PULMOTIL 90 TYPE A MEDICATED ARTICLE

ENVIRONMENTAL ASSESSMENT FOR THE USE OF PULMOTIL 90 TYPE A MEDICATED ARTICLE TO CONTROL BOVINE RESPIRATORY DISEASE IN CATTLE

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Introduction

Tilmicosin is the active ingredient in Pulmotil 90 Type A Medicated Article. Tilmicosin is already approved for oral use as Pulmotil 90 Type A Medicated Article for use in the feed of pigs for control of swine respiratory disease (NADA 141-064). Additionally, tilmicosin is approved for use in cattle for treatment of bovine respiratory disease as an injectable, Micotil 300 Injection (NADA 140-929).

The following assessment is provided to support an application for the use of tilmicosin (as Pulmotil 90 Type A Medicated Article) at a targeted dose of up to 12.5 mg/kg/day in the feed of cattle for the control of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni.*

This environmental risk assessment has been conducted for the use of Pulmotil 90 Type A Medicated Article in cattle based on the VICH guidelines for both phase I (VICH GL6) and phase II (VICH GL38) assessments and on normal use of cattle manure as fertilizer in the United States.

Pattern of Use and Relevant Exposure Routes

Pulmotil 90 Type A Medicated Article (Pulmotil 90 Premix) will be administered to cattle via the feed during the first 45 days following arrival at the feedlot. The treatment duration will be 14 days during this window of susceptibility to BRD.

Pulmotil 90 Premix for cattle will be added to the feed to provide a targeted daily dose of tilmicosin of 12.5 mg/kg bodyweight for a period of 14 days. Even though the annual incidence of BRD is less than 50%, all cattle at a given feedlot could be treated.

The primary route of environmental exposure to tilmicosin will be from cattle manure applied to agricultural land. Spillage and breakage of containers would be handled through the waste systems and could also be applied with manure to agricultural land.

Description of the Product

Pulmotil 90 Premix (90 g tilmicosin/pound) is a formulation of tilmicosin for incorporation into feed for cattle. The active ingredient, tilmicosin, is prepared from desmycosin which itself is derived from tylosin phosphate concentrate by mild acid hydrolysis.

International Non-proprietary Name (INN): Tilmicosin

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

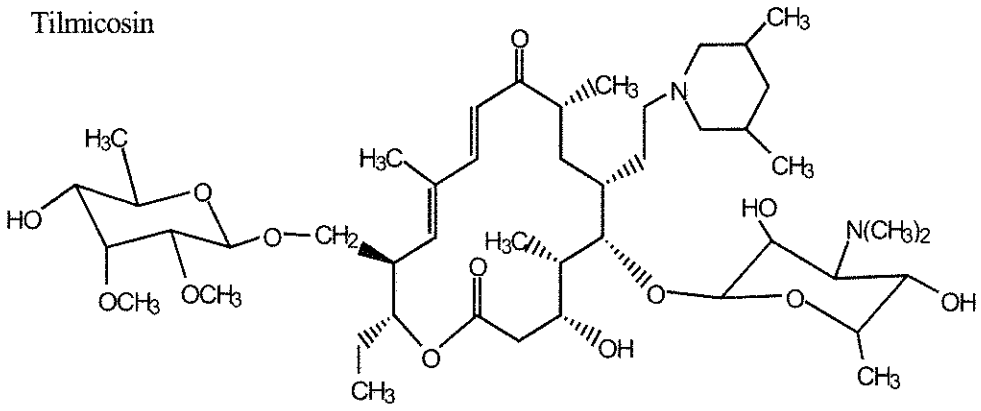
CAS Number: 108050-54-0

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

Molecular Weight: 869.15

Structural Formula:

Tilmicosin



Phase I Environmental Impact Assessment

Final Guidance for Industry #89 (CVM, 2001) published by the FDA, Center for Veterinary Medicine, and the VICH GL6 Phase I guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) were consulted to conduct the Phase I Environmental Impact Assessment for the use of Pulmotil 90 Premix in cattle. In this Phase I assessment, the maximum concentration of tilmicosin in the manure and the soil has been calculated. No metabolism or degradation in manure is assumed and a total residue approach is taken for the Phase I assessment. The initiation of a Phase II assessment is dependent upon the trigger established in the VICH GL6 guidance: if the predicted environmental concentration of the total residue in soil is greater than 100 µg/kg, a Phase II assessment is warranted.

Manure Calculation

The concentration of tilmicosin in the manure of beef cattle was estimated using the following equation:

$$[\text{Tilmicosin}]_{\text{Manure}} = \frac{\text{Dose/day} \times \text{F} \times \text{Dosing Duration}}{\text{Total Manure Production}}$$

Where:

$[\text{Tilmicosin}]_{\text{manure}}$ = tilmicosin concentration in manure (mg/kg wet weight)

Dose/day = daily dose per animal (12.5 mg/kg X 300 kg/animal)

F = fraction of animals treated (1.0)

Dosing Duration = number of days animals treated (14 days)

Total manure production = 27.3 kg manure/animal/day X 130 day production period

$$[\text{Tilmicosin}]_{\text{Manure}} = \frac{12.5\,\text{mg}_{\text{tilmicosin}}/\text{kg} \times 300\,\text{kg} \times 1.0 \times 14\,\text{days}}{130\,\text{days} \times 27.3\,\text{kg}_{\text{manure}}/\text{day}} = 14.8\,\text{mg/kg}$$

After the 130 day production period, the concentration of tilmicosin in the manure will be 14.8 mg/kg.

Concentrations in the Environment

The maximum concentration of tilmicosin in the soil has been calculated using typical agronomy practices for application of cattle manure to land. Manure from beef cattle is applied to soil at a rate of 27,200 kg/acre. Assuming a plow depth of 15 cm, the weight of the soil in an acre is approximately 910,500 kg. Assuming no degradation in soil, the concentration of tilmicosin in soil after application of cattle manure could be as high as 442 µg/kg.

$$[\text{Tilmicosin}]_{\text{Soil}} = \frac{14.8\,\text{mg}_{\text{tilmicosin}}/\text{kg}_{\text{manure}} \times 27200\,\text{kg}_{\text{manure}}}{910{,}500\,\text{kg}_{\text{soil}}} = 0.442\,\text{mg/kg}$$

Since the initial concentration in the soil is more than 100 µg/kg, a Phase II environmental risk assessment was conducted, as per the VICH GL6 Final Guidance.

Phase II Environmental Impact Assessment

Since an initial soil concentration of 442 µg/kg was calculated in the Phase I assessment, a Phase II environmental risk assessment has been conducted. Final Guidance for Industry #166 (CVM, 2006) published by the FDA, Center for Veterinary Medicine, and the VICH GL38 Phase II guidance for Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) were consulted to conduct the Phase II Environmental Impact Assessment for the use of Pulmotil 90 Premix in cattle.

Tier A

Summary of Available Data

Physical and Chemical Properties

The physical and chemical properties of tilmicosin indicate that the molecule is very water soluble and exists as a solid at normal environmental temperatures.

Melting Point	107 to 112°C		
Aqueous Solubility (Study RMK8701, 1988)		5°C	25°C
	pH 5	NT	Extremely viscous due to high solubility
	pH 7	NT	566 mg/mL
	pH 9	72.5 mg/mL	7.7 mg/mL
Thermogravimetric Analysis (Study RMK8701, 1985)	Only 1.6 percent weight loss from about 23 to 129 °C due to loss of water and minor volatile impurities. At about 167°C, another loss begins and continues through decomposition.		
n-Octanol/Water Partition Coefficient (Study AAC8728, 1988)	pH 5	pH 7	pH 9
	< 10	< 10	376
Dissociation Constant	7.4 and 8.5, respectively, for *Cis* and *Trans* isomers in 66% dimethylformamide		
UV/Visible Absorption	Peak absorption occurs at 283 nm in ethanol		

NT: Not tested

Fate

Metabolism and Excretion

The metabolism and excretion of radiolabeled tilmicosin by cattle has been described in two studies. In the first (Study ABC-0383, 1988), following injection of ^{14}C-tilmicosin, approximately 74% of the dosed radioactivity is excreted within 14 days. About 54% of the dose was found in the feces and 20% in the urine. Approximately 22% of the fecal residue was tilmicosin and 22% was the demethylated metabolite, T1. The remainder of the fecal residue was made up of low level metabolites and non-extractable residue. Urinary residue was approximately 75% tilmicosin.

In a second study (Study T5C969601, 1998), 78% of the orally administered dose was recovered in excreta; about 66% in feces and 12% in urine. Approximately 23% of the fecal residue was tilmicosin, 11.5% was T4 and 10% was T1. Approximately 51% of the urinary residue was tilmicosin and the Cis-8-epimer and 10% was T1. Other residues in urine and feces were low level and non-extractable.

The percent dose recovered was normalized to 100% and it was assumed that the unrecovered radioactivity would have the same profile as the recovered radioactivity. Therefore, in both studies, tilmicosin and major metabolites (comprising at least 10% of the administered dose) in cattle excreta totaled approximately 50% of the administered dose.

Degradation

At pH values of 4 and 7, tilmicosin is 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.

The biodegradation of tilmicosin in clay loam, loam and sandy loam soils has been examined for up to 64 days (Studies ABC-0404, 1988; T5C749301, 1993). These studies indicate that the half-life of tilmicosin is longer than 64 days. By the end of one study, measurable levels of $^{14}CO_2$ (<1% to 6.75%) had evolved. Using methanol with ammonium hydroxide, only 53 to 79% of the radioactivity could be extracted from the soils at the end of the studies. Between about 66 and 90% of the extracted radioactivity in both studies was tilmicosin. A significant percentage of the applied radioactivity remained in soils after extraction. It is likely that unextractable radioactivity is not bioavailable, or even tilmicosin. The results of these studies demonstrate that tilmicosin does degrade in soil, and is slowly degraded to CO_2.

Based on the highest percentage (90%) of tilmicosin recovered in the soil extract, there was at least 10% biodegradation of tilmicosin in the studies. If as little as 6% of the tilmicosin was degraded in 64 days, the half-life of tilmicosin in soil would be about 2 years. It would have a degradation rate constant of 0.3465/year.

Soil Adsorption

The adsorption of tilmicosin to soil was evaluated in 4 different soils (Studies ABC-0396, 1988; ABC-0450, 1990). Freundlich binding isotherms were constructed for each soil at a range of concentrations and the resulting Freundlich Kd coefficients were converted to Koc values on the basis of the organic carbon fraction in the soils. The Freundlich Kd coefficients ranged from 86 to 318 while the Koc values ranged from 4244 to 36150. The magnitude of these values as well as the difficult extraction of tilmicosin from soil during the soil degradation studies indicate that tilmicosin will be strongly absorbed to the soil and will be unlikely to move extensively through soil.

Photolysis

Tilmicosin undergoes very rapid photolysis in water exposed to summer sunlight at 40 degrees north latitude, with a half-life of about 0.82 hours (Study JJL8704, 1987). Further, chromatographic analysis demonstrated that even the initial degradation products disappeared within one day.

Bioconcentration

Tilmicosin has a very low n-octanol/water partition coefficient (Kow), so it is not likely to significantly bioconcentrate in aquatic organisms. Veith et al. (1979) generated a linear model to predict the bioconcentration factor for chemicals in fathead minnows:

$$\log BCF = 0.85 \times \log Kow - 0.70$$

Using this equation and the highest log Kow value for tilmicosin (2.575), the estimated bioconcentration factor (BCF) for tilmicosin is 31.

Environmental Fate Studies

Soil Adsorption/Desorption (ABC-0396, 1988 and ABC-0450, 1990)		Freundlich Coefficient	Koc
	Clay Loam pH 6.9	318	36150
	Sandy Loam pH 5.7	129	8214
	Loam pH 8.9	86	4244
	Loam pH 6.5	181	16667
Hydrolysis (RMK8702, 1987)	Hydrolytically stable at pH values 4 and 7. Half-life of 156 days at pH 9 at 25°C.		

Degradation in Soil (ABC-0404, 1988; T5C749301, 1993)	Biodegradation to $^{14}CO_2$ ranged from <1% to about 7% over 64 days in loam, clay loam and sandy loam soils. 53 to 79% of the radioactivity was extracted from the soil with methanol and with 1% ammonium hydroxide. 66 to 90% of the extract was tilmicosin. As little as 6% degradation in 64 days yields a half-life for tilmicosin in soil of about 2 years, with a degradation rate constant of 0.3465/yr.		
Photolysis – DT50 values, hours (JJL8704, 1987)	pH 5	pH 7	pH 9
	0.84	0.82	0.82

Toxicity

Soil Organisms

The toxicity of tilmicosin to soil microflora, plants, and earthworms has been evaluated (Studies Inveresk 389681, 1997; ABC-0399, 1988; ABC-42631, 1995; W00788; 1982.6336).

No effects on respiration or nitrogen fixation by soil microflora were observed with tilmicosin at the highest concentration tested, 20,000 µg/kg.

In a seedling germination test with four crop species, only cucumber seeds were affected by exposure to tilmicosin via filter paper saturated with aqueous tilmicosin solutions. There were no effects on germination for any of the four species. However, at the highest concentration, 100,000 µg/L, the cucumber radicle length was reduced 45.5%. Thus NOEC for cucumbers was 10,000 µg/L.

In a seedling growth test, six crop species seedlings transplanted into sandy loam soil or sand-only substrate treated with tilmicosin. In the sandy-loam soil test, no effects were observed in corn, ryegrass, soybeans, tomatoes and wheat up to the highest concentration tested, 300,000 µg/kg. In cucumbers, the NOEC was 30,000 µg/kg. In the sand-only test, all species showed reduced growth in a dose-response fashion. Ryegrass, soybean, cucumber and tomato all had the lowest NOEC value of 3,000 µg/kg.

Taken together, the two plant studies with tilmicosin indicate that cucumbers have a particular sensitivity to tilmicosin; the mechanism for this sensitivity is unknown. Additionally, comparison of the NOEC in the seed germination study, in which the exposure medium was tilmicosin-saturated filter paper, with the NOEC in the seedling growth study, in which the exposure medium was sand, indicates that seedling growth is a slightly more sensitive endpoint than radicle development (NOEC of 3,000 vs 10,000). Finally, binding to soil reduces bioavailability and phytotoxicity.

The effects of tilmicosin on earthworms were evaluated in two studies. In Study W00788, *Lumbricus terrestris* were exposed to tilmicosin for 28 days with no adverse effects on survival, growth, or behavior observed at concentrations up to 918,000 µg/kg in the test media. In a chronic study including a reproduction endpoint (Study 1982.6336), *Eisenia fetida* were exposed to tilmicosin in soil up to a concentration of 1,000,000 µg/kg. After 4 weeks, adults were removed, leaving cocoons and any juveniles. After a second 4-week period, juvenile worms were counted to assess reproduction. No effects were observed on survival, growth, or reproduction and the NOEC was a tilmicosin concentration ≥ 1,000,000 µg/kg.

Aquatic Organisms

The toxicity of tilmicosin to algae, daphnia, and fish has been evaluated (Studies J00693, 1993; C00189, 1989; F00189, 1989; F00289, 1989).

Psuedokirchineriella subcapitata (formerly *Selenastrum capricornutum*) were exposed to tilmicosin in a static toxicity test for 14 days at initial assayed concentrations ranging from 12 to 1173 µg/L. Concentration-dependent decreases in biomass and growth rate were observed, and terminal biomass of algae was significantly reduced at initial tilmicosin concentrations ≥ 112 µg/L. At the end of the 14 day study, the five lowest test concentrations had no detectable levels of tilmicosin, and tilmicosin concentrations at the remaining two highest test levels had decreased substantially. The decline in tilmicosin concentrations was probably due to photolysis. Since light is required for algae to grow, an algal toxicity test cannot be conducted without the possibility of photolysis. Accurate calculation of the median effective concentration, the lowest-observed effect concentration, and the no-observed effect concentration for the entire study was not possible. Results from this study confirm that tilmicosin photodegrades rapidly.

Daphnia magna were exposed to tilmicosin in a static toxicity test for 48 hours at average concentrations ranging from 2,600 to 95,000 µg/L. Hypoactivity and immobilization were observed at concentrations of 9,000 µg/L and higher. The EC50 was 57,300 µg/L and the NOEC was 2,600 µg/L.

Bluegill (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) were exposed to tilmicosin in static toxicity tests for 96 hours at mean measured concentrations ranging from 214,000 to 679,000 µg/L (bluegill) and 98,000 to 875,000 (rainbow trout). For bluegill, the LC50 of tilmicosin was 716,000 µg/L and the NOEC was 214,000 µg/L. For rainbow trout, the LC50 of tilmicosin was 851,000 µg/L and the NOEC was 534,000 µg/L.

Terrestrial Effects Studies

Respiration and Nitrogen Transformation Tests (28 days) (Inveresk 389681, 1997)	NOECs ≥ 20,000 µg/kg	
Terrestrial Plants – Seedling Germination (ABC-0399, 1988)		NOEC µg/L
	Corn	≥ 100,000

	Cucumber	10,000	
	Soybean	≥ 100,000	
	Wheat	≥ 100,000	
Terrestrial Plants – Seedling Growth (ABC-42631, 1995)		NOEC µg/kg	
		Soil	Sand
	Ryegrass	≥ 300,000	3,000
	Wheat	≥ 300,000	100,000
	Maize	≥ 300,000	30,000
	Soybean	≥ 300,000	3,000
	Cucumber	30,000	3,000
	Tomato	≥ 300,000	3,000
Earthworm 28-day Growth and Survival (Study W00788, 1988) Earthworm Reproduction (Study 1982.6336, 2008)	NOEC ≥ 918,000 µg/kg NOEC ≥ 1,000,000 µg/kg		

Aquatic Effects Studies

Algal Growth Inhibition (J00693, 1993)	Significant photolysis precluded accurate calculation of an EC50, LOEC and NOEC. Concentration-related effects on growth and biomass were observed, with statistically significant reduction in terminal biomass at initial tilmicosin concentrations ≥ 112 µg/L.
Daphnia immobilization (C00189, 1989)	EC50: 57,300 µg/L NOEC: 2,600 µg/L
Fish Acute Toxicity	Bluegill

(F00189 and F00289, 1989)	LC50: 716,000 µg/L NOEC: 214,000 µg/L Rainbow Trout LC50: 851,000 µg/L NOEC: 534,000 µg/L

PEC Calculations and Refinements (Exposure Assessment)

Soil

The PECsoil-initial was calculated in the Phase I assessment as 442 µg/kg. Per the VICH guideline (GL38), this value can be refined based on the actual composition of the dose excreted by the treated animal by adding the active substance and the relevant metabolites (those that are 10% or more of the administered dose). Calves excrete approximately 50% of the administered dose as tilmicosin or relevant metabolites. Thus the $PEC_{refined\text{-}soil}$ is 221 µg/kg.

Tilmicosin slowly degrades in soil, with a half-life that is estimated to be no longer than 2 years. Therefore, it is possible that with annual application of manure containing tilmicosin, the concentration in soil could increase over time. With a half-life of 2 years (degradation rate constant of 0.3465/yr) and an annual application of tilmicosin resulting in an initial $PEC_{refined\text{-}soil}$ of 221 µg/kg ($C_{initial}$), an asymptotic concentration (C_{max}) of tilmicosin in soil would be 754 µg/kg ($C_{max} = C_{initial}/1\text{-}e^{-0.3465}$). The asymptotic concentration is about 3.4 times higher than the concentration in soil after a single application.

Groundwater

The strong adsorption of tilmicosin to soil will inhibit extensive movement in soil, such that groundwater contamination is not expected. However, a calculated expected concentration of tilmicosin in pore water was used as a worst-case estimate of potential groundwater contamination. The following calculation was used for deriving pore water concentration (ECB 2003):

$$PEC_{porewater} = \frac{PEC_{soil} \times BulkDensity_{wetsoil}}{Kd \times 1000}$$

Using a wet soil bulk density value of 1700 kg/m^3 and the lowest measured Freundlich partition coefficient for tilmicosin of 86, the tilmicosin concentration in soil pore water after a single concentration can be estimated as 4.4 µg/L in soil after a single application. Considering the asymptotic concentration in soil, the pore water concentration of tilmicosin could be as high as 14.9 µg/L.

Surface Water

Movement of tilmicosin from soil to surface water may occur through runoff following rainfall events. A scenario of 1% runoff of compound from 10 acres of soil into a one-acre pond which is 2 m deep was considered. A one-acre pond that is 2 m deep has a volume of 8,100,000 L. Inserting the concentration of timicosin residues in manure and the application rate of manure per acre, the following calculation was performed to estimate the concentration of tilmicosin residues in the pond:

$$\frac{[\text{Tilmicosin}]_{\text{manure}} \times \text{kg manure/acre} \times 10 \text{ acres} \times 0.01}{8{,}100{,}000\text{L}} = [\text{Tilmicosin}]_{\text{pond}}$$

The concentration of total residues of tilmicosin in cattle manure is 14.8 mg/kg. A total of 27,200 kg of cattle manure is applied per acre, such that 4,025,600 mg of tilmicosin residues will be applied per 10 acres. Therefore, 40,256 mg will enter the pond. A one-acre pond that is 2 m deep has a volume of 8,100,000 L. Therefore, the concentration of total tilmicosin residues in the pond would be 5 µg/L.

If metabolism and excretion are considered, the concentration of tilmicosin and relevant metabolites in manure is only 7.4 mg/kg and a total of 20,128 mg would enter the pond. Therefore the $PEC_{surfacewater}$ refined for metabolism and excretion is 2.5 µg/L.

The concentration in the water can also be refined by the propensity of tilmicosin to adsorb to soil and sediment. Since the tilmicosin that runs off soil is bound to soil particles, the Kd values of tilmicosin for soil were used. The measured Kd values for tilmicosin to soil ranged from 86 to 318. For purposes of the risk assessment, the lowest Kd value of 86 was used. The PEC refined for adsorption is calculated by the following equation:

$$PEC_{\text{water-adsorption refined}} = \frac{\text{mass}_{\text{tilmicosin}}}{\text{mass}_{\text{water}} + (\text{mass}_{\text{sediment}} \times K_d)}$$

The mass of tilmicosin and relevant metabolites that enters the pond is 20,128 mg, the mass of water in the pond is 8,100,000 kg, and the mass of the sediment, assuming mixing into the top 5 cm of sediment, is 300,000 kg. Therefore the PEC for surface water refined for metabolism and excretion and adsorption to sediment is 0.6 µg/L.

After accumulation following repeated annual application, the amount of tilmicosin and relevant metabolites available from runoff is about 3.4 times that after a single application. Therefore, the $PEC_{surfacewater\text{-}refined}$ is 2.0 µg/L (0.6 µg/L x 3.4 for accumulation). The surface water concentration would decline over time due to photolysis.

PNEC Calculations (Effect Assessment)

Terrestrial

The assessment factors used and the PNECs calculated for terrestrial species are tabulated below.

	Toxicity endpoint	Assessment Factor	PNEC
Soil Microflora	≤25% change from control = 20,000 µg/kg	1	20,000 µg/kg
Plants, germination – filter paper	NOEC = 10,000 µg/L	10	1,000 µg/L
Plants, growth – soil	NOEC = 30,000 µg/kg	10	3,000 µg/kg
Plants, growth – sand	NOEC = 3,000 µg/kg	10	300 µg/kg
Earthworms	NOEC = 1,000,000	10	100,000 µg/kg

Aquatic

The assessment factors used and the PNECs calculated for aquatic species are tabulated below.

	Toxicity endpoint	Assessment Factor	PNEC
Algal Growth	NOEC not determined due to photolysis Lowest initial concentration that affected algae: 112 µg/L	10	Not determined
Daphnia acute	EC50 = 57,300 µg/L	1000	57.3 µg/L
Fish Acute	LC50 = 716,000 µg/L	1000	716 µg/L

Risk Characterization

Soil

The predicted concentration of tilmicosin residues in soil ($PEC_{refined\text{-}soil}$) after a single application is 221 µg/kg. After repeated annual applications to the same soil, the highest concentration in soil could be 754 µg/kg (assuming a degradation half-life in soil of 2 years).

Plants were the most sensitive terrestrial species. The lowest PNEC for plants grown in sand was 300 µg/kg, which is higher than the initial predicted concentration of tilmicosin residues in soil (221 µg/kg), but not the possible asymptotic concentration in soil (754 ug/kg). However, sand is an unrealistic medium for crops that will be fertilized with cattle manure. The next-highest PNEC calculated for plants was 1000 µg/L, based on the seedling germination test. However, the exposure in that study also was unrealistic as it was conducted with tilmicosin-saturated filter paper and not in soil. Therefore, the PEC

values in soil were compared to the PNEC value for plants grown in sandy-loam soil. These comparisons indicate that, for single and repeated applications of tilmicosin-containing manure, there is no significant risk to plants or other terrestrial species. The PNECs for all terrestrial species tested are all higher than the predicted soil concentration after repeated annual applications of 754 µg/kg.

PEC/PNEC Ratios for Terrestrial Compartment

Species	$PEC_{refined\text{-}soil}$	PNEC	PEC/PNEC Ratio
Plants	Refined, single application-initial: 221 µg/kg	3000 µg/kg	0.07
	Refined, annual application: 754 µg/kg		0.25

Because the lowest PNEC for terrestrial species is greater than the $PEC_{refined\text{-}soil}$, even after routine annual applications, there is no significant risk to soil-dwelling organisms.

Groundwater

The strong adsorption of tilmicosin to soil will inhibit extensive movement in soil, such that groundwater contamination is not expected. However, the PEC in the pore water (as an estimate of the maximum possible groundwater concentration) was compared to the lowest aquatic PNEC as a worst-case estimate of the risk.

PEC/PNEC Ratios for Pore water Compartment

Species	$PEC_{pore\ water}$	PNEC	PEC/PNEC Ratio
Daphnia	Refined, single application-initial: 4.4 µg/L	57.3 µg/L	0.08
	Refined, annual application: 14.9 µg/L		0.26

Because the lowest PNEC for aquatic species is greater than the $PEC_{pore\ water}$, even after routine annual applications, there is no significant risk to organisms exposed to predicted environmental concentrations of tilmicosin in groundwater.

Surface Water

The maximum predicted concentration of tilmicosin and relevant metabolites in surface water after repeated annual applications of manure ($PEC_{refined\text{-}surface\ water}$) is 2.0 µg/L. The lowest PNEC that could be calculated for tilmicosin was 57.3 µg/L, and was determined from the daphnid acute toxicity study. The greatest PEC/PNEC ratio for aquatic organisms is 0.03.

PEC/PNEC Ratios for Surface water Compartment

Species	$PEC_{refined\text{-}surface\ water}$	PNEC	PEC/PNEC Ratio
Daphnia	Refined, single application-initial: 0.6 µg/L	57.3 µg/L	0.01
	Refined, annual application: 2.0 µg/L		0.03

Algae actually appeared to be the most sensitive aquatic species tested, but a definitive PNEC value could not be calculated for algae because tilmicosin concentrations declined during the study due to photolysis. The lowest initial concentration in the test that actually caused a significant decrease in algal biomass was 112 µg/L, a concentration that is about 56 times higher than the highest concentration expected in surface water ($PEC_{surfacewater\text{-}refined}$ for metabolism, excretion, soil accumulation and sediment sorption).

There does not appear to be any significant risk to aquatic species from exposure to predicted concentrations of tilmicosin in surface waters.

Conclusions Tier A

The environmental impact from the use of tilmicosin at 12.5 mg/kg in cattle to prevent bovine respiratory disease in high intensive rearing situations has been evaluated. The pathway for introducing tilmicosin residues into the environment considered in this risk assessment was via the application of cattle manure as fertilizer to soil. Leaching to groundwater and runoff to surface water from that soil was also considered.

The predicted environmental concentrations were refined to consider metabolism and excretion of tilmicosin and major (>10% of dose) metabolites as well as potential accumulation in the environment subsequent to annual application of cattle manure to soil. The predicted environmental concentration of tilmicosin and relevant metabolites in soil is 221 µg/kg after a single application of manure to soil, and 754 µg/kg after repeated annual applications to soil. The maximum predicted environmental concentrations in groundwater are 4.4 and 14.9 µg/L after single and repeated applications, respectively. The predicted environmental concentrations in surface water following rainfall events are 0.6 and 2.0 µg/L after single and repeated applications, respectively.

Plants were the most sensitive terrestrial species tested with a predicted no-effect concentration for tilmicosin of 3000 µg/kg. The lowest no-observed-effect concentration in aquatic species was in daphnids and the predicted no-effect concentration in that species was 57.3 µg/L. The predicted environmental concentrations in soil, surface water and groundwater are all lower than the predicted no-effect concentrations, even after repeated annual application of cattle manure to soil. Algae are likely more sensitive to tilmicosin than daphnids, but rapid photolysis precluded definition of a no-observed-effect concentration. However, the lowest initial concentration to cause a decrease in algae growth (112 µg/L) is almost 200 times greater than the predicted environmental concentration after a rainfall event following a single application of manure to soil, and almost 60 times greater than the concentration following routine annual application. Given its susceptibility to photolysis, no long-term adverse effects on algae are expected from the use of tilmicosin.

Treatment of cattle with Pulmotil 90 Type A Medicated Article to prevent bovine respiratory disease is not expected to result in effects on terrestrial or aquatic organisms exposed to tilmicosin residues from cattle manure used to fertilized cropland soil.

Information on Environmental Assessment Expert

The following individual is responsible for the information in the Environmental Assessment Report for tilmicosin used as Pulmotil 90 Type A Medicated Article to control bovine respiratory disease:

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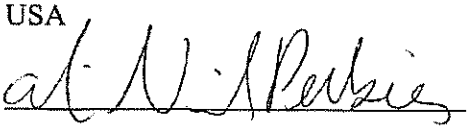
Signature: 

Date: 22 October 2008

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Degrees:

BS	Chemistry, Tulane University	1988
PhD	Pharmacology/Toxicology, University of Mississippi	1996

Current and Previous Appointments:

Senior Research Scientist, Environmental Risk Assessment, Lilly Research Laboratories (2005 to present)
Senior Toxicologist, Nonclinical Safety Assessment, Lilly Research Laboratories (2003 to 2005)
Senior Toxicologist, Environmental Science, Lilly Research Laboratories (2000 to 2002)
Editorial Board of Environmental Toxicology and Chemistry
Research Scientist, Research Institute of Pharmaceutical Sciences, U. Mississippi (1996 to 1999)

Publications:

Fifteen publications and numerous presentations and posters in the field of environmental toxicology.

References

(ECB) European Commission - Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau. 2003. Technical guidance document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances, Commission Regulation (EC) No 1488/94 on risk assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the council concerning the placing of biocidal products on the market. Luxembourg: Office for Official Publications of the European Communities. Part II. 328 p.

Veith GD, DeFoe DL, Bergstedt BV. 1979. Measuring and Estimating the Bioconcentration Factor of Chemicals in Fish. J Fish Res Board Can 36:1040-1048.

VICH 2000, Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) – Phase I, VICH GL6 Final Guidance.

VICH 2006, Environmental Impact Assessments (EIAs) for Veterinary Medicinal Products (VMPs) – Phase II, VICH GL38 Final Guidance

Appendix A Study RMK8701, Water Solubility 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 Study RMK8701, Thermogravimetric Analysis 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 Study AAC8728, Octanol-Water Partition Coefficient 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 Study ABC-0383, Metabolism and Excretion by Cattle Report Summary

Title: Characterization of Residues in Excreta of ^{14}C Tilmicosin-Treated Cattle

Name and Address of Investigators:
A.L. Donoho, J. S. Peloso, and T.D. Thomson
Lilly Research Laboratories
A Division of Eli Lilly and Company
P. O. Box 708
Greenfield, Indiana 46140-0708

Study Number: ABC-0383

Study Dates: May 26, 1987 to January 8, 1988

Test Article: ^{14}C tilmicosin

Test System: Cattle injected with ^{14}C tilmicosin

Summary of Experimental Design:

Cattle weighing approximately 200 kg were injected with 14C tilmicosin at a dose of 10 mg/kg body weight to determine the quantitative and qualitative pattern of residues in tissues. The 14C tilmicosin, Lot 702-SZ0-23, had equimolar distribution of radioactivity in the macrolide rind and piperidine ring. As part of this study, urine and feces were collected from two treated animals to determine the pattern of excretion of radioactive residues and the composition of excreted residues.

Summary of Results:

Most of the dose was excreted in feces, 55.56% and 51.71% for the two steers over a collection period of 14 days. Urinary excretion accounted for 17.69% and 21.18% for the same two animals, respectively. Very little of the dose remained in the edible tissues of the cattle. Of the excreted radioactivity, approximately 50% was excreted during the first day after injection.

Chromatographic characterization indicated that urinary radioactivity was approximately three-fourths parent tilmicosin. Fecal radioactivity was approximately 22% parent tilmicosin and 22% metabolite T-1 which is tilmicosin with one methyl group removed from the mycaminose sugar. The remainder of fecal residue was minor metabolites and nonextractable radioactivity.

Appendix E Study T5C969601, Metabolism and Excretion by Cattle Report Summary

Title: 14C Tilmicosin Milk Tissue Residue Decline and Metabolism Study in Calves

Name and Address of Investigators: DJ Sweeney, KM Ehrenfried, SC Fossler, DE Kiehl, CM Stobba-Willey, Elanco Animal Health, 2001 W Main Street, Greenfield, IN 46140

Study Number: T5C969601

Study Dates: October 1997

Test Article: 14C-Tilmicosin

Test System: Three- to Five-Day Old Dairy Calves

Summary of Experimental Design: Calves were dosed orally twice daily with 12.5 mg/kg tilmicosin for five consecutive days. Excreta were collected throughout dosing and for 7 days after the end of dosing.

Results: In the 12 days of excreta collection following initiation of dosing, 78% of the dosed radioactivity was recovered in urine (12%) and feces (66%). Seven days after the end of dosing, the mean percent of dosed radioactivity still being recovered was approximately 2% per day. The radioactivity in the urine and feces was profiled by radiochromatography and the results are presented in the table as percent of radioactivity in the excreta.

	Parent and Metabolites as An Average Percentage of Total Radioactivity Found in Excreta	
	Urine	**Feces**
Tilmicosin	-	23
Tilmicosin + Cis-8-epimer	51	-
T-1	10	10
T-3 + T-12	-	5.5
T-4	-	11.5
T-8	-	1
T-11	4.5	9
T-12	9	-
T-13	-	5

The percent of dosed radioactivity recovered in urine (12%) and feces (66%) was normalized to total dosed radioactivity (e.g. 15 and 85% in urine and feces, respectively). Assuming that the composition of the unrecovered radioactivity has the same profile as that characterized, the residue composition as a percentage of the total dose can be calculated as below:

	Parent and Metabolites as Percentage of Total Dose	
	Urine	**Feces**
Tilmicosin	-	19.55
Tilmicosin + Cis-8-epimer	7.65	-
T-1	1.5	8.5
T-3 + T-12	-	4.7
T-4	-	9.8
T-8	-	0.85
T-11	0.68	7.7
T-12	1.35	-
T-13	-	4.25

Appendix F Study RMK8702, Hydrolysis 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 Study ABC-0404, Degradation in Soil 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 G: Continued

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

	Tilmicosin-treated			Glucose-treated		
Fraction	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 H Study T5C749301, Degradation in Soil 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 I Studies ABC-0396 and ABC-0450, Soil Sorption 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

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 $Ca(OH)_2$.

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.

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 J Study JJL8704, Photolysis 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.

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.

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 K Study 389681, Soil Microflora Toxcity Report Summary

Title: Effect of Tilmicosin (177370) on Soil Microflora

Name and Address of Investigator: S Chapleo, Inveresk Research, Tranent, EH33 2NE, Scotland

Study Number: 389681

Study Date: May 1997 to July 1997

Test Article: Tilmicosin Aqueous N.I.

Test System: Tilmicosin incorporated into sandy loam and sandy silt loam soils

Experimental Design: The study design complied with the BBA Guidelines for the Official Testing of Plant Protection Products, Part VI, Section 1-1 and was consistent with the OECD Guidelines 216 and 217. Tilmicosin was incorported into sandy loam and sandy silt loam soils at nominal concentrations of 2 and 20 mg/kg. Soils were incubated under aerobic conditions for 28 days. Throughout the test microbial respiration rates were determined. At 28 days post-treatment, concentrations of ammonia, nitrite and nitrate were measured.

Results: Rates of microbial respiration in treated soil of both types deviated from those in the untreated soil controls by less than 15%. Concentrations of ammonia, nitrite and nitrate in treated soil of both types deviated from those in untreated soil controls by less than 15%.

Tilmicosin did not adversely affect respiration, mineralization of organic nitrogen, or nitrification activity of soil microflora at concentrations up to 20 mg/kg. The NOEC for soil microflora exposed to tilmicosin is $\geq$ 20 mg/kg.

Appendix L Study ABC-0399, Seed Germination 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.

Appendix M Study ABC-42631, Seedling Growth 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 transplanted into 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 two levels 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. Thus, the no-observed effect concentration for the study is 30 mg/kg.

Appendix N Study W00788, 28-Day Earthworm 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*)

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.

Results: No mortality or physical signs of toxicity were observed in earthworms at any tilmicosin concentration tested. 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 but there were no differences in body weight gain in the other concentrations.

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 O Study 1982.6336, Earthworm Reproduction Report Summary

Title: Chronic Toxicity and Reproduction Test Exposing the Earthworm *Eisenia fetida* in Artificial Soil, Based on OECD Guideline 222

Name and Address of Investigator: Michael R Patnaude, Springborn Smithers Laboratories, 790 Main Street, Wareham, MA 02571-1037

Study Number: 1982.6336

Study Date: June 2008 to August 2008

Test Article: Tilmicosin premix (formulated with corncobs)

Test System: Earthworms exposed to tilmicosin in artificial soil

Experimental Design: Artificial soil was spiked with nominal concentrations of tilmicosin of 95, 170, 310, 560, and 1000 mg/kg. Adult worms (10 per replicate, 8 replicates for the control and 4 replicates per treatment level) were incubated for 4 weeks under fed conditions. After 4 weeks adults were removed from soil, assessed for health and weighed. Vessels were incubated for an additional 4 weeks. After 4 weeks, reproduction was assessed by carefully sifting through the soil in each vessel and removing and counting offspring.

Results: Survival and growth in adult worms in the control treatment was 100% and +27%, respectively. Survival and growth of the adult worms in the treatment groups ranged from 98 to 100% and +27 to +32%, respectively, and there were no statistical differences from control. The mean number of offspring per replicate in the control group was 98 ± 16. The mean number of offspring in the treatment groups ranged from 73 to 108 offspring per replicate and there were no statistical differences from control.

The LC50 and EC50 values were estimated to be > 1000 mg tilmicosin/kg. The NOEC for earthworm survival, growth, and reproduction was ≥ 1000 mg/kg.

Appendix P Study J00693, Algal Growth Inhibition 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 initial 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 Q Study C00189, Daphnid Immobilization 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. 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 R Study F00189, Bluegill Toxicity 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. 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 S Study F00289, Rainbow Trout Toxicity 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. 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.