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ENVIRONMENTAL ASSESSMENT

OF THE USE OF SEMDURAMICIN SODIUM PREMIX

IN THE FEED OF BROILER CHICKENS

FOR THE PREVENTION OF COCCIDIOSIS

PFIZER INC.

APRIL 1993

ENVIRONMENTAL ASSESSMENT

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ENVIRONMENTAL ASSESSMENT

OF

THE USE OF SEMDURAMICIN SODIUM PREMIX IN THE FEED
OF BROILER CHICKENS FOR THE PREVENTION OF COCCIDIOSIS

1. DATE: April 5, 1993

2. APPLICANT: Pfizer Inc.
(Sponsor #000069)

3. ADDRESS: 235 East 42nd Street
New York, N.Y. 10017

4. DESCRIBE THE PROPOSED ACTION:

Pfizer Inc. is filing a New Animal Drug Application requesting approval for the use of a premix containing semduramicin sodium in broiler feeds. Feed containing semduramicin activity at the recommended use level of 25 ppm (corresponding to a concentration of 25.6 ppm of semduramicin sodium) would be fed continuously to broilers for the prevention of coccidiosis.

The bulk drug will be produced at one or more of Pfizer's existing manufacturing plants in Groton, Connecticut and Taketoyo, Japan. The premix, a 5.13% Type A Medicated Article, will be formulated and packaged at Pfizer's existing Lee's Summit, Missouri plant and will be blended to Type C medicated broiler feeds at feedmills in broiler-producing areas. The finished feeds will be used in commercial broiler houses. These are located primarily in rural areas of the states of Alabama, Arkansas, California, Delaware, Georgia, Maine, Maryland, Mississippi, North Carolina, Pennsylvania, Texas, and Virginia.

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

A. Semduramicin Sodium

Semduramicin sodium is an antibiotic produced by a strain of Actinomadura roseorufa var. Huang. It belongs to a class of antibiotics known as ionophores or polyether carboxylic acids. It will be produced as a crystalline sodium salt.

Generic Name: Semduramicin sodium

Trade Name: AVIAX

Chemical Name: (2R,3S,4S,5R,6S)-tetrahydro-2,4-dihydroxy-6-[(1R)-1-[(2S,5R,7S,8R,9S)-9-hydroxy-2,8-dimethyl-2-[(2R,5S)-tetrahydro-5-methyl-5-[(2R,3S,5R)-tetrahydro-5-[2S,3S,5R,6S)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2H-pyran-2-yl]-3-[[(2S,5S,6R)-tetrahydro-5-methoxy-6-methyl-2H-pyran-2-yl] oxy]-2-furyl]-2-furyl]-1,6-dioxaspiro [4.5] dec-7-yl]ethyl]-5-methoxy-3-methyl-2H-pyran-2-acetic acid· sodium

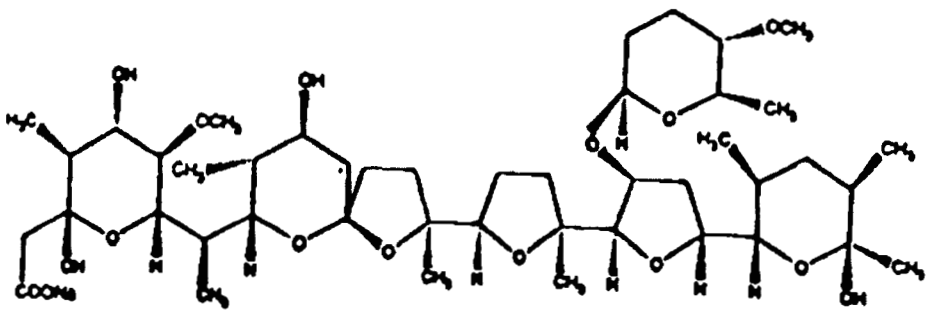
CAS Registry Number (sodium salt): 119068-77-8

Pfizer Code Number: UK-61,689-2

Molecular Formula: $C_{45}H_{75}O_{16}Na$

Molecular Weight: 895

Structural Formula:



Physical Description: White Solid, m.p. 170°C

B. Other Premix Ingredients:

In addition to semduramicin sodium, premixes may contain diluents commonly used in feed premixes, such as soybean mill run, rice hulls, calcium carbonate, sodium carbonate, sodium aluminosilicate, and mineral oil.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

A. From the sites where bulk drug is produced:

The manufacture of semduramicin sodium will be carried out in one or two general purpose fermentation and recovery plants designed to have minimal environmental impact and be in compliance with all applicable emissions requirements. Either of these sites would operate in accordance with local environmental regulations. The plants are located in Groton, Connecticut and Taketoyo, Japan.

1. Production/Processing Overview

Semduramicin sodium is a fermentation-produced ionophore that is primarily associated with the solid mycelial portion of the fermentation broth.

Recovery of semduramicin sodium from the fermentation broth involves standard unit operations including: separation of broth solids, solvent extraction, carbon treatment, evaporative concentration, crystallization, drying, and milling.

The aqueous portion of the fermentation broth (i.e. the broth filtrate) contains a small percentage of semduramicin sodium, and is treated as a waste stream during recovery processing.

All effluent/waste streams, regardless of production site, will be treated in a manner to reduce residual semduramicin sodium content in discharged waste to less than 0.1 ppm.

2. Manufacturing and Occupational Safety

a. Material Safety Data Sheets

The manufacturing site(s) will make available to employees the appropriate detailed Material Safety Data Sheets (MSDS) essentially similar to OSHA Form 20. The MSDS for semduramicin sodium and semduramicin sodium 5.13% premix will contain the information shown in the attached examples (Appendix a-1), though the format and local language will vary from one site to another.

b. Hazard Evaluation Studies

Dermal and occular irritation studies were conducted in rabbits (Appendix C-15). Only mild dermal irritation was observed following a 24 hour exposure to 0.5 g semduramicin sodium applied to intact skin. Abraded skin exposed to the same dose for 24 hours showed well defined erythema which

subsided completely within 4-5 days. Instillation of 21.5 mg semduramicin sodium to the conjunctival sac caused slight reddening of the conjunctivae, slight chemosis and/or slight discharge as well as circumcorneal injection and a small, localized area of iritis in one rabbit. Within 24 hours of dosing, treated eyes appeared normal.

Steps have been taken to minimize occupational and user exposure to semduramicin at Pfizer bulk drug and premix manufacturing sites. Facilities for production of semduramicin bulk are equipped with appropriate physical isolation and air handling facilities to minimize worker exposure. Many of the production operations are automated. Pfizer workers will also wear appropriate protective equipment including gowns, gloves and protective masks as circumstances require. The 5% semduramicin sodium premix product (Aviax) will be manufactured in a new, automated premix plant located in Lee's Summit, Missouri, which has been specifically designed to minimize worker exposure. Exposure to semduramicin will be minimized by means of both the physical isolation of operators from automated drum dumping stations, mixers and packaging operations, and by the design of the air handling systems. Furthermore, workers will wear appropriate protective clothing, as required.

To determine exposure to airborne semduramicin sodium, air samplers were placed at key manufacturing stations, either adjacent to equipment or fitted to operators to sample air close to the breathing zone. Membrane filters from the air samplers were analyzed for the presence of semduramicin sodium and expressed as mg per cubic meter of air. The results (below) show that airborne semduramicin was either not detected or was present only in extremely minute quantities.

Testing Area	Sampling Period	mg semduramicin sodium/ cubic meter of air
Bulk drug dump station	29 minutes	0.08
Vrieco Conical Premix Blender (15 min. mix)	9 minutes	non detected
Blend Sifter	9 minutes	non detected
Premix Bag Filler	18 minutes	0.07

Semduramicin premix has been specifically formulated to protect feed mill operators from exposure to airborne semduramicin. The low dusting characteristics of semduramicin premix were demonstrated by results obtained with a Heubach dustmeter. In this test, a sample of premix in a rotating drum is dropped repeatedly through a moving air stream, which carries any dislodged respirable dust to a capture filter for analysis. The method is useful for comparing the relative dustiness of premix formulations.

In this test, semduramicin commercial premix showed <2 micrograms semduramicin dust/g premix. The results are also significantly lower than those obtained for four other contemporary, commerically available poultry premix products, which gave results of 30 (Coban), 12 (Cygro), 24 (Monteban) and 50 (Biocox) mcg active/g under the same conditions.

3. Emissions

The substances which could be emitted and/or discharged from specific production units, and the respective exposure limits (when available) for the Groton site, are as follows:

Substance	Chemical Abstracts Registry No.	TWA[1] ppm	mg/m3
Semduramicin sodium	113378-31-7(acid form)		
Extracted mycelium	--		
Unconsumed fermentation nutrients (i.e. hydrolyzed starch, molasses, yeast extracts, soybean oil, soybean meal, blood meal, cotton seed meal, corn starch liquor, etc.)			
Solvents			
Methanol	67-56-1	200	260
Isopropanol	67-63-0	400	980
Acetone	67-64-1	750	1800
Methyl isobutyl ketone	108-10-1	50	205
Ethyl acetate	141-78-6	400	1400
n-Butyl acetate	123-86-4	150	710
Methylene chloride	75-09-2	50	175
Chloroform	67-66-3	2	9.78
Heptane	142-82-5	400	1600
Hexane	110-54-3	50	180
Petroleum ether	8032-32-4	300[2]	
Toluene	108-88-3	100	375
1,1,1-trichloroethane	71-55-6	350	1900
Isopropyl ether	108-20-3	250	1050
Acids/Bases			
Hydrochloric acid	7647-01-0	5	7 [ceiling]
Sulfuric acid	7664-93-9	1	
Sodium hydroxide	1310-73-2	2 [ceiling]	
Calcium hydroxide	1305-62-0	5	
Calcium oxide	1305-78-8	5	
Potassium hydroxide	1310-58-3	2 [ceiling]	
Ammonium hydroxide	1336-21-6		
Other			
Filteraid (silica)	68855-54-9	15	
Carbon	7440-44-0	3.5	
Sodium chloride	7647-14-5		
Ammonium carbonate	8000-73-5		
Calcium carbonate	471-34-1	15 total dust	
Ammonium nitrate	6484-52-2		
Calcium nitrate	13477-34-4		
Ammonium phosphate, monobasic	7722-76-1		
Ammonium phosphate, dibasic	7783-28-0		
Potassium phosphate, monobasic	7778-77-0		
Potassium phosphate, dibasic	7758-11-4		

Substance	Chemical Abstracts Registry No.	TWA[1] ppm	mg/m3
Sodium phosphate, monobasic	10049-21-5		
Sodium phosphate, dibasic	7782-85-6		
Sodium sulfate	7727-73-3		
Ammonium sulfate	7783-20-2		
Magnesium sulfate	7487-88-9		
Manganese sulfate	10034-96-5		
Ferrous sulfate	7782-63-0		
Cobalt chloride	7791-13-1		
Potassium chloride	7447-40-7		
Urea	57-13-6		
Formaldehyde	50-00-0	1.0	
Hydrogen peroxide	7722-84-1	1.0	1.4

[1] Allowable 8-hour time-weighted average exposure according to OSHA Air Contaminants 29 CFR 1910.1000

[2] Limit set by ACGIH.

4. Groton Site

The Groton plant site is a large, multi-product, pharmaceutical and specialty chemical manufacturing facility. Through its Environmental Control Department, the plant maintains an integrated program for management of solid, liquid and air-borne wastes. The plant has available a number of waste disposal systems and, depending upon the magnitude and concentration of each stream as well as the overall plant product mix, the most efficient treatment system is selected for each stream. Where more than one treatment is possible for a particular waste stream, each alternative treatment plan is described below. Each treatment alternative is capable of insuring that the plant remains in compliance with all emissions requirements.

Solid Wastes

Broth solids will be disposed of by incineration/pyrolysis:

Incineration/pyrolysis facilities will operate under Resource Conservation and Recovery Act permits and employ technology sufficient to destroy more than 99.99% of the waste.

These broth solids will be handled in compliance with Federal requirements of U.S. Environmental Protection Agency Regulations 40 CFR Parts 260-267 and with Connecticut Department of Environmental Protection Section 25-54cc(c).

Activated carbon solids will be either incinerated/pyrolyzed as above or sent to a contracted disposal firm in compliance with Federal requirements of 40 CFR Parts 260-267 and Connecticut Department of Environmental Protection Section 25-54cc(c).

Liquid Wastes

Broth Filtrate and Aqueous Crystallization Mother Liquors: These primary aqueous streams will be disposed of by one of the following methods:

1) The stream will be treated as necessary in a chemical pre-treatment operation (acid treatment) for degradation of semduramicin sodium. The treated filtrate stream will be sent to the Pfizer site effluent treatment facility and discharges will have semduramicin sodium concentration below 0.1 ppm. Effluent is disposed of from this facility into the Thames River under all limitations in plant site NPDES permit CT 00000957 issued in accordance with U.S. Environmental Protection Agency Regulation 40 CFR Parts 124 and 125 and administered by the Connecticut Department of Environmental Protection. This permit is being extended administratively by the Connecticut DEP until it issues the renewal permit, for which a timely application has been submitted by Pfizer Inc. (Appendix a-2).

   The limitations include a pH between 5.0 and 9.0, a BOD not to exceed 21,500 kg/day, absence of a visible oil sheen, foam or floating solids, no discoloration of the receiving waters and a rise in the temperature of the receiving stream of no more than 4^{0}F and to no higher than 83^{0}F.

2) The stream will be incinerated/pyrolyzed as described above for the broth solids.

Other aqueous waste streams, such as tank and floor washings, will be chemically pre-treated (acid treatment) as needed for degradation of semduramicin sodium, and, where necessary, stripped of residual organic solvent in appropriate evaporative distillation equipment. The treated aqueous streams will be sent to the Pfizer site effluent treatment facility (as above) prior to disposal under all limitations specified in plant site NPDES permit CT 00000957.

Solvent streams will be recovered/recycled as much as practicable via distillation systems. Disposal of unusable solvent streams will be via waste heat recovery or pyrolysis in compliance with the following regulations administered by the Connecticut Department of Environmental Protection: Connecticut General Statutes Air Regulations Title 22a, Environmental Protection, Chapter 439 and U.S. Environmental Protection Agency Regulations 40 CFR Parts 264 and 265.

These regulations require that Resource Conservation and Recovery Act permits be obtained and that the technology employed ensures destruction of more than 99.99% of the applicable waste.

Air Emissions

Evaporation of organic solvents will be controlled as appropriate with condensers or scrubbers. Air emissions from process vessels will be controlled by vent condensers and/or conservation vents. All air emissions will be in compliance with Connecticut General Statutes Air Regulations Title 22a, Environmental Protection, Chapter 439.

Occupational exposure to air contaminants during the bulk manufacturing process will be limited, since most of the operations will be contained within a closed system. Emission of particulate matter during bulk drying and milling will be controlled by local exhaust ventilation and filter dust collectors.

Monitoring of the work area to ascertain occupational exposure will be regularly carried out and all exposure limits (see table under 6A3, above) will be in compliance according to OSHA Air Contaminants 29 CFR 1910.1000

The attached statement (Appendix a-3) certifies compliance with all Federal, State and local emissions requirements.

5. Taketoyo, Japan Site

Solid Wastes

Broth solids will be disposed of by incineration of solids:

Incineration facilities will operate under the permits of the Agreement with Taketoyo Town and employ technology sufficient to destroy more than 99.99% of the waste.

These broth solids will be handled in compliance with National requirements of Environmental Protection Agency Regulations, Article 12 of the Industrial Waste Disposal Control Law and with the Taketoyo Town Environmental Protection Regulations, Articles 20-30.

Activated carbon solids will be sent to a contracted disposal firm in compliance with Prefectural requirements of Environmental Protection Regulations, Article 19.

Liquid Wastes

Broth Filtrate: The aqueous broth filtrate stream, which contains a small percentage of total broth semduramicin sodium content, will be treated as necessary in a chemical pre-treatment operation (acid treatment) for degradation of semduramicin sodium. The treated filtrate stream will be sent to the Pfizer site effluent treatment facility and discharges will have semduramicin sodium concentration below 0.1 ppm. Effluent is disposed of from this facility into Kinuura Bay under all limitations imposed by the Environmental Protection Agreement with Taketoyo Town, Articles 16-20 and by the National Water Pollution Prevention Law, Article 3.

Other aqueous waste streams, such as tank and floor washings, will be chemically pre-treated (acid treatment) as needed for degradation of semduramicin sodium, and, where necessary, stripped of residual organic solvent in appropriate evaporative distillation equipment. The treated aqueous streams will be sent to the Pfizer site effluent treatment facility (as above) prior to disposal under all limitations imposed by the Agreement with Taketoyo Town and the National Water Pollution Prevention Law (described above).

Disposal of other solvent streams will be by an outside contracted firm certified by the Prefectural Government in compliance with the Environmental Protection Regulations, article 19.

These regulations require that the technology employed ensures destruction of more than 99.99% of the waste.

Air Emissions

Evaporation of organic solvents will be controlled as appropriate with condensers. Air emissions generated during evaporation will be controlled by vent condensers and/or conservation vents. All air emissions will be in compliance with Air Pollution Prevention Law, Article 3, Prefectural Environmental Protection Regulations, Article 19 and the Agreement with Taketoyo Town, Article 16.

Occupational exposure to air contaminants during the bulk manufacturing process will be limited, since most of the operations will be contained within a closed system. Emission of particulate matter during bulk drying and milling will be controlled by local exhaust ventilation and filter dust collectors.

Monitoring of the work area to ascertain occupational exposure will be regularly carried out and the exposure limits (see table under 6A2, above) will be in compliance with Industrial Safety and Health Law, Article 28.

The attached statement (appendix a-3) certifies compliance with all National, Prefectural and Local emissions requirements.

B. From the site where premix is produced:

Semduramicin sodium will be incorporated into feed premixes and packaged for sale at Pfizer Inc.'s general purpose plant for the manufacture of animal health products located at 1107 SE Missouri 291, in Lee's Summit, Missouri. The plant is designed to have minimal environmental impact and be in compliance with all Federal, State, and local emission requirements.

The premix manufacturing operation will involve only the blending of semduramicin sodium bulk with inert ingredients in equipment constructed of non-reactive product contact parts.

Particulate emissions from the manufacturing process will be controlled via cyclones and baghouse filters. While the particulate emissions will result principally from the transfer and processing of soybean mill run, there will be emission controls on all manufacturing areas. Particulate emissions will be controlled in compliance with the Missouri Air Pollution Control Regulations 10CBR10.2.

Particulate concentrations in the workplace will be monitored and maintained below the Permissible Exposure Limits (PEL's) according to the OSHA standard in 29CFR1910.1000.

Wastewater discharges from the manufacturing process will consist of wash water from equipment clean-out operations. Since the manufacturing processes will be dry operations, equipment clean-outs will typically be done by scouring with dry feed. Components of the equipment will be cleaned with water infrequently. This wastewater discharge may contain semduramicin sodium, sodium carbonate, mineral oil, sodium aluminosilicate and soybean dust. The quantity of product present in the wastewater discharge will be minimal because of the nature of the process and ordinary cleaning procedures. Wastewater from the site is discharged to the Little Blue Valley Sewer District publicly owned wastewater treatment plant. This discharge is authorized by an operating permit issued to the Pfizer Lee's Summit plant by the Missouri Water Pollution Control Board. All discharges will be in compliance with the standards set by this Board.

Solid wastes will consist of the fiber drums with empty inner plastic liners which may contain trace quantities of semduramicin sodium, other paper packaging from raw materials, floor sweepings, dust collector rejects and some dry mill material from equipment cleaning. Production of the premix will not generate hazardous wastes as defined by the Federal Regulations 40CFR261.4, nor will it generate hazardous waste as defined by the Missouri Hazardous Waste Management Law Title 16 Chapter 260. The solid wastes will be disposed by landfill under the Missouri Solid Waste Rules 10CSR80, or by incineration.

The Lee's Summit site makes available to its employees the appropriate detailed Material Safety Data Sheets (MSDS) essentially similar to OSHA form 20. The MSDS sheets for semduramicin sodium and the premix will each have the same content as the appended copies (Appendix a-1), though they will be in a slightly different format.

The attached statement (Appendix a-4) certifies compliance with all Federal, State, and local emissions requirements.

C. Introduction of substances as a result of use:

Use of semduramicin sodium as an anticoccidial agent for broilers would result in introduction of semduramicin sodium into the environment through excretion by broilers which have consumed medicated feed and subsequent application of the broiler manure to soil as fertilizer.

1. Concentration of Semduramicin in Broiler Excreta

The concentration of semduramicin sodium in unaged manure from broilers which consumed feed containing 25 ppm of semduramicin has been determined to average 1.6 ppm (Appendix c-1). Most of the ingested drug (about 93%) is broken down by broilers into many polar metabolites. All but one of the metabolites were inactive in a test for biological activity that is highly sensitive to ionophores (Appendix c-1). The sole active metabolite was present in very low concentration, only about 1.3 times that of unchanged semduramicin sodium. The concentration of this metabolite in fresh excreta is therefore about 2.1 ppm.

$$1.3 \times 1.6 \text{ ppm} = 2.1 \text{ ppm}$$

It is related in structure to semduramicin, and has molecular weight of the same order of magnitude. Although it is the only metabolite producing a biological response, the present calculation assumes it to be as active as semduramicin sodium, and its concentration has been added to that of semduramicin sodium to give a combined concentration equivalent to 3.7 ppm of semduramicin sodium in broiler excreta.

1.6 ppm (unchanged drug) + 2.1 ppm (metabolite) = 3.7 ppm in excreta

2. Potential Concentration of Semduramicin in Soil

Poultry manure is commonly used as a fertilizer. Use of manure containing semduramicin sodium as fertilizer would result in introduction of the drug into the soil. The resulting initial concentration of drug in soil can be estimated from the concentration of drug in manure and the rate of application of manure to soil.

The recommended rate of application for poultry manure ranges up to 7.5 short tons (6.8 metric tons) per acre, with incorporation into the top six inches in soil (Reference 3) The top six inches of soil in one acre weigh about 909,000 kg. Use of broiler manure containing 3.7 ppm semduramicin sodium (Section 6.C.1) would result in initial concentration in soil of up to only about 0.03 ppm.

$$3.7 \text{ mg/kg} \times 6{,}800 \text{ kg/acre} = 25{,}160 \text{ mg/acre}$$
$$25{,}160 \text{ mg/acre} \div 9.09 \times 10^{5} \text{ kg/acre} = 0.0276 \text{ mg/kg or about } 0.03 \text{ ppm}$$

The calculations above assume no adjustment for dilution of manure with bedding materials (litter) and assume that semduramicin will remain stable in the manure until the latter is applied to soil. In reality, the concentration of semduramicin in manure is diluted by bedding materials and is likely to degrade gradually in this litter and manure mixture. The projected concentrations therefore probably represent exaggerated estimates.

3. Amount of Semduramicin Introduced into the Environment

It is possible to estimate the amount of semduramicin sodium that will be introduced into the environment by a commercial broiler raising facility which uses semduramicin sodium as its anticoccidial agent. A large commercial broiler operation can consist of four to five broiler houses containing 80,000-100,000 birds in total. There are usually 6 production cycles per year. Broiler chickens are raised to an average weight of about 4.2 pounds (Reference 1). It takes a modern broiler about 42 days to reach this weight (Reference 2). During this time, it excretes about 0.054 kg of waste per day (Reference 3), or a total of 2.268 kg.

$$0.054 \text{ kg/day} \times 42 \text{ days} = 2.268 \text{ kg}$$

A facility containing 100,000 broilers produces 226,800 kg of manure during each growout cycle:

$$2.268 \text{ kg/broiler} \times 100{,}000 \text{ broilers} = 226{,}800 \text{ kg}$$

If the facility has 6 growout cycles per year, it produces about 1,400 metric tons of manure per year:

$$226{,}800 \text{ kg/cycle} \times 6 \text{ cycles} = 1{,}360{,}800 \text{ kg}$$

This amount of manure would contain about 5.0 kg of semduramicin sodium:

$$1{,}360{,}800 \text{ kg} \times 3.7 \text{ mg/kg} = 5{,}034{,}960 \text{ mg} = 5.035 \text{ kg}$$

When applied at the rate of 7.5 short tons (6.8 metric tons) per acre, 1,400 tons of manure and 5.0 kg of semduramicin sodium would be distributed over about 200 acres:

$$1{,}360{,}800 \text{ kg manure} \div 6{,}800 \text{ kg/acre} = 200 \text{ acres}$$

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

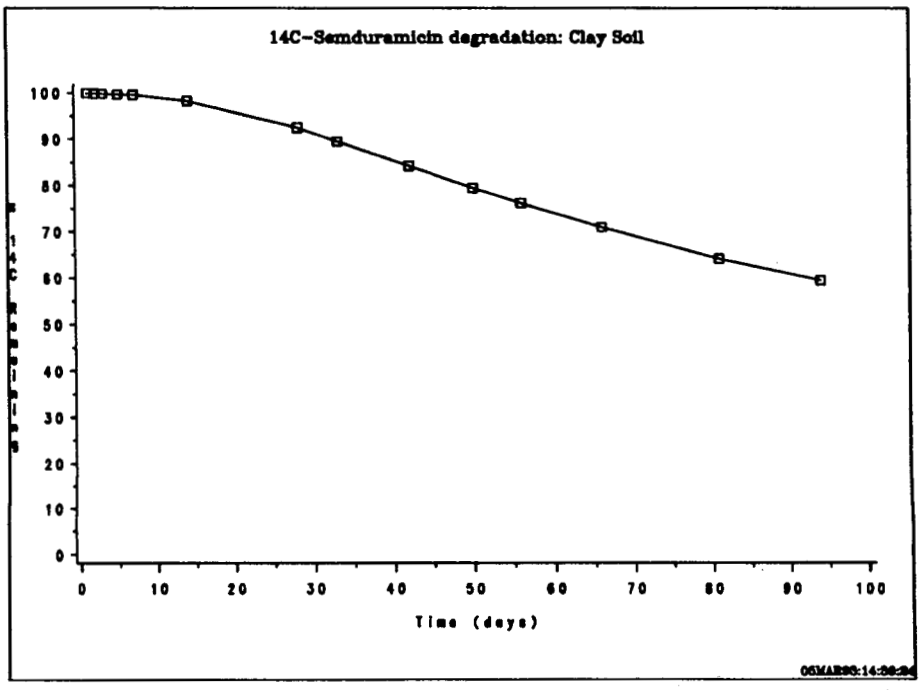
A. Air

Semduramicin sodium is a high molecular weight (895), high melting (m.p. 170°C), ionic solid and would be expected to be non-volatile. In agreement with this expectation, data where obtained which confirm that the vapor pressure must be less than 10^{-8} torr at 20°C (Appendix c-2). Therefore, semduramicin sodium would not be expected to partition into the atmosphere under the conditions of production or use.

B. Terrestrial Ecosystems

As discussed above under 6.C.2, low concentrations (about 0.03 ppm) of semduramicin sodium would be introduced into the soil as a result of use of the product. This initial concentration of semduramicin would decline by aerobic biodegradation as demonstrated in a laboratory test (Appendix c-7). Semduramicin was observed to degrade to CO_2 in 3 soils of varying characteristics that were acquired from different geographical regions within the US. Fifty percent biodegradation was observed in approximately 94 and 42 days, respectively, for Ohio and Iowa soils, and 40% degradation for North Dakota soil in approximately 94 days. The estimated (vs. experimentally measured) time to 50% biodegradation for Ohio, Iowa, and North Dakota soils was 79, 42, and 104 days, respectively.

Although the kinetics of semduramicin in soils cannot be predicted from the studies conducted and are likely to be complex (Reference 4), first order kinetics will be used to illustrate how semduramicin might be eliminated from soils. First order kinetics have been found applicable for describing degradation of a variety of chemicals present at very low (e.g. ppm) concentrations (Reference 4) and describe a significant portion of the multiphase kinetics of semduramicin degradation observed in the soil biodegradation study (e.g. clay soil).



The concentration of drug in soil at any defined time after its application to soil with manure can be determined by the following equation, assuming that the initial drug concentration in soil and depletion half life are known:

$$C_{\text{at time } t} = C_o e^{-kt}$$

If C_0 equals the concentration of semduramicin in soils immediately following the application of manure containing the drug (0.03 ppm, Section 6C3), k equals the depletion rate constant and e is the natural logarithm, then C equals the concentration of semduramicin in the soil at time t, i.e. one year later when the soil is refertilized. Employing the estimated 50% biodegradation rates for 3 soils of 42, 79 and 104 days, yearly fertilization of soils with poultry manure containing semduramicin would not lead to accumulation of increasing concentrations of drug in soils.

Depletion rates (k) are established from the estimated 50% biodegradation values listed above:

Soil type	t1/2 (Days to 50% Biodegradation)	k ($Days^{-1}$)
Silty clay loam (Iowa)	42	0.017
Clay loam (Ohio)	79	0.0088
Clay (North Dakota)	104	0.0067

The concentration (C) of semduramicin at 365 days (t):

$$\log C_0 - k \cdot t/2.3$$

Soil Type	C (ppm) at 365 days
Silty clay loam (Iowa)	6.1×10^{-5}
Clay loam (Ohio)	1.2×10^{-3}
Clay (North Dakota)	2.6×10^{-3}

As shown, one year after depositing 0.03 ppm semduramicin in soil with manure, only 61 ppt to 1.2 ppb would remain at the time the soil was refertilized. There would be no significant increase in concentration of semduramicin above the 0.03 ppm level over time; therefore, the projected maximum environmental concentration of semduramicin in soils would remain 0.03 ppm.

C. Aquatic Ecosystems

Although semduramicin sodium is appreciably soluble in water (solubility 1,900 ppm), with an acid dissociation constant of 5.39 (Appendix c-2), soil sorption/desorption experiments indicate that it would largely remain bound to soil rather than partition into aquatic systems (Appendix c-4).

A maximum potential concentration of semduramicin in surface water can be estimated using a 40 acre (16.2 hectares) watershed with a 2.5 acre pond (average depth, 2.5 ft) as the surface water receiving runoff. As has been demonstrated, the highest potential concentration of semduramicin in soil resulting from use is 0.03 ppm. The 16.2 hectare watershed would contain, at most, 1.091 kg of semduramicin sodium (0.03 mg/kg x 9.09 x 10^5 kg/acre x 2.47 acres/hectare X 16.2 hectares = 1.091 kg). The pond would contain 7.71 x 10^6 liters of water (6.25 acre ft x 43,560 ft^3/acre ft x 28.32 liters/ft^3). Assuming a worst case scenerio that all the drug runs off the water shed, the maximum potential concentration of semduramicin sodium in the pond would be only 0.14 mg/L or 0.14 ppm (1.091 X 10^6 mg ÷ 7.71 X 10^6 L).

Actually, the amount in run off would decline as the runoff traversed uncontaminated soil and semduramicin sodium was absorbed or deposited onto its surface. The resulting concentration in the pond would not persist, as semduramicin sodium decomposes in aqueous solution, particularly in acidic waters (Appendix c-5), and decomposition is accelerated by exposure to sunlight (Appendix c-6).

The logarithm of the octanol-water partition coefficient (log K_{ow}) of semduramicin sodium is 2.21 to 2.58, depending on pH (Appendix c-3). By comparison, compounds that are known to bioaccumulate, such as DDT (log K_{ow} 6.19), have log K_{ow} values of 4 or greater (Reference 5). Thus the value for semduramicin sodium suggests that significant accumulation is not predicted. Testing has demonstrated that semduramicin sodium does not persist and accumulate in living beings, but is readily converted to more polar metabolites and excreted. Rapid and extensive metabolic degradation has been demonstrated in chickens, dogs, and rats (Appendix c-1).

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

A. Terrestrial Species

As discussed above under 6C, the projected maximum concentration of semduramicin in soil is 0.03 ppm. This maximum concentration could only occur when fresh poultry manure had just been mixed into the soil, i.e. no degradation had taken place in manure or soil. The greatest possible potential for adverse effects is assessed below by comparing the projected maximum concentration in soil to the results of exposure studies with terrestrial organisms.

Semduramicin sodium was tested in the laboratory for its ability to inhibit the growth of six different species of soil microorganisms (Appendix c-8). The minimum inhibitory concentration (MIC) in each case was found to be above 100 ppm, which is more than 3,300 times the projected maximum concentration in soil.

When graded concentrations of semduramicin sodium were tested on six species of crop seeds in the laboratory (Appendix c-9), the highest concentration which did not affect seed germination or root elongation and the ratio of this concentration to the projected maximum concentration in soil were found to be as follows: corn, 17 ppm, 567 times; cucumber, 34 ppm, 1,133 times; pinto beans, 6.3 ppm, 210 times; rye, 13 ppm, 433 times; wheat, 6.3 ppm, 210 times. The lowest concentration of semduramicin that affected soybean germination was 1 ppm in the preliminary test. No effects were observed at concentrations ranging between 0.36 and 2.2 ppm in the definitive test. A no observable effect concentration (NOEC) was not established for soybeans.

The lowest concentrations that had any detectable adverse effect on the growth of seedlings of the same six crop species during a 21-day observation period (Appendix c-10) and the ratios of these concentrations to the projected maximum concentration in soil were as follows: corn, 4.2 ppm, 140 times; cucumber 2.2 ppm, 73 times; pinto beans, 2.2 ppm, 73 times; soybean, 0.77 ppm, 25 times; NOECs were established for all parameters in ryegrass and wheat except for root weight (NOECs $\leq$ 0.31 and 0.77 ppm respectively). These values are 10-26 times the projected maximum concentration in soil.

The above results indicate that use of semduramicin sodium as directed would not be expected to have any significant adverse effects on terrestrial organisms in the environment.

B. Aquatic Species

The potential exposure of aquatic organisms to semduramicin sodium is expected to be intermittent, since it depends on rain runoff from soil fertilized with broiler excreta containing semduramicin sodium; and short-lived, since the concentration of semduramicin in water would decline due to hydrolysis and photodecomposition. The greatest possible potential for adverse effects is assessed below by comparing the projected maximum concentration in surface waters of 0.14 ppm (Section 7C) to the results of acute exposure studies with aquatic organisms.

In rainbow trout (Appendix c-14), the concentration of semduramicin sodium which causes 50% mortality after 96 hours of exposure (the 96-hour LC_{50}) was found to be 32 ppm, which is approximately 230 times the projected maximum concentration. The highest concentration which did not produce any observable adverse effects was 11 ppm, or about 80 times the projected maximum concentration.

In bluegill (Appendix c-13), the corresponding numbers are 38 ppm for the 96-hour LC_{50}, which is 270 times the projected maximum concentration, and 13 ppm for the highest concentration which did not produce any observable adverse effects, or 93 times the projected maximum concentration.

In the water flea, Daphnia magna (Appendix c-12), the concentration which caused immobilization of 50% of daphnids exposed for 48 hours (the 48-hour EC_{50}) was found to be 38 ppm, approximately 270 times the projected maximum concentration. The highest concentration which did not produce any observable adverse effects was 19 ppm, 136 times the projected maximum.

In a freshwater algae (Appendix c-11), the lowest concentration which produced a significant reduction in growth rate and maximum cell density was 19 ppm, 136 times the projected maximum concentration, and the highest concentration which had no significant effect on growth rate or culture density was 10 ppm, or 71 times the projected maximum concentration.

Based on these data, the contemplated use of semduramicin sodium is highly unlikely to result in any significant adverse effects on aquatic organisms in the environment.

9. USE OF RESOURCES AND ENERGY

Manufacturing semduramicin sodium bulk and premix will require amounts of resources and energy similar to those used to produce and formulate any fermentation-derived additive for animal feeds, such as other ionophores. Disposal of wastes generated from production will not require use of unusual amounts of energy or natural resources.

No effects are anticipated upon endangered or threatened species nor upon properties listed in or eligible for listing in the National Register of Historic Places.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. The label for semduramicin sodium premix will instruct users to wear protective clothing and a dust mask when mixing medicated feed and to wash hands thoroughly afterwards. Other than these precautions listed on the label, no mitigation measures are necessary for semduramicin sodium.

11. ALTERNATIVES TO THE PROPOSED ACTION

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

12. LIST OF PREPARERS

The following are all members of the staff of Pfizer Central Research, Pfizer Inc., Groton, Connecticut:

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M.A. in Organic Chemistry
8 years experience in laboratory studies; 8 years experience in present position.

Larry R. Chappel, Ph.D.

Manager of Environmental Safety
Animal Health Product Development
20 years experience in R&D on animal health drugs.

Roderick B. Dougherty, D.V.M.

Manager of Regulatory Affairs, Animal Health Product Development
6 years veterinary practice experience; 11 years experience animal health R&D.

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Assistant Director, Fermentation Process R&D
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Richard C. Koch, Ph.D.

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Ph.D. in Organic Chemistry
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Manager, Department of Drug Metabolism
M.A. in Analytical Chemistry
M.B.A. in Management
20 years of experience in studies of the fate of drugs in mammals.

Jon L. Schaeffer, D.V.M, Ph.D.

Senior Regulatory Affairs Scientist
Animal Health Product Development
3 years veterinary practice and 3 years experience in R&D on animal health drugs.

Jeffrey A. Richards, Ph.D.

Manager, Analytical R&D
Ph.D. in Analytical Chemistry
14 years of experience in analytical chemistry.

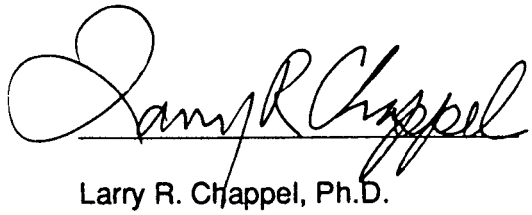
The following individual is a member of the production staff of Pfizer's U.S. Animal Health Operations at Lee's Summit, MO.:

Richard H. Bartel, B.S., P.E.

Environmental Affairs Supervisor, Lee's Summit plant. B.S. in Chemical Engineering; 30 years of experience in chemical manufacturing and environmental engineering.

13. CERTIFICATION

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



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Larry R. Chappel, Ph.D.
Manager, Environmental Safety
Animal Health Product Development
Pfizer Central Research
Pfizer Inc.

Date

14. REFERENCES

1. Broiler Industry. May 1987. USDA figures in for 1986 broiler sales.

2. Arbor Acres. 1988. Broiler Feeding and Management Guide. Arbor Acres, Glastonbury, CT, pp. 11-12.

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4. Alexander, M. and K. M. Scow. 1989. Kinetics of biodegradation in soil pp. 243-269 in Reactions and Movement of Organic Chemicals in Soils. Sawhney, B. L. and K. Brown, Eds. Soil Science of America Inc. Special Publication Number 22, American Society of Agronomy.

5. Food and Drug Administration. 1987. Environmental Assessment Technical Assistance Handbook. Technical Assistance Document 3.02. Food and Drug Administration, Washington, D.C.

Appendix a-1

Material Safety Data Sheets

Central Research Division
Eastern Point Road
Groton, Connecticut 06340
Emergency Telephone: (203)441-4100

June, 1992 **MSDS # 0109**
(Superceedes April 1989)

SEMDURAMICIN SODIUM

[UK-61,689-2; Ionophore]

SECTION I: *PHYSICAL DATA*

Appearance:	White solid
Melting Point:	169°-170°C
Molecular Formula:	$C_{45}H_{75}O_{16}Na$
Molecular Weight:	895
Chemical Family:	Polyether Carboxylic Acid (ionophore).
Solubility:	Soluble in common organic solvents; sparingly soluble in water and hexane.
Description:	Semduramicin sodium is an animal health drug for use as an anticoccidial agent in poultry. The material is orally active and all forms of ingestion or inhalation must be avoided. Semduramicin sodium is pure, undiluted material.

SECTION II: *FIRE AND EXPLOSION HAZARD*

The dust explosibility rating has not been determined. As with any organic solid, precautions to minimize dust generation are required. Process equipment for the handling of dry Semduramicin sodium should be provided with proper explosion relief devices.

Semduramicin sodium does not present any unusual or significant fire hazards. If the material becomes involved in a fire, the latter may be suppressed with appropriate extinguishing medium, including water.

SECTION III: *HEALTH HAZARD INFORMATION*

Semduramicin sodium is an orally active animal health drug In toxicology studies, the no observed effect level was 0.3 mg/kg in dogs (1 year) and 1.0 mg/kg in rats (3 months). Semduramicin sodium had no effect upon reproduction or fertility of rats in a 3-generation toxicity study. There was no evidence of teratogenicity in appropriate animal studies, and no evidence of mutagenic potential in a standard battery of tests for genetic toxicity. Carcinogenicity testing is underway, but related compounds which have been tested are not carcinogenic.

Semduramicin sodium has been tested for skin and eye irritation and it is not an irritant to intact or abraded rabbit skin, and is not an ocular irritant to unrinsed rabbit eyes.

Semduramicin sodium, like similar ionophore antibiotics, causes muscle damage in dogs (2 mg/kg), detectable by serum creatinine phosphokinase (CPK) elevations and eventually by affected stance and gait. A dose of 0.5 mg/kg in dogs produces retinal lesions after 6 months. No significant cardiovascular changes in dogs were observed after oral administration up to 3 mg/kg.

SECTION IV: *FIRST AID INFORMATION*

Ingestion: In the event of ingestion of Semduramicin sodium (solid or liquid solutions), medical attention should be summoned immediately.

Inhalation: Personnel who have inhaled Semduramicin sodium should be removed to fresh air and observed by medical personnel.

Skin Contact: Skin contacted with solids or solutions of Semduramicin sodium should be washed thoroughly with water. Contaminated clothing should be removed. If any effects are observed, medical attention should be sought. In case of eye contact, immediately wash with water for 15 minutes and seek medical attention.

SECTION V: *REACTIVITY DATA*

Semduramicin sodium is stable in bulk form for 12 weeks at temperatures up to 50°C. It is unstable in acidic and strongly alkaline conditions.

SECTION VI: *SPILL OR LEAK PROCEDURE*

Spills of Semduramicin sodium should be collected (scooped) into an appropriate recovery container. Personnel involved in clean-up of spills, particularly solids, must wear respiratory protection, gloves and eye protection.

SECTION VII: *PRECAUTION INFORMATION*

When handling Semduramicin sodium avoid contact with skin, eyes or mucous membranes. Avoid inhaling dust. Wear a dust mask, safety glasses and gloves when handling the undiluted material. Wear gloves and eye protection when handling the material in a fume hood. All animal health drugs and/or medicated feeds may produce undesirable physiological effects to the worker, if handled improperly. It is recommended that good manufacturing practices be observed at all times and that further precautions be followed to minimize exposure to the active drug. These include minimizing the generation of dusts, avoiding contact with the skin, eyes, and mucous membranes, and providing adequate ventilation. Personal protective equipment including gloves and safety glasses should be worn when handling Semduramicin sodium and additional protective equipment, such as a dust mask, or use of a fume hood, may be appropriate for certain handling activities.

issued by: D. P. Brannegan

Central Research Division
Eastern Point Road
Groton, Connecticut 06340
Emergency Telephone: (203)441-4100

June, 1992 **MSDS # 0135**
(Superceedes April 1989)

SEMDURAMICIN SODIUM 5% PREMIX

[UK-61,689-2; Ionophore]

SECTION I: *PHYSICAL DATA*

Appearance:	Finely ground meal
Composition:	Semduramicin Sodium 5% Premix contains semduramicin sodium, sodium carbonate and mineral oil in a soybean mill run base.
Description:	The premix is intended for use as an anticoccidial agent in poultry after suitable dilution into poultry feed.
Chemical Family:	Semduramicin sodium is a polyether carboxylic acid ionophore.
Typical Use Level:	400 g Semduramicin Sodium 5% Premix per 1,000 kg of feed, providing a final semduramicin level of 20 ppm.

SECTION II: *FIRE AND EXPLOSION HAZARD*

The dust explosivity rating has not been measured. As with any organic solid, precautions to minimize dust generation are required. Mineral oil has been included as a premix component to minimize dusting.

Semduramicin Sodium 5% Premix does not present any unusual or significant fire hazards. If the material becomes involved in a fire, it may be suppressed with appropriate extinguisher media, including water.

SECTION III: *HEALTH HAZARD INFORMATION*

Semduramicin sodium is an orally active animal health drug. In toxicology studies, the no observed effect level was 0.3 mg/kg in dogs (1 year) and 1.0 mg/kg in rats (3 months). Semduramicin sodium had no effect upon reproduction or fertility of rats in a 3-generation toxicity study. There was no evidence of teratogenicity in appropriate animal studies, and no evidence of mutagenic potential in a standard battery of tests for genetic toxicity. Carcinogenicity testing is underway, but related compounds which have been tested are not carcinogenic.

Semduramicin sodium has been tested for skin and eye irritation and it is not an irritant to intact or abraded rabbit skin, and is not an ocular irritant to unrinsed rabbit eyes.

Semduramicin sodium, like similar ionophore antibiotics, causes muscle damage in dogs (2 mg/kg), detectable by serum creatinine phosphokinase (CPK) elevations and eventually by affected stance and gait. A dose of 0.5 mg/kg in dogs produces retinal lesions after 6 months. No significant cardiovascular changes in dogs were observed after oral administration up to 3 mg/kg.

SECTION IV: *FIRST AID INFORMATION*

Ingestion: In the event of ingestion of Semduramicin Sodium 5% Premix, medical attention should be summoned immediately.

Inhalation: Personnel who have inhaled Semduramicin Sodium 5% Premix should be removed to fresh air and observed by medical personnel.

Skin Contact: Skin contacted with solids or solutions of semduramicin sodium should be washed thoroughly with water. Contaminated clothing should be removed. If any effects are observed, medical attention should be sought. In case of eye contact, immediately wash with water for 15 minutes and seek medical attention.

SECTION V: *REACTIVITY DATA*

Semduramicin Sodium 5% Premix is stable in bulk form for 12 weeks at temperatures up to 50°C and at least 2 years at room temperature. Semduramicin sodium is unstable in acidic and strongly alkaline conditions.

SECTION VI: *SPILL OR LEAK PROCEDURE*

Spills of Semduramicin Sodium 5% Premix should be collected (scooped) into an appropriate recovery container. Personnel involved in clean-up of spills, particularly solids, must wear respiratory protection, gloves and eye protection.

SECTION VII: *PRECAUTION INFORMATION*

When handling Semduramicin Sodium 5% Premix avoid contact with skin, eyes or mucous membranes. Avoid inhaling dust. Wear a dust mask, safety glasses and gloves when handling the undiluted material. Wear gloves and eye protection when handling the material in a fume hood. All animal health drugs and/or medicated feeds may produce undesirable physiological effects to the worker, if handled improperly. It is recommended that good manufacturing practices be observed at all times and that further precautions be followed to minimize exposure to the active drug. These include minimizing the generation of dusts, avoiding contact with the skin, eyes, and mucous membranes, and providing adequate ventilation. Personal protective equipment including gloves and safety glasses should be worn when handling Semduramicin Sodium 5% Premix and additional protective equipment, such as a dust mask, or use of a fume hood, may be appropriate for certain handling activities.

issued by: D. P. Brannegan

Appendix a-2

NPDES Permit Explanation

NPDES PERMIT EXTENSION

The paragraph that follows explains the administrative extension by the Connecticut DEP of the NPDES permit that was to expire in 1985 and provides the name of the person at the DEP who is familiar with the situation. A copy of the NPDES permit is found on pages 8066-8079 (NADA 140-940).

The plant's NPDES permit was issued by the Connecticut Department of Environmental Protection (CT DEP), the delegated agent of the United States Environmental Protection Agency, on May 20, 1980 with a life of five years or until May 19, 1985. Pfizer submitted a timely permit renewal application at least 180 days prior to the permit expiration date. Under the conditions set forth in 40 CFR 124 and 125 the NPDES permits in place at the time of expiration continue to be in effect during the permit renewal process, therefore, the permit issued in 1980 remains in effect in the Groton plant. This situation is not unusual. There is a significant backlog of pending permits within Connecticut and throughout the U.S. Over the years there have been a number of meetings with the CT DEP and the USEPA regarding this permit. The plant environment manager contacts the CT DEP on a periodic basis to remind the agency of the pending permit application. According to the agency, the pending permit is under active review, and will be issued in the near future.

Joseph Holmes of the Water Management Bureau, Department of Environmental Protection, Hartford, Connecticut is familiar with the situation.

Appendix a-3

Certification of Compliance - Bulk Manufacturing Sites

February 23, 1993

TO WHOM IT MAY CONCERN:

This is to certify that, to the best of our knowledge, Pfizer Inc's manufacturing plant at Groton, Connecticut is in compliance with all applicable federal, state, and local emissions requirements and is expected to remain in compliance when semduramicin sodium bulk is produced at the site.

Robert T. Pfisterer

Robert T. Pfisterer
Plant Environmental Director
Food Science Group

DATE: April 9, 1993

TO WHOM IT MAY CONCERN:

This is to certify that to the best of our knowledge, Pfizer's manufacturing plant at Taketoyo, Aichi Prefecture, Japan is in compliance with all applicable national and local emissions requirments and is expected to remain in compliance when semduramicin sodium is produced at the site.

H. Kikuchi

H. Kikuchi
Vice President, Manufacturing
Pfizer Pharmaceuticals Inc.
Nagoya Plant

Appendix a-4

Certification of Compliance - Premix Manufacturing Site

April 5, 1993

TO WHOM IT MAY CONCERN:

This is to certify that, to the best of our knowledge, Pfizer Inc's manufacturing plant at Lee's Summit, Missouri is in compliance with all applicable federal, state and local emissions' requirements and is expected to remain in compliance when the Semduramicin Type A Medicated Article is produced.

Richard H. Bartel
Manager, Environmental Compliance
North American Animal Health Division

Appendix b

Data Summary Charts

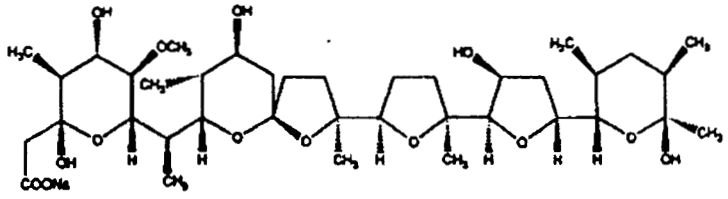
APPENDIX b

DATA SUMMARY CHARTS

PHYSICAL-CHEMICAL AND ENVIRONMENTAL FATE DATA

Generic Name: Semduramicin sodium

Structural Formula:



Molecular Formula: C_{45} H_{75} O_{16} Na

Molecular Weight: 895

Solubility in water:	pH	Solubility (mg/ml)
	6	1.7
	7	1.9
	9	1.9

N-Octanol-Water Partition Coefficient:

pH	Log K_{ow}
6	2.58
7	2.37
9	2.21

Vapor Pressure: Non-volatile

Dissociation Constant: pKa = 5.39 in 1:1 acetone:water

Ultraviolet-Visible Absorption Spectrum: No detectable absorption at 290 to 800 nm and pH 5, 7 or 9.

Melting Temperature: 170°C.

Absorption to Soil:

Soil Type	K_d	K_{oc}
Mississippi Silty Clay Loam	25.7	1800
Arkansas Silty Loam	10.6	1400
Iowa Sandy Loam	4.65	150

Hydrolysis:

pH:	5	6	7	8	9
Half-life (days):	11.1	36.1	89.9	115	77.1

Photodegradation:

pH:	6	7	9
Half-life (days):	7.1	8.76	11.32

Biodegradation in Soil:

Soil Type	Estimated Half-life (Days) At 25 ppm
Iowa Silty Clay Loam	42
Ohio Clay Loam	79
North Dakota Clay	104

ACUTE AND SUBACUTE TOXICITY STUDIES

TERRESTRIAL ORGANISMS

ORGANISM	ENDPOINT
Soil Microbes -	Minimum Inhibitory Concentration (MIC)
Clostridium novyi	145
Bacillus stearothermophilus	170
Flavobacterium meningosepticum	170
Nostoc	125
Trichoderma viride	145
Penicillum italicum	240
Crop Seeds -	NOEC for Seed Germination and Root Elongation (ppm)
Corn	17
Cucumber	34
Pinto Bean	6.3
Soybean	Not determined
Rye	13
Wheat	6.3
Crop Seedlings (ppm)	LOEC For Survival, Root Weight, Shoot Weight, and Shoot Length
Corn	4.2
Cucumber	2.2
Pinto Bean	2.2
Soybean	0.77
Ryegrass	0.31
Wheat	0.77

ACUTE AND SUBACUTE STUDIES

AQUATIC ORGANISMS

ORGANISM	ENDPOINT
Freshwater algae	MIC = 19 ppm
Daphnia	48-hour EC_{50} = 38 ppm
Bluegill	96-hour LC_{50} = 38 ppm
Rainbow trout	96-hour LC_{50} = 32 ppm

Appendix c-1

Excretion of Semduramicin and its Metabolites by Broilers

Report Summary: EXCRETION OF SEMDURAMICIN AND ITS METABOLITES BY BROILERS

Study Numbers: 1515N-60-87-003, 18,027-97 and 17499-97

Test Material: Excreta from broilers medicated with radiolabeled semduramicin

Studies were conducted to assess the levels, mass balance and identity of products excreted by poultry dosed with carbon-14 labeled semduramicin sodium.

A. **Concentrations of Semduramicin in Poultry Excreta.**

Thirty-seven day old broilers consumed feed containing 24 ppm of C-14 radiolabeled semduramicin sodium for 7 days. On days 4 through 7, excreta from 15 males and 14 females were collected and pooled by sex and date. Triplicate samples of each collection were assayed for total radioactivity by combustion and scintillation counting.

Samples of the day 6 collection from males and of the day 7 collection from females were extracted with solvents. The extracts were fractionated by high performance liquid chromatography (HPLC), and the amount of radioactivity in each sample was measured. The fractions obtained from the excreta of male broilers were also examined by the most sensitive known test for a biological activity characteristic of polyether antibiotics, an *in vitro* anticoccidial assay.

The balance between ingestion and excretion of radiolabeled material was calculated for these two collections of excreta from the measured concentration of drug in feed (f), measured feed intake (i), standard ratio of excreta to feed intake (r), and measured concentration of total radioactivity in the excreta (e). This made it possible to calculate the percentage of the ingested dose represented by each of the most abundant HPLC fractions.

$$\frac{[(e) \times (r) \times (i) \times 100]}{[(f) \times (i)]} = \% \text{ Dose ingested}$$

e = the determined concentration of total residues (radioactivity) in excreta.
r = the standard ratio of excreta to feed intake = 0.054 kg excreta per day for consumption of 0.064 kg feed/day, (Reference 2, this summary)
i = the measured feed intake.
f = the determined concentration of semduramicin in feed.

In the data provided:

e = 27 mg/kg semduramicin equivalents, the actual measured concentration of radioactivity in excreta from female broilers on day 7 (Study: 1515N-60-87-003);

r = 0.054/0.064, a literature value (Reference 2, this summary);

i = 0.142 kg of feed (mean measured value of feed consumed by 14 female broilers on day 7 of study 1515N-60-87-003), and

f = 24 mg/kg, the actual measured concentration for semduramicin (corrected for 97% recovery) in feed (Study: 1515N-60-87-003).

Accordingly, inserting these values in the above equation shows 95% of the dose ingested was found in excreta of female broilers during the seventh day of medication.

$$\frac{[(27) \times (0.054/0.064) \times (0.142) \times 100]}{[(24) \times (0.142)]} = 95\%$$

A similar calculation for male broilers revealed 105% of the ingested dose was excreted on day 6 of the study by male broilers. In this case, the measured concentration in excreta (e) on day 6 for male birds is 29.8 mg/kg (Study: 1515N-60-87-003), and (i) the measured feed intake for 15 male broilers is 0.165 kg, (Study: 1515N-60-87-003). Thus the percent of dose ingested =

$$\frac{[(29.8) \times (0.054/0.64) \times (0.165) \times 100}{[(24) \times (0.165)]} = 105\%$$

Thus an excellent mass balance for the dose ingested was calculated for male and female broilers in the semduramicin study.

Separately, small samples of the most abundant metabolites were isolated from the bile of medicated broilers for structure identification by mass spectral and nuclear magnetic resonance analysis.

Summary of Results:

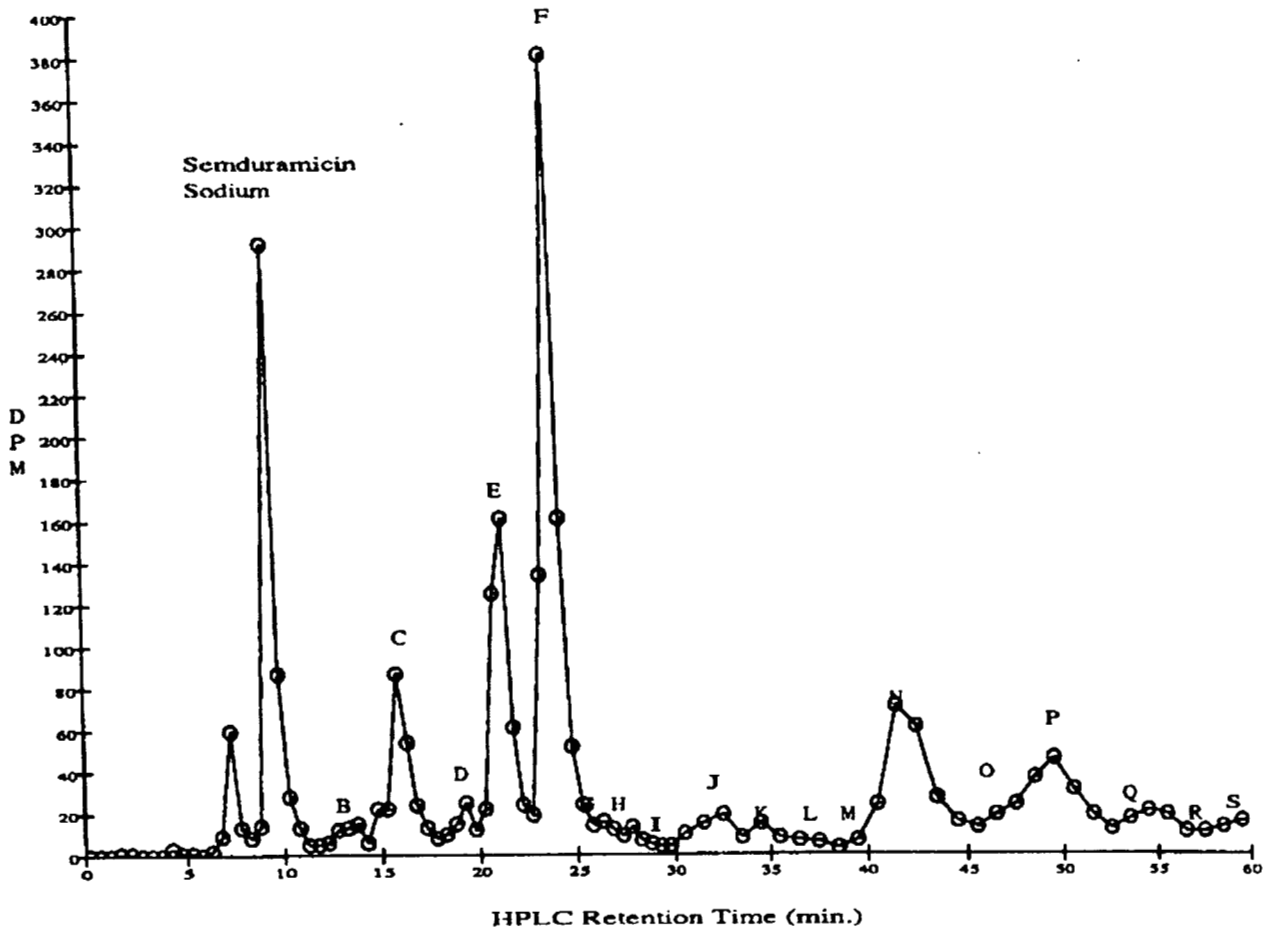
The following levels of radioactive material (in ppm) were measured in the eight separate collections of excreta:

24-Hour Collection	Males	Females	Mean
Day 4	29.8	19.1	24.5
Day 5	22.2	16.6	19.4
Day 6	29.8	23.3	26.6
Day 7	18.7	27.0	22.9
Mean	25.1	21.5	23.4

The HPLC profile of extracts from excreta is illustrated in the accompanying figure. The most prominent HPLC peaks, in order of increasing polarity, were as follows:

	% of Ingested Dose	
HPLC Peak	Females	Males
Semduramicin	7.8	3.7
C	4.0	4.8
E	5.9	7.8
F	11.5	10.7

Figure 1. HPLC-LSC profile obtained from pooled extract derived from the 24-hour collection and last day of 14 female broilers fed carbon-14 labeled semduramicin sodium in feed at 25 ppm for 7 days. Experiment: 1515N-60-87-003.



The abundance of unchanged semduramicin shown above translates into concentrations of 2.1 ppm and 1.1 ppm in the excreta of female and male broilers, respectively. These results in 6-week old broilers agree with the level of 1.6 ppm of unchanged semduramicin measured by a specific assay in the excreta of 2-week old medicated broilers (Report 17499-97).

The remainder of the radiolabeled material in excreta consisted of an array of polar metabolites. Peak F was the only metabolite that exceeded 10% of the ingested dose. It and peak E were shown to represent O-desmethyl metabolites of semduramicin. Neither E nor F was active in the sensitive *in vitro* assay; this is consistent with the greatly reduced biological activity in several tests, including the *in vitro* anticoccidial assay, that results from O-demethylation of the polyether ionophore monensin (Reference 1, this summary). Peak C, representing about 4-5% of the ingested dose, was the only component of the extracts other than unchanged semduramicin that was active in the *in vitro* assay. Its structure could not be established with certainty, but probably corresponds to ring-opening of the terminal cyclic hemiketal, followed by reduction of the resulting ketone. A similar metabolite of monensin has been described (Reference 3, this summary) and was reported to be about half as active as monensin in a microbiological assay.

B. Mass Balance in Semduramicin Excretion Studies in Broilers

A mass balance of radiolabeled material ingested and excreted by medicated broilers is calculated below for both excreta collections from experiment 1515N-60-87-003 that were used to obtain a chromatographic profile of excreted metabolites.

1) Female Broilers

The percentage of the excreted dose represented by various peaks in the HPLC was determined in excreta collected during the last day of dosing from 14 female broilers which for 7 days consumed feed medicated with radiolabeled semduramicin.

Determination of the actual amount of semduramicin ingested and excreted

Females housed in two separate pens consumed feed medicated with radiolabeled semduramicin for 7 days. During the seventh day, they consumed 0.93 kg of feed in one pen and 1.06 kg in the other, or an average of 0.995 kg per pen. Each pen contained 7 broilers, so on average each broiler consumed 0.142 kg of feed:

$$0.995 \text{ kg/pen} \div 7 \text{ broilers/ pen} = 0.142 \text{ kg/broiler}$$

The intended level of semduramicin in feed was 25 ppm; the actual measured level was 23 ppm. The mean recovery in the assay procedure is 97%. When the measured level of 23 ppm is corrected for 97% recovery in the assay, a level of 24 ppm in feed is obtained:

$$23 \text{ ppm} \div 0.97 = 24 \text{ ppm}$$

Therefore, the broilers in these pens ingested an average of 3.41 mg of semduramicin on day 7:

0.142 kg/broiler x 24 mg/kg = 3.41 mg/broiler

Total Amount of Drug and Metabolites Excreted

The total amount of waste excreted was estimated from a formula which related feed consumed to waste excreted. During growout, a broiler consumes an average of 0.064 kg feed/day and excretes 0.054 kg of raw waste/day (Reference 2, this summary). Applying these relative proportions of consumed feed and excreted waste to the semduramicin study leads to an estimate of 0.120 kg of raw waste excreted on day 7:

0.142 kg feed/broiler x 0.054 kg excreta/0.064 kg feed = 0.120 kg excreta/broiler.

The measured concentration of radioactivity in excreta collected from female broilers on day 7 was 27.0 ppm of semduramicin equivalents (Study: 1515N-60-87-003). Therefore, the total amount of radiolabeled material excreted by each broiler on day 7 was 3.24 mg of semduramicin equivalents:

0.120 kg/broiler x 27 mg/kg = 3.24 mg/broiler

Mass Balance of Radiolabeled Material

Of the 3.41 mg ingested by each broiler on day 7, 3.24 mg (95%) can be accounted for by radioactivity measured in excreta.

2) Male Broilers

Radioactivity and *in vitro* anticoccidial activity were determined for HPLC fractions obtained from material excreted by 15 male broilers which consumed medicated feed for 6 days. Only two fractions were found to possess anticoccidial activity: unchanged semduramicin sodium and a slightly more polar material. The relative amounts of this more polar material and unchanged drug were 6.4% and 4.9%, respectively, a ratio of 1.3:1.0. Even though it was present in a larger amount, the polar metabolite was less potent *in vitro* than semduramicin sodium.

Feed consumption on day 6 (Study: 1515N-60-87-003):

Pen 1 M: 1.14 kg ÷ 7 broilers = 0.163 kg/broiler
Pen 2 M: 1.33 kg ÷ 8 broilers = 0.166 kg/broiler
Average: 0.165 kg/broiler

Drug level in feed: 24 mg/kg

Amount of drug ingested on day 6:

24 mg/kg x 0.165 kg/broiler = 3.96 mg/broiler

Amount of excreta produced on day 6:

0.165 kg/broiler x 0.054 kg/0.064 kg = 0.139 kg/broiler

Measured concentration of radioactivity in excreta collected from male broilers on day 6 (Study 1515N-60-87-003):

29.8 mg/kg

Amount of radiolabeled material in day 6 excreta:

29.8 mg/kg x 0.139 kg/broiler = 4.14 mg/broiler

Mass balance: 4.14 mg ÷ 3.96 mg x 100 = 105%

3) Conclusion: An excellent mass balance can be calculated for excreta that were used to investigate the abundance and polarity of metabolites.

C. Identification of residues in poultry excreta and comparative metabolism with the dog and rat.

Comparative radiotracer metabolism studies were conducted with carbon-14 labeled semduramicin sodium in poultry and laboratory species to elucidate its biotransformation among species, and to provide data supporting the use of this anticoccidial compound in broiler chickens.

These metabolism studies complement earlier radiotracer studies that defined the target tissue, and the quantitative assessment and depletion of total and marker residues in edible tissues and excreta of broilers that were fed and withdrawn from carbon-14 labeled semduramicin sodium under projected use conditions.

In comparative metabolite identification studies, liver, bile and excreta were obtained from poultry fed ^{14}C-semduramicin sodium in feed at projected use level of 25 ppm for 7 to 11 days. The rat and dog were dosed by aqueous gavage with ^{14}C-semduramicin sodium at 1 mg/kg/day SID x 5, and samples of liver recoverd from each species at sacrifice.

As defined by HPLC, liver of poultry sacrified six hours after withdrawal, is comprised mainly of unchanged semduramicin sodium (-45%) and an array of more polar, low-level (<0.1 ppm) metabolites. None of these metabolites represent more than 10 percent of total radioactivity. HPLC peaks corresponding to these metabolites are detected in the liver of the rat and the dog receiving aqueous oral gavages of the drug, tentatively confirming exposure of the toxicology species to the metabolites of semduramicin sodium in the use animal target tissue. Several of these metabolites are also found in bile and excreta of the chicken at levels sufficient for identification. When isolated from chicken bile two metabolites were tentatively identified by FAB mass spectrometry as the A-ring O-desmthyl, and the G-ring O-desmethyl compounds. A third and lower abundant metabolite was tentatively identified by FAB MS as a ring-opened product of semduramicin. The mass spectral assignments of the desmethyl compounds were confirmed by proton NMR.

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Appendix c-2

Physical-chemical Properties of Semduramicin Sodium

Report Summary: PHYSICO-CHEMICAL PROPERTIES OF SEMDURAMICIN SODIUM

Solubility in Aqueous Buffers. The solubility of semduramicin sodium was determined by stirring 50 ml volumes of pH 6, 7, and 9 acetate or ammonium aqueous buffers maintained at 25 ± 1°C containing an excess (210 mg) of crystalline semduramicin sodium. Samples (4 ml) were removed periodically, filtered, and assayed by HPLC until a constant solubility value was reached. The pH values remained constant over the course of the experiment. There were three to five replicates at each pH. Solubility results are summarized in Table 1.

It was observed that the solubility of semduramicin sodium is essentially constant over the pH range six to nine. There was no evidence of aggregation or supersaturation. The solubility of semduramicin sodium was reduced upon addition of sodium ions (common ion effect) and enhanced upon addition of calcium or magnesium ions.

Solubility results were not obtained at pH 5 due to instability of semduramicin at low pH over a long period of time; however, below pH 5 the solubility of semduramicin sodium falls off due to the intrinsic solubility (about 0.06 mg/mL) of the free acid form (pKa is 4.2).

Dissociation Constant. The dissociation constant (pKa) of semduramicin was determined by potentiometric titration, in quintupicate, of approximately 6.25 mM solutions [200 mg/40 ml] of semduramicin sodium dissolved in 1:1 acetone:water using 0.5N hydrochloric acid as titrant at 23 ± 2°C. The theoretical neutral equivalent was obtained. The pKa value was computed from the standard relationship, pH=pKa at the half titrated point. An apparent pKa value of 5.39 ± 0.01 was obtained. From this result, the pKa in aqueous solution was estimated as 4.2. This value is reasonable, considering semduramicin as a substituted acetic acid. An example titration curve is provided in Figure 1.

Ultraviolet-Visible Absorption Spectrum. The absorption spectra of 0.1 mg/mL solution of semduramicin sodium in 1:1 methanol:pH buffer were determined in triplicate at 5, 7 and 9. There were no detectable absorption bands (with an extinction coefficient exceeding 25 L/mole-cm) over the wavelength range of 290 to 800 nm. Absence of absorption is expected, based on the structure of semduramicin, which does not contain any common chromophoric groups such as double bonds, conjugated π systems, etc.

Melting Temperature. A capillary tube containing semduramicin sodium and another tube containing a melting point reference standard were placed into the heating well of a melting point apparatus. The temperature was raised at a constant rate (1°C/min) and the temperatures were noted at which changes were observed in either material. The determination were carried out in quadruplicate for the sample and in triplicate for the standard. The average melting temperature for semduramicin sodium was 170°C; the replicates gave a range of 168 to 170°C. The average melting point for the standard was 173°C, which fell within the manufacturer's specification (Thomas Standard E, 172.5-173.5°C). Since the melting point of semduramicin is 170°C, no change of state is expected for the compound during its transport, storage, use or disposal.

Thermogravimetric Analysis. A sample of semduramicin sodium was heated (30°C/min) under nitrogen in a commercial, Perkin-Elmer TGA instrument, which continuously and accurately monitors the weight of the sample. The sample was run in triplicate. The samples exhibited a small weight loss corresponding to water content to a temperature of approximately 150°C. Beginning at about 162°C, there was a rapid loss of weight caused by thermal degradation of semduramicin to volatile decomposition products. The TGA plot is shown in Figure 2.

These results indicate that semduramicin sodium has very low vapor pressure and is non-volatile, as expected for a salt of high molecular weight. Additional thermogravimetric data were obtained which confirm that the vapor pressure must be less than 10^{-8} torr at 20°C. One gram samples of semduramicin sodium and pyrene (for which the vapor pressure has been reported as 7×10^{-7} torr at 20°C) were examined for weight loss at a severe challenge condition of 100°C for 24 hours under vacuum. The semduramicin sodium sample did not lose any significant weight beyond solvated water, but the pyrene sample was nearly completely volatilized.

Table 1. Solubility Results of Semduramicin Sodium

Sampling Time (hours)	Solubility (mg semduramicin/ml)*		
	pH=6	pH=7	pH=9
0.5	1.32	1.69	1.83
1	1.29	1.73	1.83*
3	1.38	1.70	1.86*
6	1.40	1.69	1.84*
24	1.44	1.75*	1.93*
96		1.92*	1.97*
120		1.96*	
144		1.95*	
168			1.95*
193	1.77*		
216	1.67*		
240	1.68*		
Solubility	1.7	1.9	1.9

* Values averaged to calculate the solubility

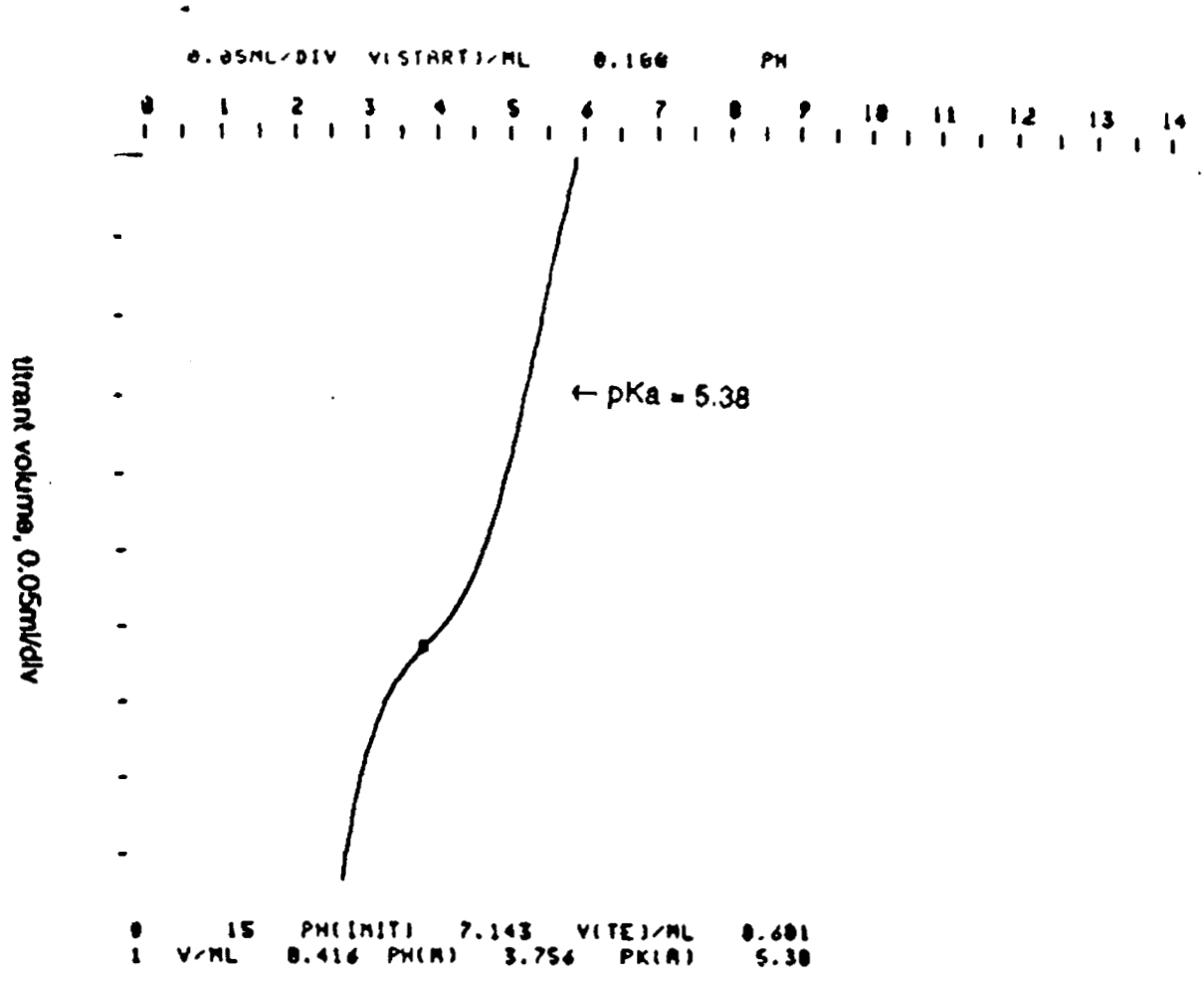

Figure 1. Potentiometric titration plot for semduramicin sodium.

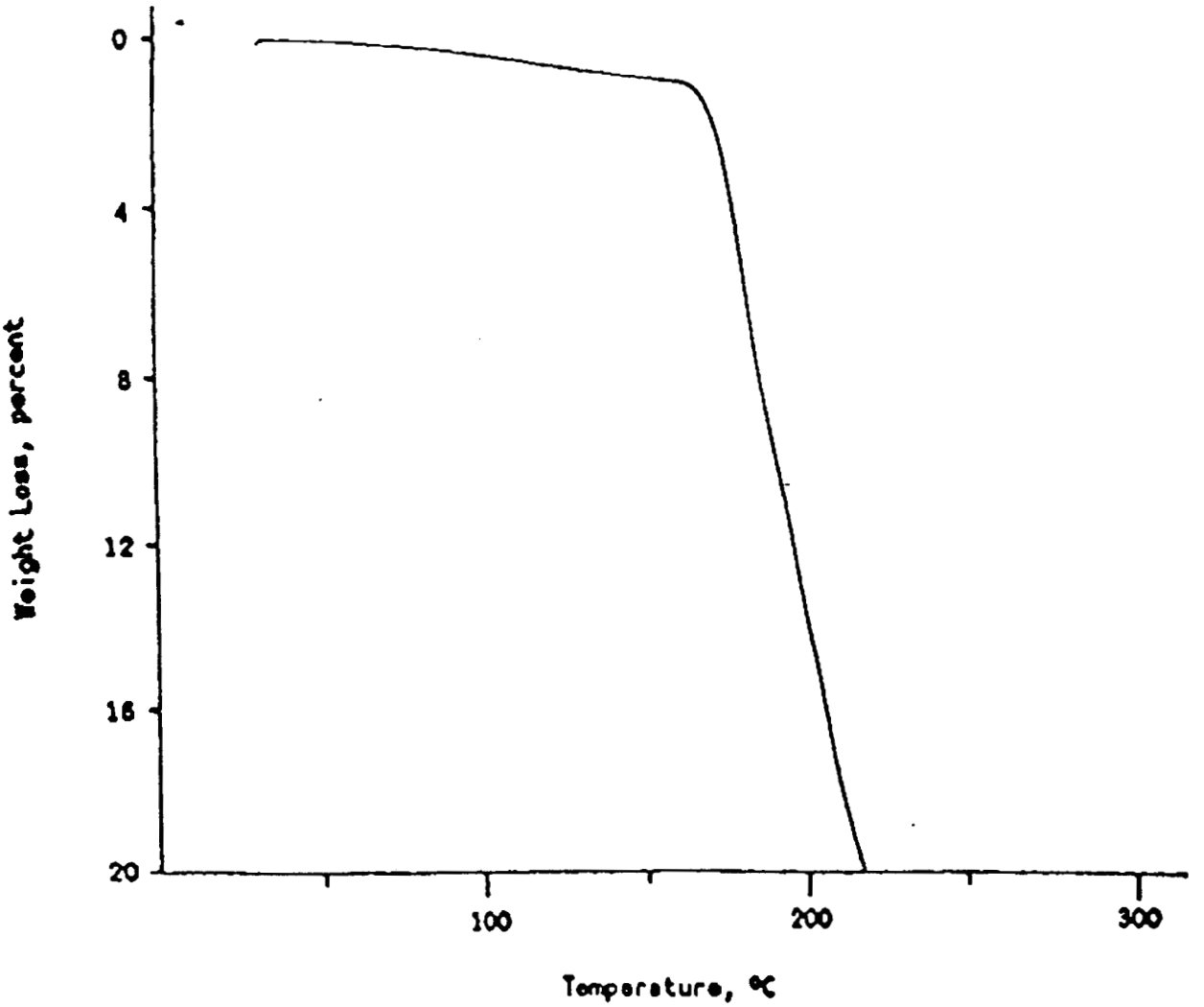

Figure 2. Thermogravimetric plot of semduramicin sodium

Appendix c-3

The Octanol-water Partition Coefficient of Semduramicin Sodium

Report Summary: THE OCTANOL-WATER PARTITION COEFFICIENT OF SEMDURAMICIN SODIUM

Study Number: 2438-1287-6114-705

Test System: Two-phase solvent system

Summary of Experimental Design: Solutions of radiolabelled semduramicin sodium were prepared at $6x10^{-6}$ and $7x10^{-7}$ molar concentrations in pH 6, 7, and 9 aqueous buffers. Each combination of pH and concentration was prepared in triplicate. (Solutions in pH 5 buffer were not studied because they were known to be unstable.) A 40 ml volume of each solution was shaken gently for two hours at 25 ± 1°C in a centrifuge tube with 2 ml of n-octanol. It had been determined in a preliminary experiment that this length of time was sufficient to attain equilibrium.

The phases were then separated by centrifugation and the amount of radiolabel present in each phase was determined by liquid scintillation counting of aliquots. The radiocount for each aliquot was divided by the volume of the aliquot, and the resulting radiocount per unit volume of the phase was divided by the specific radioactivity of the test material to obtain the final concentration of semduramicin sodium in the phase.

The partition coefficient (K_{ow}) for semduramicin sodium in each system was calculated by dividing the final concentration of semduramicin sodium in the octanol phase by its final concentration in the aqueous buffer phase. The partition coefficient was converted to its logarithm, log K_{ow}.

The radiometric mass balance was checked by multiplying the radiocount per unit volume of each phase by the volume of the phase, summing the resulting total radiocount per phase for the two phases of each system, and dividing the total by the amount of radioactivity originally added to the system in the buffer solution. The result was expressed as a percentage.

Summary of Results: The table below lists the mean values of the final concentration in the octanol phase, final concentration in the aqueous phase, partition coefficient, logarithm of partition coefficient, and percent radiolabel recovered, for each combination of pH and initial concentration in the aqueous buffer. Semduramicin demonstrated a slight preference for the n-octanol phase at all three pH's. Based on the low $_{p}K_{ow}$ of less than 3, significant accumulation of semduramicin in aquatic organisms is not predicted.

pH	Concentration (mg/ml)			Kow	log Kow	%Recovery
	Initial	Octanol	Water			
6	$6.3x10^{-4}$	0.010	$3.3x10^{-5}$	291.60±2.27	2.47±0.01	96.37±0.51
6	$5.4x10^{-3}$	0.097	$1.9x10^{-4}$	499.49±10.22	2.70±0.01	98.87±1.64
7	$6.3x10^{-4}$	0.009	$5.4x10^{-5}$	172.38±9.83	2.24±0.02	97.18±1.75
7	$5.4x10^{-3}$	0.092	$2.9x10^{-4}$	326.53±9.78	2.51±0.01	97.54±2.18
9	$6.3x10^{-4}$	0.009	$7.6x10^{-5}$	119.15±3.48	2.08±0.01	98.03±1.14
9	$5.4x10^{-3}$	0.089	$4.0x10^{-4}$	222.24±7.69	2.35±0.01	95.84±1.35

Appendix c-4

Soil Sorption and Desorption of Semduramicin Sodium

Report Summary: SOIL SORPTION AND DESORPTION OF SEMDURAMICIN SODIUM

Study Number: 2438-6115

Test System: Three types of soil in contact with aqueous solutions.

Summary of Experimental Design: The same general procedure was used to conduct a screening test, a soil kinetics test, and an isotherm determination. All tests were conducted in triplicate. An Iowa Sandy Loam, an Arkansas Silty Loam, and a Mississippi Silty Clay Loam were used in the isotherm determination. The characteristics of these soils are shown in Table 1.

To study sorption, samples of each soil were shaken in capped centrifuge tubes with solutions of radiolabelled semduramicin sodium in 0.01 M aqueous calcium chloride. The ratio of solution to soil was 5:1 in the screening test and 5:1 or 20:1 in the soil kinetics and isotherm tests. For every combination of soil type and initial concentration, the concentration remaining in the aqueous phase (C_e) was determined by radioassay, and the amount sorbed onto soil (x) was calculated from the difference between the initial and final concentration in the aqueous phases.

To study desorption, soil samples containing sorbed semduramicin sodium were equilibrated twice in succession with fresh 0.01 M aqueous calcium chloride, and the concentrations in the aqueous phases were again determined by radioassay. In the screening test, a separate set of sorption and desorption experiments was carried out with deionized water as the aqueous vehicle.

Calculations: The logarithm of the experimentally determined equilibrium concentration, log C_e, was plotted against log (x/m) for each soil, where x/m is the concentration in the soil. The points on the graph were fitted to a logarithmic transformation of the Freundlich isotherm equation:

$$\log (x/m) = \log (K_d) + 1/n \log (C_e)$$

where K_d is the Freundlich sorption coefficient and 1/n, an empirical constant, is the slope of the graph. Log K_d was read off the graph as the intercept. The antilog, K_d, was then calculated and converted to K_{oc}, the sorption constant adjusted for the organic carbon content of the soil, according to the equation:

$$K_{oc} = (K_d \times 100)/\% \text{ organic carbon}$$

For the two soils that were studied at a ratio of solution to soil of 20:1 (Arkansas and Mississippi), the percent of the initially added semduramicin sodium that would be sorbed from aqueous solution onto each of the soils at a solution ratio of 5:1 was calculated from the K_d values determined for sorption in the isotherm test:

$$\% \text{ sorbed} = [K_d/(K_d + 5)] \times 100$$

Similarly, the percent of the semduramicin sodium sorbed onto soil that would be desorbed when the soil is exposed to fresh 0.01M calcium chloride solution, at a solution:soil ratio of 5:1, was calculated from the K_d values determined for desorption:

$$\% \text{ desorbed} = [5(K_d + 5)] \times 100$$

Summary of Results: The results of the screening test showed that calcium chloride did not significantly interfere with the sorption or desorption of semduramicin as compared to distilled water. Semduramicin readily sorbed (<25%) and did not readily desorb (>75% of the fraction sorbed), indicating that an advanced test should be conducted. Equilibrium was attained at approximately 72 hours for all three soil types.

The results of the isotherm test confirmed that the sorption of semduramicin sodium in soil is moderately strong though reversible (Tables 2, 3, and 4). As shown below, the value of K_d, the Freundlich sorption coefficient, in these three soils ranged from 4.65 to 25.7 for sorption and from 2.41 to 16.2 for desorption. The corresponding ranges of K_{oc} were 150 to 1,750 and 79 to 1,100, respectively. Compounds having a K_{oc} value of 1,000 or larger are considered relatively immobile in soil and have a low potential for leaching into the water table or into runoff water. Thus, semduramicin sodium would be expected to be relatively immobile in two of the three soils (MS STCYLM and AR SYLM) and to be only moderately mobile even in a soil that is predominantly sand (IA SDLM).

	Sorption			Desorption		
Soil Type	K_d	K_{oc}	1/n	K_d	K_{oc}	1/n
MS STCYLM	25.7	1750	0.949	16.2	1100	1.016
AR STLM	10.6	1400	0.843	6.35	840	0.798
IA SDLM	4.65	150	0.824	2.41	79	0.765

It was calculated that a solution:soil ratio of 5:1 from 48 to 84% of the initially added semduramicin sodium will be sorbed onto these soils from the aqueous solution, and 24 - 67% will be desorbed upon subsequent exposure of the soils to fresh calcium chloride solution.

SOIL TYPE	% SORBED	K_s	% DESORBED	K_{des}
MS STCYLM	84	25.7	24	16.2
AR STLM	68	10.6	44	6.35
IA SDLM	48	4.65	67	2.41

Table 1. Characterization of the Soils Used in the Sorption/Desorption Coefficient Determination

Source Texture	Iowa Sandy Loam	Arkansas Silty Loam	Mississippi Silty Clay Loam
% Sand	53.2	16.0	72.0
% Silt	37.6	58.0	50.0
% Clay	9.2	26.0	28.0
% Organic Matter	5.2	1.3	2.5
pH	5.6	6.2	7.7
Cation Exchange Capacity (meq/100g)	12.5	6.0	12.2
% Field Moisture Capacity	23.9	16.9	24.97

Table 2. Isotherm Test: Concentrations of Semduramicin in Soil and in Solution
Mean of three values and standard deviation

Mean Measured Initial Concn. µg/ml		Soil type/concentration: IOWA SANDY LOAM Aqueous, ug/ml	IOWA SANDY LOAM Soil, ug/g	ARKANSAS SILTY LOAM Aqueous, ug/ml	ARKANSAS SILTY LOAM Soil, ug/g	MISSISSIPPI SILTY CLAY LOAM Aqueous, ug/ml	MISSISSIPPI SILTY CLAY LOAM Soil, ug/g
				SORPTION STUDY			
9.7	Mean	5.62	20.45	6.76	59.13	4.21	107.9
	SD	0.12	0.59	0.26	5.23	0.21	---[a]
4.6	Mean	2.66	9.83	3.40	24.55	2.00	52.45
	SD	0.07	0.33	---[a]	---[a]	0.14	2.71
2.3	Mean	1.24	5.28	1.48	16.31	1.13	23.31
	SD	0.05	0.23	0.13	2.55	0.05	0.90
0.93	Mean	0.44	2.46	0.59	6.82	0.37	11.32
	SD	---[a]	---[a]	0.01	0.20	---[a]	---[a]
				DESORPTION STUDY			
9.7	Mean	3.16	6.91	2.14	17.60	2.75	54.27
	SD	0.03	0.17	0.22	7.49	---[a]	---[a]
4.6	Mean	1.70	2.71	1.22	4.74	1.57	21.97
	SD	0.05	0.75	---[a]	---[a]	0.04	2.11
2.3	Mean	0.75	2.17	0.67	3.91	0.71	10.17
	SD	0.05	0.03	0.06	0.90	0.01	1.35
0.93	Mean	0.31	1.05	0.23	2.38	0.31	5.46
	SD	---[a]	---[a]	0.03	0.66	---[a]	0.94

[a]One or two samples lost due to broken centrifuge tubes, hence standard deviation not applicable.

Table 3. Linear regression analysis of the Sorption Data using the Freundlich Isotherm [$\log_{10}(x/m) = \log_{10}(K_d) + 1/n \log_{10}(C_e)$] for UK 61,689-2 with three soil types.

	Soil Type					
Mean Measured Concentration mg/L	MS Silty Clay Loam		AR Silt Loam		IA Sandy Loam	
	$\log_{10} C_e$	$\log_{10} x/m$	$\log_{10} C_e$	$\log_{10} x/m$	$\log_{10} C_e$	$\log_{10} x/m$
9.7	0.62	2.03	0.83	1.77	0.75	1.31
4.6	0.30	1.72	0.53	1.39	0.42	0.99
2.3	0.05	1.37	0.17	1.21	0.09	0.72
0.93	-0.43	1.05	-0.23	0.83	-0.36	0.39
Correlation:	0.989		0.988		0.998	
Slope (1/n):	0.949		0.843		0.824	
Int. (Log K_d):	1.41		1.026		0.667	
n	1.05		1.186		1.214	
K_d	25.7		10.6		4.65	
% Organic Carbon*	1.47		0.76		3.06	
K_{oc}	1800		1400		150	

*% Organic Carbon = % Organic Matter/1.7.

Table 4. Linear Regression analysis of the Desorption Data using the Freundlich Isotherm [$\log_{10}$ (x/m) = $\log_{10}$ (K_d) + 1/n $\log_{10}$ (Ce)] for UK 61,689-2 with three soil types.

Mean Measured Concentration mg/L	Soil Type: MS Silty Clay Loam		AR Silt Loam		IA Sandy Loam	
	$\log_{10} C_e$	$\log_{10}$ x/m	$\log_{10} C_e$	$\log_{10}$ x/m	$\log_{10} C_e$	$\log_{10}$ x/m
9.7	0.44	1.73	0.33	1.25	0.50	0.84
4.6	0.20	1.34	0.09	0.68	0.23	0.43
2.3	-0.15	1.01	-0.17	0.59	-0.12	0.34
0.93	-0.51	0.74	-0.64	0.38	-0.48	0.02
Correlation:	0.986		0.891		0.963	
Slope (1/n):	1.016		0.798		0.765	
Int. (Log K_d):	1.210		1.803		0.383	
n	0.984		1.253		1.307	
K_d	16.2		6.35		2.41	
% Organic Carbon*	1.47		0.76		3.06	
K_{oc}	1100		840		79	

* % Organic Carbon = % Organic Matter/1.7.

Appendix c-5

Hydrolysis of Semduramicin Sodium

Report Summary: HYDROLYSIS OF SEMDURAMICIN SODIUM

Study Number: 2438-0189-6142-715

Test Systems: • Solutions in aqueous buffers

Summary of Experimental Design:

Sterile solutions containing 10 ppm of semduramicin sodium in pH 5, 6, 7, 8 and 9 aqueous buffers were prepared and three replicates of each solution were placed into glass volumetric flasks. The flasks were fully covered with aluminum foil to shield them from light and placed in a shaker water bath. In a preliminary experiment, this bath was held at 49.8 $\pm$ 0.4°C; in the definitive experiment, it was maintained at a constant temperature of 25.0 $\pm$ 0.2°C. An aliquot of each replicate at each pH was analyzed by a high pressure liquid chromatography assay specific for semduramicin sodium at the initiation of the experiment and then at periodic intervals. The logarithms of the observed concentrations at each pH were plotted as a function of time and correlated with time by linear regression analysis according to the equation for first-order reaction kinetics:

$$\log C = - (k/2.30)t + \log Co$$

Where C is the concentration at time t, Co is the initial concentration, and k is the rate constant. The half-life (t1/2) of semduramicin sodium was then calculated from the equation:

$$t1/2 = \ln 2/k$$

In addition, at initiation and near termination of the definitive experiment each solution was tested for microbiological activity against *Bacillus stearothermophilus* by the Kirby-Bauer Method. Zones of inhibitation that surrounded discs containing either semduramicin sodium in buffered solution or penicillin G were compared following incubation of plates at 65° for 2 hours and 45 minutes.

Summary of Results:

The following rate constants and half-lives were calculated from the preliminary experiment conducted at 50°C:

pH	Rate Constant (days $^{-1}$)	Half-life (days)
5	0.328	2.11
6	0.140	4.94
7	0.0637	10.9
8	0.0330	21.0
9	0.0615	11.3

The corresponding results from the definitive test at 25°C are as follows:

pH	Rate Constant (days $^{-1}$)	Half-life (days)
5	6.23×10^{-2}	11.1
6	1.93×10^{-2}	36.1
7	7.85×10^{-3}	89.9
8	6.02×10^{-3}	115.0
9	1.00×10^{-2}	77.1

Mean differences in zone sizes between semduramicin sodium and the penicillin G standard for each pH at the initiation and conclusion of the definitive test are as follows:

	pH:	5	6	7	8	9
Difference at initiation	(mm)	4.4	6.1	6.5	7.5	7.0
Difference on day 23	(mm)	-0.4	3.1	4.3	6.2	6.0

The hydrolysis study supports an observation that UK-61,689-2 is hydrolyzed rapidly at pH 5 and pH 6. The microbial inhibition data indicate that the inhibitory activity of UK-61,689-2 was lower than the inhibitory effect of penicillin at pH 5 toward the target microbe. At the other pHs tested, the inhibitory effect of UK-61,689-2 was greater than or equal to the inhibitory effect of penicillin toward the target organism. While the data provide an indication that semduramicin reacts under the conditions of the test, a more extensive study would be required to determine the extent of degradation.

Appendix c-6

Photodegradation of Semduramicin Sodium in Aqueous Solution

Report Summary: PHOTODEGRADATION OF SEMDURAMICIN IN AQUEOUS SOLUTION

Study Number: 2438-0488-6125-720

Test System: Irradiation of Aqueous Solutions with Simulated Sunlight.

Summary of Experimental Design:

Solutions containing 10 ppm of semduramicin sodium in pH 6, 7, and 9 buffers were prepared. Samples of each buffered solution were used to fill four sets of 13 X 100 mm quartz glass tubes. Three of the sets were saturated with air, and one of these was shielded from light by covering the tubes with aluminum foil. The fourth set was saturated with nitrogen and covered with foil. All the tubes were placed in an instrument that simulates natural sunlight by means of a Xenon arc lamp and appropriate filters. A fifth set filled with a solution containing an actinometer (reference material), 10^{-5} M p-nitroacetophenone and 1.5×10^{-3} M pyridine, was also placed in the instrument. Irradiation was started, the temperature was maintained at 34°C, and a sample from each set was analyzed by HPLC for semduramicin sodium or p-nitroacetophenone at initiation and at periodic intervals thereafter. At initiation and termination, a sample of each set containing semduramicin sodium was also assayed microbiologically. For each pH, the ratio of the concentration of semduramicin, C, to the initial concentration, Co was plotted versus time, t, according to the equation for first order decomposition.

$$\ln C/Co = -kt$$

The slope of the line, k, was obtained from the graph and used to calculate the half-life, t 1/2, according to the relation:

$$t\ 1/2 = \ln 2/k$$

Summary of Results:

The concentration of semduramicin sodium in the irradiated samples declined fairly rapidly, more so at lower than at higher pH:

Measured Concentration (mg/L) of UK-61689-2 and p-nitroacetophenone (PNAP).

Sampling Interval (Hour)	pH 6	pH 7	pH 9	PNAP
0	9.30	9.94	10.0	1.64
21	6.14	7.82	7.81	1.48
38	6.39	7.65	8.12	1.41
48	4.42	6.03	6.16	1.34
66	3.63	5.72	6.59	1.31
90	4.59	4.77	5.69	1.24
136	2.69	3.78	5.58	1.10
166	3.03	3.40	5.17	1.15
187	2.37	3.56	3.54	1.08

Values for UK-61,689-2 represent a mean of three replicates on day 0 and a mean of two replicates for all subsequent sampling intervals. Values for PNAP are for a single unaerated replicate.

The following rate constants and half-lives were determined; the half-lives determined under continuous irradiation were multiplied by two to obtain the half-lives in days with 12-hour exposure to light:

pH	Rate Constant (1/hours)	Half-Life (days)	Half-Life (Days, 12-hour exposure/day)
6	8.14×10^{-3}	3.55	7.10
7	6.59×10^{-3}	4.38	8.76
9	5.10×10^{-3}	5.66	11.32
PNAP	2.55×10^{-3}	11.3	22.6

The concentration in the aerated samples kept in the dark also declined, but much more slowly than in the irradiated samples. The concentration in the nitrogenated samples kept in the dark declined even more slowly. The reference material decomposed at the expected rate.

The microbiological activity of the irradiated solutions of semduramicin sodium was markedly lower at termination than at initiation for all three pH's, indicating that activity against the indicator organism was lost as semduramicin sodium decomposed. In a disc assay, at each pH, the initial solution produced a larger zone of inhibition than a fixed concentration of a reference standard at the same pH. The decline in the activity of the irradiated solutions manifested itself as a reduced diameter of their zones of inhibition and consequently, a smaller difference from the reference standard.

	pH	6	7	9
Difference at initiation	(mm)	4.6	5.1	4.9
Difference after 187 hours	(mm)	0	0.5	0.6

Semduramicin sodium was found to undergo photodegradation in aqueous solution even though if does not show detectable absorption in the UV/visible spectrum. This appears to be a case of photo-induced degradation, a type of indirect photolysis in which a reactive chemical species formed by the action of light reacts chemically with a compound, which does not itself absorb light, so as to decompose it (**Choudry, G.G.** and **G.R.B. Webster.** 1985. Protocol guidelines for the investigations of photochemical fate of pesticides in water, air, and soils. I. Introduction. Residue Reviews 96:80-81). While there is evidence that oxidant species such as singlet oxygen have been reported in a variety of natural waters, there is no direct evidence that these oxidatively degrade organic pollutents in natural waters. Due to the lack of absorption of semduramicin, it was not possible to calculate a quantum yield.

Appendix c-7

Biodegradation of Semduramicin Sodium in Soil

Report Summary: AEROBIC BIODEGRADATION OF SEMDURAMICIN SODIUM IN SOIL

Study Number: SC910074

Test System: ^{14}C semduramicin sodium admixed with soils at 25 ppm.

Summary of Experimental Design:

Characteristics of 3 soils employed in the study are as follows:

Soil Identification (Location)	Cation Exchange Capacity (meq/100g)	Organic Matter (%)	pH	Field Moisture Capacity (%)	Texture (%) Sand	Silt	Clay
Clay Loam (West Jefferson, OH)	9.3	2.6	5.7	24.93	24	48	28
Clay (Casselton, ND)	28.6	5.6	6.4	41.80	22	38	40
Silty Clay Loam (Iowa)	25.8	3.9	7.3	31.83	10	56	34

Three treatments were employed: 1) ^{14}C semduramicin at a final concentration of 25 ppm in soil (5.5×10^6 DPM activity), 2) glucose (a combination of ^{14}C and unlabeled) at a final concentrations of 10 mg C/50 g soil (6.03×10^6 DPM activity), 3) untreated control. Each treatment was evaluated in triplicate for each of the 3 soils. A series of 27 incubation flasks, each containing 50 g of soil, were arranged in a system modified from Marinucci and Bartha (Apparatus for monitoring the mineralization of volatile ^{14}C-labelled compounds. *Appl. Environ. Microbiol.* **38**: 1020-1022) for trapping $^{14}CO_2$ and where appropriate, organic volatiles. Flasks were incubated in the dark at 22±3°C. The amount of radiolabeled carbon dioxide in the traps was measured periodically by liquid scintillation counting. All treatments were monitored for 66 days; two of the soils (Ohio and North Dakota) were monitored an additional 28 days (94 days total) because CO_2 production in the semduramicin treatment had not plateaued by day 66.

Material balance was computed for the glucose and semduramicin treatments at the end of the incubation period. Ohio and North Dakota soils containing semduramicin were extracted with organic solvents followed by base and acid hydrolysis to recover as much radioactivity from the soils as possible. Methylene chloride and ethylacetate extracts were analyzed by high pressure liquid chromatography (HPLC) and thin layer chromatography (TLC) to determine the relative proportions of unchanged drug and metabolites.

Summary of Results:

The semduramicin treatment demonstrated significant degradation to CO_2 with 50% biodegradation in approximately 94 and 42 days, respectively, for Ohio and Iowa soils, and 40% degradation for North Dakota soil in approximately 94 days.

The glucose treatment demonstrated rapid degradation to CO_2 in all three soils with 50% biodegradation achieved in approximately 7, 28, and 14 days, respectively, for Ohio, North Dakota and Iowa soils.

For semduramicin, the estimated (vs experimentally measured) time to 50% biodegradation for Ohio, Iowa, and North Dakota soils was 79, 42 and 104 days, respectively:

Soil Type	% Degraded			Days to 50% Biodegradation
	Day 14	Day 66	Day 94	
Clay Loam	1.00	----	51.95	79
Clay	1.76	----	40.30	104
Silty Clay Loam	1.41	63.38	----	42

The untreated control demonstrated no CO_2 evolution in any of the soils. No appreciable quantities of organic volatiles (~0.2%) were trapped during the test period from any treatment.

At the termination of the experiment, material balance achieved for the glucose treatment was 92% (66.5% degraded, 25.3% bound to soil), 93% (58.5% degraded, 34.7% bound to soil), and 94% (61.6% degraded, 31.7% bound to soil), respectively, for Ohio, North Dakota, and Iowa soils. For the semduramicin treatment, it was 95% (52.0% degraded, 10.8 extracted by methylene chloride and 32.1% bound to soil); 98% (40.3% degraded, 19.3% extracted by methylene chloride and 38.0% bound to soil), and 97% (63.5% degraded, 4.9% extracted by methylene chloride and 28.5% bound to soil), respectively, for the same three soils as follows:

^{14}C glucose	Clay Loam (West Jefferson, OH) (94 days)	Clay (Casselton, ND) (94 days)	Silty Clay Loam (Iowa) (66 days)
% degraded	66.46	58.55	61.61
Methylene chloride extract %	0.14	0.13	0.36
Bound to soil (%)	25.30	34.70	31.70
Mass balance	92±1	93±1.95	94±0.66
^{14}C semduramicin			
% degraded	51.95	40.30	63.38
Methylene chloride extract %	19.3	10.8	4.9
Bound to soil (%)	38.0	32.1	28.5
Mass balance	98±1.02	95±1.95	97±0.54

The Ohio and North Dakota soils in the semduramicin treatment were monitored 94 days for CO_2 evolution. At trial termination, these soils contained 48 and 60%, respectively, of the total applied radioactivity. Mutlisolvent extractions, followed by base and acid hydrolysis released 22% and 35% total applied radioactivity from the two respective soils. Methylene chloride extractions of the two soils contained an average of 11 and 19% of total applied radioactivity. Ethyl acetate extractions of the same soils contained an average of 6 and 12% total applied radioactivity. Both extractions were analyzed by HPLC to determine the relative proportion of unchanged drug and metabolites. Methylene chloride extracts contained three major components in Ohio clay loam and 3 to 5 components in North Dakota clay soil, with the largest of the major components being semduramicin. No single component accounted for more than 0.3% of the total applied radioactivity or more than 0.05 ppm in either soil. Ethyl acetate extracts contained 5 components each in Ohio and North Dakota soils. No single component accounted for more than 0.05% of the total applied radioactivity or more than 0.011 ppm in either soil.

Appendix c-8

Effect of Semduramicin Sodium on Soil Microbes.

Report Summary: EFFECT OF SEMDURAMICIN SODIUM ON SOIL MICROBES

Study Number: 2438-0189-6143-795

Test Species: Soil-dwelling microbes

Summary of Experimental Design:

The lowest concentrations of semduramicin sodium that will inhibit the growth of pure cultures of representative soil bacteria, ascomycetes, fungi, and blue-green algae were determined by the agar plate dilution technique. The following organisms were used:

Clostridium novyi, a free-living nitrogen-fixing bacterium
Nostoc, a blue-green alga
Bacillus stearothermophilus and *Flavobacterium meningosepticum*, soil bacteria
Trichoderma viride, an ascomycete
Penicillium italicum, a mold

Each of the above organisms was maintained in pure culture under conditions appropriate for the species. A preliminary range-finding study was conducted at widely spaced concentrations, approximately 1,000, 100, 10 and 1 ppm. The results were used to select a series of four closely spaced concentrations.

In the definitive test, semduramicin sodium was introduced into molten agar at concentrations of 0, 100, 125, 145, 167 and 186 ppm, except that concentrations of 0, 186, 205, 233, 240, and 256 ppm were used for *Penicillium italicum*. All concentrations were prepared in triplicate. Each agar preparation was then poured into a Petri dish, allowed to cool and solidify, inoculated with one of the organisms, and incubated at an appropriate temperature. When colony growth was well developed on the plates which did not contain any drug, the plates containing semduramicin sodium were examined visually for microbial growth. All three replicates were evaluated at each concentration, and if microbial growth was found to have been prevented in one or more replicate, that concentration was recorded as inhibitory (I); if growth (more than a single colony) was observed in all three replicates, the result was recorded as "growth" (G).

Summary of Results:

The recorded observations are shown in the following table:

Species	100mg/L	125 mg/L	145 mg/L	167 mg/L	186 mg/L	Blank Control	Solvent Control
Clostridium novyi	G	G	I	I	I	G	G
Bacillus stearothermophilus	G	G	G	I	I	G	G
Flavobacterium meningosepticum	G	G	G	I	I	G	G
Nostoc	G	I	I	I	I	G	G
Trichoderma viride	G	G	I	I	I	G	G

Species	186mg/L	205 mg/L	233 mg/L	240 mg/L	256 mg/L	Blank Control	Solvent Control
Penicillium italicum	G	G	G	I	I	G	G

These observations indicate that the lowest concentration which inhibits growth, the minimum inhibitory concentration (MIC), is:

Species	MIC (mg/kg)
Clostridium novyi	145
Bacillus stearothermophilus	167
Flavobacterium meningosepticum	167
Nostoc	125
Trichoderma viride	145
Penicillium italicum	240

Appendix c-9

Effect of Semduramicin Sodium on Seed Germination and Root Elongation

Report Summary: EFFECT OF SEMDURAMICIN SODIUM ON SEED GERMINATION AND ROOT ELONGATION

Study Number: 2438-1287-6117-600

Test Species: Six species of plant seeds

Summary of Experimental Design:

Seeds of the following species were used:

Monocotyledons: Lolium perenne L. - rye
Triticum aestivum L. - wheat
Zea mays L. - corn

Dicotyledons: Cucumis sativus L. - cucumber
Glycine max L. - soybean
Phaseolus vulgaris L. - pinto bean

After soaking in distilled water, the seeds were placed on two sheets of filter paper saturated with test solution and contained in a glass Petri dish, 50 seeds of a given species to a dish. Preliminary tests were conducted with each species using nominal concentrations of semduramicin sodium of 0.96, 9.6, 96 and 960 ppm. Based on the results of the preliminary test, the following measured concentrations were evaluated. The drug was dissolved in distilled water and added to six replicate dishes:

Corn:	0, 17, 34, 56 and 86 ppm
Cucumber:	0, 17, 34, 56 and 86 ppm
Pinto bean:	0, 6.3, 13, 16 and 31 ppm
Rye:	0, 4.0, 6.3, 13, and 31 ppm
Soybean (germination):	0, 0.36, 0.61, 0.93, and 2.2 ppm
Soybean (elongation):	0, 17, 34, 56 and 86 ppm
Wheat:	0, 4.0, 6.3, 16, and 31 ppm

The dishes were incubated at 30°C and 100% humidity for 3 to 4 days. Every day during the test, the number of seeds that had germinated was determined, and the seeds were examined for any morphological abnormalities such as discoloration, swelling or lesions. Upon termination of the exposure, the radicle (primary root) lengths of ten impartially selected, germinated seeds from each dish were measured.

Summary of Results:
For soybean, test concentrations between 0.36 and 2.2 ppm had no effect upon germination; however, because germination was reduced statistically in the preliminary test at 1 ppm, the lowest concentration evaluated, a NOEC was not established. In the preliminary test, root elongation was reduced statistically at 960 and 96 ppm but not at 9.6 or 0.96 ppm. In the definitive test, all concentrations tested (86-17 ppm) reduced root length statistically and a NOEC was not established.

For other species tested, the highest concentrations at which no adverse effects on germination, radicle length or appearance which were observed were as follows: for corn, 17 ppm; cucumber, 34 ppm; pinto bean, 6.3 ppm; rye, 13 ppm; and wheat, 6.3 ppm. Higher concentrations inhibited germination and/or root elongation.

No adverse effects were observed in any species on appearance such as discoloration, swelling or lesions. For all species except soybean, the lowest concentration at which seed germination or root elongation were inhibited (the LOEC) and the highest concentration that had no statistically significant effect as the exposed seeds as compared to controls (the NOEC) are summarized below:

	Germination		Root Elongation	
Species	NOEC[a]	LOEC[a]	NOEC[a]	LOEC[a]
Corn	17[b]	34	34	56
Cucumber	56	86	34	56
Pinto Bean	6.3[b]	13	6.3	13
Rye	13	31	13	31
Wheat	16	31	6.3	16
Soybean[c]				

[a]NOEC and LOEC shown are measured concentrations (ppm)
[b]Lowest concentration tested.
[c]NOEC and LOEC not established.

The following two tables show the effects of various concentrations of semduramicin on germination and on radicle length (root elongation):

Mean Number of Germinated Seeds and Percent Germination of Plant Seeds Exposed to Semduramicin Sodium in the Definitive Test.

Plant Species	Germination Time (days)		Germination Data				
Corn	4	Concentration (ppm):	0	17	34	56	86
		No. germinated seeds:	48±1	46±2	42±2[a]	40±4[a]	39±4[a]
		% germination:	95±2	93±4	84±4	81±7	78±8
Cucumber	4	Concentration (ppm):	0	17	34	56	86
		No. germinated seeds:	50±0	50±1	49±1	48±2	42±3[a]
		% germination:	100±0	99±1	98±2	97±3	85±5
Pinto Bean	3	Concentration (ppm):	0	6.3	13	16	31
		No. germinated seeds:	43±3	43±4	37±3[a]	27±4[a]	27±4[a]
		% germination:	86±6	86±7	73±5	54±8	54±8
Ryegrass	3	Concentration (ppm):	0	4.0	6.3	13	31
		No. germinated seeds:	47±2	46±2	46±1	47±1	32±7[a]
		% germination:	94±3	92±5	91±3	94±3	64±15
Soybean	3	Concentration (ppm):	0	0.36	0.61	0.93	2.2
		No. germinated seeds:	22±10	17±6	25±3	33±9	29±5
		% germination:	44±20	35±13	50±5	65±19	57±10
Wheat	3	Concentration (ppm):	0	4.0	6.3	16	31
		No. germinated seeds:	48±1	48±1	47±2	46±1	41±5[a]
		% germination:	96±1	96±2	94±4	93±2	82±11

[a]Statistically different (P≥0.05) from mean for the control.

Radicle Lengths (mm) of Six Plant Species After Exposure to Semduramicin in the Definitive Test.

	Measured Test Concentration (ppm)*				
Species	**Control**	**17**	**34**	**56**	**86**
Corn	29 (12)	28 (3)	26 (5)	18 (6)[a]	16 (4)[a]
Cucumber	22 (6)	15 (3)	17 (2)	12 (3)[a]	8 (2)[a]
	Control	**6.3**	**13**	**16**	**31**
Pinto Bean	31 (4)	29 (3)	19 (7)[a]	14 (3)[a]	13 (3)[a]
	Control	**4.0**	**6.3**	**13**	**31**
Rye	20 (1)	20 (2)	20 (2)	18 (4)	8 (2)[a]
	Control	**17**	**34**	**56**	**86**
Soybean	25 (8)	17 (3)[a]	10 (2)[a]	9 (2)[a]	6 (1)[a]
	Control	**4.0**	**6.3**	**16**	**31**
Wheat	47 (2)	43 (5)	40 (10)	35 (6)[a]	33 (3)[a]

*• Values shown are means for six replicates per test concentration with standard deviation in parentheses.

[a] Treatment data were significantly different ($P \geq 0.05$) from the control data.

Appendix c-10

Effect of Semduramicin Sodium on Seedling Growth

Report Summary: EFFECT OF SEMDURAMICIN SODIUM ON SEEDLING GROWTH

Study Number: 2438-0288-6116-620

Test Species: Six species of plant seedlings

Summary of Experimental Design:

Seedlings of the following species were used:

Monocotyledons: *Lolium perenne* L. - rye
Triticum aestivum L. - wheat
Zea mays L. - corn

Dicotyledons: *Cucumis sativus* L. - cucumber
Glycine max L. - soybean
Phaseolus vulgaris L. - pinto bean

Groups of five sprouted seedlings grown on-site from seed were transplanted into washed silica sand held in plastic containers and housed in an environmental chamber, where favorable conditions for growth were maintained. A nutrient solution containing semduramicin sodium, or nutrient solution devoid of drug, was added daily to each group of seedlings. Preliminary tests were conducted with each species using nominal concentrations of semduramicin sodium of 1.0, 10, 100, and 1000 ppm. Based on the results of the preliminary test, the following measured concentrations of semduramicin sodium were tested.

Corn:	0, 0.77, 2.2, 4.2, and 7.0 ppm
Cucumber:	0, 0.23, 0.31, 0.77, and 2.2 ppm
Pinto bean:	0, 0.77, 2.2, 4.2, and 7.0 ppm
Rye:	0, 0.31 0.77, 2.2, and 4.2 ppm
Soybean:	0, 0.23, 0.31, 0.77, and 2.2 ppm
Wheat:	0, 0.77, 2.2, 4.2 and 7.0 ppm

Each treatment was replicated 5 times. Seedling shoot lengths were measured on days 1, 3, 5, 7, 14, and 21. At test termination, dry weights of the shoots and roots were measured separately and any abnormal appearance was noted. The percent elongation of the shoots was calculated from the equation:

% elongation = (length of treated tissue-length of control) (x100) ÷ (length of control).

Summary of Definitive Test Results:

Corn: The highest concentration at which no significant effects on plant survival, root weight, shoot weight, shoot length, or appearance were noted was 2.2 ppm. At 4.2 ppm, the number of surviving seedlings was reduced and at 7.0 ppm, the average root weight was lower than in the seedlings that were not exposed to semduramicin sodium. Shoot weight and shoot length were not affected by the highest concentration tested, 7.0 ppm.

Cucumber: The highest concentration at which no significant effects were observed was 0.77 ppm. At 2.2 ppm, both shoot weight and shoot length were reduced. Survival and root weight were not affected by the highest level tested, 2.2 ppm.

Pinto Bean: The highest concentration at which no significant effects were observed was 0.77 ppm. At 2.2 ppm, root weight was reduced, and at 4.2 ppm, there were reductions in survival, shoot length and shoot weight.

Ryegrass: The lowest concentration at which an effect on root weight was still observed was 0.31 ppm. Survival, shoot length and shoot weight were reduced at 2.2 ppm.

Soybean: The highest concentration at which no significant effects were observed was 0.31 ppm. At 0.77 ppm, shoot length was reduced. Survival, root weight and shoot weight were not affected by the highest concentration tested, 2.2 ppm.

Wheat: The concentration at which an effect was still observed on root weight was 0.77 ppm. At 2.2 ppm, shoot weight and length were reduced and so was survival at 7.0 ppm. Survival was not affected at 2.2 ppm and shoot length and weight were not affected by 0.77 ppm.

The next three pages contain the following information:

- The lowest concentration that had a statistically significant effect on a particular parameter (the LOEC) and the highest concentration that had no statistically significant effect on the exposed plants as compared to the controls (the NOEC);

- The mean shoot length of the seedlings after various periods of exposure;

- A summary of percent mortality, mean shoot and root weight, and observed abnormalities in exposed seedlings.

LOEC and NOEC Values for All Species of Plants Exposed to Semduramicin.

Species		LOEC[a]	NOEC[a]
Corn			
	Mortality	4.2	2.2
	Root Weight	7.0[b]	4.2
	Shoot Weight	>7.0[b]	7.0[b]
	Shoot Length	>7.0[b]	7.0[b]
Cucumber			
	Mortality	>2.2[b]	2.2[b]
	Root Weight	>2.2[b]	2.2[b]
	Shoot Weight	2.2[b]	0.77
	Shoot Length	2.2[b]	0.77
Pinto Bean			
	Mortality	4.2	2.2
	Root Weight	2.2	0.77
	Shoot Weight	4.2	2.2
	Shoot Length	4.2	2.2
Ryegrass			
	Mortality	2.2	0.77
	Root Weight	0.31[c]	<0.31[c]
	Shoot Weight	2.2	0.77
	Shoot Length	2.2	0.77
Soybean			
	Mortality	>2.2[b]	2.2[b]
	Root Weight	>2.2[b]	2.2[b]
	Shoot Weight	>2.2[b]	2.2[b]
	Shoot Length	0.77	0.31
Wheat			
	Mortality	4.2	2.2
	Root Weight	0.77[c]	<0.77[c]
	Shoot Weight	2.2	0.77[c]
	Shoot Length	2.2	0.77[c]

[a]Based on test concentrations (mg/kg) measured prior to 0-hour.
[b]The highest measured concentration of UK-61,689-2 tested.
[c]The lowest measured concentration of UK-61,689-2 tested.

Mean Shoot Lengths of Seedlings Exposed to Semduramicin

Plant Species	Concentration (ppm)	Day/Shoot Length (cm) 1	3	5	7	14	21
Corn	0	11.5±2	19.0±2.1	23.9±3.0	28.6±4.8	38.5±4.8	43.8±5.1
	O(S)	9.6±1.2	15.6±3.2	20.9±4.6	25.2±6.3	34.0±8.3	38.9±9.8
	0.77	9.8±0.8	16.3±1.1	21.3±1.1	26.8±2.0	36.9±3.3	41.6±5.0
	2.2	8.6±0.8	12.7±0.7	17.4±1.1	23.3±3.1	34.3±4.2	39.2±2.6
	4.2	9.3±0.7	10.0±0.9	10.3±1.7	14.2±2.0	34.3±0.2	35.9±1.6
	7.0	9.3±1.5	9.8±1.6	11.3±5.0	15.1±7.3	26.0±5.7	27.2±4.0
Cucumber	0	4.1±0.5	4.4±0.4	4.9±0.5	5.1±0.4	6.4±0.5	10.2±0.6
	O(S)	4.1±0.5	4.5±0.6	4.8±0.6	5.4±0.6	6.2±0.5	9.4±0.6
	0.23	4.5±0.4	4.8±0.3	5.2±0.3	5.6±0.2	6.6±0.4	10.3±0.9
	0.31	4.3±0.4	4.7±0.5	5.2±0.5	5.7±0.5	6.5±0.3	10.1±0.6
	0.77	4.0±0.5	4.3±0.5	4.7±0.5	5.1±0.5	5.7±0.6	10.0±1.2
	2.2	4.3±0.4	4.4±0.5	4.7±0.5	4.9±0.6	5.5±0.5[a]	7.2±0.4
Pinto Bean	0	11.5±0.8	17.0±1.1	23.9±1.9	31.5±3.3	55.6±5.8	62.7±5.9
	O(S)	11.9±0.8	16.6±1.2	21.1±2.7	27.0±3.3	58.3±8.5	65.3±8.8
	0.77	9.6±1.4	15.0±0.9	19.4±1.4	25.1±1.4	53.9±4.7	39.0±5.1
	2.2	10.3±1.8	15.6±1.9	18.8±1.8	23.6±2.3	49.4±6.7	53.6±5.3
	4.2	9.2±1.6	11.6±1.6	14.9±2.4	18.4±3.6	31.2±10.8	36.0±8.8
	7.0	9.4±1.6	11.1±1.6	12.7±2.1	15.7±2.5	29.2±8.3	35.8±12.3
Ryegrass	0	5.3±0.7	7.8±0.9	9.2±1.3	11.6±1.5	21.1±1.5	29.7±2.5
	O(S)	4.3±0.5	6.7±0.6	8.0±0.5	11.3±0.7	20.8±1.6	27.3±1.6
	0.31	3.5±0.5	5.6±0.6	6.9±0.6	10.1±1.7	19.1±2.1	26.8±1.1
	0.77	3.3±0.5	5.4±0.8	6.4±0.9	9.0±1.1	17.2±1.1	25.2±1.0
	2.2	4.4±0.8	5.9±1.1	6.6±1.2	7.6±1.7	12.3±4.0	19.9±3.0[a]
	4.2	4.3±0.7	4.4±0.8	5.6±0.8	5.8±1.0	-----	-----
Soybean	0	8.3±0.8	12.3±0.5	17.2±0.4	22.0±0.7	41.7±1.6	65.0±4.7
	O(S)	9.4±0.8	13.1±1.6	18.2±1.1	22.8±1.3	41.0±1.9	57.3±4.1
	0.23	9.7±0.8	13.6±0.4	18.9±0.7	24.0±0.9	43.4±2.2	59.1±3.5
	0.31	8.7±1.5	12.0±1.8	16.8±2.1	22.6±1.5	40.2±2.7	57.1±2.2
	0.77	8.4±0.9	12.3±2.0	17.6±2.6	23.5±3.3	38.4±3.2	51.0±4.2[a]
	2.2	7.7±1.2	10.9±1.7	15.5±1.6	21.2±1.3	34.6±1.6	48.9±4.4[a]
Wheat	0	9.0±1.1	12.5±0.6	15.7±1.5	21.1±1.7	27.9±2.6	32.8±1.8
	O(S)	11.1±1.8	14.1±1.7	17.1±1.2	22.1±2.8	31.4±2.0	37.0±1.4
	0.77	10.2±1.4	13.7±1.6	16.4±0.5	21.5±0.7	29.9±2.1	34.9±1.7
	2.2	8.7±0.8	11.5±0.8	14.6±1.4	18.4±2.6	25.4±2.0	30.5±2.3[a]
	4.2	8.3±1.2	9.3±1.7	9.9±1.6	10.7±1.3	14.7±2.4	22.1±1.9[a]
	7.0	8.7±1.6	8.9±1.4	9.5±1.9	10.3±2.1	12.3±3.4	19.1±4.5[a]

[a]Mean is significantly different (P≥0.05) from the control.
O(S) is a solvent control.

Percent Survival, Mean Shoot and Root Weight, and Observed Abnormalities of Seedlings Exposed to Semduramicin

Plant Species	Concentration	Percent Mortality	Mean Dry Weight(mg) Root	Shoot	Observed Abnormalities
Corn	0	20.0	369.1 ±100.0	137.7±30.9	
	O(S)	24.0	376.7±195.6	102.1±44.8	
	0.77	8.0	208.1±67.0	111.0±21.0	
	3.2	28.0	165.8±48.6	100.1±15.7	
	4.2	92.0	468.3±14.3	75.7±29.0	wilted plants
	7.0	88.0	130.4±78.1[a]	77.4±40.5	wilted plants
Cucumber	0	0.0	174.6±106.9	368.4±40.0	
	O(S)	0.0	133.9±62.1	278.7±48.3	
	0.23	0.0	124.9±62.1	303.6±36.1	
	0.31	0.0	97.2±43.0	319.9±40.7	
	0.77	0.0	97.2±43.0	311.6±29.2	
	2.2	0.0	74.1±51.4	192.0±28.6[a]	Dried out, reduced size
Pinto Bean	0	4.0	901.9±603.7	530.8±38.8	
	O(S)	4.0	953.1±473.6	449.3±59.8	
	0.77	0.0	552.8±285.3	404.0±36.2	
	2.2	16.0	321.3±92.0[a]	384.2±52.8	
	4.2	44.0	364.0±151.1[a]	162.3±88.4[a]	yellow colored leaves,
	7.0	44.0	275.1±110.2[a]	216.1±78.9[a]	brown tipped leaves, wilted
Ryegrass	0	0.0	32.9±6.2	29.3±3.7	
	O(S)	0.0	20.5±8.4	20.6±2.1	
	0.31	0.0	7.7±3.1[a]	19.8±2.4	
	0.77	4.0	8.1±4.3[a]	17.1±2.6	yellow colored leaves,
	2.2	40.0	5.5±1.0[a]	12.7±2.9[a]	brown tipped leaves,
	4.2	100.0	---	---	wilted plants
Soybean	0	0.0	141.2±49.2	360.3±49.8	
	O(S)	4.0	82.8±44.1	248.3±21.9	
	0.23	0.0	83.1±33.5	237.7±28.9	
	0.31	0.0	74.0±14.5	278.7±14.3	None
	0.77	4.0	77.7±19.0	236.0±35.3	
	2.2	0.0	54.3±13.9	230.9±27.2	
Wheat	0	0.0	199.4±68.3	58.8±7.3	
	O(S)	0.0	223.7±55.6	68.9±11.2	
	0.77	0.0	74.1±12.4[a]	60.8±7.6	
	2.2	4.0	70.2±24.6[a]	43.0±3.1[a]	
	4.2	28.0	54.5±11.5[a]	24.2±3.4[a]	
	7.0	64.0	39.6±6.5[a]	17.0±6.7[a]	6/9 wilted

[a]Mean is significantly different (P≥0.05) from control.
O(S) is a solvent control.

Appendix c-11

Effect of Semduramicin Sodium on Algae

Report Summary: THE EFFECT OF SEMDURAMICIN SODIUM ON FRESHWATER ALGAE

Study Number: 2438-1287-6113-550

Test Species: *Selenastrum capricornutum*, a freshwater green alga

Summary of Experimental Design:

The test was conducted in 125 ml flasks, each containing 50 ml of Algal Assay Procedure (AAP) medium. The following average measured concentrations of semduramicin sodium were tested in triplicate: 150, 70, 39, 19, 10, and 0 (negative control) ppm. Each flask was inoculated with about 10^4 algal cells per ml and placed on a gyrotory shaking table in an environmental chamber. Light and temperature favorable to algal growth were maintained. At 24 hours and at each subsequent 48 hour interval, triplicate cell counts were conducted on each flask using a hemocytometer and a compound microscope. The test was continued until day 13 when cell density in all flasks increased by less than 5% per day.

Test endpoints were 1) cell density and 2) growth rate (μ)

1) Cell density = Number of Cells ÷ (Number of Microscopic Fields x Field Volume)

Field Volume = Volume of hemocytometer grid (0.1 x 0.1 x 0.01 cm)

2) growth rate (μ) was calculated using the formula:

$$\mu = \frac{\ln(X_2/X_1)}{t_2 - t_1}$$

where ln = natural logarithm, X_1 and X_2 are cell density measured at times t_1 and t_2 and μ is expressed in units of days $^{-1}$. The maximum growth rate ($^{\mu}$ max) for each culture vessel is the highest value for μ calculated for any 24 hour interval during the test.

From the observed values for maximum culture density and the calculated values for maximum growth rate, the highest test concentration that caused no significant growth inhibition or stimulation (No Observed Effect Limit, NOEL) and the lowest test concentration that caused significant inhibition (Minimum Inhibitory Concentration, MIC) were determined using one-way analysis of variance (Sokal and Rohl 1981) and Dunnett's Procedure (Dunnett 1955, 1964).

Summary of Results:

The concentration of semduramicin in AAP media was determined by HPLC analysis with post-column derivatization and found on day 13 to average 104% of the level found on day 0. As shown in Table I, growth of *Selenastrum capricornutum* was completely inhibited by concentration at at above 39 mg/L semduramicin. For culture density and for $^{\mu}$ max, Dunnett's Test indicated that the Minimum Inhibitory Concentration (MIC) of semduramicin was 19 mg/L and the No Observed Effect Limit (NOEL) was 10 mg/L.

Table I. Cell Density and Growth Rate of Algae Exposed to Semduramicin Sodium

	Observation Time (hours)								
	0	24	72	120	168	216	264	312	MAXIMUM
Control									
cell density	1.30	4.00	36.39	241.72	266.56	643.61	744.17	626.11	744.17
S.D.	0.00	0.75	8.82	38.82	32.26	30.10	21.03	42.45	21.03
Growth rate		1.334	1.100	0.942	0.051	0.448	0.076	0.089	1.363
S.D.		0.228	0.178	0.134	0.022	0.054	0.027	0.049	0.185
10 mg/L									
cell density	1.30	2.86	26.58	224.81	289.44	530.28	576.67	549.17	590.28
S.D.	0.00	0.19	1.34	47.96	18.33	84.60	62.07	50.26	48.29
Growth rate		0.945	1.115	1.049	0.134	0.302	0.046	-0.025	1.136
S.D.		0.79	0.046	0.100	0.090	0.105	0.026	0.055	0.024
19 mg/L									
cell density	1.30	2.39	11.28	65.97	149.16	393.06	413.33	390.56	430.28
S.D.	0.00	0.19	2.00	45.64	85.80	96.77	47.35	60.12	59.99
Growth rate		0.727	0.772	0.780	0.433	0.555	0.035	0.031	0.911
S.D.		0.099	0.127	0.322	0.076	0.260	0.076	0.058	0.129
39 mg/L									
cell density	1.30	2.50	0.94	0.64	0.31	0.00	0.00	0.00	2.50
S.D.	0.00	0.22	0.10	0.61	0.53	0.00	0.00	0.00	0.22
Growth rate		0.781	-0.487	-0.362	-0.187			0.781	
S.D.		0.108	0.089	0.563				0.108	
70 mg/L									
cell density	1.30	1.61	0.44	0.00	0.00	0.00	0.00	0.00	1.63
S.D.	0.00	0.32	0.05	0.00	0.00	0.00	0.00	0.00	0.29
Growth rate		0.241	-0.639						0.241
S.D.		0.251	0.096						0.251
150 mg/L									
cell density	1.30	1.36	0.81	0.00	0.00	0.00	0.00	0.00	1.52
S.D.	0.00	0.54	0.34	0.00	0.00	0.00	0.00	0.00	0.35
Growth rate			-0.012	-0.264					0.080
S.D.			0.501	0.201					0.363

Appendix c-12

Acute Toxicity Study with Semduramicin Sodium in Daphnia

Report Summary: ACUTE TOXICITY STUDY WITH SEMDURAMICIN SODIUM IN DAPHNIDS

Study Number: 2438-1287-6113-110

Test Species: Water Flea (*Daphnia magna*)

Summary of Experimental Design:

The test was conducted in 250 ml glass beakers, each containing 200 ml of test solution. The following measured concentrations of semduramicin sodium were present in four beakers each: 48, 31, 19, 11, 6.2, and 0 (negative control) ppm. Daphnids which were no more than 24 hours old were obtained from a laboratory culture and distributed impartially, five to a beaker. Test solution temperatures, oxygen content and pH were monitored and when necessary, adjusted. At 0, 24, and 48 hours after initiation, the number of immobilized daphnids in each beaker and any other signs of toxicity were recorded.

Summary of Results: The test concentrations remained stable during the experiment. A mean of 85% of the daphnids exposed to 48 ppm and 20% of those exposed to 31 ppm were found to be immobile, and hence presumed dead, after 48 hours (see Table). From the test data, the concentration of semduramicin sodium which causes immobilization in 50% of exposed daphnids (the EC_{50}) was estimated by standard statistical procedures to be as follows:

	EC_{50}	95% Confidence Interval
24-hour	42	38-49
48-hour	38	31-48

The no-observed effect concentration (NOEC) through 48 hours was 19 mg/L. The NOEC is the highest concentration of the test material that has no statistically significant adverse effect on the exposed organisms as compared to the controls (15). One out of 20 daphnids (5%) was immobilized at 19 ppm and at 11 ppm; also, one daphnid was caught on particulate matter at 11 ppm. However, any apparent difference between a test group and the controls would have to be at least 15% to be statistically significant. Furthermore, although immobilization and entrapment on particulate matter did not occur in the control group in this particular test, they are observed randomly at a low incidence in control groups and are not unusual for daphnids. Therefore, the observations at 19 and 11 ppm are not considered drug-related. No other physical or behavioral abnormalities, such as flared carapace, were observed.

Concentrations Tested, Corresponding Cumulative Percent of Immobilized Organisms and Observations During the 48-hour Static Exposure of Daphnids (*Daphnia magna*) to UK 61,689-2 (N=20).

Mean Measured Concentration	Cumulative Number of Immobilized Organisms[a]									
	24-hour					48-hour				
(mg/L)	A	B	C	D	Mean	A	B	C	D	Mean
48	40	60[b]	80	100	70	60[d]	80[d]	100	100	85
	(2)	(3)	(4)	(5)	(4)	(3)	(4)	(5)	(5)	(4)
31	20	0	20	0	10	40	0[e]	20	20	20
	(1)	(0)	(1)	(0)	(1)	(2)	(0)	(1)	(1)	(1)
19	0	0	0	0	0	0	0	0	20	5
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
11	0	0[c]	0	0	0	0	0	0	20	5
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
6.2	0	0	0	0	0	0	0	0	0	0
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Control	0	0	0	0	0	0	0	0	0	0
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

[a]Cumulative percent mortalities are listed with the corresponding number of dead organisms in parentheses.
[b]One of the surviving daphnids was lethargic and caught on particulate matter.
[c]One of the surviving daphnids was caught on particulate matter.
[d]One of the surviving daphnids was lethargic.
[e]Two of the surviving daphnids were lethargic.

Appendix c-13

Acute Toxicity Study with Semduramicin Sodium in Bluegill

Report Summary: ACUTE TOXICITY STUDY WITH SEMDURAMICIN SODIUM IN BLUEGILL

Study Number: 2438-0188-6113-100

Test Species: Bluegill (*Lepomis macrochirus*)

Summary of Experimental Design:

Groups of ten bluegill were selected impartially from a population which had been maintained in a common holding tank and ranged from 0.21 to 1.56 g in weight. Each group was placed in 14 liters of test solution contained in an 18.9 liter glass aquarium. Duplicate groups were exposed for 96 hours to mean measured concentrations of 100, 62, 37, 22, 13, 7.6, and 0 (negative control) ppm of semduramicin sodium. Test solution temperatures, oxygen content, and pH were monitored and when necessary, adjusted. At 0, 24, 48, 72 and 96 hours after initiation, mortalities were recorded, dead fish were removed, and surviving fish were observed for signs of toxicity.

Summary of Results: The test concentrations remained stable throughout the experiment. Mortalities were observed at test concentrations of 22 ppm and above (see Table). From the test data, the concentration of semduramicin sodium in the test solution which causes 50% mortality in exposed bluegill (the LC_{50}) was calculated by standard statistical methods to be as follows:

	LC_{50}	95% Confidence Interval
24-hour	>100	-- - --
48-hour	40	31 - 51
72-hour	38	31 - 47
96-hour	38	31 - 47

No mortalities and no physical or behavioral abnormalities, such as lethargy, loss of equilibrium, or darkened pigmentation were observed in bluegill exposed to 13 or 7.6 ppm.

Concentrations Tested, Corresponding Cumulative Percent Mortalities and Observations Made During the 96-hour Static Exposure of Bluegill (*Lepomis macrochirus*) to UK 61,689-2 (N=20).

Mean Measured Concentration (mg/L)	Cumulative Mortality (%)[a]			
	24-hour	48-hour	72-hour	96-hour
100	45(9)[b]	75(15)	85(17)	85(17)[d]
62	15(3)	60(12)	60(12)	60(12)
37	45(9)[b]	50(10)	50(10)	50(10)
22	45(9)[b]	50(10)	50(10)	50(10)
13	0(0)	0(0)	0(0)	0(0)
7.6	0(0)	0(0)	0(0)	0(0)
Control	0(0)[c]	5(1)	5(1)	5(1)

[a]cumulative percent mortalities are listed with the corresponding number of dead organisms in parentheses.

[b]One of the surviving fish exhibited a complete loss of equilibrium.

[c]One of the surviving fish was at the surface of the test solution.

[d]One of the surviving fish exhibited darkened pigmentation.

Appendix c-14

Acute Toxicity Study with Semduramicin Sodium in Rainbow Trout

Report Summary: ACUTE TOXICITY STUDY WITH SEMDURAMICIN SODIUM IN RAINBOW TROUT

Study Number: 2438-0188-6113-103

Test Species: Rainbow trout (*Onchorhynchus mykiss*)

Summary of Experimental Design:

Groups of ten trout were selected impartially from a population which had been maintained in a common holding tank and ranged from 0.39 g to 1.58 g in weight. Each group was placed in 14 liters of test solution contained in an 18.9 liter glass aquarium. Duplicate groups were exposed for 96 hours to mean measured concentrations of 50, 30, 18, 11, 6.4 and 0 (negative control) ppm of semduramicin sodium. Test solution temperatures, oxygen content, and pH were monitored and when necessary, adjusted. At 0, 24, 48, 72 and 96 hours after initiation, mortalities were recorded, dead fish were removed, and surviving fish were observed for signs of toxicity.

Summary of Results: The test concentrations remained stable throughout the experiment. A significant incidence of mortality was observed at test concentrations of 30 and 50 ppm; surviving fish exhibited loss of equilibrium, lethargy, and/or darkened pigmentation (see Table). From the test data, the concentration of semduramicin sodium in the test solution which causes 50% mortality in exposed rainbow trout (the LC_{50}) was calculated by standard statistical methods to be as follows:

	LC_{50}	95% Confidence Interval
24-hour	43	---
48-hour	39	30-50
72-hour	33	18-50
96-hour	32	18-50

The no-observed effect concentration (NOEC) through 96 hours was 11 ppm. The NOEC is the highest concentration of the test material that has no statistically significant adverse effect on the exposed organisms as compared to controls (15). At 18 ppm, there was no mortality, but several fish exhibited darkened pigmentation. One out of 20 trout (5%) exposed to 11 ppm died and one exposed to 6.5 ppm also showed abnormal pigmentation. However, the difference between a test group and the controls would have to be at least 15% to be statistically significant. Furthermore, although no deaths or abnormal pigmentation occurred in the control group in this particular study, they occur randomly at a low incidence in controls (up to 10% is considered acceptable) and are not unusual for trout. Therefore, the observations at 11 and 6.4 are not considered drug-related. No other physical or behavioral abnormalities, such as loss of equilibrium or lethargy, were observed at 11 or 6.4 ppm.

Concentrations Tested, Corresponding Cumulative Percent Mortalities and Observations Made During the 96-hour Static Exposure of Rainbow Trout (*Onchorhynchus mykiss*) to UK 61,689-2 (N=20).

Mean Measured Concentration (mg/L)	Cumulative Mortality (%)[a]			
	24-hour	48-hour	72-hour	96-hour
50	70(14)[b]	85(17)[be]	90(18)[be]	95(19)[i]
30	5(1)[cd]	15(3)[cf]	40(8)[bh]	40(8)[bf]
18	0(0)	0(0)[g]	0(0)[g]	0(0)[cf]
11	5(1)	5(1)	5(1)	5(1)
6.4	0(0)	0(0)	0(0)	0(0)[g]
Control	0(0)	0(0)	0(0)	0(0)

[a]Cumulative percent mortalities are listed with the corresponding number of dead organisms in parentheses.

[b]All of the surviving fish exhibited darkened pigmentation.

[c]Several of the surviving fish exhibited darkened pigmentation.

[d]Two of the surviving fish exhibited a complete loss equilibrium.

[e]all of the surviving fish were lethargic.

[f]One of the surviving fish exhibited a complete loss of equilibrium.

[g]One of the surviving fish exhibited darkened pigmentation.

[h]One of the surviving fish exhibited a partial loss of equilibrium.

[i]All of the surviving fish exhibited a partial loss of equilibrium.

Appendix c-15

Acute Dermal and Ocular Irritation Studies with Semduramicin Sodium in Rabbits

Report Summary: ACUTE DERMAL AND OCULAR IRRITATION STUDIES WITH SEMDURAMICIN SODIUM IN RABBITS

Study Number: 88-564-09

Test Species: Albino rabbit (New Zealand White)

Summary of Experimental Design:

1) Dermal Irritation: One male and two female rabbits were used. Their bodyweights ranged from 3.35 to 3.66 kg. A dose of 0.5 gram of semduramicin sodium was applied to one intact and one abraded site on the back of each rabbit and was held in continuous contact with the skin under an occlusive patch for 24 hours. Each test site measured approximately two inches square. During the dosing procedure, both the compound and the skin were thoroughly wetted with distilled water until an aqueous paste of the compound was formed. The total dose of 1 gram applied to each animal was equivalent to a dose of 273 to 299 mg/kg of semduramicin sodium. All rabbits were observed for 7 days after dosing.

2) Ocular Irritation: One male and two female rabbits were used. Their bodyweights ranged from 3.66 to 3.97 kg. A dose of 21.5 mg semduramicin sodium, equivalent to the 0.1 ml volume of solid specified in the procedure, was introduced into the conjunctival sac of the left eye. The treated eye of each rabbit was not rinsed after dosing. The animals were observed for 7 days. On the day of dosing (day 0), the eyes were evaluated with minimal manipulation and without the use of fluorescein.

Skin reactions and ocular changes were evaluated visually according to the standard Draize scoring system, in which a score of zero denotes no effect and higher scores denote increasingly severe reactions. A Primary Irritation Score for skin was calculated as the sum of the mean erythema scores at 24 and 72 hours, divided by 4.

Summary of Results:

1) Only mild dermal irritation was observed. Following a 24-hour exposure to the compound, the skin at all intact sites appeared normal, and no gross tissue changes were observed at any of these sites during the remainder of the 7-day observation period.

   At 24 hours, well-defined (value of 2) erythema, but no edema, was apparent at each of the abraded sites. The erythema was confined primarily to an area approximately 2 mm to either side of the abrasion lines. Also, at 24 hours, the skin along the abrasion marks was slightly separated and appeared reddish or golden-brown. By 48 hours post dose, dry, crusty (scab-like) tissue was apparent along the abrasions.

   The erythema at each of the abraded sites subsided completely within 4 or 5 days of dosing. Sloughing on the dry tissue along the abrasions occurred, but at the time of sacrifice on day 7, there was still some scab-like tissue along the lines of abrasion at each site. (Erythema and subsequent scab formation along and confined to the abrasion lines are not uncommon or unexpected findings following abrasion of rabbit skin.)

The mean values obtained from the individual scores were as follows:

Condition of skin	Time after application (hours)	Mean value of score Erythema	Edema
Intact	all observations	0	0
Abraded	24	2.00	0
	48	2.00	0
	72	1.67	0
	96	0.67	0
	120-168	0	0

The Primary Irritation Score calculated from the data is 0.92. (The maximum possible score would be 8).

All rabbits were alert and active throughout the test period, and there were no obvious clinical signs of toxicity. However, each animal exhibited decreased food consumption on two or three days at various times during the study and weighed slightly less at the time of sacrifice than it did prior to dosing.

These results indicate that semduramicin sodium is not a primary skin irritant.

2) Immediately after dosing, each rabbit held its eye closed for only a few seconds, and within one minute, two animals were briefly rubbing the treated eye; however, none of the rabbits exhibited signs of obvious pain or discomfort. Within 1 to 5 hours of dosing, slight reddening of the conjunctivae, slight chemosis, and/or slight discharge were apparent. In addition, circumcorneal injection was noted in each animal, and a small, localized area of iritis was observed in one of the three rabbits. By 24 hours after dosing and throughout the remainder of the study, the treated eye of each rabbit appeared normal.

The mean values obtained from the individual scores are summarized below.

Time After Application (Hours)	Mean Scores Cornea	Iris	Conjunctivae	Total
1	0	0	2.00	2.00
3	0	1.67	3.33	5.00
5	0	1.67	3.33	5.00
24-168	0	0	0	0
Maximum possible score	80	10	20	110

All rabbits were asymptomatic throughout the 7-day test period, and they gained weight.

The results of this test indicate that semduramicin sodium is not an ocular irritant.