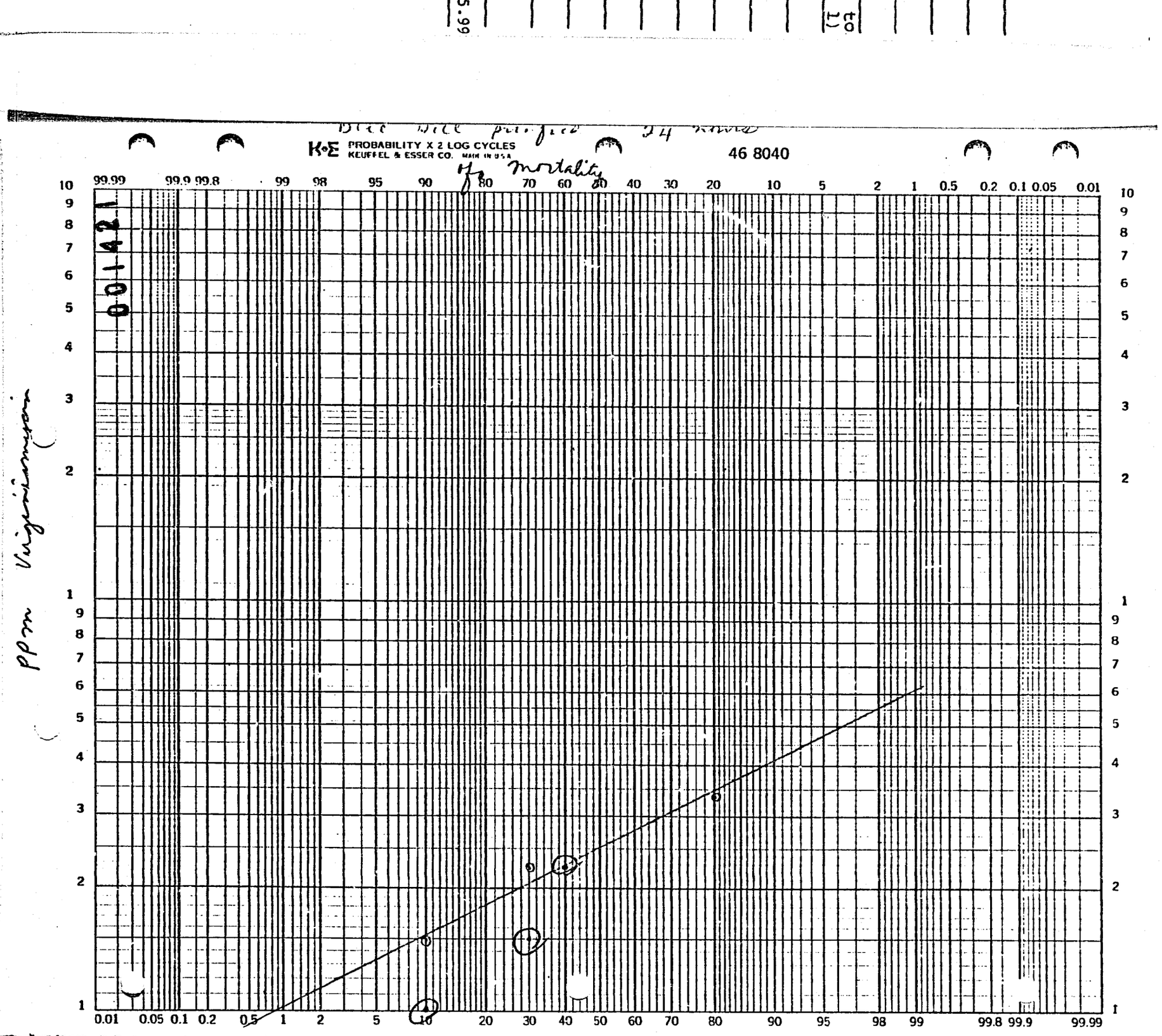
$fLC_{50} = S\,[2.77/\sqrt{N'}]$ 1.475

fLC_{50} = 1.275

LC_{50}/fLC_{50} = lower limit = 198 ppm

$LC_{50} \times fLC_{50}$ = upper limit = 321 ppm

Analysis By: John A. Buccafusco — Date: 5/14/77


K•E PROBABILITY X 2 LOG CYCLES
KEUFFEL & ESSER CO. MADE IN U.S.A.
46 8040
% Mortality
99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01
0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99
10 9 8 7 6 5 4 3 2 1

WARF INSTITUTE, INC.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

001422

Chemical Virginiamycin — Date Tested 4/12/77

Test Animal Bluegill Sunfish — Date Reported 5/12/77

Lot Number #AFV/206/75 — Temperature 74°F

Exposure Period 48 Hours — Water Quality Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
100	1/10	10	10.0	0	0.000
150	3/10	30	24.0	6	0.020
225	4/10	40	46.0	6	0.015
338	10/10	100(91.7)	69.0	22.7	0.240

Total animals = 40

Total Contribution to Chi 0.275

K, No. of Doses = 4

Chi^2 = contribution to Chi x $\frac{\text{total animals}}{K}$ = 2.75

LC_{84} = 470 ppm

Chi^2 (p=.05) for (K-2) 2 deg. of freedom = 5.99

LC_{50} = 240 ppm

LC_{16} = 127 ppm

Confidence limits (.05) for S:

R = ____ S = ____ A = ____

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2} = 1.93$

$fs = A\ [10\ (K-1)/K\sqrt{N'}]$

fs =

S = 1.93

S/fs = lower limit =

S x fs = upper limit =

Confidence limits (.05) for LC_{50}

N' = 30 — 0.5055

$fLC_{50} = S\ [2.77/\sqrt{N'}]$ 1.93

fLC_{50} = 1.40

LC_{50}/fLC_{50} = lower limit = 171 ppm

$LC_{50} \times fLC_{50}$ = upper limit = 336 ppm

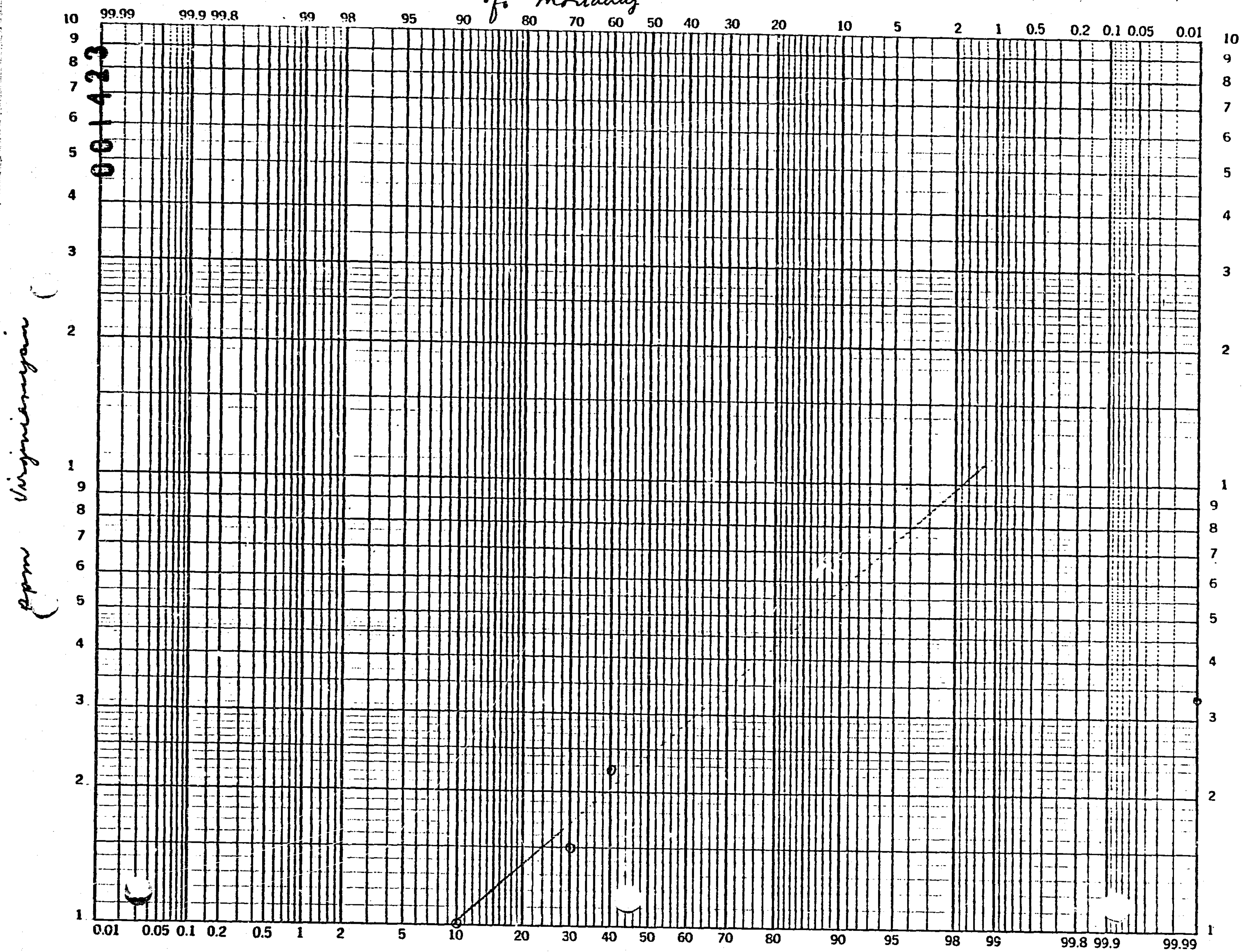
Analysis By: John A. Brennan — Date: 5/14/77

Olive Hill perfusion 48 hours

K•E PROBABILITY X 2 LOG CYCLES
KEUFFEL & ESSER CO. MADE IN U.S.A.

46 8040

001423



WARF INSTITUTE, INC.

MADISON, WISCONSIN

PROBIT ANALYSIS WORK SHEET

Chemical Virginiamycin

Test Animal Bluegill Sunfish

Lot Number #AFV/206/75

Exposure Period 96 Hours

Date Tested 05/12/77

Date Reported 5/12/77

Temperature 74°F

Water Quality Standard

ppm Concentration	No. Dead / Total No.	Observed % Mortality	Expected % Mortality	O-E	Contribution to Chi (Nomo No. 1)
100	2/10	20			
150	3/10	30			
225	4/10	40			
338	10/10	100			

Total animals = _____

K, No. of Doses = _____

LC_{84} = _____

LC_{50} = Between 225 and 338 ppm

LC_{16} = _____

$S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}$ =

S =

Confidence limits (.05) for LC_{50}

N' = _____

$fLC_{50} = S^{[2.77/\sqrt{N'}]}$

fLC_{50} =

LC_{50}/fLC_{50} = lower limit =

$LC_{50} \times fLC_{50}$ = upper limit =

Total Contribution to Chi _____

Chi^2 = contribution to Chi $\times \frac{\text{total animals}}{K}$ = _____

Chi^2 (p=.05) for (K-2) ___ deg. of freedom = ___

Confidence limits (.05) for S:

R = _____ S = _____ A = _____

$fs = A^{[10\ (K-1)/K\sqrt{N'}]}$

fs =

S/fs = lower limit =

S x fs = upper limit =

Analysis By: John A. Breeseman Date: 5/14/77

K·E PROBABILITY X 2 LOG CYCLES
KEUFFEL & ESSER CO. MADE IN U.S.A.

46 8040

% Mortality

99.99 99.9 99.8 99 98 95 90 80 70 60 50 40 30 20 10 5 2 1 0.5 0.2 0.1 0.05 0.01

0.01 0.05 0.1 0.2 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.8 99.9 99.99

1 2 3 4 5 6 7 8 9 10

0014425

ppm Viability

Enclosure A

Dr. C. John Di Cuollo
Smith Kline Animal Health
September 16, 1976

001427

GENERAL PROTOCOL

FOR THE EVALUATION ON THE POTENTIAL TOXICITY OF VIRGINIAMYCIN RESIDUES CONTAINED IN PIG MANURE AND BROILER LITTER TO EARTHWORMS

MATERIALS AND METHODS: Red worms (Eisenia foetida), or another common variety will be employed in this study. Fresh manure will be obtained from pigs on regular feed medicated at 50 g/ton virginiamycin and a companion control manure specimen from pigs on control basal ration. A similar study will be performed with chicken litter obtained from 50 broilers receiving non-medicated and a medicated feed ration containing virginiamycin at 20 g/ton. A negative/negative control will also be employed with a 5 replicate design. Application rates will be based on a wet basis as shown in Table I. Minor variations to these application rates are acceptable.

TABLE I

Preparation of Test Soil

Tons/Acre (based on wet weight) of Manure or Litter*	Species
3	Broiler
8	Broiler
10	Pig
22	Pig
50	Pig

Aliquots of the above mixed soil preparations for each manure and litter sample are placed in 1 quart clear styrene plastic containers. One hundred (100) earthworms are added to each container and the soil covered with a layer of damp cheesecloth and held at 50oF. The worms will be checked daily for activity and sensitivity to external stimulation. After 14 days exposure, 1) the number of worms, 2) their general condition and 3) the reproductive activity will be recorded.

*The lowest application rates for litter and manure should be consistent with their use in the field as fertilizer. If not, these should be readjusted.

CJD:baa

Dr. C. John Di Cuollo
Smith Kline Animal Health
September 15, 1976

GENERAL PROTOCOL

FOR THE PERFORMANCE OF FIELD PHYTOTOXICITY STUDY ON LITTER FROM VIRGINIAMYCIN-TREATED BROILERS

PROCEDURE: During the fall of 1976, litter will be collected from pens of broilers which will be fed either a basal ration or a medicated ration containing levels of virginiamycin at 20 g/ton of feed.

Litter specimens from these studies will be air-dried and ground with a Waring blender and incorporated into all of the soils. Moisture determinations to be performed by WARF on fresh and air-dried samples. Application rates will be calculated on a wet basis equivalent to 0, 3, and 8 tons per acre of fresh litter.* The test materials will be incorporated into all soils including the covering soil to a depth of approximately 2½ inches. The following two source samples will be tested on the plants listed in Table I:

1. Litter from floor pens containing chicks fed virginiamycin at 20 g/ton (5 replicates).
2. Litter from floor pens containing chicks fed basal ration only (5 replicates).
3. Negative/negative control (8 replicates to be employed).

REPORT: Evaluate the crops according to growth or vigor between untreated blank litter and virginiamycin 20 g/ton litter plots.

TABLE I

Alfalfa	Wheat
Cucumbers	Corn
Soybeans	Fescue
	Green beans

ADDENDUM: Please add, delete or alter crop selection/protocol to adequately explore the purpose of these studies.

*The lowest application rate should be consistent with the use of litter in the field as a fertilizer. If not, this rate should be readjusted.

CJD:baa

Dr. C. John Di Cuollo
Smith Kline Animal Health
September 15, 1976

001429

PROTOCOL

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

The effect of virginiamycin on the development of the housefly is proposed in this study. Both litter containing broiler manure and pig manure will be tested for adverse activity against housefly eggs and larvae. Litter and feces will be collected for this study from caged broilers and pigs fed virginiamycin at the rate of 20g/ton and 50 g/ton respectively, for an appropriate period of time. Companion litter and manure specimens from broilers and pigs on basal ration will be used as controls in these same studies. Fecal and litter samples will be shipped air-dried from Smith Kline. In addition, Smith Kline will also send five samples each (approximately 50 g each) of fresh litter and manure for moisture determinations. This will allow for reconstitution of the samples to their original water content prior to starting the study.

Control and medicated litter and manure samples will be seeded with housefly eggs. The development of the eggs into larvae and complete adult houseflys will be observed. The eggs will be seeded onto standard CSMA housefly rearing media. A 5 replicate design will be employed with a negative/negative control.

Report adverse effects, if any, of the above manure collections against any stage of the housefly.

CJD:baa

001430

Enclosure B

cc: Dr. Di Cuollo
File

001431

TO: James A. Miller

FROM: Pat Kraeer

SUBJECT: Virginiamycin Environmental - Report of Test from U.S. Testing Company, Inc., Memphis, TN

DATE: November 3, 1976

REF: PMK 8526 - pp. 138, 139, 146, 147

Attached are the results of analysis of a swine dirt and chicken litter sample sent to the U.S. Testing Company on 9/28/76 for analysis according to EPA established guidelines. In a telephone conversation with Mr. Philip Coop of the company prior to his issuing the test results, he informed me that erroneous texture measurements were being obtained on the chicken litter sample due to its high organic matter content. In order to correct the problem, the company first performed the Lawford ignition test (550°C for 3 to 4 hours) to remove the interfering organic matter (and also quantitate organic matter by weight difference); the Wokley-Black hydrometer texture test was then applied to the remaining non-organic residue.

Date: 11/3/76

Patrick M. Kraeer
James A. Miller

PK:baa

United States Testing Company, Inc.
MEMPHIS LABORATORY
3765 PREMIER COVE • MEMPHIS, TENNESSEE 38118 • 901-794-8800

REPORT OF TEST
October 18, 1976

001432
760808-292-1A
NUMBER

CLIENT: Mrs. Patricia Kraeer
Smith Kline Animal Health Products
1600 Paoli Pike
West Chester, PA 19380

SUBJECT: Analysis of two samples according to pesticide registration guidelines.

Parameter	Chicken Litter	Swine Dirt
pH	7.7	6.4
Organic Matter, %	55.8	3.8
Cation Exchange Capacity, meq/100g	72.9	16.9
1/3 Bar Moisture, %	103.6	17.0
Texture	silt loam	silt loam
Sand, %	14.8	23.2
Silt, %	57.4	74.0
Clay, %	27.8	2.8

Note: The texture and percentages of sand, silt, and clay are for the mineral fraction of the chicken litter after destruction of the organic matter.

APPLEBROOK
OCT 22 1976
RECEIVED

SIGNED FOR THE COMPANY
BY W. P. Bonner, Ph.D.

001433

Appendix II

January 7, 1977

001434

To: James Miller

From: R. P. Supplee

Subject: Procedure Used for Environmental Impact Study 1976

Poultry:

Entire feces collection was taken from 1 control and 1 medicated group of chickens each consisting of 100 broiler size chickens (4-5 lbs.) received from Truslow Farms September 28, 1976. Same diet was fed to both groups of birds throughout, except that the medicated feed contained virginiamycin @ 23 gm/ton in pre-mix (see attachment 1 for medicated diet). Birds were put on proper diets upon arrival and fed for one week to assure proper adaptation to feed. On October 5, 1976 pens were thoroughly cleaned, clean dried sawdust added to pens for bedding and actual collection was started. Fourteen (14.8) kg bedding was added to control pen and 14.9 kg was added to medicated pen. No additional bedding was added and collection ended on October 29, 1976 when fecal material was separated, spread out to not more than 2 inches in depth and air-dried on plastic in B-wing Building @ average temperature of 63° until it reached as low moisture content as reasonably possible in this atmosphere. On December 6, 1976, following amounts of feces were shipped in cardboard plastic lined drums to: Client Services, WARF Institute Incorporated, Madison, Wisconsin.

Drum	Control Poultry Manure	Medicated Poultry Manure
1	33.9 kg. net wt.	36.0 kg. net wt.
2	37.2 kg. net wt.	40.1 kg. net wt.
3	38.3 kg. net wt.	36.7 kg. net wt.

All control poultry manure drums were marked #4 and all medicated poultry drums were marked #3.

Note: Of 112.8 kg medicated manure, 10.8 kg was packaged separately in 40.1 kg drum as it was slightly more moist than other feces due to leakage from broken waterer.

Swine:

Swine feces were collected from 2 groups of pigs. Both control and medicated pigs from both groups were fed SK&F formula "T" swine grower (see attachment #2), except that the medicated feed contained virginiamycin @ 50 gm/ton in pre-mix.

Group #1 consisted of 8 control and 8 medicated hogs each weighing 200 to 240 lbs. which were received from Willow Glen Farm September 20, 1976. Pigs were put on proper diets upon arrival and fed for one week to assure proper adaptation to feed. On September 28, 1976, feces collection was started on a daily basis, with no bedding added, and ended on November 11, 1976. Entire collection from first group was used for study.

OCT 4 9 5

Group #2 consisted of 10 control and 30 medicated pigs weighing 170 to 180 lbs. each, received from Willow Glen Farm on November 11, 1976. Pigs were put on proper diets upon arrival and fed for one week to assure proper adaptation to feed. Feces collection was started on November 18, 1976, on a daily basis with no bedding used and ended on December 3, 1976. Only part of collection was needed to complete the study. On November 23, 1976 one pig was removed from control group due to a prolapsed rectum. All swine fecal material used was separated, spread out to a depth of not more than 2 inches and dried on plastic in B-wing Building @ average temperature of 63° until it reached as low moisture content as reasonably possible in this atmosphere. On December 6, 1976 and December 9, 1976 following amounts of swine feces from respective groups were sent in plastic lined cardboard drums to: Client Services, WARF Institute Incorporated, Madison, Wisconsin.

December 6, 1976 Shipment

Control Swine Feces
Net Wt. Kg

Drums	Group #1	Group #2	Total	Group #1	Group #2	Total
1	35.1	0	35.1	33.3	0	33.3
2	35.4	0	35.4	35.1	0	35.1
3	34.0	0	34.0	16.6	18.4	35.0
4	23.7	9.4	33.1	--	--	---
	128.2	9.4	137.6	85.0	18.4	103.4

December 9, 1976 Shipment

All from Group #2 pigs

Drum	Control Swine Feces	Medicated Swine Feces
1	39.1 Kg. Net W	38.7
2	--	35.6
	39.1 Kg. Net Wt.	74.3 Kg. Net Wt.

All medicated swine drums were marked #1 and all control swine drums were marked #2.

Attachment #1

Virginiamycin Medicated Feed (23 gm/ton) Color: Red

001436

N.B. Ref: DB 8532, 139

Ingredient	% W/W	Amt./2700 lbs.
Medium Ground Shelled Corn	58.00	1566.00
Soybean Meal, 44%	27.00	729.00
Fish Meal, Ad-Sol (Adams Labs. Fairfax, VA)	3.00	81.00
Dehydrated Alfalfa Meal, 17%	5.00	135.00
Distiller's Dried Grains with Solubles (Solulac)	2.00	54.00
Dicalcium Phosphate	1.50	40.50
Ground Limestone	1.50	40.50
Plain Salt	0.50	13.50
DL-Methionine	0.05	1.35
*Broiler Vitamin/Mineral Premix #1 (Xtra Factors)	0.45	12.15
Medicated Premix (for 23 gm/ton)	1.00	27.00

*Contains finished feed equivalents of the following:

Vitamin A	6928 IU/kg.
Vitamin D_3	1584 IU/kg.
Vitamin B-1 (Thiamine)	0.22 mg/kg.
Bitamin B-12	0.009 mg/kg.
Vitamin K	3.22 mg/kg.
Riboblavin	4.04 mg/kg.
Niacin	29.2 mg/kg.
Pantothenic Acid	9.9 mg/kg.
Choline Chloride	395 mg/kg.
Folic Acid	0.11 mg/kg.
Copper	3.56 mg/kg.
Iodine	1.29 mg/kg.
Iron	36.03 mg/kg.
Manganese	58.89 mg/kg.
Magnesium	8.99 mg/kg.
Zinc	49.68 mg/kg.
Cobalt	0.33 mg/kg.
Vitamin B-6	1.12 mg/kg.

BASIC SWINE GROWER RATION

Formula 'T'

13%

001437

Ingredient	% w/w (unit/lb.) in FF
Medium Ground Shelled Corn	79.50
Soybean Meal, 44%	13.35
Dehydrated Alfalfa Meal, 17%	4.50
Calcium Propionate	0.15
Miller Swine Min-Vit 10 with E & K	2.50
Vitamin A	1500 IU
Vitamin D-3	400 IU
Vitamin E	5 IU
Vitamin K	1 mg.
Riboflavin	2 mg.
Niacin	9 mg.
D-Pantothenic Acid	4 mg.
Vitamin B-12	0.01 mg.
Calcium (Ca) min.	0.44 %
Calcium (Ca) max.	0.49 %
Phosphorous (P) min.	0.23 %
Salt (NaCl) min.	0.475 %
Salt (NaCl) max.	0.525 %
Iodine (I) min.	0.000032 %
Iron (Fe) min.	0.011 %
Copper (Cu) min.	0.000475 %
Manganese (Mn) min.	0.006 %
Zinc (Zn) min.	0.0099 %
Magnesium (Mg) min.	0.0034 %

APPLEBROOK RESEARCH CENTER
SMITHKLINE ANIMAL HEALTH PRODUCTS
DIVISION OF SMITHKLINE CORPORATION

SK NUMBER
SK&F 7988-C

GENERIC NAME
Virginiamycin

PROTOCOL OR TEST NO.
V-4005-77

DATE OF REPORT
August 4, 1978

DISTRIBUTION LIST
Dr. DiCuollo
Ms. DePaolantonio
Ms. Kirby
Mr. Lewis
Mr. Miller
Dr. Wang
Files
Archives

REPORT

SECTION: BIOANALYTICAL

DATE STUDY STARTED: September 1, 1976

DATE STUDY COMPLETED: December 31, 1976

SUBJECT: Stability of Virginiamycin in Poultry Excreta and Litter

ABSTRACT: 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 70% degradation occurring within a 3 day period and 95% degradation occurring by 14 days.

STUDY DIRECTOR: P. M. Kraeer

SIGNATURE AND INITIALS OF STUDY DIRECTOR: Patricia M. Kraeer PMK

SIGNATURE AND INITIALS OF REPORT AUTHOR: Patricia M. Kraeer PMK

SIGNATURE AND INITIALS OF COWORKERS: J. J. DePaolantonio J.J.D.

David O. Lewis D.O.L.

APPROVAL: Richard Wang

TITLE: Manager, Bioanalytical

NOTEBOOK REFERENCES:

JJD 8553, 58, 59, 64, 71, 72-74, 78-81, 134, 227-235.

CJD/mgd
8/2/78

Smith Kline Animal Health Products

001440

STABILITY OF VIRGINIAMYCIN IN POULTRY EXCRETA AND LITTER

Smith Kline Animal Health Products

TABLE OF CONTENTS

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Smith Kline Animal Health Products

LIST OF TABLES

I. 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 and conditions in order to better duplicate temperatures and environmental factors encountered during actual use.

II. MATERIALS AND 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 at a level of 30 PPM using 1.0 ml of a 600 µg/ml water solution of virginiamycin. 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 with 30 ml of 0.1 M citric acid and 30 ml acetone. This extract was then diluted and assayed microbiologically for virginiamycin using the disc method[1].

Procedures for final calculations, together with sample calculations are provided as footnotes to the various tables to be referred to in Section III of this report.

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.

Replicate 20 g samples of the pooled excreta were weighed into

---

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

1. NADA 96-762, Part 5, vii 2, Microbiological Assay, Pages 1620-1621.
NADA 91-513, Part 5, E(3), Microbiological Assay, Pages 2052-2057.

polypropylene bottles. The samples were fortified at a level of 30 PPM using 1.0 ml of a 600 µg/ml water solution of virginiamycin. The fortified samples were stored loosely capped at room temperature (18-22°C).

After the appropriate degradation period, samples were extracted with 35 ml of 0.1 M citric acid and 35 ml of acetone. The extract was then 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 at a level of 30 PPM using 1.0 ml of a 600 µg/ml water solution of virginiamycin. 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 re-weighed 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 with 35 ml of 0.1 M citric acid and 35 ml of acetone. The extract was then diluted and assayed microbiologically using the disc method.

III. DATA AND RESULTS

A. POULTRY LITTER AT ROOM TEMPERATURE (STUDY 1)

Results of the room temperature degradation studies in poultry litter are summarized in Table 1. The rate of degradation was rapid with 83.2% of the virginiamycin degraded in a 7 day period.

B. POULTRY EXCRETA AT ROOM TEMPERATURE (STUDY 2)

Table 2 provides data for the room temperature stability study employing poultry excreta. In this case, after 14 days at room temperature, only 5.6% of the initial virginiamycin concentration remained.

C. POULTRY EXCRETA AT AMBIENT TEMPERATURE (STUDY 3)

The results of the virginiamycin stability study in poultry excreta under ambient conditions are displayed in Table 3. Under these conditions, which better simulate actual "use" conditions, the antibiotic degrades rapidly, with 94.7% degraded in 14 days.

IV. CONCLUSIONS

Virginiamycin fortified into poultry excreta and poultry litter is unstable and degrades rapidly at room temperature or under ambient conditions with greater than 95% degradation occurring within a 14 day period.

PMK/mgd
9/12/78

FOOTNOTES TO TABLES 1, 2, 3

$^{+}$0 Time = Control sample fortified just prior to extraction to provide measure of extraction efficiency (63.8% for excreta, 90.8% for litter).

SAMPLE CALCULATION: TABLE 1, 3 DAY

1. THEORETICAL CONCENTRATION

20 Grams Litter + 40.0 ml Water + 1.0 ml Virginiamycin Standard (600 µg/ml).

Extraction Volume = 60.0 ml.
Theoretical Concentration = 5.88 µg virginiamycin/ml extract.

2. PPM-VM RECOVERED

$\frac{1.66\ \mu g/ml\ VM\ assayed}{5.88\ \mu g/ml\ Theoretical} \times 30\ \mu g/g = 8.47\ \mu g/g$ (PPM-VM recovered).

3. RECOVERY EFFICIENCY:

Measure of extraction efficiency obtained with 0 time sample = 90.84% for litter samples.

4. TOTAL PPM CORRECTED FOR EFFICIENCY:

$\frac{\text{PPM Recovered}}{0.9084}$

$\frac{8.47\ \mu g/g\ VM\ Recovered}{0.9084} = 9.33\ \mu g/g$ VM in sample.

5. % VM DEGRADED:

$100 - \left(\frac{9.33\ \mu g/g}{30.0\ \mu g/g} \times 100\%\right) = 68.92\%$ VM Degraded.

TABLE 1

STABILITY OF VIRGINIAMYCIN (VM) IN POULTRY LITTER AT ROOM TEMPERATURE

SAMPLE	VIRGINIAMYCIN CONCENTRATION (µg/ml OF EXTRACT)				(2) PPM-VM RECOVERY	(3) RECOVERY EFFICIENCY	(4) TOTAL PPM CORRECTED FOR EFFICIENCY	(5) % VM DEGRADED
	(1) THEORETICAL CONCENTRATION	ASSAYED VALUE	AVERAGE	STANDARD DEVIATION				
0 Time+ (Initial)	5.88	5.35 5.20 5.30 5.58 5.30	5.34	0.15	27.24	90.84	30	-
3 Day	5.88	1.64 1.57 1.76	1.66	0.10	8.47	90.84	9.33	68.92
7 Day	5.88	.926 .871 .901	.899	0.03	4.59	90.84	5.05	83.17

N.B. REF.: JJD 8553, 233.

TABLE 2

STABILITY OF VIRGINIAMYCIN (VM) IN POULTRY EXCRETA AT ROOM TEMPERATURE

SAMPLE	VIRGINIAMYCIN CONCENTRATION (µg/ml OF EXTRACT)				(2) PPM-VM RECOVERY	(3) RECOVERY EFFICIENCY	(4) TOTAL PPM CORRECTED FOR EFFICIENCY	(5) % VM DEGRADED
	(1) THEORETICAL CONCENTRATION	ASSAYED VALUE	AVERAGE	STANDARD DEVIATION				
0 Time+ (Initial)	8.45	5.61 5.18 5.67 5.22 5.29	5.39		19.14	63.79	30	-
3 Day	8.45	1.12 1.13 1.09	1.11	0.03	3.94	63.79	6.18	79.41
7 Day	8.45	1.07 1.08 1.09	1.08	0.01	3.83	63.79	6.01	79.96
14 Day	8.45	.305 .280 .319	.301	0.03	1.07	63.79	1.68	94.42

N.B. REF.: JJD 8553, 235.

TABLE 3

STABILITY OF VIRGINIAMYCIN (VM) IN POULTRY EXCRETA AT AMBIENT TEMPERATURE

SAMPLE	VIRGINIAMYCIN CONCENTRATION (µg/ml of EXTRACT) (1) THEORETICAL CONCENTRATION	ASSAYED VALUE	AVERAGE	STANDARD DEVIATION	(2) PPM-VM RECOVERY	(3) RECOVERY EFFICIENCY	(4) TOTAL PPM CORRECTED FOR EFFICIENCY	(5) % VM DEGRADED
0 Time+ (Initial)	8.45	5.32 5.75 5.33	5.47	0.25	19.42	64.73	30	-
7 Day	8.45	1.25 1.22 1.20	1.22	0.03	4.33	64.73	6.69	77.70
14 Day	8.45	.275 .300 .289	.292	0.02	1.04	64.73	1.60	94.66

N.B. REF.: JJD 8553, 234.

IV. Stability of Virziniamycin in Water

A. 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.

B. Materials and 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 was 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 resevoir.

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 the chemical method.(1)

This experiment was repeated utilizing the same conditions and procedures (Experiment 2).

A third experiment was conducted without the presence of swine, using the same conditions and procedures as Experiment 1. This experiment was subsequently duplicated (Experiment 4).

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 by adding $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ to deionize water 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.

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[1] NADA 96-762, Part 5, x.c., pages 1604-1605

These data were contained in Appendix I of our Environmental Impact Analysis Report which was submitted to NADA 91-467 and 91-513 with our letter dated March 28, 1978.

C. Data and Results

Results of virginiamycin degradation experiments conducted in the presence of swine are summarized in Table 8. In 22 hours, at least 37 percent of the virginiamycin had degraded.

Table 9 provides data on the degradation rate of virginiamycin without the presence of swine. An average of 25 percent of the virginiamycin had degraded at the end of 22 hours.

Data from the fifth experiment demonstrate the effect of hard water and elevated temperature on the degradation rate and are presented in Table 10. At room temperature after 23 hours, 36 percent of the virginiamycin had degraded at all pHs 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.

Table 8

Stability of Virginiamycin in Galvanized Containers in the Presence of Swine

EXPERIMENT-1				EXPERIMENT-2	
Init. mg/l	Init. pH	% Remaining 22 hrs.	pH 22 hrs.	Init. mg/l	% Remaining 22 hrs.
22.1	7.7	64.1	7.15	16.6	62.8
31.7	8.3	60.3	7.20	39.1	62.8
66.7	7.7	62.6	7.10	57.5	62.2

Notebook Reference JC 6914,119 JC 6914,120

Table 9

Stability of Virginiamycin in Galvanized Containers Without the Presence of Swine

EXPERIMENT-3				EXPERIMENT-4	
Init. mg/l	Init. pH	% Remaining 22 hrs.	pH 22 hrs.	Init. mg/l	% Remaining 22 hrs.
38.8	7.6	62.8	7.8	54.2	77.9

Notebook Reference JC 6914,121 JC 6914,123

Table 10

Stability of Virginiamycin in Synthetic Hard Water (123 mg/liter as $CaCO_3$)

(Initial virginiamycin concentration = 47 mg/l)

Experiment 5

Condition	Room Temperature			37° C		
Initial pH	6	7	8	6	7	8
% Virginiamycin Remaining after 23 hours	64	64	60	41	41	40
% Virginiamycin Remaining After 48 hours	43	47	45	21	25	31

Notebook Reference JC 6914, 199-200

D. 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.

3/10/77
Date

G Richard Unangst
R. Richard Unangst
Manager, Pharmaceutical Development

001454

Appendix A

Smith Kline Animal Health Products

cc: Jim Miller
Files

001455

TO: Dr. C. John Di Cuollo

FROM: J. DePaolantonio

SUBJECT: A Water/n-Octanol Partitioning Study On Virginiamycin

NOTEBOOK REF: 8385-27,28

DATE: March 15, 1977

Submitted in this report are the findings of an n-octanol/water partitioning study on virginiamycin. Findings of the study suggest that virginiamycin is lipophilic by virtue of its partitioning into n-octanol after extensive agitation in an n-octanol/water system.

I. Partitioning Coefficient

A. 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.

B. Materials and Methods

A water solution of virginiamycin was prepared and its concentration confirmed utilizing the disc microbiological assay procedure. In this procedure, Corynebacterium xerosis is the assay organism. 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 on to 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.

C. Data and 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. The n-octanol phase samples were assayed, but the n-octanol was toxic to the assay organism, making interpretation of results impossible.

D. 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. In Part VII of our NADAs, we described a study in swine where oral administration of a single dose of virginiamycin at 100 mg/kg b.w. resulted in the absence of significant antibiotic levels, either in serum or urine. This suggests, that in spite of its intrinsic lipophilicity, virginiamycin is, in fact, poorly absorbed across membranes.

The poor absorption exhibited by virginiamycin in swine can be explained by its high molecular weight and large cross-sectional size. The molecular weight of Factor M is 542 and that of Factor S is 809. Virginiamycin is composed of both factors, which act synergistically in producing its antibiotic activity. The structures of both factors are illustrated in Figure 1 of this report. These structures reveal the relatively large size and bulkiness of the molecules. The rate of diffusion of a compound is a function of the concentration gradient across the membrane (C_1-C_2), the surface area available for transfer (A), the thickness of the membrane (d), and the diffusion constant (R) of the substance transferred. This relationship (Fick's Law) can be expressed as follows:[1]

$$\text{Rate of diffusion} = R\,\frac{A(C_1-C_2)}{d}$$

The diffusion constant of the compound is related to its molecular weight, spatial configuration, degree of ionization and lipid solubility. As the molecular weight or cross-sectional size of the molecule increases, the rate of diffusion decreases. Apparently, in the case of virginiamycin, the high molecular weight and bulkiness of the molecule are such that absorption across lipoid membranes is minimal.

3/15/77
Date

N. A. DeFalantonio
N. DeFalantonio, Assoc. Scientist

3/15/77
Date

C. John Di Cuollo
C. John Di Cuollo, Ph.D.
Manager, Development Operations

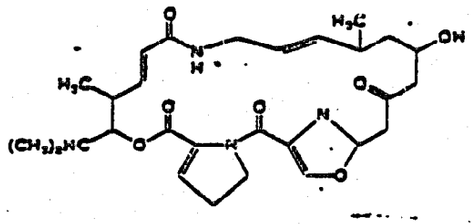
[1] Fick, A. (1855) Über Diffusion. Poggendorffs Annalen d. Physik

JD:baa

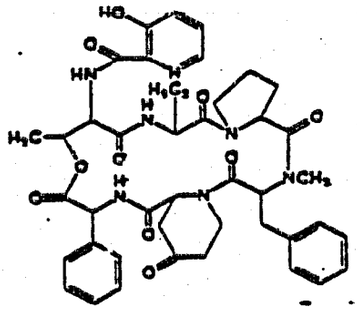
FIGURE 1

Structures of Factors M and S of Virginiamycin

VIRGINIAMYCIN M



VIRGINIAMYCIN S
$C_{43}H_{49}O_{10}N_7$



001459

March 7, 1977

TO: Helen Birkhead

FROM: H. E. Matthews

SUBJECT: Environmental Impact Summary

The production of Virginamycin begins with fermentation in an aqueous broth then extraction with MIBK and crystallization using hexane.

The solvents MIBK and hexane are recovered and reused in production. The antibiotic production facility complies with existing local and provincial regulations concerning effluent emission.

HEM/jw