ENVIRONMENTAL ASSESSMENT FOR THE USE OF PULMOTIL® TYPE A MEDICATED ARTICLE IN SWINE

Elanco Animal Health
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285

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1. **DATE** April 1996

2. **APPLICANT** Elanco Animal Health
A Division of Eli Lilly and Company

3. **ADDRESS** Lilly Corporate Center
Indianapolis, Indiana 46285

4. DESCRIPTION OF THE PROPOSED ACTION

A new animal drug approval has been requested for the use of Pulmotil® Type A Medicated Article in the feed of swine. Tilmicosin is the active ingredient in Pulmotil. Between 200 and 400 ppm (181.8 to 363.6 g/ton) of tilmicosin will be used for 21 days in the feed of swine for control of bacterial pneumonia. Approval of this new animal drug would authorize Dista Products Limited (Fleming Road, Speke, Liverpool L24 9LN), a production facility of Eli Lilly and Company in England, to formulate and package Pulmotil for sale within the United States. Production of the bulk active ingredient will be done at the facilities of Eli Lilly S.A. (Dunderrow, Kinsale, Ireland).

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

a. The environment adjacent to the manufacturing plant and the formulating and packaging plant.

b. The environment adjacent to facilities which mix Pulmotil with feed.

c. Swine facilities where Pulmotil is used and residue may be found in animal waste.

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

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

5. IDENTIFICATION OF THE CHEMICAL SUBSTANCE

A. PULMOTIL® TYPE A MEDICATED ARTICLE

Pulmotil will be incorporated into the complete feed used for swine. Twenty percent of Pulmotil will be the active ingredient, tilmicosin. Tilmicosin is a semi-synthetic macrolide antibiotic that is effective against *Pasteurella sp.* and other microorganisms (Ose, 1987). Pulmotil also contains ground corn cobs.

B. TILMICOSIN

Tilmicosin is prepared from desmycosin by reductive amination of the C-20 aldehyde with a mixture of cis and trans-3,5-dimethylpiperidine. Desmycosin is derived from tylosin phosphate concentrate by mild acid hydrolysis. The bulk drug is produced as an aqueous solution containing at least 20% tilmicosin. Characterization of a dry lot of tilmicosin indicated this technical material was 88.3% pure and was a mixture of a cis isomer and epimer and trans isomers.

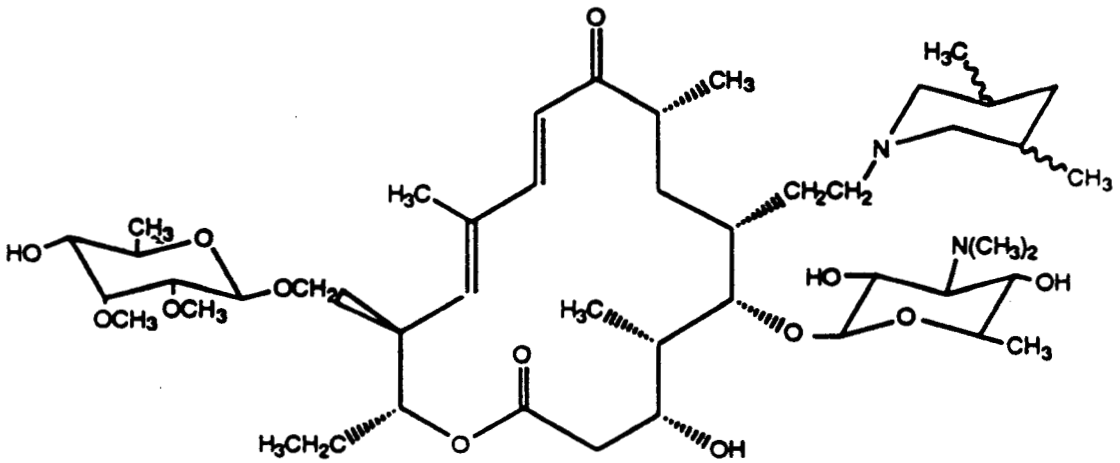
Chemical Name (Tilmicosin): 20-Deoxo-20-(3,5-dimethylpiperidin-1-yl)-desmycosin

CAS Registry Number: 108050-54-0

Molecular Formula: $C_{46}H_{80}N_2O_{13}$

Molecular Weight: 869.15

Structural Formula:



Characterization of Technical Material:

75.7%	Cis isomer and epimer of tilmicosin
12.7%	Trans isomer of tilmicosin
2.85%	Residual desmycosin
0.99%	20-dihydro-desmycosin
0.6%	Incomplete dimer of tilmicosin with only one 3,5-dimethylpiperidine ring and with only one methyl group on one mycaminose sugar.
0.46%	Tilmicosin without the mycaminose ring structure
0.36%	Tilmicosin without a methyl group on the mycinose ring
1.8%	Fifteen unknown impurities with 0.05 to 0.30% of the total chromatographic area
3.3%	Anhydrous dibasic sodium phosphate (Na_2HPO_4)
0.6%	Water

Solubility (Appendix A):

Water (25°C)	pH 9	7.7 mg/L
	pH 7	566 mg/L
	pH 5	Extremely viscous solution due to high solubility

Melting Point: 107°C to 112°C

UV Absorption: Peak absorption occurs at 283 nm in ethanol

Vapor Pressure (Appendix B): Tilmicosin is a non-volatile solid. Thermogravimetric analysis indicates a 1.6% weight loss from about 23°C to 129°C. This loss is probably due to volatilization of water and other minor impurities. At about 167°C another loss begins resulting in a continuous loss through decomposition.

n-Octanol/Water Partition Coefficient (Appendix C): The n-octanol/water partition coefficients for tilmicosin were determined to be <10 at pH levels 5 and 7, and 376 at pH 9.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCES FROM THE MANUFACTURING SITES

1. LOCATION OF FACILITIES USED FOR MANUFACTURING, FORMULATING, AND PACKAGING

The processes for manufacturing tilmicosin, the operations for formulating and packaging Pulmotil, and pollution control practices at the corresponding facilities are designed and constructed to result in minimal environmental impact. Production of tilmicosin will occur at production facilities of Eli Lilly S.A. (Dunderrow, Kinsale, Ireland). Pulmotil will be manufactured at the facilities of Dista Products Limited (Fleming Road, Speke, Liverpool L24 9LN) in England for Eli Lilly and Company. These facilities will effectively contain and control the liquid, solid, or gaseous pollutants from the production of tilmicosin or manufacture of Pulmotil.

2. ENVIRONMENTAL REGULATORY REQUIREMENTS

Treatment, storage, and disposal practices for the Kinsale facility are defined by the regulations administered by the Irish E.P.A., by the Cork County Council and, in other instances, by the Cork Corporation. The Environmental Protection Agency in Ireland issued an Integrated Pollution Control license to cover all discharges from the production facility in Ireland (Appendix O).

Treatment, storage, and disposal practices for the Dista facilities are defined by the regulations administered by the North West Water PLC, the Merseyside Waste Disposal Authority, and the Metropolitan Borough of Knowsley. The Metropolitan Borough of Knowsley grants a license allowing construction of facilities under the authority of the Town and Country Planning Act of 1990 and issues a license stipulating the allowable air emissions under the Environmental Protection Act of 1990 Part 1. The North West Water PLC issues a Consent to Discharge license under the Public Health Acts of 1937 and 1961. and the Water Act of 1973. Four consents to discharge trade effluent from the waste

treatment tanks have been provided by the North West Water PLC. These consents contain provisions which limit the total COD discharged in any 24-hour period, the total flow in any 24-hour period, the pH range of the effluent, and the concentrations of various metals. Some classes of substances are restricted or prohibited in the discharge. North West Water PLC monitors compliance with the consent conditions normally on a weekly basis and retains the right to prosecute under the Control of Pollution Act of 1974 for any breach of compliance. Only negligible amounts of COD and suspended solids will be discharged from the floor washing operation at the formulating and packaging facility. The Merseyside Waste Disposal Authority issues a Waste Disposal Facility license under the Control of Pollution Act of 1974. Permits related to formulation and packaging of tilmicosin obtained from regulatory agencies in England for the discharge of wastewater; the treatment, storage, and disposal of materials, and air emissions are listed in Appendix O.

3. PROCESS CHEMICALS AND MATERIALS USED IN MANUFACTURING

Desmycosin will be produced by mild acid hydrolysis of tylosin phosphate. Tilmicosin will be derived from an amination process where 3,5-dimethylpiperidine is added to desmycosin. Pulmotil will be manufactured by using a controlled spraying process that attaches the active ingredient via a water carrier to minigranules (ground corn cobs). A list of materials that will be used, consumed, and discharged in the process to manufacture tilmicosin is in a proprietary appendix.

4. WASTE STREAM TREATMENT, CONTROL, AND HANDLING

a. Wastes from Manufacturing at Kinsale Facility

Aqueous waste streams from processes, tank and equipment washings, and floor washings will be generated from the manufacture of tilmicosin. These aqueous wastes will be pumped to the on-site biotreatment balance tank prior to treatment through a decant

vessel, which is designed to remove traces of nonmiscible solvents that may get washed into the aqueous waste streams.

All aqueous waste streams received by the treatment plant will be treated in an activated sludge biotreatment system where organic carbonaeous material can be broken down by microorganisms to yield endproducts of carbon dioxide and water, plus new cellular material (sludge). All off-gases from the treatment plant will be collected from the roofed tanks and will be routed to a fume incinerator.

The activated sludge system consists of a 1,000 cubic meter holding tank where all incoming wastes will be jet mixed and pH adjustment will be carried out. From this tank, the wastewater will be forwarded to the biotreatment tanks at a rate based on the characteristics of the waste (average throughput is estimated to be 300 to 500 cubic meters per day). The wastewater will be forwarded through a tank with pH adjustment to a range of 6.5 to 8.0. If the pH is outside control limits, the forward feeding of wastes is controlled and the system recirculates waste streams until pH is within the control limits.

The activated sludge tanks consist of two tanks with a total usable capacity of 1,000 cubic meters. In these tanks, the mass of the microorganisms will be kept in suspension and will be supplied with oxygen by means of a jet aeration system. The air will be supplied by three 100 horsepower blowers (one on standby). In-line dissolved oxygen monitoring will be conducted to facilitate air control to the system to ensure that adequate residual dissolved oxygen is mainatined.

Effluent will be routed to clarifiers where biomass in suspension will be allowed to settle. The supernatant from the clarifiers will overflow to final holding tanks before discharge. The settled biomass (sludge) will be returned to the activated sludge system for further treatment. If excess sludge is present in the system, sludge holding tanks will be used to stabilize the material prior to dewatering with a separator. Solids produced from the treatment plant will be taken off site to an approved facility. Supernatant from the dewatering process will go to polishing clarifiers.

The facility wet well will collect the effluents from the clarifier, sludge dewatering, and the thermal oxidizer scrubber. The thermal oxidizer wastewater will contain inorganic salts and fine particulate materials. This wastewater will be passed through clarifiers and a sump before discharge to cooling ponds, which will feed the facility wet well. Before dischrage to the cooling ponds, approximately 70% of the effluent will be recycled back to the thermal oxidizer units for reuse.

The final effluent will be pumped through a 5 mile pipeline which will transport it to the outer Kinsale harbor. The pipeline extends approximately 1000 meters out to sea. The effluent will be discharged through a diffuser. Based on a survey of tidal movement and mixing rates in the general harbor area, the effluent will be diluted and have no measurable impact on the receiving waters.

Numerous samples will be taken within the treatment plant and from the final effluent, and assayed on-site. The final effluent will be assayed for the emission limit values as stipulated in the discharge license. The final effluent will be assayed for biocehmical oxygen demand (BOD), chemical oxygen demand (COD), pH, total suspended soilds (TSS), ammonia as nitrogen, phenol and cyanide. Parameters that will be measured across the treatment system include the Settled Sludge Volume Index (SSVI), the Food to Microorganism (F/M) ratio, and the percentage of COD reduction.

The manufacturing of tilmicosin requires use of 5 chemicals on the OSHA Air Contaminants List (amyl acetate, sodium hydroxide, formic acid, phosphoric acid, and sulfuric acid). Any volatile organic emissions will be incinerated.

The facility will utilize a fume oxidizer, which is a regenerative thermal oxidizer unit. It will be a tertiary treatment device operated at about 850 °C and used to incinerate fumes and to control odor. Process and scrubber vents will be ducted from the production buildings and will be routed to the fume oxidizer before discharge to the atmosphere. Within the unit, volatile organic compounds, other hydrocarbons and odor-causing constiuents will be converted to carbon dioxide and water vapor.

The facility will employ the use of one liquid thermal oxidizer and one solids incinerator onsite to treat solvent and solid waste generated by the production processes. Both units will be down-fired incinerators with vertical combustion chambers operated at 1,000 °C. The units will have a minimum residence time of 1.5 seconds. The combustion gases leaving the chambers will be quench cooled before being directed to the gas cleaning plant. The liquid thermal oxidizer will also have two stages comprised of a condenser/adsorber for acid gas removal and a hydrosonic scrubber for particulate and droplet removal. The cooled and cleaned gases form the liquid thermal oxidizer will be combined with the fume oxidizer discharge.

Both units will be computer controlled. Any deviations outside the acceptable limits will be alarmed. If an alarm on a unit is unresolved, an automatic shutdown of the unit will occur. The computer ensures that waste will only be burned when the units are running under optimum conditions.

Two types of liquid waste will be fed into the units. Primary waste will be comprised of flammable solvent and waste material. Secondary waste will be comprised mainly of water with small amounts of organics. The wastes will be stored in tanks for characterization before being fed into the units. Diesel fuel will be used to heat the units and to maintain combustion temperature if there is insufficient energy available from the wastes.

Continuous monitoring of oxygen, water vapor, carbon monoxide, total organic carbon and hydrogen chloride levels will be carried out on the stack gas. The units will also be monitored for hydrogen fluoride, particulates, sulfur dioxide, and nitrogen dioxide on a quarterly basis. Performance tests indicate a destruction removal efficiency or greater than 99.99%.

In addition, the solids unit will be provided with an inclined rotary kiln which will be used to burn soild waste (such as contaminated packaging, fiber drums, etc.) from the site.

Soild waste will automatically be loaded using a ram feeder. The combustion gas will be routed through the afterburner of the soilds unit.

The final product from this bulk manufacturing facility will be a 20 percent slurry of tilmicosin in water. This product form eliminates the need for concern about particulate air emissions of tilmicosin that might have been present if the final product were a dry bulk material.

b. Wastes from Formulating and Packaging at Dista Facility

At Dista, a new state-of-the-art facility has recently been constructed. The facility has isolation rooms, localized venting, and devices to further reduce the emissions or discharge of wastes during formulation and packaging of Pulmotil. All process air will pass through high performance particle (HEPA-like) filters and dust collectors, which will be packaged with solids, particulates, and dust for approved disposal. Where appropriate, air filtration systems are designed such that they are equipped with multiple filter systems. The HEPA-like filter systems that will be used throughout this facility will have removal efficiencies of 95 percent or more for particulates of 0.7 micron in size or larger.

These facilities will include corn grit silos equipped with filters to minimize particulate emissions. Corn grit from these silos will transfer to hopper systems that will feed wet premixers. At this point, a solution of tilmicosin will be added to the ground corn cobs. This material from the wet premixers will be transferred to a fluid bed dryer that will feed nitrogen blanketed blenders. An auger weigher will receive the product from the blenders and will feed a bagging machine. The bagging machine will be composed of a bag feed system that will use flat bags fed from laminate reels, and will employ local exhaust ventilation systems to fill the bags with little or no displacement of the final product. The filled bags will pass through a conveyor system that will leak test the bag, check the bag weight, and transfer the bag to a palletizing robot which will load the bags into boxes. The palleted bags of Pulmotil will be stretch wrapped on a rotating table and

then will be stored in the warehouse section of the packaging building. Facility engineering controls, use of wet bulk material and corn grits in the mixing process, and bags with little or no volumetric displacement will minimize the potential for emission of aerosolized particles from the formulation and packaging of Pulmotil. Authorization for processes at this contained fill/finish facility has been issued from the Metropolitan Borough of Knowsley (Appendix O).

Nitrogen will be emitted to the atmosphere from the packaging and formulating process steps. The average emission rate will be about 4,000 pounds per hour during the production period for Pulmotil, which may last for about one quarter during a year. This amount of nitrogen will be required due to the type of containment operations necessary to control the atmospheric conditions inside the formulating and packaging equipment. No specific permit requirement is necessary for this discharge of nitrogen, although authorization for the processes conducted at the facility have been approved by the Metropolitan Borough of Knowsley. The discharge point for release of the nitrogen into the atmosphere is well away from any point that would result in exposure to humans. Once released, this nitrogen becomes part of the large fraction of nitrogen that naturally exists in the atmosphere.

Packaging materials and nonrecyclable wastes from the formulation and packaging areas will be disposed of at an approved off-site facility.

Liquid wastes (i.e., floor washings) that may be used in the area will be collected into effluent storage tanks where the material will be checked before being pumped to a tanker for disposal at an approved facility or discharged to the sewer. If the liquid waste from the storage tank is discharged to the sewer, only trace amounts of tilmicosin will appear in the discharge water (i.e., < 1 ppm). Up to 125 gallons of this water could be discharged in a 15-minute period with the rest of the waste water from the entire site, which is discharged at a rate of approximately 1.2 million gallons/day. If this discharge occurred,

a maximum concentration of 0.01 ppm ((125 gal/12500 gal/15 min) x 1 ppm) could be found in waste water before dilution in receiving water.

5. COMPLIANCE WITH ENVIRONMENTAL REGULATORY REQUIREMENTS

All production, formulation, and packaging facilities will comply with applicable regulations concerning emission control and waste treatment. Since it is not the practice of the regulatory authorities to issue letters of compliance, letters from Eli Lilly S.A. and Dista Products Limited confirming the intent to comply with necessary environmental permits, the intent to comply with relevant environmental regulations, and the intent to comply with good manufacturing practices are provided in Appendix P.

B. INTRODUCTION OF SUBSTANCE FROM THE FEED MIXING LOCATIONS

Feed mixing will be done by commercial feed vendors and by swine finishing operations. Commercial feed vendors are required to meet current USDA and FDA approved Good Manufacturing Practices for feed additives. Based on the required manufacturing controls for feed inventory accountability, the potential for release of tilmicosin into the environment from feed mixing locations would be low.

An exposure monitoring study was conducted in a feed mill at Greenfield, Indiana with the premix formulation containing corn cob grits and 20% tilmicosin (Appendix Q). Fifteen batches of medicated feed were prepared from the premix and contained 400 ppm of tilmicosin. Personal and area air samples were collected during premix weighing and feed bagging operations. Dust respirators were worn by personnel during this monitoring study. Concentrations of tilmicosin ranging from <1 to 4.2 $\mu g/m^3$, with an average of 2.5 $\mu g/m^3$, were found in personal full-shift samplers. Collection of short-term samples by personal samplers during bagging operations resulted in concentrations ranging from <2.2 to 9.7 $\mu g/m^3$, with an average level of 4.1 $\mu g/m^3$. Short-term personal samples collected

during the weighing operation contained the highest levels measured, which ranged from 104 to 202 $\mu g/m^3$ and averaged about 148 $\mu g/m^3$. Area samplers used during the bagging operation and those more than 15 feet from the weighing operation all had tilmicosin concentrations ≤ 10 $\mu g/m^3$. Concentrations of tilmicosin found in area samplers near the weighing operation ranged from about 50 to 164 $\mu g/m^3$.

C. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

An exposure monitoring study for tilmicosin was conducted in a swine barn at Greenfield, Indiana, where the Pulmotil formulation containing 20% tilmicosin was blended into swine feed using a portable vertical screw mixer (Appendix X). The feed was then dispensed into swine feeders. On each of three days, one lot of medicated feed was prepared by adding the premix to a 1000 pound batch of control feed to achieve a concentration of 400 ppm tilmicosin. Feed from each lot was bagged into twenty 50-pound bags. The entire lot from each day was dispensed into 18 swine feeders. The feed in each feeder was agitated for two 30-minute time periods. Personal samples collected for 30 minutes during the premix weighing, feed mixing, and bagging operation had tilmiconsin concentrations which ranged from 21.9 to 66.7 $\mu g/m^3$, with a mean of 44.5 $\mu g/m^3$. All personal samples collected for 30 minutes during the dispensing operation contained < 4 $\mu g/m^3$, the limit of quantitation for tilmicosin in these samples. All personal samples collected for 30 minutes during the agitation of the feed in the swine feeders also contained < 4 $\mu g/m^3$. The average concentration of tilmicosin found in personal samples collected for about 8 hours, which included all mixing, bagging, dispensing, and agitating activities, ranged from < 2.3 to 3.0 $\mu g/m^3$. The overall average for this full-shift exposure was 2.7 $\mu g/m^3$. Tilmicosin levels assayed in the 36 area samples collected for 30 minutes around the dispensing operation were all below the level of quantitation (less than about 4 $\mu g/m^3$). The same was true for 35 of the 36 samples collected around the weighing, mixing and

bagging operation. One area sample collected 2 feet from the balance used to weigh the premix did yield a tilmicosin concentration of 16.7 $\mu g/m^3$.

Information from the United States Department of Agriculture (USDA Economic Reporting Service, 1988) indicates that there were about 77 million pigs raised as barrows and gilts for slaughter in the United States in 1987. About one-half of this production was centered in the states of Iowa, Illinois, Indiana, Ohio, Minnesota, Missouri, Kansas, Nebraska, North Carolina, and Georgia (USDA Agricultural Statistics Board, 1988).

There is substantial variation in the numbers of swine finished on farms and production facilities in the United States. However, hundreds of facilities produce over 5,000 head each year (Rhodes and Grimes, 1983). Assuming a pig in a growing and finishing operation eats an average of 1.2 kg of feed containing 400 ppm of tilmicosin each day for 21 days, it could consume a total tilmicosin dose of up to 10.08 g. Since pneumonia in pigs generally occurs in the Spring and late Fall, it is possible for herds to be treated up to two times in a year.

If tilmicosin was used twice in one year for all of the swine produced in the United States, at most 1,552,320 kg of tilmicosin would be used (10.08 g tilmicosin/pig x 77 x 10^6 pigs X 2). This is equivalent to the use of 7.76 x 10^6 kg of Pulmotil each year. Only about 4.2% of this total is actually expected to be used in the United States, or a total annual use of about 65,000 kg of tilmicosin.

Tilmicosin may be introduced into the environment via waste products from swine. The majority of the tilmicosin fed to pigs is eliminated as the parent material (Appendix E). Approximately 70% of a radiolabeled dose of tilmicosin was recovered in excreta from pigs. Most of the recovered material was in the feces, with only about 6 % in the urine. Two metabolites were noted in the urine (together just > 25%) and feces (together just > 10 %). Because the biological activity of these metabolites is unknown, it will be assumed that the metabolites have the same biological activity as tilmicosin.

The highest theoretical concentration can be calculated for tilmicosin in the excreta from pigs from a swine operation. Pigs in the growing and finishing stages excrete an average of 4.1 kg of wet manure each day (Midwest Plan Service, 1983), and a minimum of about 47 liters of water per pig is used to flush the pens. This results in an average of about 51.1 kg of waste slurry per pig each day, or about 18652 kg per pig in one year. A herd could be treated twice with tilmicosin in a year, resulting in up to a total of 20.16 g being excreted by a pig. The highest concentration of tilmicosin in waste slurry from a swine operation would then be about 1.1 ppm (20,160 mg tilmicosin/18652 kg waste slurry).

7. FATE OF EMITTED SUBSTANCE IN THE ENVIRONMENT

Several studies have been conducted to evaluate the characteristics of tilmicosin which may influence its fate in the environment. Hydrolysis of this compound appears to happen slowly (half-life: 156 days) and only at pH 9 out of the three pH levels tested (Appendix F). Tilmicosin is very susceptible to photolysis. The calculated half-life of tilmicosin at about 40° north latitude in summer sunlight is approximately 0.8 hr in water with pH values of 5, 7, or 9 (Appendix G). Tilmicosin strongly adsorbs to soil, with soil sorption coefficients of 318, 181, 86, and 129 in clay loam, loam, loam (at pH 8.9), and sandy loam, respectively (Appendix H). Desorption ranged from <1% for clay loam to 3% for sandy loam. Moderate to low octanol/water partition coefficients and moderate to high solubility in water indicate that tilmicosin is probably not absorbed into the organic matrix of soil. Tilmicosin is strongly sorbed onto silica gel (Appendix H), which indicates adsorption onto soil particles probably accounts for the relatively high sorption coefficients. No evidence of significant tilmicosin biodegradation could be found in an anaerobic biodegradation study with a slurry of fresh swine manure (Appendix S). Little biodegradation of ^{14}C tilmicosin to $^{14}CO_2$ occurred in clay loam, loam, or sandy loam during a 64-day experiment (Appendix I). Most of the residue extracted from these soils with methanol and NH_4OH was parent tilmicosin. However, about one-quarter of the radioactivity extracted from the soil was composed of degradation products. Similar results were found in another 8-week study (Appendix R) at a lower tilmicosin concentration (1 ppm). In this study, a small amount (< 7 %) of the radiolabel in the tilmicosin was recovered from the loam soil as $^{14}CO_2$. Only about 10 % of the radioactivity extracted from all the soils was composed of degradation products. Even though this study was not definitive due to its design, the results do indicate that tilmicosin does not generally degrade rapidly in soil.

Tilmicosin could be introduced into the environment from outdoor pens or from manure used as fertilizer on cropland. Based on thermogravimetric analysis, tilmicosin is a

non-volatile solid. Measurable concentrations of free tilmicosin would not be expected in the atmosphere. It is possible for tilmicosin to move from pens and cropland soil into adjacent aquatic systems receiving runoff water from these areas.

A. POTENTIAL CONCENTRATIONS OF TILMICOSIN IN SOIL

1. POTENTIAL CONCENTRATION OF TILMICOSIN IN SWINE OPERATIONS

Typical swine operations have enclosed confinement facilities or open-front confinement facilities on concrete. Swine excreta is collected from these facilities for later use as fertilizer on cropland. Few production facilities confine pigs in the open on soil. Open-front facilities would probably only occur in small operations. Production at these smaller operations could be limited by winter weather. Pigs in the finishing stage of growth in an open-front facility would have an average area of about 15 ft^2 each (Midwest Plan Service, 1983a). A pig could be fed up to a total of 10.08 g of tilmicosin in about one half of a year and would excrete about the same amount of residue. If all the excreta were mixed into the top 3 inches of soil of a small facility, the concentration of tilmicosin in the soil could be as high as 64 ppm (10,080 mg tilmicosin/((10.45 kg soil/ft^2) x 15 ft^2)).

2. POTENTIAL CONCENTRATION OF TILMICOSIN IN CROPLAND SOIL

The highest initial concentration of tilmicosin in cropland soil can be estimated from the highest expected concentration in the waste slurry from a swine herd (1.1 ppm, Section 6C), and from the use rate of swine excreta on cropland. A reasonable estimate of the application rate of swine excreta as fertilizer is 10 tons/acre (22.4×10^3 kg/ha). It is standard practice to incorporate manure into the top six inches of soil to avoid loss of nutrients in runoff. A six-inch deep soil layer in one hectare weighs about 2.25×10^6 kg. At this use rate, the initial concentration of tilmicosin in cropland soil can be calculated to be as high as 0.011 ppm ((1.1 mg/kg) x (22.4×10^3 kg/ha) ÷ 2.25×10^6 kg of soil/ha). Even if tilmicosin accumulates in cropland soil for up to ten years, the highest concentration

would be no more than 0.110 ppm. Tilmicosin does appear, however, to degrade somewhat in loam soil.

B. POTENTIAL CONCENTRATIONS OF TILMICOSIN IN AQUATIC SYSTEMS

Movement of tilmicosin through runoff into aquatic systems could occur from cropland soils or from swine operations. The highest possible aquatic concentrations of tilmicosin would be found in runoff water before it is diluted by streams and ponds where aquatic organisms dwell. Although the solubility of tilmicosin varies with the pH of water, the solubility levels at different pH values are high enough to have little impact on the concentration of the compound in runoff water. Tilmicosin degrades quickly in water, with a half-life from aqueous photolysis of about 0.8 hours. Based on episodic introduction of tilmicosin to surface water from runoff and the short half-life in water due to photolysis, any exposure of nontarget aquatic organisms to tilmicosin would be of short duration.

Concentrations of tilmicosin in runoff water from a swine operation or cropland can be calculated. The highest theoretical concentration of tilmicosin in two inches of runoff from a swine facility can be calculated. Assuming all the tilmicosin excreted by a pig treated once could be extracted from the soil of a swine operation into the water of one two-inch runoff event, the highest theoretical concentration in runoff would be about 142 mg/L (10,080 mg/(28.32 L/ft^3)(15 ft^2 x 2 in x 1 ft/12 in)). Well designed facilities have catchment systems for this runoff. In some cases, however, this runoff may reach surface waters. If all the tilmicosin was extracted from fertilized cropland, the highest theoretical concentration of tilmicosin in a 2-inch runoff event would be 0.48 mg/L ((1.1-mg tilmicosin/kg waste slurry x 10 tons of slurry/acre/yr x 907 kg/ton x 10 yr) ÷ (2 in. x 102,794 L/acre-in)).

More realistic concentrations of tilmicosin in runoff water can be estimated by accounting for the potential of tilmicosin to desorb from soil. If desorption of tilmicosin from manure and cropland soil was as high as its desorption from sandy loam soil, the

highest expected concentrations of tilmicosin in runoff water would be about 3% of the highest theoretical concentrations. The highest expected concentrations of tilmicosin in runoff water from a swine facility and cropland are 4.3 and 0.014 mg/L, respectively.

Any tilmicosin delivered to surface water would photodegrade rapidly and would have little propensity to bioconcentrate in aquatic organisms. The highest n-octanol/water partition coefficient for tilmicosin is 376 at pH 9. Neely, Branson, and Blau (1974) developed a regression equation for projected steady-state residue concentrations in trout muscle versus measured n-octanol/water partition coefficients for a variety of synthetic compounds.

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

Using this equation and the log K_{ow} (2.575) for tilmicosin, the predicted BCF for tilmicosin is about 33. Since tilmicosin would be photodegraded and continuously diluted in surface water, only transient exposure of aquatic organisms to tilmicosin would occur. Given the episodic nature of exposure, low exposure concentrations, and a low propensity to bioconcentrate, it is very unlikely that tilmicosin would accumulate in the tissues of aquatic organisms.

8. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY STUDIES

A testing program has been completed with various laboratory animal species and tilmicosin. Complete reports of all of these studies have been submitted to support the proposed action. Studies which help determine the safety of tilmicosin to the public and the environment are briefly described below.

Acute Studies With Tilmicosin

Oral Median Lethal Dose for Sprague-Dawley Rats: 850 mg/kg for males; 800 mg/kg for females.

Subcutaneous Median Lethal Dose for Fischer 344 Rats: 185 mg/kg for males; 440 mg/kg for females.

Subcutaneous Median Lethal Dose for ICR Mice: 97 mg/kg for males; 109 mg/kg for females.

Inhalation by Fischer 344 Rats: Two groups of 10 male and 10 female rats were exposed for 4 hours to a solid particulate aerosol of tilmicosin. Exposure concentrations were very high, 4.053 and 0.494 mg/L. Six males and eight females died during exposure to 4.053-mg tilmicosin/L of air. The predominant signs of toxicity were mortality, hypoactivity, rales, weight loss, nasal discharge, dry nasal exudate, poor grooming, and dyspnea in this group. All surviving animals appeared to be normal by Day 8 after exposure and for the remainder of the study. The predominant signs of toxicity in animals exposed to 0.494 mg/L were hypoactivity, weight loss (males), and poor grooming. All of these animals appeared to be normal by Day 4 post-exposure and for the remainder of the study. Group mean body weights exceeded pre-exposure levels upon termination of the study. Gross pathologic examination revealed soiling of the nares and congested liver and lungs in the animals which died on test. No abnormalities were detected in animals surviving the 2-week observation period. The

4-hour median lethal concentration was between 0.494- and 4.053-mg tilmicosin/L of air.

Intramuscular Injection in Monkeys: A single intramuscular dose of 30 mg/kg was fatal and 20 mg/kg caused vomiting. A single dose of 10 mg/kg caused no signs of toxicity.

Mutagenicity, DNA Repair, and Sister Chromatid Exchange: Tilmicosin was not mutagenic in mouse lymphoma cells or in the Ames Salmonella/mammalian microsome test for bacterial mutation. Tilmicosin did not induce DNA repair synthesis in cultured rat hepatocytes nor did it induce sister chromatid exchange *in vivo* in bone marrow of Chinese hamsters.

Dermal Toxicity and Irritation in Rabbits: No dermal irritation, no mortality, and no overt signs of systemic toxicity were observed for 14 days after a dose of 5000 mg/kg body weight was applied topically to the skin. No compound-related lesions were found in a gross pathological examination of test animals.

Dermal Sensitization in Guinea Pigs: An intracutaneous injection of tilmicosin did not produce a sensitization response.

Ocular Irritation in Rabbits: Corneal dullness, slight iritis, and moderate to severe conjunctivitis occurred within 1 hour after a dose of 17 mg of tilmicosin was placed in the eyes of rabbits. Treated eyes in all rabbits returned to normal within 7 days.

Subchronic and Chronic Toxicity

Three-Month Rat Study: Rats in groups of 20 of each sex received daily 50, 250, or 1000 mg/kg oral gavage doses of tilmicosin for 3 months. Evidence of toxicity in the middle-dose and/or high-dose groups included decreased survival, poor body condition, decreased body weight gains, decreased food consumption, decreased efficiency of food utilization, organ weight changes, and histopathological tissue

alterations. The no-effect level for daily administration of tilmicosin by gavage to these Crl:CD (SD) rats was 50 mg/kg.

Teratology Study with Rats: Tilmicosin was administered orally by gavage to mated female rats at dose levels of 0, 10, 70, or 500 mg/kg/day on gestation days 6 through 15. No embryo/fetal toxicity and no teratogenicity were found at the highest dose tested, 500 mg/kg/day. Maternal toxicity was indicated by depressed body weight gain during the first half of the treatment period and increased salivation at the two highest dose groups. During the post-treatment period, food consumption was elevated at these two dose groups, so their weight gains for the entire gestation period were comparable to control values. Females in the highest dose groups also displayed depressed food consumption and alopecia. No maternal toxicity was found for rats given daily doses of 10 mg/kg.

Two-Generation Reproduction Study with Rats: Two generations of male and female rats were provided tilmicosin by oral gavage at dosages of 0, 10, 45, and 200 mg/kg/day. The highest dosage resulted in excessive salivation in F_0 generation male and female rats. This highest dosage also resulted in reduced body weight gain and food consumption by F_0 generation female rats, as well as a small increase in pup mortality during the first 4 days postpartum in the F_{1a} and F_{1b} litters. Reduced body weight gain and food consumption were noted for F_0 generation females provided a daily tilmicosin dosage of 45 mg/kg. No adverse effects were found for rats given a daily tilmicosin dosage of 10 mg/kg.

One-Year Dog Study: Total oral doses of 0, 4, 12, and 36 mg tilmicosin/kg body weight were administered daily. All animals survived to the end of the study. The dogs exposed to 12 mg/kg/day did not gain weight as rapidly as the control dogs. Decreased body weight gain, tachycardia, slightly to moderately enlarged hearts, and mild depression of the ST segment in electrocardiograms were noted in dogs given a dose of 36 mg/kg/day. Histological alterations of the heart were not detected in this

high-dose group. The food consumption, hematology, clinical chemistry, and urinalysis parameters studied were not affected in any treatment group. Mild dermatitis of the pinna (ear), noted sporadically in treated dogs during the last 7 months of the study, was considered to be of no toxicological importance. The no-observable-effect level was 4 mg/kg/day.

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

1. HUMAN EXPOSURE TO TILMICOSIN DURING PRODUCTION AND USE OF PULMOTIL

Tilmicosin and Pulmotil will be manufactured in only two plants, and engineering controls and personal hygiene precautions will be effective in minimizing exposure. Tilmicosin will be produced as an aqueous solution. This eliminates the need for special containment of dust that could come from a dry bulk drug. New state-of-the-art facilities and equipment have been built to formulate and package Pulmotil. The solution side of the process is a closed system. The premixers maintain the integrity of the contained process during all phases of operation (loading, mixing, and unloading). Advances in the fluid bed dryer selected for this process, such as the metal cartridge filter, HEPA filter, and wash-in-place system allows for containment of materials. The blender inlet and outlet conveyors will be suitably sealed to prevent release of materials. Blenders are totally contained. Pulmotil will be loaded into bags with little or no air displacement. Given that tilmicosin will be used as an aqueous solution and that the formulation and bagging processes will be contained, the potential for exposure to tilmicosin is quite low. A short-term excursion limit and long-term exposure guideline of 360 $\mu g/m^3$ and 120 $\mu g/m^3$, respectively, for tilmicosin have been recommended to allow safe exposure for < 30 minutes and up to 12 hours. During the production processes, when appropriate, workers may wear personal protective gear such as foot covers, gloves, eye protection, protective clothing, or hoods attached to a fresh air supply. Based on these protective

measures, the magnitudes of the acute toxicity values, and the fact that tilmicosin is neither a mutagen nor a teratogen in laboratory animals, it can be concluded that workers producing tilmicosin and formulating Pulmotil will not be adversely affected by the proposed action.

The Pulmotil label will instruct people to routinely wear protective clothing, impervious gloves, and a NIOSH-approved dust mask when mixing and handling Pulmotil and to wash thoroughly after handling the product. The time for greatest potential exposure to tilmicosin would be during the weighing of Pulmotil, but this only requires a few minutes. Concentrations from 104 to 202 $\mu g/m^3$ have been measured in personal samplers during this weighing operation at a feed mill. At a swine facility, personal samples collected throughout the weighing, bagging, and mixing operations ranged from 21.9 to 66.7 $\mu g/m^3$. Short-term exposure levels in the bagging operation at a feed mill ranged from 2 to 9.7 $\mu g/m^3$. A dust mask should reduce concentrations during this short exposure by a factor of at least 5, to levels <40 $\mu g/m^3$. Personal samples from dispensing operations and area samples at the swine barn were normally < 4 $\mu g/m^3$. Personal samples simulating a full day of exposure averaged 2.5 $\mu g/m^3$ for weighing, mixing, and bagging in the feed mill, and 2.7 $\mu g/m^3$ for weighing, mixing, bagging and dispensing in the swine barn. Exposure levels in a feed mill and in a swine facility will be below the short-term excursion limit (360 $\mu g/m^3$) and long-term guideline (120 $\mu g/m^3$) recommended for occupational safety.

Based on proposed safety measures and expected exposure concentrations, the production, formulation, and use of tilmicosin is not expected to result in adverse affects on human health.

2. HUMAN EXPOSURE TO TILMICOSIN VIA THE FOOD SUPPLY

Exposure of humans to large amounts of tilmicosin via the food supply is quite unlikely. Since any tilmicosin residue released onto soil adsorbs strongly, it is highly

improbable that measurable amounts of tilmicosin would occur in drinking water from groundwater or surface water sources. Details of any exposure of humans to tilmicosin in meat are listed in a Freedom of Information Summary. The proposed action is not expected to adversely affect human health through the food supply.

C. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON NONTARGET ORGANISMS

Use of Pulmotil for pigs should result in very little exposure of nontarget organisms. There is some possibility that tilmicosin in pig feed could be available to birds. Low concentrations (4.3 or 0.014 mg/L) of tilmicosin are expected in runoff water from swine operations and cropland, and rapid photodegradation of tilmicosin (half-life about 0.8 hr) would result in even lower exposure levels for aquatic organisms in surface waters. Organisms associated with soil may be exposed to tilmicosin for a longer period of time. Studies have been conducted to determine the effects of tilmicosin on nontarget organisms and results of these studies are summarized below.

Aquatic Organisms

Bluegill (*Lepomis macrochirus*) were exposed to average measured tilmicosin concentrations of 0.0, 214, 524, 528, 604, and 679 ppm (Appendix J). The median lethal concentration, the 95% confidence limits for the median lethal concentration, and the slope of the concentration-response curve were 716 ppm, 635 to 807 ppm, and 12.6, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to the tilmicosin concentration of 214 ppm.

Rainbow trout (*Salmo gairdneri*) were exposed to average measured tilmicosin concentrations of 0.0, 98, 196, 424, 534, 659, and 875 ppm (Appendix K). The median lethal concentration, the 95% confidence limits for the median lethal concentration, and the slope of the concentration-response curve were 851 ppm, 784 to

988 ppm, and 12.3, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to tilmicosin concentrations ≤ 534 ppm.

Daphnia magna were exposed to average measured tilmicosin concentrations of 0.0, 2.6, 9.0, 26.4, 38.5, 58.6, and 95.0 ppm (Appendix L). The 48-hour median effective concentration, the 95% confidence limits of the median effective concentration, and the slope of the concentration-response curve were 57.3 ppm, 51.5 to 64.8 ppm, and 10.5, respectively. No immobilization or physical signs of toxicity were observed in animals exposed to the tilmicosin concentration of 2.6 ppm.

The green alga, *Selenastrum capricornutum*, was exposed to initial assayed tilmicosin concentrations of 0.0, 0.012, 0.025, 0.054, 0.112, 0.240, 0.468, and 1.173 ppm (Appendix T). The average specific growth rate and the maximum specific growth rate of the algal populations were significantly reduced from exposure to initial tilmicosin concentrations ≥0.240 ppm. Terminal biomass of the algal populations was significantly reduced at initial tilmicosin concentrations ≥0.112 ppm. However, tilmicosin concentrations declined significantly by the end of the study in all treatments, probably due to photolysis. Since light is required in this study for algae to grow, an algal toxicity test cannot be conducted without the possibility of photolysis. Because concentrations changed significantly over the time course of this study, accurate calculation of the median effective concentration and lowest observed effect concentration for the entire study is not possible. Results from this study do confirm that tilmicosin photodegrades rapidly.

The concentration of tilmicosin expected in runoff water from cropland (0.014 ppm) is about 186 times lower than the concentration (2.6 ppm) which resulted in no effects on any of the aquatic animal species tested. The highest possible concentration of tilmicosin in runoff from a swine facility (4.3 ppm) is about 122 times lower than the concentration that first resulted in acute effects on fish and is about two times lower than

the first effect concentration found for an aquatic invertebrate. Actual exposure of aquatic organisms to tilmicosin in surface waters would be episodic, depending on runoff from rainfall. Concentrations of tilmicosin in surface water would rapidly decline due to photolysis. Any exposure to tilmicosin delivered by runoff from cropland into surface waters is not expected to have adverse effects on populations of aquatic organisms. While definitive concentrations of tilmicosin which affect algae could not be determined, it is possible that runoff from swine facilities may cause a short-term reduction in the growth of algal populations until the levels of tilmicosin are significantly diminished by photolysis.

Terrestrial Organisms

Germination and radicle development were monitored for seeds of corn (*Zea mays*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) exposed to solutions containing 1, 10, or 100 ppm of tilmicosin (Appendix M). Seed germination of the four cultivars was not affected by any tilmicosin treatment. Radicle development was not affected in corn, soybeans, and wheat. The length of cucumber radicles were reduced by 45.5% at the highest treatment level, but were unaffected at 10 ppm.

A 21-day seedling growth study (Appendix Y) was conducted to determine the effects of tilmicosin on corn (*Zea mays*), cucumber (*Cucumis sativus*), perennial ryegrass (*Lolium perenne*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), and wheat (*Triticum aestivum*). Seedlings of each species were planted in sand and sandy loam that was then treated with nutrient media containing tilmicosin. Treatment levels in the sandy loam soil were 0, 1, 3, 10, 30, 100, and 300 mg/kg (mg tilmicosin per kg of dry soil). These same treatment levels and an additional treatment of 0.3 mg/kg were used to the test the effects of tilmicosin in sand as a substrate. Five plants were used for each replicate and five replicates were used for each treatment level. Shoot length, shoot weight, and root weight were measured for all plants in the study. Well-defined

dose-response relationships were evident for all species when exposed to high tilmicosin levels in sand. For the test with sand, no significant adverse effects were found for corn, cucumber, perennial ryegrass, soybean, tomato, and wheat at exposure levels of 30, 3, 3, 3, 3, and 100 mg/kg, respectively. The effects of tilmicosin on seedling growth were significantly reduced when tilmicosin was introduced into sandy loam soil. Tilmicosin strongly sorbs to soil, but least of all to sandy loam. Even so, sorption to the sandy loam soil was apparently strong enough to significantly reduce the effects of tilmicosin on the seedlings. Only cucumbers were significantly affected by tilmicosin in sandy loam soil, and only at the highest level tested. In sandy loam soil, no significant adverse effects were found for the other five species tested at a tilmicosin level of 300 mg/kg.

A 28-day study (Appendix N) was conducted to determine the effects of soil-incorporated tilmicosin on the earthworm (*Lumbricus terrestris*). Average exposure concentrations were 0, 74, 423, and 918 ppm. No mortality or physical signs of toxicity were found at any tilmicosin concentration tested. Earthworms exposed to the highest test concentration did gain more weight than control worms during the study.

A 5-day dietary toxicity study (Appendix U) was conducted with 11-day-old bobwhite *(Colinus virginianus)*. Measured concentrations of tilmicosin in the diets were 0.0 (solvent control), 1130, 2390, and 4820 ppm. Groups of 10 bobwhite were fed these diets for 5 days and untreated diets for another 3 days. No mortalities, signs of toxicity, or reductions in food consumption were found at any treament level tested. During the 5-day treatment phase of the study, a significant reduction in body weight gain occurred for birds exposed to the two highest treatment levels. No effects were noted on body weight gain at the 1130-ppm treatment level.

A 5-day dietary toxicity study (Appendix V) was conducted with 4-day-old mallards *(Anas platyrhynchos)*. Measured concentrations of tilmicosin in the diets were 0.0 (solvent control), 1120, 2370, and 4710 ppm. Groups of 10 mallards were fed these diets for 5

days and untreated diets for another 3 days. No mortalities, or signs of toxicity were found at any treament level tested. During the 5-day treatment phase of the study, a significant reduction in body weight gain occurred for birds exposed to the highest treatment level. Food consumption by these birds was also somewhat lower. No effects were noted on body weight gain or food consumption at tilmicosin levels of 1120 and 2370 ppm.

Tilmicosin does exhibit antibacterial activity (Ose, 1987). The minimum inhibitory concentrations (MIC) of tilmicosin incorporated into agar plates or Mueller-Hinton broth were determined for a group of gram-positive and gram-negative animal pathogens and gram-positive and gram-negative facultative aerobes. The MIC values for these organisms ranged from 0.024 ppm to >50 ppm. Tilmicosin is most active against *Acholeplasma* and most species of *Mycoplasma* (MIC values generally ≥0.024≤0.097 ppm). These organisms are part of the same phylum, Aphragmabacteria (Margulis and Schwartz, 1982), a group of bacteria incapable of synthesizing the polysaccharide compounds muramic acid and diaminopimelic acid. These polysaccharide compounds help form the finished cell walls of all other bacteria. Many *Mycoplasma* cause animal diseases and only exist in animal tissues. Tilmicosin was also active against *Actinomyces*, part of a group of fungus-like bacteria that are generally gram-positive. The majority of gram-negative bacteria tested by Ose (1987) were not sensitive to tilmicosin. The least sensitive organisms (MIC values >50 ppm) tested were *Fusobacterium*, part of a group of fermenting bacteria that cannot tolerate oxygen; *Treponema*, part of the Spirochaetae phylum (Margulis and Schwartz, 1982); and *Eschĕrichia* and *Salmonella*, both part of a large and diverse group (Omnibacteria) of enterobacteria and other facultatively aerobic gram-negative heterotrophic bacteria.

Exposure of pure cultures of an ascomycete *(Chaetomium globosum)*, a mold *(Aspergillus flavus)*, two soil bacteria *(Comamonas acidovorans* and *Azotobacter chroococcum)*, and a blue-green algae *(Nostoc sp.)* demonstrated only some sensitivity to tilmicosin

(Appendix W). Both *Aspergillus* and *Chaetomium* had MIC values that were >1000 ppm, while the MIC for *Comamonas* was 250 ppm. *Azotobacter* and *Nostoc* were the most sensitive organisms tested in these studies with MIC values of 5 and 0.5 ppm, respectively. The MIC value for *Nostoc* may be an overestimate due to the potential for photolysis of tilmicosin in the study, but this value reflects the general level of sensitivity found in the study with a green algal species.

Based on these results, tilmicosin has the potential to inhibit microorganism activity in the soil on which a swine facility is operated. The highest concentration of tilmicosin in soil of a swine facility would probably be about 64 ppm (Section 6C). This level was lower than the MIC values for a mold, fungus, and soil bacteria, but higher than the MIC values for *Azotobacter*, *Nostoc* and some gram-positive and gram-negative animal pathogens and facultative aerobes. The highest expected concentration in the waste slurry at a swine facility is 1.1 ppm (Section 6C). While several groups of microbes have been shown to be sensitive to tilmicosin at this or lower levels, soil bacteria, a fungus, and a mold would be unaffected by this tilmicosin concentration. Total gas production by bacteria exposed to tilmicosin (1 ppm and 79 ppm) in swine waste under anaerobic conditions was not significantly different than control gas production during the course of a 73-day study. Inhibition of microbial activity in cropland soil at tilmicosin concentrations up to 0.110 ppm is not likely.

Higher terrestrial organisms should not be affected by exposure to levels of tilmicosin that may be found in swine feed or in cropland soil. No effects were found for birds at a dietary level of 1,130 ppm, a concentration that is almost three times higher than the highest tilmicosin level proposed for swine feed. Worms were not adversely affected at tilmicosin concentrations in soil up to 918 ppm, a level that is about 8,300 times higher than the highest possible tilmicosin concentration in cropland soil. Terrestrial plants grown in sandy loam soil were not adversely affected by exposure to a tilmicosin concentration of

100 ppm, a level about 909 times higher than the possible level in cropland soil. Even plants grown in sand were not adversely affected at a tilmicosin concentration of 3 ppm, a level that is 27 times higher than the level of tilmicosin that could possibly accumulate in soil after 10 years.

The proposed action would not be likely to result in any sustained adverse effects on terrestrial or aquatic nontarget organisms outside the soil in a swine facility.

9. UTILIZATION OF NATURAL RESOURCES AND ENERGY

Production and formulation of tilmicosin will occur at facilities designed for the production and formulation of other animal drugs and pharamaceuticals. These facilities will be operated according to Good Manufacturing Practices.

In general, process streams from the production of tilmicosin only utilize a portion of the waste treatment or recovery facilities already installed for these and other process wastes. Disposal of waste from the manufacturing processes and operations will not require use of unusual amounts of energy or natural resources.

Estimation of natural resources and energy used in the production of Pulmotil Type A Medicated Article included fixed costs and other miscellaneous energy usage not directly related to production, such as administrative office use. Production for the final liquid bulk drug substance at the facility in Kinsale will use about 9 percent of the natural resources (i.e., electricity, fuel oil, and propane gas) used by the entire production facility. At the Dista facility, formulation and packaging Pulmotil Type A Medicated Article will use about 4 percent of the natural resources (i.e., electricity, oil, and coal) used by the entire facility.

Production, formulation, and use of Pulmotil will not affect endangered or threatened species, nor will these actions affect 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 Pulmotil label will instruct people to routinely wear protective clothing, impervious gloves, and a NIOSH-approved dust mask when mixing and handling Pulmotil, and to wash thoroughly after handling the product. The label will also instruct users to immediately rinse eyes if accidental contact occurs with Pulmotil. If eye irritation persists, the label will indicate that medical attention should be sought.

Tilmicosin bulk drug and Pulmotil Type A Medicated Article will be manufactured in only two plants, and engineering controls and personal hygiene precautions will be effective in minimizing exposure at both sites. The bulk tilmicosin will be produced from raw ingredients as an aqueous solution and transferred to the premix manufacturing site in this form. This eliminates the need for special containment of dust that could come from handling a dry bulk drug. New state-of-the-art facilities and equipment have been designed and built to formulate and package Pulmotil. The solution side of the process is a closed system. The premixers maintain the integrity of the contained process during all phases of operation (loading, mixing, and unloading). Advances in the fluid bed dryer selected for this process, such as the metal cartridge filter, HEPA filter, and wash-in-place system also allow for containment of materials. The blender inlet and outlet conveyors are suitably sealed to prevent release of materials. Blenders are totally contained. Pulmotil will be loaded into bags with little or no air displacement in a "form-fill-seal" bagging system. Given that tilmicosin will be used as an aqueous solution and that the formulation and bagging processes will be contained, the potential for exposure to tilmicosin by workers is extremely low.

All manufacturing facility workers in both the bulk drug and premix manufacturing plants are trained to safely work in production areas with active materials. On-site industrial hygienists evaluate new processes such as those for Pulmotil and will ensure that all workers are informed about appropriate handling practices. As for all new products,

appropriate exposure guidelines have been established for tilmicosin. Engineering controls and personal protective gear are used to minimize exposure. During the production processes, when appropriate, workers may wear personal protective gear such as foot covers, gloves, eye protection, protective clothing, or hoods attached to a fresh air supply. A material safety data sheet that lists hazard data, exposure limits, and safe handling practices is available to all workers.

Based on these engineering controls, protective measures, implementation of exposure guidelines, and worker safety communications, it can be concluded that workers will safely produce tilmicosin and formulate Pulmotil.

11. ALTERNATIVES TO THE PROPOSED ACTION

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

12. LIST OF PREPARERS

The following personnel of Eli Lilly and Company are responsible for the preparation of the Environmental Assessment:

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April 17, 1996
Date

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Environmental Affairs

April 18, 1996
Date

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Senior Research Scientist
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April 18 1996
Date

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.

Gregory S. Probst, Ph.D.
Executive Director
Toxicology and Drug Disposition

April 22, 1996
Date

14. REFERENCES

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Appendix A: Report Summary

Title: Water Solubility of Tilmicosin

Name and Address of Investigator: J. S. Peloso; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, Indiana 46140-0708

Study Number: RMK8701

Study Dates: July 23, 1987 to April 20, 1988

Test Article: Characterized tilmicosin

Test System: Laboratory apparatus, including assay by high-performance liquid chromatography (HPLC).

Summary of Experimental Design: Excess tilmicosin was added to water solutions maintained at pH levels of 5, 7, and 9 by addition of phosphoric acid. The test temperature was 25°C for all three pH levels, and also 5°C for pH 9. The samples were filtered to remove undissolved tilmicosin and assayed by HPLC.

Summary of Results: The test results show that the water solubility of tilmicosin is very dependent on temperature and pH. At pH 9, solubilities of 7.7 and 72.5 mg/ml were obtained at temperatures of 25°C and 5°C, respectively. Tilmicosin is considerably more soluble as the pH is lowered, having a solubility of 566 mg/mL at pH 7 and 25°C. At pH 5, the solubility is so great that a sticky paste is formed.

Appendix B: Report Summary

Title: Thermogravimetric Analysis of Tilmicosin

Name and Address of Investigators: J. J. Lewis; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Number: JJL8910

Study Date: May 6, 1985

Test Article: Tilmicosin Reference Standard Material

Test System: Thermogravimetric Analyzer

Summary of Experimental Design: Approximately 10 mg of tilmicosin was weighed onto a sample pan of a thermogravimetric analyzer. The initial temperature of the analyzer was 20°C. The heating rate was set at 5°C/min under a nitrogen flow of 40 cc/min. A thermogram representing percent weight loss versus temperature was recorded.

Summary of Results: The thermogram indicated a weight loss of only 1.6% from 23°C to about 129°C. This loss represents a loss of water and other minor volatile impurities. No losses were observed around the tilmicosin melting point range, 107°C to 112°C. A continuous loss through decomposition of tilmicosin was observed to begin at 167°C. These results indicate that tilmicosin is a non-volatile solid.

Appendix C: Report Summary

Title: n-Octanol/Water Partition Coefficients of Tilmicosin

Name and Address of Investigator: J. S. Peloso; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, Indiana 46140-0708

Study Number: AAC8728

Study Dates: December 1, 1987 to July 28, 1988

Test Article: Purified ^{14}C tilmicosin

Test System: Laboratory apparatus for mixing and partitioning phases in centrifuge tubes, including liquid scintillation analysis of each phase.

Summary of Experimental Design: Solutions of ^{14}C-radiolabeled tilmicosin in n-octanol were equilibrated with aqueous buffers having pH levels of 5, 7, and 9 at a temperature of 25°C. The concentration of tilmicosin in each phase was determined by radiochemical analysis.

Summary of Results: The n-octanol/water partition coefficients (K_{ow}) were determined to be <10 at pH levels of 5 and 7 and 376 at pH 9. These low values indicate that tilmicosin would not bioaccumulate in lipoid tissue.

Appendix D: Material Safety Data Sheet for PULMOTIL® Type A Medicated Article

ELANCO

MATERIAL SAFETY DATA SHEET

PULMOTIL® TYPE A MEDICATED ARTICLE AF0472; QA358V

Pulmotil® is a Type A medicated article used in swine for the control of bacterial pneumonia caused by *Actinobacillus pleuropneumoniae* and *Pasteurella multocida.*

I. MANUFACTURER/EMERGENCY INFORMATION

A. Manufacturer

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

B. Emergency Telephone Numbers

Eli Lilly and Company (317) 276-2000
CHEMTREC (800) 424-9300

C. General Information Telephone Number

Elanco Animal Health (800) 428-4441

D. Issued: 10/93; Revised: 7/95, 8/95

II. MATERIAL IDENTIFICATION

A. Generic Name

Tilmicosin (as tilmicosin phosphate)	20%

1. Chemical Abstract Registry Number (CAS#): 137330-13-3

B. Other Ingredients

Ground corn cobs (inert ingredients)	80%

There are no hazardous or carcinogenic inert ingredients.

III. PHYSICAL AND CHEMICAL PROPERTIES

A. Normal Physical State, Odor, Appearance: Yellowish-tan to reddish-tan free-flowing granular material

B. pH (aqueous 50/50): 5.5-7

C. Solubility in Water: Tilmicosin phosphate is very soluble in water. The ground corn cobs (inert ingredients) are insoluble in water.

D. Melting Point: Not applicable

IV. FIRE AND EXPLOSION HAZARD DATA

A. Auto-Ignition Temperature (Dust Layer): 374°F (190°C)

B. Flashpoint: Not applicable

C. Flammable Limit

1. Lower Explosive Limit (LEL): 0.90 oz/cu ft (dust layer)
2. Upper Explosive Limit (UEL): Not applicable

D. Unusual Fire and Explosion Hazards: None known; however, as a finely divided material, may form dust mixtures in air which could explode if subjected to an ignition source.

E. Fire Fighting Information: Use water, CO_2, dry chemical, foam, or Halon.

V. NATIONAL FIRE PROTECTION ASSOCIATION (NFPA 704)

(4=Extreme: 3=High: 2=Moderate: 1=Slight: 0=Insignificant)

A. **Health:** 2

B. **Flammability:** 1

C. **Reactivity:** 0

VI. SHIPPING REQUIREMENTS

A. **DOT:** Not regulated.

B. **ICAO:** Not regulated.

C. **IMO:** Not regulated.

VII. REACTIVITY DATA

A. **Stability and Storage:** Stable at normal temperatures and pressures. Store at room temperature. Product should not be used after the date printed on the bag.

B. **Hazardous Decomposition:** None known.

C. **Hazardous Polymerization:** Will not occur.

D. **Incompatibility:** None known.

VIII. HEALTH HAZARD DATA

A. **Toxicology (Animal Toxicity Data)**

1. Acute Exposure (PULMOTIL)
   a. Eyes - Rabbit, irritant
   b. Skin - Rabbit, 1000 mg/kg, no deaths, slight irritant.
   c. Inhalation - This formulation was not tested. Tilmicosin phosphate - Rat, median lethal concentration 3200-4400 mg/m^3 for four hours, reduced activity, labored breathing.
   d. Ingestion - Rat, 500 mg/kg, no deaths or toxicity.
   e. Sensitization - This formulation was not tested. Tilmicosin phosphate was not a contact sensitizer in the guinea pig.
2. Chronic Exposure (tilmicosin phosphate)
   a. Target Organ Effects - Based on studies in dogs, rats, and cattle, increased heart weight and size, heart muscle degeneration characterized by small areas of cell death, severe and persistent increase in heart rate with changes in electrocardiogram ST, Q, and T wave forms occurred generally at higher oral or injection doses where some mortality occurred.

      Increased adrenal, kidney, and liver weights, increased cell size in adrenal cortex, lymphoid depletion, increased liver enzyme activity, mucosal edema of the gallbladder, and subretinal fluid accumulation were seen at higher oral or injection doses where some mortality occurred and were likely secondary effects of stress resulting from high doses.

      Other Effects - Decreased food consumption and body weight gains, slightly decreased urine pH, occult blood in urine, increased serum alanine transaminase.
   b. Teratology & Reproduction - Not teratogenic or fetotoxic. No impairment of reproductive capacity. Slight increase in offspring mortality at maternally toxic doses.
   c. Mutagenicity - Not mutagenic in bacterial or mammalian cells.
   d. Carcinogenicity - No lifetime animal study data available. Not listed as a carcinogen by American Conference of Governmental Industrial Hygienists (ACGIH), National Cancer Institute/National Toxicology Program (NCI/NTP), International Agency for Research on Cancer (IARC), or Occupational Safety and Health Administration (OSHA).

B. Effects of Human Exposure

1. Signs and Symptoms of Exposure

   a. Occupational (tilmicosin phosphate powder) - Allergic reactions in a manufacturing setting have been reported. Allergy symptoms may include skin rash, watery eyes, shortness of breath, nasal congestion, choking, coughing, and wheezing.

   b. Occupational (PULMOTIL) - No allergic reactions in a manufacturing setting have been reported. Based on animal data, this material may be irritating to the eyes.

2. Medical Conditions Generally Aggravated by Exposure

   Sensitivity to tilmicosin and/or tylosin.

C. Exposure Guidelines

1. Permissible Exposure Limit (PEL): Not established.
2. Threshold Limit Value (TLV): Not established.
3. Lilly Exposure Guideline (tilmicosin): 0.12 mg/m^3 for a 12-hour exposure.

D. Primary Route of Entry: Inhalation and skin contact.

IX. FIRST AID (Statement of Practical Treatment)

A. Eyes

Hold eyelids open and flush with steady, gentle stream of water for 15 minutes. See an ophthalmologist (eye doctor) or other physician immediately.

B. Skin

Remove contaminated clothing and clean before reuse. Wash all exposed areas of skin with plenty of soap and water. Get medical attention if irritation develops.

C. Inhalation

Move individual to fresh air. Get medical attention if breathing difficulty occurs. If not breathing, provide artificial respiration assistance (mouth to mouth) and call physician immediately.

D. Ingestion

Call a physician or Poison Control Center immediately (see emergency telephone numbers listed in I.B.). If available, administer activated charcoal (6-8 heaping teaspoonfuls) with two to three glasses of water OR give 1-2 tablespoons syrup of ipecac and drink one or two glasses of water to induce vomiting. Do not give anything by mouth to an unconscious person. Immediately transport to a medical care facility and see a physician.

X. PRECAUTIONS FOR SAFE HANDLING AND USE

A. Spill Handling Information

Do not contaminate any body of water. For spills, sweep up and place the material, and any damaged unusable containers, in landfill in accordance with applicable regulations. Large spills due to traffic accidents, etc., should be reported immediately to CHEMTREC and Eli Lilly and Company for assistance. Prevent spilled material from spreading onto adjacent land or into streams, ponds or lakes.

B. Waste Disposal

Dispose of any cleanup materials and waste residue according to applicable federal, state, and local regulations.

C. Container Disposal

No special package disposal required.

D. Storage: (See Section VII, Paragraph A)

XI. PROTECTIVE EQUIPMENT REQUIREMENTS

During manufacture, wear goggles to protect eyes, wear impermeable gloves and protective equipment to avoid direct contact with skin. Use approved respirator.

When mixing and handling Pulmotil use protective clothing, impervious gloves, and a NIOSH approved dust mask. Operators should wash thoroughly with soap and water after handling.

XII. OTHER INFORMATION

A. NADA Number: Not assigned.

B. PULMOTIL® (tilmicosin phosphate, Elanco)

Appendix E: Report Summary

Title: Tilmicosin Metabolism Study in Tissues and Excreta of Pigs Fed 400 ppm ^{14}C-Tilmicosin

Name and Address of Investigators: A. L. Donoho, J. M Darby, S. L. Helton, D. J. Sweeney, J. L. Occolowitz, and D. E. Dorman; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, IN 46140-0708

Study Number: T5C759201

Study Dates: June 12, 1992 through January 12, 1993

Test Article: ^{14}C Tilmicosin

Test System: Six crossbred swine (Three castrated males and three females)

Summary of Experimental Design: Six crossbred swine fed with ^{14}C tilmicosin equivalent to 400 ppm in the feed for 5 days. Pigs were dosed on the assumption that they would consume 4.5 % of their body weight under normal feeding conditions. At each feeding, a portion of the treated feed (800 ppm) was fed first. Subsequently, the pigs were given equal portions of untreated feed. Twice daily dosing of the pigs continued for 5 days. As part of this metabolism study, urine and feces were collected from the pigs. Urine and feces from two pigs were assayed to determine the rate and route of excretion of the ^{14}C tilmicosin. Urine and feces were collected from one male and one female during the period from 1 day predose to 7 days after the last dose. Urine and feces were frozen for storage before analysis. Fecal samples were assayed for radioactivity by combustion and urine samples were analyzed by liquid scintillation counting. Distribution of radioactivity and metabolite pattern were determined for feces after extraction and fractionation. Urine samples were injected into the HPLC without prior fractionation.

Summary of Results: Approximately 70% of the tilmicosin administered to the pigs was recovered in the feces and urine. About 6% of this material was found in the urine. Concentrations of total residue in urine and feces were about 6 ppm and 860 ppm, respectively. Fractionation of radioactivity in feces revealed that residue was primarily tilmicosin. An uncharacterized tilmicosin metabolite equivalent to approximately 10 percent of the total was also found. Isolation and characterization of this metabolite indicated a compound equivalent to tilmicosin plus one mole of hydrogen, and one sulfate group. The proposed structure of this metabolite is tilmicosin with one carbon-carbon double bond reduced and addition of SO_3H in the macrolide ring moiety. A small amount of another metabolite, N-demethyl tilmicosin, was also found in the feces. Radioactivity in the urine was mostly tilmicosin. About 25 % of the radioactivity was the previously uncharacterized metabolite, with a small amounts of the N-demethyl tilmicosin metabolite, and seco acid isomers of tilmicosin.

Appendix F: Report Summary

Title: Hydrolysis of Tilmicosin in Aqueous Buffer Solutions

Name and Address of Investigator: J. S. Peloso; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, IN 46140-0708

Study Number: RMK8702

Study Dates: August 3, 1987 to June 1, 1988

Test Article: Characterized tilmicosin

Test System: Laboratory test with sterile buffer solutions and high-performance liquid chromatographic (HPLC) assay of samples.

Summary of Experimental Design: Sterile, aqueous buffer solutions of pH 4, 7, and 9 were fortified with 250 mg/ml tilmicosin and maintained in the dark at 50°C. The solutions were assayed for tilmicosin 5 days after initiation of the study. To further define the extent of base promoted hydrolysis, sterile aqueous buffer solutions of pH 9 were fortified with 250 mg/mL tilmicosin and maintained in the dark at 25°C. Samples were periodically removed and assayed by HPLC during the 28-day test period.

Summary of Results: At pH 4 and 7, tilmicosin was hydrolytically stable in water, with a calculated half-life of 1 year or more at 25°C. At pH 9, the average hydrolysis rate constant at 25°C was calculated to be 18.53×10^{-5} hours. This corresponds to a half-life of 156 days and indicates a moderate degree of hydrolytic instability.

Appendix G: Report Summary

Title: Aqueous Photodegradation Study of Tilmicosin

Name and Address of Investigator: J. J. Lewis; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, IN 46140-0708

Study Number: JJL8704

Study Dates: July 20 through 30, 1987

Test Article: Tilmicosin Reference Standard Material

Test System: Aqueous sample solutions in quartz tubes under actual summer sunlight conditions at approximately 40° north latitude.

Summary of Experimental Design:

Buffer solutions were prepared at pH 5, 7, and 9 with sterile, air-saturated, HPLC-grade water. Reaction solutions were prepared by dissolving tilmicosin reference standard material in each buffer solution to a final concentration of 8.7 mg/mL (10^{-5} M). Aliquots of these sterile test solutions were poured into sterile quartz tubes, sealed, and exposed to summer sunlight at approximately 30° from the vertical.

Based on initial data on samples exposed to sunlight for 0, 1, 3, and 7 days, triplicate sample sets for each pH were exposed to sunlight at shorter intervals of 0, 1, 2, and 4 hours. At each pH, identical positive control solutions contained in quartz tubes were wrapped in aluminum foil to exclude sunlight and were sampled at the same time intervals as the exposed samples. Blank buffer solutions were also exposed to sunlight to check for any interferences. The concentration of tilmicosin in the samples was determined by high-performance liquid chromatography with UV detection at 280 nm.

Summary of Results: No degradation of tilmicosin was observed in the positive control solutions and no interferences were observed in the blank buffer solutions. Tilmicosin was determined to undergo rapid, aqueous photodegradation under sunlight conditions at all three pH levels tested. Degradation products observed in the 1-, 2-, and 4-hour chromatograms essentially disappear within 1 day as evidence by the 1-day chromatograms. The aqueous photodegradation rate constants (k) for tilmicosin at pH 5, 7, and 9 were 0.83 ± 0.11, 0.84 ± 0.09, and 0.84 ± 0.12 hrs $^{-1}$, respectively. Using these calculated rate constants, the corresponding half-life values ($t_{1/2}$) for tilmicosin at pH 5, 7, and 9 were 0.84 ± 0.11, 0.82 ± 0.10, and 0.82 ± 0.12 hours, respectively. These results are quantitatively accurate for the test conditions and should qualitatively reflect photodegradation rates at other latitudes. Based on these data, tilmicosin and its degradation products should not accumulate in the aquatic environment.

Appendix H: Report Summary

Title: Tilmicosin Soil Sorption/Desorption and ^{14}C Tilmicosin Supplementary Soil Sorption Study

Name and Address of Investigators: A. L. Donoho; Agricultural Chemistry Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, IN 46140-0708

Study Number: ABC-0396 and ABC-0450

Test Article: ^{14}C Tilmicosin

Summary of Experimental Design:

Study ABC-0396

Eight grams of sandy loam (pH 5.7), loam (pH 6.5), and clay loam (pH 6.9) were equilibrated in glass centrifuge tubes with 40 ml of 0.01 M $CaCl_2$ solution containing various concentrations of ^{14}C tilmicosin. Equilibration was done by mixing on a mixing wheel at 25 ± 1°C. The ^{14}C tilmicosin, Lot 702-SZ0-23, had equimolar distribution of radioactivity in the macrolide ring and piperidine ring. The specific activity was 1.29 mCi/mg and the purity was approximately 95%. Samples were run in triplicate with appropriate blanks and controls. After mixing for the appropriate interval, samples were centrifuged at 2230 x g and aliquots of solution were assayed for radioactivity by liquid scintillation counting.

Study ABC-0450

The same methodology was used as in Study ABC-0396 with eight gram samples of silica gel (for dry column chromatography, Activity III/30 mm, Woelm 04530) and with samples of loam soil adjusted to pH 8.9 using $Ca(OH)_2$.

Summary of Results:

A preliminary experiment was conducted using a concentration of 1 mg/ml ^{14}C tilmicosin equilibrated as described above for 24, 48, and 72 hours, to determine the time required for equilibration. After equilibration, two desorption steps were performed with fresh $CaCl_2$ solution to determine the degree of desorption. Results are summarized in Table 1. A 24-hour mixing time was sufficient to achieve equilibration. Almost all (>95%) of the radioactivity was adsorbed to the soils. Very little (<3%) was desorbed by mixing with fresh $CaCl_2$ solution.

A second set of samples was run for 24 hours at concentrations of 0.2 to 25 mg/ml to determine the Freundlich sorption coefficients (K) for the three soils. The results are summarized in Table 2. The sorption coefficients were 318, 181, and 129 for clay loam, loam, and sandy loam soils, respectively. Thus, tilmicosin was tightly sorbed to all three soil types.

Appendix H: Continued

In a third study (ABC-0450), a Freundlich sorption coefficient (K) was determined for loam soil adjusted to pH 8.9 using three concentrations of ^{14}C tilmicosin ranging from 1 to 25 ppm. The data is listed in Table 2. This study demonstrated that even at a high soil pH, tilmicosin is strongly sorbed to soil, with a K value of 86. When a 1-ppm solution of ^{14}C tilmicosin was mixed with silica gel, only 2% was recovered from the supernate. The silica gel adsorbed 98% of the tilmicosin from the solution.

Table 1. Results from Preliminary Sorption Experiment.

	Radioactivity in Supernate (%)				
	Sorption			Desorption	
Sample	24-hr	48-hr	72-hr	First	Second
Loam	1.6	1.4	1.6	1.0	0.8
Clay loam	0.4	0.3	0.7	0.3	0.2
Sandy loam	3.9	4.5	4.2	3.0	2.6

Table 2. Radiochemical Counting Results from Sorption Isotherm.

Conc.	Soil, log dpm/g				Supernate, log dpm/ml			
(ppm)	C Loam	Loam	Loam(pH 8.9)	S Loam	C Loam	Loam	Loam(pH 8.9)	S Loam
25	5.571	5.566	5.525	5.528	2.529	3.084	3.669	3.870
5	4.870	4.865	4.812	4.849	1.813	2.326	2.921	2.887
1	4.114	4.110	4.111	4.099	1.255	1.634	2.228	2.037
0.2	3.468	3.462	---	3.439	0.845	1.176	---	1.653

Equation: log dpm/g = m log dpm/ml + log K
From this equation, the Freundlich adsorption coefficients K were 318, 181, 86, and 129, for clay loam, loam, loam (pH 8.9), and sandy loam soils, respectively.

Appendix I: Report Summary

Title: Biodegradation of ^{14}C Tilmicosin in Soil

Name and Address of Investigators: A. L. Donoho and D. E. Ruggles; Agricultural Chemistry Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140-0708

Study Number: ABC-0404

Study Dates: February 8 to August 22, 1988

Test Article: ^{14}C Tilmicosin, Lot 702-SZ0-23 (See Appendix H)

Test System: Soils contained in closed incubation flasks.

Summary of Experimental Design: The biodegradation study was conducted according to procedures described in the Environmental Assessment Technical Assistance Handbook, FDA, CVM. Clay loam, loam, and sandy loam soils were fortified with ^{14}C glucose (positive controls) or with unlabeled glucose plus 10 ppm ^{14}C tilmicosin. The samples were adjusted to moisture levels of 75% of field capacity and incubated at room temperature (ca 22°C) in the dark for 64 days. The flasks were fitted with traps to collect organic volatiles and $^{14}CO_2$ and the sample trains were aerated twice each day. Radioactivity in the traps was determined by liquid scintillation counting (LSC) using samples from a negative control for backgrounds. At the end of the study, radioactivity remaining in the soils was determined by extraction and then by combustion of the spent soil, coupled with LSC.

Summary of Results: Results are summarized in Table 1. Recovery of $^{14}CO_2$ from the ^{14}C glucose positive controls ranged from 31% to 62%, indicating that the soils were viable. Recovery of $^{14}CO_2$ in the ^{14}C tilmicosin treatment samples was <1%, indicating a low degree of biodegradation. Neutral solvent extraction of the soils recovered <10% of the radioactivity. Extraction with methanol containing 1% ammonium hydroxide recovered approximately 62% to 79% of the radioactivity and most of this fraction appeared to be parent tilmicosin. The spent soils contained 14% to 24% of the original ^{14}C tilmicosin radioactivity. These results indicate that the half life for degradation of tilmicosin in the soils was longer than 64 days, since approximately two-thirds to three-fourths of the tilmicosin remained as parent compound.

Appendix I: Continued

Table 1. Radioactivity Distribution (%) Among Various Fractions from ^{14}C Tilmicosin and ^{14}C Glucose Treated Soils[a/]

Fraction	Tilmicosin-treated			Glucose-treated		
	Loam	C Loam	S Loam	Loam	C Loam	S Loam
Volatiles	0	0	0	2	1	<1
$^{14}CO_2$	0	<1	<1	31	43	62
Neut. Solv.	8	9	4	2	1	2
Meth./NH_4OH	78	79	62	<1	<1	<1
Spent Soil	19	14	24	36	31	27

a/ Values are given as % of the total added to the sample.

Appendix J: Report Summary

Title: The Acute Toxicity of Tilmicosin to Bluegill in a Static Test System

Name and Address of Investigators: D. W. Grothe and J. R. Smith; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; Box 708; Greenfield, IN 46140

Study Number: F00189

Study Dates: January 9 through 13, 1989

Test Article: Tilmicosin

Lot Number: X-44606

Species: Bluegill (*Lepomis macrochirus*)

Experimental Design: Groups of 20 juvenile bluegill (mean individual weight, 0.87 g) were exposed to average measured tilmicosin concentrations of 0.0 (water control), 214, 524, 528, 604, and 679 ppm. Aquaria with 30 L of test or control solution were used to contain each group of 20 fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Total alkalinity, total hardness, and conductivity of the dilution water were determined. Behavioral signs of toxicity (sluggishness, hypoactivity, minimal swimming behavior, labored respiration, and prostration) and mortality were monitored for fish in each aquarium on a daily basis.

Results: Water quality characteristics were as follows: pH, 8.1 to 9.3; dissolved oxygen at least 87% saturation; temperature, 21.0°C to 21.8°C; total hardness, 137 mg/L (as $CaCO_3$); alkalinity, 138 to 150 mg/L (as $CaCO_3$); and conductivity, 311 to 350 mS/cm. Detailed records of behavioral observations and mortalities are shown in Tables 1-4. Fish exposed to tilmicosin concentrations $\geq$ 524 ppm exhibited sluggishness, hypoactivity, or prostration. The 96-hour median lethal concentration, its 95% confidence limits, and the slope of the concentration-response curve were 716 ppm, 635 to 807 ppm, and 12.6, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to a tilmicosin concentration of 214 ppm.

Appendix J: Continued

Table 1. Physical Condition/Behavior of Bluegill Exposed for 24 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 24 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
214	20	--	--	--	--	--	--
524	20	--	--	--	--	--	--
528	--	10	9	--	--	--	1
604	--	8	9	--	--	--	3
679	--	4	10	--	--	--	6

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix J: Continued

Table 2. Physical Condition/Behavior of Bluegill Exposed for 48 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 48 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
214	20	--	--	--	--	--	--
524	19	--	--	--	--	--	1
528	--	9	10	--	--	--	1
604	--	7	10	--	--	--	3
679	--	3	10	--	--	1	6

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix J: Continued

Table 3. Physical Condition/Behavior of Bluegill Exposed for 72 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 72 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
214	20	--	--	--	--	--	--
524	19	--	--	--	--	--	1
528	--	9	10	--	--	--	1
604	--	6	11	--	--	--	3
679	--	3	9	--	--	--	8

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix J: Continued

Table 4. Physical Condition/Behavior of Bluegill Exposed for 96 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 96 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
214	20	--	--	--	--	--	--
524	19	--	--	--	--	--	1
528	18	--	1	--	--	--	1
604	15	2	--	--	--	--	3
679	2	8	2	--	--	--	8

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix K: Report Summary

Title: The Acute Toxicity of Tilmicosin to Rainbow Trout in a Static Test System

Name and Address of Investigators: D. W. Grothe and J. R. Smith; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Number: F00289

Study Dates: February 20 through 24, 1989

Test Article: Tilmicosin

Lot Number: X-44606

Species: Rainbow Trout (*Salmo gairdneri*)

Experimental Design: Groups of 20 juvenile rainbow trout (mean individual weight, 0.53 g) were exposed to average measured tilmicosin concentrations of 0.0 (water control), 98, 196, 424, 534, 659, and 875 ppm. Aquaria with 30 L of test or control solution were used to contain each group of 20 fish. Dissolved oxygen concentrations, pH, and temperature of the solutions were recorded daily. Total alkalinity, total hardness, and conductivity of the dilution water were determined. Behavioral signs of toxicity (sluggishness, hypoactivity, minimal swimming behavior, labored respiration, and prostration) and mortality were monitored for fish in each aquarium on a daily basis.

Results: Water quality characteristics were as follows: pH, 8 to 9.2; dissolved oxygen, at least 78% saturation; temperature, 12.0°C to 13.4°C; total hardness, 120 mg/L (as $CaCO_3$); alkalinity, 124 to 130 mg/L (as $CaCO_3$); and conductivity, 181 to 186 mS/cm. Detailed records of behavioral observations and mortalities are shown in Tables 1-4. Fish exposed to tilmicosin concentrations ≥659 ppm exhibited sluggishness, hypoactivity, or prostration. The 96-hour median lethal concentration, its 95% confidence limits, and the slope of the concentration-response curve were 851 ppm, 784 to 988 ppm, and 12.3, respectively. No mortalities and no behavioral signs of toxicity were found for fish exposed to a tilmicosin concentration of 534 ppm.

Appendix K: Continued

Table 1. Physical Condition/Behavior of Rainbow Trout Exposed for 24 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 24 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
98	20	--	--	--	--	--	--
196	20	--	--	--	--	--	--
424	20	--	--	--	--	--	--
534	20	--	--	--	--	--	--
659	--	12	8	--	--	--	--
875	--	--	12	--	--	3	5

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix K: Continued

Table 2. Physical Condition/Behavior of Rainbow Trout Exposed for 48 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 48 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
98	20	--	--	--	--	--	--
196	20	--	--	--	--	--	--
424	20	--	--	--	--	--	--
534	20	--	--	--	--	--	--
659	--	10	10	--	--	--	--
875	--	--	6	--	--	3	11

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix K: Continued

Table 3. Physical Condition/Behavior of Rainbow Trout Exposed for 72 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 72 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
98	20	--	--	--	--	--	--
196	20	--	--	--	--	--	--
424	20	--	--	--	--	--	--
534	20	--	--	--	--	--	--
659	--	9	10	--	--	1	--
875	--	--	6	--	--	3	11

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix K: Continued

Table 4. Physical Condition/Behavior of Rainbow Trout Exposed for 96 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Individual Fish Condition/Behavior Value at 96 Hr[a]						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
0.0 (Control)	20	--	--	--	--	--	--
98	20	--	--	--	--	--	--
196	20	--	--	--	--	--	--
424	20	--	--	--	--	--	--
534	20	--	--	--	--	--	--
659	--	10	8	--	--	--	2
875	--	--	8	--	--	1	11

[a]Expressed as the number of fish that exhibited one of the following condition/behavior patterns:
1.0 Normal, equal to controls.
1.5 Sluggish, less active than controls, darted away from probe.
2.0 Hypoactive, could be touched with probe.
2.5 Swimming impaired, could be turned on their sides with probe.
3.0 Labored respiration, minimal voluntary movement, sometimes dark in color.
3.5 Prostrate, movement barely detected.
4.0 Dead.

Appendix L: Report Summary

Title: The Acute Toxicity of Tilmicosin to *Daphnia magna* in a Static Test System

Name and Address of Investigators: D. W. Grothe, Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, Indiana 46140

Study Number: C00189

Study Dates: January 17 through 19, 1989

Test Article: Tilmicosin

Lot Number: X-44606

Species: *Daphnia magna*

Number of Animals: 5/replicate, 4 replicates/treatment

Experimental Design: A group of 20 *Daphnia*, <24 hours old, were exposed for 48 hours to control water and to solutions of tilmicosin with average measured concentrations of 0.0 (water control), 2.6, 9.0, 26.4, 38.5, 58.6, and 95.0 mg/L. Each replicate beaker contained 200 ml of test solution. Temperature, dissolved oxygen, and pH of the test solutions were measured daily. Total alkalinity, total hardness, and conductivity were measured in the diluent water and the test solutions. *Daphnia* were assessed for hypoactivity, prostration, and immobility.

Results: The water quality characteristics were as follows: pH, 8.0 to 8.5; dissolved oxygen concentration, at least 90% of saturation; temperature, 19.4°C to 21.6°C; total alkalinity, 142 to 152 mg/L (as $CaCO_3$); total hardness, 137 mg/L (as $CaCO_3$); and conductivity, 282 to 301 mS/cm. At tilmicosin concentrations ≥9.0 ppm, exposure-related signs of toxicity ranged from hypoactivity to immobility (Table 1). The 48-hour median effective concentration, the 95% confidence limits, and the slope of the concentration-response curve were 57.3 ppm, 51.5 to 64.8 ppm, and 10.5, respectively. No immobilization or physical signs of toxicity were observed in animals exposed to a tilmicosin concentration of 2.6 mg/L.

Appendix L: Continued

Table 1. Physical Condition/Behavior of *Daphnia magna* Populations Exposed for 48 Hours to Tilmicosin.

Average Assayed Tilmicosin Concentration (mg/L)	Physical Condition[a]							
	24 Hr				48 Hr			
	N	H	P	I	N	H	P	I
0.0 (water control)	20	--	--	--	20	--	--	--
2.6	20	--	--	--	20	--	--	--
9.0	20	--	--	--	--	20	--	--
26.4	20	--	--	--	--	20	--	--
38.5	20	--	--	--	--	19	--	1
58.6	--	19	--	--	--	10	--	10
95.0	--	13	--	--	--	--	--	20

[a]Expressed as the number of test organisms that exhibited one of the following general physical conditions: N - normal, H - hypoactive, P - prostrate, I - immobilized.

Appendix M: Report Summary

Title: Determination of the Effect of Tilmicosin on Seed Germination

Name and Address of Investigators: J. E. Dalidowicz; Agricultural Chemistry Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P. O. Box 708; Greenfield, IN 46140-0708

Study Number: ABC-0399

Study Dates: February 16 to October 28, 1988

Test Article: Tilmicosin

Test System: Seeds germinated in the dark in Petri dishes.

Summary of Experimental Design: Seeds of corn (*Zea mays*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) were soaked for 1 hour in distilled water. They were then germinated in Petri dishes in filter paper saturated with solutions of 1, 10, or 100 ppm of tilmicosin. After germination, the percent germination and the radicle length of seedlings were determined.

Summary of Results: The results show that tilmicosin did not have an effect on seed germination in any of the four cultivars or on radicle development of corn, soybean, or wheat. The development of the cucumber radicle was not affected at tilmicosin concentrations of 1 or 10 ppm, but there was a 45.5% reduction in radicle length at 100 ppm. The no-effect concentration of 10 ppm is higher than the highest soil concentration expected from product use. Therefore, no phytotoxicity problems are expected from the agricultural use of tilmicosin.

Appendix N: Report Summary

Title: The Toxicity of Soil-Incorporated Tilmicosin to the Earthworm in a 28-Day Test

Name and Address of Investigators: D. W. Grothe and J. L. Seacat; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Number: W00788

Study Dates: August 17 to September 14, 1988

Test Article: Tilmicosin

Lot Number: X-44606 (purity, 85.98%)

Species: Earthworm (*Lumbricus terrestris*)

Summary of Experimental Design: Tilmicosin was blended with pulverized rabbit feces, sandy loam soil, and water to achieve average measured tilmicosin concentrations of 0.0, 74, 423, and 918 ppm. Four replicates, each containing 2.0 kg of test media and 10 earthworms, were used for a control and at each treatment level. Every 7 days the earthworms were observed (normal, flaccid, prostrate or dead). Worms were weighed at the beginning and end of the study. Earthworms were exposed to the test media for 28 days.

Summary of Results:

No mortality or physical signs of toxicity were observed in earthworms at any tilmicosin concentration tested (Table 1). The mean body weight gain (35.6%) by earthworms at the tilmicosin concentration of 918 ppm was significantly higher than the mean body weight gain (28.6%) by control worms after 28 days of exposure (Table 2).

It was concluded that no behavioral effects or reductions in body weight gain resulted when earthworms were exposed for 28 days to soil containing concentrations of tilmicosin as high as 918 ppm.

Appendix N: Continued

Table 1. Physical Condition and Survival of Earthworms Exposed to Tilmicosin for 7, 14, 21, and 28 Days. Study W00788.

Analyzed Tilmicosin Concentration (mg/kg)	Physical Condition[a]																			
	Day 7					Day 14					Day 21					Day 28				
	1	2	3	4	% Surv.	1	2	3	4	% Surv.	1	2	3	4	% Surv.	1	2	3	4	% Surv.
0.0 (Control)	40	0	0	0	100	39	0	0	1[b]	97.5	39	0	0	1	97.5	39	0	0	1	97.5
74	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100
423	40	0	0	0	100	40	0	0	0	100	39	0	0	1[b]	97.5	39	0	0	1	97.5
918	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100	40	0	0	0	100

[a]Physical condition expressed as the number of earthworms that exhibited one of the following conditions: 1 - normal, 2 - flaccid, 3 - prostrate, 4 - dead.
[b]Accidentally killed when the worm was being physically separated from the test media.

Appendix N: Continued

Table 2. Body Weight of Earthworms Exposed to Tilmicosin for 28 Days. Study W00788.

Analyzed Tilmicosin Concentration (mg/kg)	Body Weight (g) and Body Weight Gain (g)[a]		
	Day 0	Day 28	
	Body Weight	Body Weight	Body Weight Gain
0.0 (Control)	3.3841 ± 0.1601	4.7456 ± 0.2433	1.3615 ± 0.1691
74	3.5550 ± 0.1913	4.7066 ± 0.4074	1.1516 ± 0.2972
423	3.7197* ± 0.1642	5.2561* ± 0.1937	1.5364 ± 0.1079
918	3.6069 ± 0.1112	5.6073* ± 0.2721	2.0004* ± 0.2227

[a]Mean ± SD for four replicates.
*Statistically significant difference between this value and the control ($p \leq 0.05$).

Appendix O: Environmental Regulations for the Dista and Eli Lilly S.A. Facilities

Appendix O: Environmental Regulations Affecting the Dista Products, Ltd. Formulation Facility and the Eli Lilly S.A. Manufacturing Facility.

ENVIRONMENTAL REGULATIONS - DISTA PRODUCTS LTD.

Legislation	Licence	Licence Ref No.	Date of Issue	Admin. Agency
Town and Country Planning Act 1990	Planning Permission	K/APP/14656	21st Jan 1993	Metropolitan Borough of Knowsley
Environmental Protection Act 1990 Part 1	Environmental Protection (Applicatons, Appeals & Registers) Regs 1991 Sl No. 507	B3/1194/6.9/ 24	13th Jan 1995	Metropolitan Borough of Knowsley
Publice Health (Drainage of Trade Premises) Act 1937 Publice Health Act 1961 Water Act 1973	Consent to Discharge	-	1st June 1981	North West Water PLC (formerly Authority)
Control of Pollution Act 1974[1]	Waste Disposal Facility Licence[2]	WDL 292/02	13th Mar 1989	Merseyside Waste Disposal Authority

[1] Solids will be disposed of in accordance with the requirements of this act.

[2] Not specifically required for the formulating and packaging operations for Pulmotil, but applicable to other operations at the facility.

Appendix O (cont.): Environmental Regulations Affecting the Dista Products, Ltd. Formulation Facility and the Eli Lilly S.A. Manufacturing Facility.

ENVIRONMENTAL REGULATIONS - Lilly S.A.*

Legislation	Licence	Licence Ref No.	Date of Expiration	Admin. Agency
Air Pollution Control of 1987	Air Pollution Control License	A.P. 3/93(R)	9/1/96	Cork Co. Council
Local Government (Water Pollution) Act 1977	Water Pollution Control License	WP(W) 6/91	11/23/95	Cork Co. Council
European Communities (Toxic and Dangerous Waste Regulations 1982)	Toxic and Dangerous Waste Disposal Permit	2TDW	9/28/95	Cork Co. Council
European Communities (Waste) Regulations 1979	Non Toxic Waste Permit	NTW	12/31/94*	Cork Co. Council

* Note: These licenses ceased to have effect in 1995 and the Eli Lilly S.A. Facility was then be operating under an Integrated Pollution Control (IPC) License issued by the Irish E.P.A. The Eli Lilly S.A. Facility IPC License has the Reference Number 9. The IPC License will last for a period of 3-5 years.

NORTH WEST WATER AUTHORITY

PUBLIC HEALTH (DRAINAGE OF TRADE PREMISES) ACT 1937

PUBLIC HEALTH ACT 1961

WATER ACT 1973

Serial No 77/4/185a

NOTICE OF DIRECTION CONCERNING DISCHARGE OF TRADE EFFLUENT

TO Kodak Ltd.,

of Hemel Hempstead. Herts.,

The North West Water Authority (hereinafter referred to as "the Authority") HEREBY GIVE YOU NOTICE as Owner(s) ~~Occupier(s)~~ of the trade premises situate and known as

Kodak Ltd., Accrefield Road, Kirkby, Liverpool L33.

(hereinafter referred to as "the said premises") that the Authority in exercise of the powers conferred upon them by Section 60 of the Public Health Act 1961 and the Water Act 1973 DIRECT that as from First day of July 1977 the conditions attaching to their Consent dated the Twenty-third day of May 19 73 to the discharge of trade effluent into the public sewer from the said premises shall be ~~rescinded~~ and the following conditions shall be substituted, namely:-

Sewer affected

1. The sewer(s) into which the trade effluent may be discharged and the point(s) of discharge is/are the sewer(s) situate at Accrefield Road - (South) and more particularly shown on the plan attached hereto.

Maximum amount to be discharged in any day

2. The maximum amount of the trade effluent discharged on any one day of twenty-four hours shall not exceed 1365 m^3 without the prior written consent of the Authority.

Maximum rate of discharge

3. The highest rate at which the trade effluent may be discharged shall not exceed 91 m^3/hour

Times of Discharge

~~4. No trade effluent shall be discharged except between the hours ... in any day.~~

Matters to be eliminated prior to discharge to the sewer

5. There shall be eliminated from the trade effluent before it is discharged into the sewers of the Authority the following matters:-

(a) petroleum spirit:

(b) calcium carbide:

with the following formula:—

$$C = R + V + \frac{Ot}{Os} B + \frac{St}{Ss} S$$

Where C = Total charge in pence per cubic metre.

R = Reception and conveyance charge per m^3 of trade effluent.

V = Preliminary treatment charge per m^3 of trade effluent.

B = Biological oxidation cost per m^3 of settled sewage. (including the cost of secondary sludge disposal).

S = Treatment and disposal cost of primary sludges per m^3 of sewage.

Ot = The Chemical Oxygen Demand (COD) in mg/l of the trade effluent after settlement for one hour at pH 7.0 or at the pH of the mixed sewage.

Os = The COD in mg/l of average strength settled sewage.

St = Total suspended solids content of the trade effluent at pH 7.0 in mg/l (or at the pH of the mixed sewage).

Ss = Total suspended solids content of average strength crude sewage in mg/l.

A minimum charge of Ten pounds (£ 10) per annum or such higher charge as the Authority may from time to time fix will be payable.

For the purpose of this condition a year is the period from the First day of April to the Thirty-first day of March following.

Inspection Chamber

11. (i) An inspection chamber or manhole shall be provided and maintained by the Trader in a suitable position in connection with each pipe through which the trade effluent is discharged and shall be so constructed and maintained as to enable a person readily to obtain samples of the trade effluent so discharged, to the approval of the Authority in all respects.

Measurement of the discharge

(ii) Suitable apparatus shall be provided for measuring and automatically recording the volume of trade effluent discharged and maintained in working order by the Trader in connection with every such pipe, unless otherwise exempted, in writing, by the Authority.

(iii) If the measuring and recording apparatus as aforesaid ceases to function satisfactorily, then the Authority shall have the right to make estimates of the volume and composition of the trade effluent until such time as the said apparatus is again operating to the satisfaction of the Authority.

(iv) Records shall be kept by the Trader of the volume, rate of discharge, nature and composition of the trade effluent discharged to the sewer. Such records shall be kept available for inspection at all reasonable times by an authorised officer of the Authority and copies shall be sent to the Authority on demand.

(v) The foregoing provisions of this condition shall be deemed to be complied with if other methods of sampling the trade effluent, determining its nature and composition, and measuring and recording the discharge are agreed and confirmed in writing by the Authority.

Your attention is directed to the following provisions of the Public Health Act 1961 relating to Appeals to the Secretary of State for the Environment.

SECTION 60

(4) A water authority shall give to the owner and occupier of the trade premises to which the consent relates notice of any direction and the notice shall include information as to the right of appeal conferred by the next following subsection.

(5) The owner or occupier of the trade premises may within two months of the giving of the notice to him or with the written permission of the water authority at any later time appeal to the Secretary of State against the direction.

(6) The notice shall state the date on which the direction is to take effect and if an appeal is brought under this section before that date the direction shall not take effect until the appeal is withdrawn or finally disposed of:

Provided that so far as a direction relates to the making of charges payable by the occupier of the trade premises, it may take effect on any date after the giving of the notice.

(7) On an appeal under this section the Secretary of State shall have power to annul the direction given by the water authority or to substitute for it any other direction and any direction given by the Secretary of State may include provision as to the charges to be made for any period between the giving of the notice by the water authority and the determination of the appeal.

Dated this First day of May 19 77

Divisional Office

North West Water Authority,
Western Division,
Marton House, Stanley Road,
Bootle, Merseyside,
L20 3NH.
Telephone No. 051-922-7260

DIVISIONAL MANAGER for and on behalf of NORTH WEST WATER AUTHORITY

Appendix P: Letters from Manufacturing Facilities Assuring Compliance with Regulations and Permits
(Letters from Dista Facility and Eli Lilly S.A. refer to Environmental Regulations listed in Appendix O)

Dista Products Limited

Fleming Road, Speke
Liverpool L24 9LN
Telephone (051) 486 3939
Telex 627178
Fax (051) 486 8740

Direct No. (051) 448

12th March 1993.

TO: Dr. Derek Johnson,
Paylean/Pulmotil Project Manager,
Dista Products Limited.
Speke Operations.

FROM: Mr. A.F. Tinsley.

Dear Dr. Johnson,

This letter of confirmation is provided in answer to the request in connection with the Paylean/Pulmotil submission to the U.S. Food & Drug Administration. The request was for official confirmation that our planned facility at Dista Products, Speke for the manufacture of Paylean/Pulmotil, will comply with the relevant environmental regulations of the U.K., especially as detailed in Appendix A of this letter.

From our enquiries, it appears that it is not possible to obtain such official confirmation. We can however, confirm that we have received no official notification that our operations in the manufacture of Paylean/Pulmotil will not comply with the relevant environmental laws of the U.K. as detailed, and that we intend in the manufacture of Paylean/Pulmotil, to comply with the relevant environmental laws of the U.K. especially those mentioned in Appendix A of this letter. It is also our intention that the production of Paylean/Pulmotil will comply with Good Manufacturing Practices.

Yours sincerely,

MR. ALAN TINSLEY,
Director H.R. & Environmental Services
Dista Products Limited,
Speke Operations.

ly S.A. – Irish Branch, Dunderrow, Kinsale, Co. Cork.

Lilly

ELI LILLY S.A. – IRISH BRANCH

Mr. Mark Owens.
Director , Corporate Environmental Affairs,
Eli- Lilly and Co.,
Indiannapolis,
Indiana, 96285.

19/7/95

Dear Mr. Owens,

This letter of confirmation is provided in answer to the request in connection with the Tilmicosin submission to the U.S. F.D.A. The request was for official confirmation that our planned facility at Eli-Lilly, Kinsale for the manufacture of Tilmicosin will comply with the relevant environmental regulations of Ireland.

We can confirm that our operations in the manufacture of Tilmicosin will comply with the requirements set out in our current Environmental licence, namely, our Integrated Pollution control licence (Ref. No. 9), as issued by the Environmental Protection Agency. It is also our intention that the production of Tilmicosin will comply with Good Manufacturing Practices.

Yours Sincerely,

Dr. Anne Chandler.
Director of Operations

(021) 772699
76900 LILY EI
(021) 775152

Appendix Q: Report Summary

Title: Exposure monitoring study for tilmicosin in a feed mill at Greenfield, Indiana

Name and Address of Investigators: M. A. Moreman, Eli Lilly and Company, Indianapolis, IN 46285

Study Number: T5C759301

Study Dates: July 21 through 23, 1993

Test Article: Pulmotil premix containing 20 % tilmicosin (lot number X49253)

Summary of Experimental Design: This study was designed to assess worker exposure to tilmicosin while preparing a feed containing 400 ppm tilmicosin from a 20% tilmicosin premix. Three types of monitoring were conducted: full shift (8-hr) personal monitoring for comparison with the Lilly Exposure Guideline (LEG) of 120 $\mu g/m^3$; short term (<30-min) personal monitoring for comparison with the excursion limit of 360 $\mu g/m^3$ (based on the American Conference of Governmental Industrial Hygienists guidance on excursion limits) and area monitoring to assess the concentration of tilmicosin in ambient air in various locations. The feed mill at the Eli Lilly and Company Greenfield Laboratories was the site of the study. To simulate an exposure environment to the worker employed in a commercial feed mill with minimal or no engineering controls, all engineering exposure control measures were disarmed, including local exhaust ventilation and weighing hoods. A working regimen was followed that simulated commercial feed mill practice by weighing five lots of tilmicosin premix followed by mixing and bagging of each individual medicated feed lot. Full-shift personal monitoring encompassed all tasks, while short-term personal monitoring was conducted on the tasks of premix weighing and medicated feed bagging. Mixing was not assessed with short-term personal monitoring or area monitoring due to the short duration of the task (dumping the premix into the mixer lasted approximately 5 seconds).

Weighing of the premix was conducted on an electronic platform scale. The scale was housed in a cement-block room (7 ft. X 7 ft.) situated in a feed storage warehouse. After weighing, the premix was poured through a small door at the top of the mixer for blending. The mixer was a 1.5 ton capacity, horizontal double-ribbon, tilt-tub device made by Wenger. The mixer was totally enclosed (one cloth dust bag for air displacement) with dust exhaust lines attached. The mixer was located in an area that was about 35 ft. X 55 ft. Once the mixing cycle was completed, the blended feed was dumped into a surge bin directly below the mixer and transported pneumatically to the finished feed bin for bagging. The feed was discharged from the finished feed bin down a metal tube to the hopper on the baghouse scale. Finished feed (50 pounds) was released from the scale into a bag. The top of the bag was stitched closed and placed on a pallet for transport to a warehouse.

The premix was mixed into fifteen separate 750-pound batches of a 16% crude protein corn-soy swine grower diet (ration no. 31) with no liquid additions. Each batch of feed was mixed to contain 400 ppm (364 g/ton) of tilmicosin. Each batch of feed was mixed, bagged, and stacked before the next batch was started. Exposure monitoring was conducted during premix weighing, and medicated feed bagging operations by pumping ambient air through glass fiber filters and analyzing them for their tilmicosin content. The actual ambient levels of tilmicosin in air were calculated based on the amount found on the filter, the volume of air pumped through the filter, and the survey recovery value.

Monitoring was conducted on July 21, 22, 23, 1993 at the Eli Lilly and Company Greenfield Feed Mill. For short-term monitoring (<30 minutes), samples were collected on glass fiber filters fitted in 37 mm open face dust sampling cassettes attached by tygon tubing to GAST Model DOA-104-AA high volume air sampling pumps. The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 20.1 liters per minute (l/min.). For full-shift monitoring (approximately 8 hours), glass fiber filters in 37 mm closed face dust sampling cassettes were attached by tygon tubing to DuPont P2500B air sampling pumps. The pumps were calibrated with a Kurz electronic flowmeter and had an average air flow rate of approximately 2.3 l/min. For personal monitoring, the filter cassettes were located in the worker's breathing zone.

A 3M9920 dust, fume, and mist respirator and latex gloves were worn while weighing the premix.

Summary of Results:

Full-shift personal monitoring (Table 1) was conducted for an average of 455 minutes at a flow rate ranging from 2.2 l/min to 2.5 l/min. Nine samples were taken, seven of which yielded detectable breathing zone concentrations of tilmicosin with a minimum of 1.3 $\mu g/m^3$, a maximum of 4.2 $\mu g/m^3$, and a geometric mean of 2.5 $\mu g/m^3$ with a standard deviation of 1.7. The remaining two samples yielded concentrations below the limit of quantitation (LOQ) (sample LOQs were approximately 1 $\mu g/m^3$).

Short-term personal monitoring (Table 1) for the premix weighing operation was conducted for the duration of the task, lasting an average of 13 minutes at a flow rate ranging from 20.1 l/min to 20.3 l/min. Three samples were taken with a minimum of 104.1 $\mu g/m^3$, a maximum of 202.2 $\mu g/m^3$, and a geometric mean of 147.8 $\mu g/m^3$ with a standard deviation of 1.4.

Short-term personal monitoring (Table 1) for the medicated feed bagging operation was conducted for an average of 24 minutes at a flow rate ranging from 19.5 l/min to 20.8 l/min. Six samples were taken, four of which yielded detectable breathing zone concentrations with a minimum of 2.0 $\mu g/m^3$, a maximum of 9.7 $\mu g/m^3$, and a geometric mean of 4.1 $\mu g/m^3$ with a standard deviation of 2.1.

Area monitoring (Table 2) was conducted to determine the ambient concentration of tilmicosin while the tasks of premix weighing and medicated feed bagging were occurring. Samples were taken from areas contiguous to the task and approximately 15 feet from the task. Area samples were collected for approximately the same period of time as the corresponding personal samples (i.e., area samples at the bagging task were collected for an average of 24 minutes, as the personal samples at the same task were collected for an average of 24 minutes). Sampling flow rates ranged from 19.5 l/min to 20.6 l/min. Three area samples were taken near the premix weighing operation (approximately 1 ft. away) yielding concentrations ranging from 50.4 $\mu g/m^3$ to 163.5 $\mu g/m^3$. Four samples were collected at a site approximately 15 feet from the premix weighing operation yielding one quantifiable concentration of 10.0 $\mu g/m^3$. Eight area samples were taken near the bagging operation (approximately 2 ft. away) of which one yielded a quantifiable concentration of 3.2 $\mu g/m^3$. Three samples collected at a site approximately 15 feet from the feed bagging operation were below the LOQ (<2.6 $\mu g/m^3$).

This study was designed to assess the worker exposure to tilmicosin while weighing premix and mixing and bagging medicated feed in a feed mill. Full shift monitoring demonstrated exposure levels of tilmicosin to be approximately 2.1% of the exposure guideline of 120 $\mu g/m^3$. The mean breathing zone air concentrations of tilmicosin during premix weighing were approximately 41% of the 3X LEG excursion limit of 360 $\mu g/m^3$. The mean breathing zone air concentrations of tilmicosin during medicated feed bagging were approximately 1% of the 3X LEG excursion limit of 360 $\mu g/m^3$.

These data demonstrate that atmospheric tilmicosin exposure to workers weighing tilmicosin premix or bagging medicated feed was below recommended exposure guidelines.

Table 1: Results of Personal Monitoring for Feedmill Exposure Study - 20% tilmicosin formulation - study number T5C759301

Full-Shift Personal Results		
Sample Duration (min).	**Result ($\mu g/m^3$)**	**Exposure Guideline ($\mu g/m^3$)**
549	3.0	120
403	1.4	120
467	<1.0	120
471	<1.0	120
403	1.3	120
465	4.2	120
396	4.0	120
473	1.5	120
470	4.0	120
Short-Term Personal Results - Weighing		
15	104.1	360
13	153.6	360
11	202.2	360
Short-Term Personal Results - Bagging		
21	5.7	360
21	<2.6	360
25	2.6	360
25	<2.2	360
27	9.7	360
27	2.0	360

Table 2: Results of Area Monitoring for Feedmill Exposure Study - 20% tilmicosin formulation - study number T5C759301

Task	Location *	Result ($\mu g/m^3$)
weighing	near	163.5
weighing	near	50.4
weighing	near	161.4
weighing	far	<3.5
weighing	far	<4.1
weighing	far	10.0
bagging	near	<3.6
bagging	near	<2.5
bagging	near	<3.7
bagging	near	<3.3
bagging	near	<2.1
bagging	near	<3.6
bagging	near	3.2
bagging	near	<3.6
bagging	far	<2.6
bagging	far	<2.1
bagging	far	<2.0

*'near' samples were located contiguous to the task; 'far' samples were located approximately 15 feet away

Appendix R: Report Summary

Title: Biodegradation of ^{14}C Tilmicosin in Soil

Name and Address of Investigators: A. S. Kennington and A. L. Donoho; Agricultural Chemistry Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140-0708

Study Number: T5C749301

Study Dates: January through November, 1993

Test Article: ^{14}C Tilmicosin, Lot 702-AFF-2

Test System: Soils contained in closed incubation flasks.

Summary of Experimental Design: The biodegradation study was generally conducted according to procedures described in the Environmental Assessment Technical Assistance Handbook, FDA, CVM. Clay loam, loam, and sandy loam soils were fortified with ^{14}C glucose (positive controls), unlabeled glucose (negative control), or 1 ppm ^{14}C tilmicosin. The samples were adjusted to moisture levels of 75% of field capacity and incubated at room temperature in the dark for 8 weeks. The flasks were fitted with traps to collect organic volatiles and $^{14}CO_2$. Radioactivity in the traps was determined by liquid scintillation counting (LSC). At the end of the study, radioactivity remaining in the soils was determined by extraction with LSC and by combustion of the spent soil.

Summary of Results: Biodegradation of the ^{14}C glucose to $^{14}CO_2$ ranged from 50 to 68 percent in the three soils. Minimal biodegradation of ^{14}C tilmicosin to $^{14}CO_2$ occurred during the experimental period. Organic volatiles and $^{14}CO_2$ traps accounted for less than 1 percent of the total ^{14}C tilmicosin radioactivity in clay loam and sandy loam soils. In loam soil, about 7 percent of the total ^{14}C tilmicosin radioactivity was recovered as $^{14}CO_2$. Extraction of subsamples of tilmicosin-treated soils with methanol containing 1 percent ammonium hydroxide recovered 53% to 68% of the radioactivity. HPLC analysis showed that the radioactivity extracted from the soils by ammoniac methanol was predominantly unchanged tilmicosin (88% -90%).

Appendix S: Report Summary

Title: Determination of Biodegradation Potential of Tilmicosin (EL-870) under Anaerobic Conditions

Name and Address of Investigators: Karen P. Christensen, Springborn Laboratories, Inc. Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts 02571

Study Number: 1982.1094.611.727

Study Dates: January 13, 1995 to March 27, 1995

Test Article: Tilmicosin (Lot No. RS0164) and ^{14}C Tilmicosin (Lot 702-AFF-2)

Test System: Slurry of fresh swine manure held under anaerobic conditions

Summary of Experimental Design: The biodegradability of tilmicosin was evaluated in anaerobic test systems consisting of fresh swine manure diluted 1:10 with anaerobic mineral medium. Test procedures were adapted from U.S. EPA TSCA guidelines. Tilmicosin was tested at concentrations of 1.0 mg/L using radiolabeled material and 78.6 mg/L using nonradiolabeled material. Sodium benzoate (a combination of nonradiolabeled amd ^{14}C material) was used as a reference article. The systems were incubated at approximately 35 °C for 73 days. Vessels were amended with an additional source of carbon (glucose and ethanol) on day 59. Gas production was monitored in all treatments. The biotransformation of ^{14}C tilmicosin was monitored by extraction and analysis after 14, 28, 42, and 73 days of incubation. Concentrations of sodium benzoate were also monitored during the study.

Summary of Results: Gas was produced in the untreated control, as well as in the vessels treated with sodium benzoate and tilmicosin. No statistical difference was found in total gas production among any of the untreated control or treatment vessels for the entire study. Gas production in the tilmicosin treated vessels was, however, statistically lower than gas production in the untreated control during the first 3 days of the study. Cumulative net gas production indicated that tilmicosin and sodium benzoate did not mineralize to CO_2 and/or methane during the study.

HPLC analysis of reference compound vessels for sodium benzoate showed that all of the sodium benzoate added at the study initiation was present after 73 days of incubation, indicating that the compound did not degrade under the test conditions. The distribution of radioactivity in extracts from test vessels containing 1 mg ^{14}C tilmicosin showed that the extractable radioactivity was accounted for as tilmicosin after 73 days of incubation. These data indicated that tilmicosin did not degrade significantly under the test conditions.

Active microbial counts and production of gas in control, reference, and test material vessels indicated that a viable microbial population was present in all vessels. Under the conditions of this study, tilmicosin was not degraded in swine waste maintained under anaerobic conditions for 73 days.

Appendix T: Report Summary

Title: The 14-Day Acute Toxicity of Tilmicosin to the Freshwater Green Alga (*Selenastrum capricornutum*) in a Static Test System

Name and Address of Investigators: D. W. Poage and W. H. Jordan; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; Box 708; Greenfield, IN 46140

Study Number: J00693

Study Dates: April 14 through 28, 1993

Test Article: Tilmicosin

Lot Number: X-48986

Species: Green algae (*Selenastrum capricornutum*)

Experimental Design: A static toxicity test was conducted to evaluate the effects of tilmicosin on the green alga, Selenastrum capricornutum. Algal cells were cultured for 14 days in a liquid nutrient medium that contained tilmicosin at initial assayed concentrations of 0.0, 12, 25, 54, 112, 240, 468, and 1173 µg/L. Tilmicosin was assayed at the end of the tested at each treatment level. Each treatment consisted of three replicate 500-ml Erlenmeyer flasks containing 100 ml of nutrient medium with an initial algal density of 1000 cells/ml. The algal population of each flask was quantified on Days 2, 3, 4, 5, 7, 10, and 14.

Summary of Results: Terminal cell count was significantly reduced relative to water control cultures at inital analyzed tilmicosin concentrations ≥ 468 µg/L, while maximum cell count was significantly reduced at initial assayed concentrations ≥ 240 µg/L. The average specific growth rate, calculated over the first 4 days of the study, was significantly reduced at initial tilmicosin concentrations ≥ 240 µg/L. Maximum specific growth rate was also significantly reduced at initial concentrations ≥ 240 µg/L. Terminal biomass was significantly reduced at tilmicosin initial concentrations ≥ 112 µg/L. Definitive calculations could not be done for a median effective concentration or the lowest effective concentration of tilmicosin because the tilmicosin rapidly degraded in the test systems, probably due to photolysis. Tilmicosin concentrations were below detectable levels in all but the highest two treatments. Dramatic declines were also found at these highest two treatment levels. Since light is required for this type of study, it is unlikely that definitive estimates for a median effective concentration or lowest effective concentration can be found for an algal toxicity study. This study does support data which indicate that tilmicosin is rapidly degraded by photolysis.

Appendix U: Report Summary

Title: The Toxicity of Tilmicosin to Juvenile Bobwhite in a 5-Day Dietary Study

Name and Address of Investigators: D. W. Grothe and J. L. Seacat; Toxicology Division; Lilly Research Laboratories; Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Number: A00589

Study Dates: February 9 through 17, 1993

Test Article: Tilmicosin

Lot Number: X-44606

Species: Bobwhite (*Colinus virginianus)*

Summary of Experimental Design: The 5-day dietary toxicity of tilmicosin was determined for 11-day-old bobwhite. Measured concentrations of tilmicosin in the diets were 0.0 (solvent control), 1130, 2390, and 4820 ppm. Groups of 10 bobwhite were fed these diets for 5 days and untreated diets for another 3 days.

Summary of Results: No mortalities, signs of toxicity, or reductions in food consumption were found at any treament level tested. During the 5-day treatment phase of the study, a significant reduction in body weight gain occurred for birds exposed to the two highest treatment levels. No effects were noted on body weight gain at the 1130-ppm treatment level.

Appendix V: Report Summary

Title: The Toxicity of Tilmicosin to Juvenile Mallards in a 5-Day Dietary Study

Name and Address of Investigators: D. W. Grothe and J. R. Smith; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Number: A00489

Study Dates: February 2 through 10, 1993

Test Article: Tilmicosin

Lot Number: X-44606

Species: Mallard (*Anas platyrhynchos)*

Summary of Experimental Design: The 5-day dietary toxicity of tilmicosin was determined for 4-day-old mallards. Measured concentrations of tilmicosin in the diets were 0.0 (solvent control), 1120, 2370, and 4710 ppm. Groups of 10 bobwhite were fed these diets for 5 days and untreated diets for another 3 days.

Summary of Results: No mortalities, or signs of toxicity were found at any treament level tested. During the 5-day treatment phase of the study, a significant reduction in body weight gain occurred for birds exposed to the highest treatment level. Food consumption by these birds was also somewhat lower. No effects were noted on body weight gain or food consumption at tilmicosin levels of 1120 and 2370 ppm.

Appendix W: Report Summary

Titles: Microbial (*Aspergillus*, *Chaetomium*, *Comamonas*) Growth Inhibition from Exposure to Tilmicosin.
Microbial (*Nostoc*, *Azotobacter*) Growth Inhibition from Exposure to Tilmicosin.

Name and Address of Investigators: J. L. Newsted and R. Mohr; Toxicology Division; Lilly Research Laboratories; A Division of Eli Lilly and Company; P.O. Box 708; Greenfield, IN 46140

Study Numbers: Z00193, Z00493

Study Dates: April and May, 1993

Test Article: Tilmicosin (Lot number X-48986)

Test System: Agar-based media

Summary of Experimental Design: Pure cultures of *Azotobacter chroococcum*, *Nostoc sp.*, *Comamonas acidovorans*, *Chaetomium globosum*, and *Aspergillus flavus* were used to inoculate agar-based media in flasks containing several concentrations of tilmicosin. Two flasks were inoculated at each test concentration. *Comamonas* was exposed to tilmicosin concentrations of 62.5, 125, 250, 500, and 1000 ppm. *Nostoc* was exposed to tilmicosin concentrations of 0.0625, 0.125, 0.25, 0.5 and 1.0 ppm, and *Azotobacter* were exposed to 1.0, 2.5, 5.0, and 10.0 ppm. *Chaetomium* and *Aspergillus* were exposed to tilmicosin concentrations of 500 and 1000 ppm. The minimum inhibitory concentration was determined to be the lowest concentration that inhibited growth based on visual inspection.

Summary of Results: MIC values from these studies were as follows: *Aspergillus flavus*, > 1000 ppm; *Chaetomium globosum*, > 1000 ppm; *Comamonas acidovorans*, 250 ppm; *Azotobacter chroococcum*, 5.0 ppm; and *Nostoc sp.*, 0.5 ppm. The MIC value for *Nostoc* may be an overestimate because tilmicosin may have photolyzed during the study.

Appendix X: Report Summary

Title: Exposure Monitoring Study of Tilmicosin in a Swine Barn

Name and Address of Investigators: R. S. Readnour, M. A. Moreman, J. L. Taylor, and P. A. Shoaf, Lilly Research Laboratories, 2001 W. Main St., Greenfield, IN 46140

Study Number: T5C619402

Study Dates: 1/4/95 to 1/6/95

Test Article: Tilmicosin Type A Medicated Article (Lot No. X-49252); potency - 203.5 g tilmicosin/kg formulation

Summary of Experimental Design: This study was designed to assess worker exposure to tilmicosin while preparing (mixing and bagging) a feed containing 400 ppm tilmicosin from a 20 percent tilmicosin premix, dispensing the medicated feed into swine feeders, and agitating medicated feed in the swine feeders. Three types of monitoring were conducted: full shift (8-hr.) personal monitoring for comparison with the Lilly Exposure Guideline (LEG) of 120 $\mu g/m^3$; short term (30-min.) personal monitoring for comparison with the excursion limit of 360 $\mu g/m^3$ (based on the American Conference of Governmental Industrial Hygienists guidance on excursion limits) and area monitoring to assess the concentration of tilmicosin in ambient air in various locations. The swine barn at the Eli Lilly and Company Greenfield Laboratories was the site of the study. To simulate an exposure environment to the worker employed in a commercial swine barn with minimal or no engineering controls, all engineering exposure control measures were disarmed, including pit ventilation. Full-shift personal monitoring encompassed all tasks, while short-term personal monitoring was conducted on the tasks of premix weighing and medicated feed mixing and bagging, dispensing of medicated feed into swine feeders, and agitating of medicated feed in the swine feeders. These tasks were also monitored by area samplers.

This exposure monitoring study for tilmicosin was conducted on each of three days in a swine barn where the agent was blended into feed using a portable vertical screw mixer, and the feed dispensed into swine feeders. On each of three days, one lot of medicated feed was prepared by adding a premix containing 20 percent tilmicosin to a 1000 pound batch of control feed to achieve a concentration of 400 ppm tilmicosin. Feed from each lot was bagged into twenty 50-lb bags. Further, the entire lot of 400 ppm tilmicosin medicated feed from each day was dispensed into 18 swine feeders. Finally, the 400 ppm medicated feed in each feeder was agitated for two 30-minute time periods.

Samples were collected on glass fiber filters fitted in 37 mm opened-faced dust sampling cassettes for background, short term, and clearance monitoring (<30 minutes). The open-faced dust sampling cassettes were attached by Tygon tubing to a GAST Model DOA-104-AA high volume air sampling pump having an approximate flow rate of 20 liters/minute. The pumps were calibrated using an electronic flowmeter prior to each day of use. On the first day of analysis, the background, short term, and clearance samples were mistakenly collected with closed-faced sampling, while on the second and third day open-faced sampling was used. This deviation in the sample monitoring had no adverse effect on the sample results. Spiked samples were used to assure the recovery and stability of tilmicosin during the handling operations. Air monitoring samples were submitted for both quality control and actual sample analysis to the analytical laboratory for each day of analysis. Samples were extracted using a method developed for tilmicosin on filters.

Results: The personal sampling results were organized by homogenous exposure groups (HEG) and statistically analyzed assuming a log-normal distribution with the exception of the HEG for full-shift personal results which more closely fit a normal distribution. The data were organized into four HEGs: one for full-shift personal monitoring; one for short-term personal monitoring assessing the premix weighing and feed mixing task; one for short-term personal monitoring assessing the dispensing of medicated feed into swine feeders task; and one for short-term personal monitoring assessing agitation of the feed to mimic the feeding action of swine. Where applicable, a geometric mean and geometric standard deviation (SD) were calculated for each HEG. Full-shift personal monitoring was conducted for an average of 469 minutes at a flow rate ranging from 2.0 L/min. to 2.1 L/min. Three samples were taken, two of which yielded detectable breathing zone concentrations of tilmicosin with a minimum of 2.7 $\mu g/m^3$, a maximum of 3.0 $\mu g/m^3$, and an arithmetic mean of 2.7 $\mu g/m^3$. The remaining sample yielded a concentration below the limit of quantitation (LOQ) (sample LOQ was approximately 2.3 $\mu g/m^3$).

Short-term personal monitoring for the premix weighing and feed mixing operation was conducted for 30 minutes at a flow rate ranging from 19.4 L/min. to 20.7 L/min. Three samples were taken with a minimum of 21.9 $\mu g/m^3$, a maximum of 66.7 $\mu g/m^3$, and a geometric mean of 44.5 $\mu g/m^3$.

Short-term personal monitoring for the dispensing of medicated feed to swine feeders operation was conducted for 30 minutes at a flow rate ranging from 19.4 L/min. to 20.7 L/min. Three samples were taken, all yielding concentrations below the LOQ (sample LOQs were approximately 4.0 $\mu g/m^3$).

Short-term personal monitoring for the agitating of medicated feed in swine feeders to mimic the feeding action of swine operation was conducted for 30 minutes at a flow rate ranging from 19.4 L/min. to 20.7 L/min. Six samples were taken, all yielding concentration below the LOQ (sample LOQs were approximately 4.0 $\mu g/m^3$).

Area monitoring was conducted to determine the ambient concentration of tilmicosin before any tasks involving the use of tilmicosin began; while tasks involving the use of tilmicosin were occurring; and after tasks involving the use of tilmicosin were completed. Thirty six samples were taken in areas contiguous to the premix weighing and feed mixing task, before, during, and after conducting the task. All samples were collected for approximately 30 minutes. Sampling flow rates ranged from 19.8 L/min. to 21.1 L/min. All samples yielded concentrations below the LOQ (sample LOQs were approximately 4.0 $\mu g/m^3$), with the exception of one sample located approximately 2 feet from the scale which yielded a concentration of 16.7 $\mu g/m^3$.

Another thirty six samples were also taken in areas contiguous to the dispensing of medicated feed into swine feeders, and agitation of the feed tasks. All samples were collected for approximately 30 minutes. Sampling flow rates ranged from 19.6 L/min. to 21.1 L/min. All samples yielded concentrations below the LOQ (sample LOQs were approximately 4.0 $\mu g/m^3$).

Full shift monitoring demonstrated exposure levels of tilmicosin to be approximately 2.3 percent of the exposure guideline of 120 $\mu g/m^3$. The mean breathing zone air concentrations of tilmicosin during premix weighing and feed mixing were approximately 12.4 percent of the excursion limit of 360 $\mu g/m^3$. The mean breathing zone air concentrations of tilmicosin during dispensing of medicated feed into swine feeders were < 1.2 percent of the excursion limit of 360 $\mu g/m^3$, as were those during agitation of the feed.

These data demonstrated that atmospheric tilmicosin exposure to workers weighing tilmicosin premix, mixing medicated feed, and dispensing medicated feed into swine feeders was below recommended exposure guidelines.

Appendix Y: Report Summary

Title: Determining the Effects of Tilmicosin on the Seedling Growth of Terrestrial Plants

Name and Address of Investigators: E. Feutz and C. Lochhaas, ABC Laboratories, Inc. 7200 E. ABC Lane, Columbia, MO 65202

Study Number: 42631

Study Dates: 5/17/95 to 6/9/95

Test Article: Tilmicosin (Lot X-48986); potency - 31.1%

Test System: Seedling plants grown in sand or sandy loam soil and provided liquid nutrient media

Summary of Experimental Design: A 21-day seedling growth study was conducted to determine the effects of tilmicosin on corn (*Zea mays*), cucumber (*Cucumis sativus*), perennial ryegrass (*Lolium perenne*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), and wheat (*Triticum aestivum*). Seedlings of each species were planted in sand and sandy loam that was then treated with nutrient media containing tilmicosin. Treatment levels in the sandy loam soil were 0, 1, 3, 10, 30, 100, and 300 mg/kg (mg tilmicosin per kg of dry soil). These same treatment levels and an additional treatment of 0.3 mg/kg were used to the test the effects of tilmicosin in sand as a substrate. HPLC analysis of the treatment solutions was performed to insure the soil and sand substrates were dosed with the appropriate amount of tilmicosin. Five plants were used for each replicate and five replicates were used for each treatment level. The seedlings were cultured in an environmentally controlled room for 21 days. Seedlings were subirrigated on an as needed basis with half strength Hoagland's nutrient solution. Shoot lengths were measured for all seedlings on days 0, 7, and 14. Shoot lengths, shoot weights, and root weights were measured for all plants at the end of the study.

Results: Well-defined dose-response relationships were evident for all species when exposed to high tilmicosin levels in sand. For the test with sand, no significant adverse effects were found for corn, cucumber, perennial ryegrass, soybean, tomato, and wheat at exposure levels of 30, 3, 3, 3, 3, and 100 mg/kg, respectively. The effects of tilmicosin on seedling growth were significantly reduced when tilmicosin was introduced into sandy loam soil. Tilmicosin strongly sorbs to soil, but least of all to sandy loam. Even so, sorption to the sandy loam soil was apparently strong enough to significantly reduce the effects of tilmicosin on the seedlings. Only cucumbers were significantly affected by tilmicosin in sandy loam soil, and only at the highest level tested. In sandy loam soil, no significant adverse effects were found for the other five species tested at a tilmicosin level of 300 mg/kg.