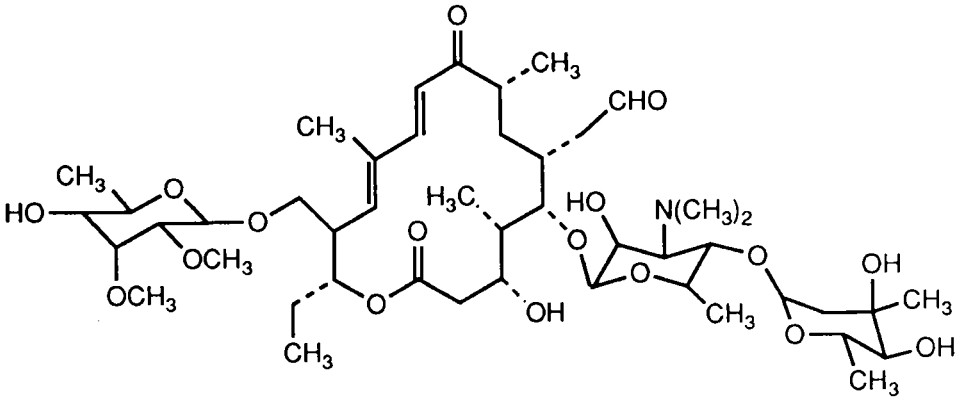
TYLOSIN

IDENTITY

Chemical: [4R-(4R*,5S*,6S*,7R*,9R*,11E,13E,15R*,16R*)]-15-{[(6-deoxy-2,3-di-O-methyl-b-D-allopyranosyl)oxy]methyl}-6-{[3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-a-L-ribo-hexo pyranosyl)-3-dimethylamino-b-D-glucopyranosyl]oxy}-16-ethyl-4-hydroxy-5,9,13-trimethyl-2,10-dioxooxacyclohexa deca-11,13-diene-7-acetaldehyde.

Synonyms: Tylan, Tylon, CAS-1401-69-0

Structural formula:



Molecular formula: $C_{46}H_{77}NO_{17}$

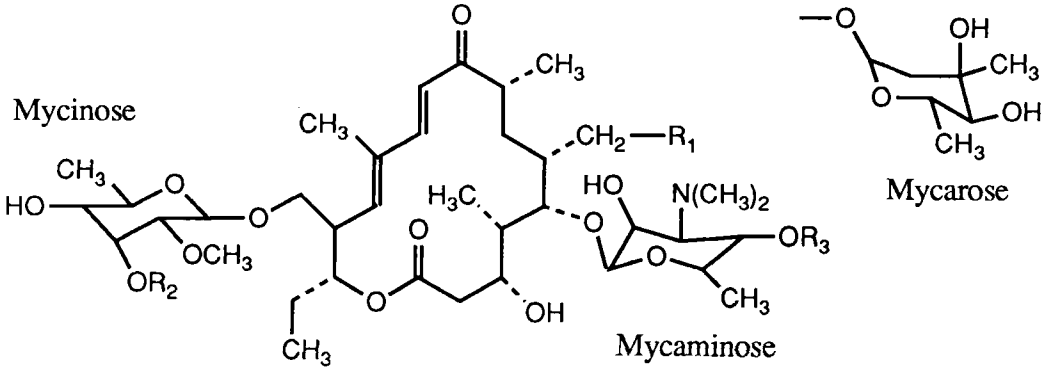
Molecular weight: 916.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Tylosin represents not less than 80% of the total area of all the peaks observed by the HPLC method, excluding the solvent front. Tylosin, desmycosin, macrocin and relomycin represent not less than 95% of the total area of all the peaks observed by the HPLC method excluding the solvent front.

Tylosin and the related compounds included above are characterized by the following structure and table found below:



Compound	R_1	R_2	R_3
Tylosin (A)	-CHO	$-CH_3$	Mycarose
Desmycosin (B)	-CHO	$-CH_3$	-H
Macrocin (C)	-CHO	-H	Mycarose
Relomycin (D)	$-CH_2OH$	$-CH_3$	Mycarose
Dihydrodesmycosin	$-CH_2OH$	$-CH_3$	-H

Appearance: White crystalline solid

Melting point: 128-132°

Solubility: Slightly soluble in water, 5 mg/ml at 25°. Soluble in lower alcohols, esters and ketones, in chlorinated hydrocarbons, benzene, ether.

UV Absorption: Maximum at 282 nm. Extinction coefficient, $E^{1\%}$ at 282 nm is 245.

pH Stability: Solutions are stable at pH 4-9. Below pH 4, another active compound, desmycosin is formed.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Tylosin is used exclusively in veterinary medicine and has never been used in human medicine. It has a broad spectrum of activity, being active against most Gram-positive bacteria and mycoplasmas, but is also active against some Gram-negative organisms, and members of the Chlamydia group. It is ineffective against the Enterobacteriaceae. Tylosin can be administered in feed, drinking water, or injected intramuscularly. It has been used in broiler and replacement chickens for the prevention and treatment of chronic respiratory disease, in broilers to improve weight gain and feed efficiency, and in layers to improve feed efficiency and increase egg production. In pigs and cattle, tylosin has been used for increased weight gain and improved feed efficiency. Tylosin

in feed is used to prevent and treat swine dysentery and to reduce the incidence of liver abscesses in cattle. As a treatment for infectious sinusitis and prevention of the respiratory form of the disease, tylosin is used in turkeys. For therapeutic purposes, injectable tylosin is used in sheep and goats.

Dosage

The dosage forms of tylosin include feed premixes, soluble powders, and formulations for intramuscular injections. In broiler chickens, the recommended dosage of tylosin varies greatly with the desired benefits. For increasing weight gain and improving feed efficiency, a dose of 5-55 ppm in the feed is recommended. A similar dose of 22-55 ppm in feed is used for increased egg production in layers. For prevention, treatment, and control of chronic respiratory disease in broilers, the use level is 880-1100 ppm in feed, 20 or more times the dose recommended for production claims.

In pigs, for use as a production aid, the dose is 10-100 ppm in the feed. For both prevention and treatment of swine dysentery, 40-100 ppm in the feed is recommended. Tylosin is used in cattle at a dose level of 50-100 mg per animal per day for increasing weight gain, improving feed efficiency, and reducing the incidence of liver abscesses.

Soluble powders are added to the drinking water at the use rate of 0.5 g/liter for treatment of respiratory disease in broilers, replacement chickens, and turkeys. Pigs receive 0.25 g/liter to aid in the treatment of swine dysentery. For prevention of pneumonia associated with bovine respiratory mycoplasma and *Pasturella multocida*, calves are treated with 1.0 g/animal twice daily.

Intramuscular injections of tylosin (5-10 mg/kg body weight/day) are used to treat pneumonia, foot rot, diptheria, metritis, and acute mastitis in cattle and for arthritis, pneumonia, erysipelas, and dysentery in pigs. Sheep and goats are treated with 10 mg/kg body weight/day for contagious agalactia and caprine pleuropneumonia.

METABOLISM

Food Animals

Swine-Metabolism and Excretion

Studies were conducted with ^{14}C tylosin to determine the metabolic fate in swine tissues. The lactone ring (position unspecified) of tylosin was labeled with ^{14}C to avoid any of the uncertainty associated with studies using tritium. Analysis by TLC showed the radiopurity of labeled tylosin to be 88.5% tylosin factor A and the specific activity was 0.462 μCi/mg. One castrated male pig, which weighed 59 kg, was conditioned on feed containing 100 mg/kg unlabeled tylosin for two weeks. Following conditioning, the labeled feed containing 110 mg of ^{14}C tylosin per kg, was fed twice daily for three days. This resulted in a dose of 3.3 mg tylosin/kg body weight/day. Each dose contained 45.7 μCi. The pig was sacrificed within 4 hours after the last dose and samples of muscle, liver, kidney, backfat, brain, lung, spleen, heart, pancreas, and intestinal contents collected for assay (Sieck et al., 1978b). The assay results for muscle, liver, kidney and backfat are listed in table I.

Feces. Of the tylosin excreted by swine, 99% was found in the feces and 1% in the urine. The total extractable radioactivity was 85% for swine feces. Samples of feces were extracted with solvents that included water, methanol, heptane, chloroform, and butanol. Extracts were further purified by column chromatography in preparation for TLC.

Tylosin factor A is extensively metabolized in swine with the primary metabolic pathway being chemical reduction of tylosin factor A at the aldehyde group to tylosin factor D, the major metabolite excreted. TLC analysis showed 33% of total radioactivity to have similar chromatographic mobility as tylosin factor D, 8% in the same zone as dihydrodesmycosin (DDM), and 6% with similar mobility as tylosin factor A. These data show factor D as the major ^{14}C tylosin residue in swine feces. At least ten minor metabolites representing 5% or less of total residues are present in swine excreta. No factor B was identified in the metabolic profile for swine or rat. Identification of tylosin factor D and DDM was confirmed by mass spectroscopy.

Liver. In swine liver, total extractable ^{14}C residue was made up of at least four major metabolites with no single major tissue metabolite present. Of total residues recovered by TLC, 15% had R_f values close to that of tylosin factor C and dihydrodesmycosin. Dihydrodesmycosin has microbiological activity with an estimated potency of 31% of that of tylosin factor A, making it detectable by microbiological assays for tylosin (Sieck et al., 1978b).

Additional metabolite profile

Tissues from the total residue study for swine reported later in the section on radiolabeled residue depletion studies (see Table II) were also used to profile the major metabolites in swine tissue. Three pigs were used and treated as described on page 110 (Mertz et al., 1982).

Feces. A series of extractions and repeated TLC procedures were used to identify dihydrodesmycosin and tylosin factors A and D in feces. Structural identity was stated as confirmed for these compounds with mass spectroscopy, although the spectral data were not provided.

Liver. Multi-step TLC procedures confirmed the presence of dihydrodesmycosin in swine liver. After considering that only 60% of total radioactivity was extractable from liver, if none of the DDM was in the bound residue, then it represents approximately 4% of total residue. Tylosin factor A was present and estimated to be about 5% of total extractable residue. Using the TLC procedures, presence of tylosin factor D in liver could not be confirmed.

Toxicological Test Species

Rats

Four male rats weighing approximately 200 g were preconditioned on unlabeled tylosin at a dose level of approximately 10 mg/kg of body weight for 3 days. On the fourth through the eighth day, each rat was dosed (10 mg/kg) by gavage with 2 ml of the

solution containing ^{14}C tylosin. The specific activity of the labeled tylosin was 0.223 μCi/mg. Urine and feces were collected and the animals were sacrificed four hours after the last dose. Samples of feces and tissue were ground and combusted to CO_2 and counted by liquid scintillation counting. Rat feces was subjected to an elaborate scheme of solvent extraction and two column chromatographic procedures similar to that employed for swine feces prior to thin layer chromatography in which known factors of tylosin and its metabolites were identified (Sieck et al., 1978b).

In rat feces, 10% of total ^{14}C residues had a similar chromatographic mobility as tylosin factor D, 6% was found within the same zone as factor A, and 4% had the same mobility as factor C and DDM. These results show that the profile of metabolites in rat feces differs from that of swine feces. Metabolites in rat liver were not characterized because the total radioactivity and residues were too low to allow measurement of individual compounds.

Tissues from these four rats were combusted, counted and were used to calculate the total residue for comparison with similar data from one pig (see table I).

Table I. Comparison of Net Radioactivity (Total Residues) in Tissues of Swine[1] and Rats[2]

	Swine		Rats
Tissue	Net ppm	Sensitivity	Net ppm
Muscle	0.013	0.012	*
Liver	0.178	0.004	0.234
Kidney	0.183	0.006	0.175
Fat	0	0.006	0.078

* - Results not given
1 - Swine recieved feed containing 110 mg ^{14}C-tylosin/kg twice per day for three days
2 - Rats were given ^{14}C-tylosin orally by gavage at a dose of 10 mg/kg for five days

Comparison of Rats with Swine

Residue concentrations in rat kidney and liver were strikingly similar to those reported in swine, in both cases approximately 0.2 ppm. Absorption of tylosin may be different, however, because in the case of swine, the dose was only one third that given to rats.

There are quantitative differences in the metabolic profile for swine and rat feces, although the qualitative analysis appears similar. Metabolites in rat are predominantly more polar compounds, as evidenced by the relative amounts that partitioned into butanol or chloroform. Of the residues in rat feces, only 33% partitioned into chloroform and 56% into butanol, while 63% of the residues from swine feces partitioned into chloroform and 18% partitioned into butanol. The ^{14}C residues in swine liver have similar chemical characteristics to the metabolites excreted by swine and rat as evidenced by similar liquid-liquid partitioning and column chromatographic characteristics. Because

the metabolic profile in rat liver was not obtainable from this study, a complete comparison between rats and swine can not be made beyond the similarities outlined here (Sieck et al., 1978b).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Swine

Depletion of total residue of ^{14}C tylosin was studied in three male pigs. The pigs, weighing from 66 to 69 kg, were fed a ration containing 110 mg of ^{14}C tylosin per kg, twice daily, for four days, resulting in a dose of approximately 3.2 mg/kg body weight/day. All the test animals were sacrificed within 4 hours following the last dose. Labeling with ^{14}C was in the lactone ring. Preparation of the tylosin dose required solubilizing the labeled drug in methanol, followed by dilution with pH 6.5 phosphate buffer and distilled water. The mean specific activity of the solutions fed to the pigs was 0.504 μCi/mg. Due to limitations in the analytical TLC method, it was difficult to determine the radiochemical purity of the ^{14}C tylosin but it was estimated as 90% or better. Following slaughter, samples of muscle, liver, kidney, and fat tissues were collected to assay for ^{14}C content. The ^{14}C content of tissues was determined by combustion, addition of solvents and liquid scintillation solution, followed by counting with a scintillation counter (Sieck et al., 1978a).

Table II shows the results of the total residue depletion study.

Table II. Total ^{14}C Radioactivity, Expressed as ppm Tylosin Equivalents in Tissues of Pigs at Four Hours After the Final Dose.

Tissue	Control	Treated			Mean(± SD)
Muscle	0.011	0.026	0.011	0.023	0.020±.008
Liver	0.013	0.273	0.231	0.213	0.239±.031
Fat	0.021	0.035	0.039	0.036	0.037±.002
Kidney	0.0003	0.245	0.152	0.139	0.179±.058

Results in table II show that total residues of tylosin following oral dosing were greatest in liver and kidney, with mean levels of 0.24 ppm for liver and 0.18 ppm for kidney within four hours of the last tylosin treatment.

Because liver had the highest concentration of tylosin residues, further analysis was conducted to characterize the ^{14}C residues. The results are presented later in the monograph under the metabolism section. Of the total ^{14}C residue, only 60% was extractable from the liver. A series of organic extractions, separations, and column chromatography were used for sample clean-up before analysis by TLC. After TLC, the plates were evaluated by radioautography. Evaluation of the ^{14}C residue recovered from the TLC plates showed 8.6% (5% of total ^{14}C residue) had chromatographic mobility similar to tylosin factor A, 10.4% (6% of total ^{14}C residue) was found in the same zone as factor D, and 11.6% (7% of total ^{14}C residue) migrated to the same zone as

dihydrodesmycosin and factor C. The remainder of total ^{14}C radioactivity was not attributed to any of the five major tylosin factors.

Residue Depletion Studies with Unlabeled Drug

Cattle-Oral-Microbiological Analysis

The objective of this study was to follow decline of tylosin residues in tissues following oral dosing of calves with tylosin tartrate. Calves weighing 36 to 45 kg and no more than 10 days of age were given tylosin tartrate in milk replacer for 14 days at a dose level of 1 g/animal twice a day. The calculated dosage rates ranged from 22.2 to 27.8 mg/kg calf given twice daily. A total of 28 calves were used for the study, 5 controls and 23 treated animals. Three calves were slaughtered within 1 hour of the last dose and the remainder slaughtered in groups of three at 1, 3, 5, 7, 9, and 12 days withdrawal. Liver, kidney, and lean muscle were analyzed for tylosin residues by a microbiological cylinder plate procedure, sensitive to 0.1 ppm, using *Sarcina lutea* (ATCC 9341) as the test organism. Table III summarizes the residue results. (Handy and Matsuoka, 1978).

Table III. Tylosin Residues in Calves Following Oral Dosing with Tylosin, Mean (± SD) in ppm as Determined by Microbiological Assay.

Withdrawal Time (Days)	Kidney	Liver	Muscle
0(1 hour)	3.5(±2.2)	7.5(±5.7)	0.2(±0.06)
1	3.0(±1.1)	5.5(±1.2)	0.2(±0.06)
3	0.6(±0.3)	1.6(±0.3)	NSR
5	NSR	0.1(±0.06)	NSR
7	NSR	0.2(±0.15)	NSR
9	NSR	0.1	
12	NSR	NSR	

NSR = No Significant Residue (< 0.1 ppm)

These results showed that residues were higher and persisted longest in liver. In young calves that received 1 g tylosin in milk replacer twice a day for 14 days, residues in all tissues depleted to below the detection limit of 0.1 ppm by 12 days post-treatment.

Cattle-Injection-Microbiological Analysis

The results of two nearly identical studies are presented, the main difference being the frequency and amount of drug given per day. The objectives of these studies were to follow residue decline at the injection site, in liver, and kidney, and then use those data to determine the withdrawal time. In the first study, calves (sex and breed not specified) received intramuscular injections twice daily for five days at the dose rate of 8.8 mg/kg of body weight. A total of 33 calves weighing approximately 227 kg each were used, 5 controls and 27 treated animals. Three calves were slaughtered at 0, 7, 10, 14, 21, 28, 35, 42, and 49 days past the last treatment (Matsuoka and Johnson, Undated a).

In a second study, calves (sex and breed not specified) weighing approximately 227 kg received intramuscular injections once daily for five days at the dose rate of 17.6 mg/kg of body weight. Twenty-five calves were used, 4 controls and 21 treated animals. Slaughter withdrawal times were 0, 14, 21, 35, 42, and 49 days post-treatment (Matsuoka and Johnson, Undated b).

For both studies, liver, kidney, and muscle from the final injection sites were analyzed for tylosin residues. Tylosin activity was determined by an agar plate microbiological assay using *Sarcina lutea*. Results of the tissue residue withdrawal studies appear in the table IV below.

Table IV. Tylosin Microbiological Residues in Three Calves Per Withdrawal Time, Mean in ppm(± SD).

	Study 1 (4 mg/lb Twice Daily)			Study 2 (8 mg/lb Once Daily)		
Time Days	Inj. Site (Muscle)	Kidney	Liver	Inj. Site (Muscle)	Kidney	Liver
0	1800(±43)	44.1(±9.0)	7.7(±3.9)	7633±1387	17.4(±3.3)	10.0(±3.1)
7	11.0(±4.5)	6.0(±0.8)	0.31(±0.09)			
10	6.4(±5.9)	2.9(±0.4)	0.20(±0.03)			
14	4.7(±2.5)	1.7(±0.5)	0.16(±0.06)	4.3(±1.5)	0.33(±0.06)	0.16(±.06)
21	1.7(±1.1)	1.8(±0.7)	< 0.1	0.99(±0.35)	0.11(±0.02)	< 0.1
28	0.6(±0.87)	0.18(±0.14)	< 0.1	0.57(±0.30)	< 0.1	0.11(±.01)
35	0.19(±0.12)	< 0.1		0.11(±0.01)	ND	< 0.1
42	ND	ND		0.11(±0.02)	ND	< 0.1
49	ND			< 0.1		

ND = Not detected

In both studies, residue persisted longest at the injection site and it was not until 42 to 49 days that all samples were below the assay sensitivity of 0.1 ppm. Residues at the injection site persisted longer in calves that had been treated with the higher dose once per day compared to the animals that were dosed twice daily. Of the other tissues, tylosin residues were highest and depleted most slowly in kidney with residues below the quantitation limits at 28 to 35 days withdrawal. However, a comparison of the kidney values shows an unexplainable striking difference, although it may be because of variations in drug uptake relating to the dose level and whether it was administered once or twice daily.

Cattle-Injection-HPLC Analysis

The purpose of this study was to follow residue depletion in calves after an intramuscular injection with tylosin. Crossbred calves (breeds unspecified) weighing approximately 240 kg were injected with 10 mg/kg body weight per day for five days. After the last treatment, calves (3 males and 3 females) were sacrificed and tissue collected at 0 (6 hours), 3, 7, 14, and 21 days withdrawal. Muscle, fat, liver, kidney, and injection site

were analyzed for tylosin. Analysis of samples was by HPLC with detection at 280 nm. The method has a limit of quantitation of 0.05 ppm and limit of detection of 0.02 ppm in tissue (Moran et al., 1990a).

The residue data are summarized in table V.

Table V. Tylosin Residues in Six Calves Per Withdrawal Time Following Intramuscular Injection, Mean (± SD) in ppm by HPLC Analysis.

Withdrawal Time (Days)	Muscle	Fat	Liver	Kidney	Inj Site-Day 5
0	0.47(±0.14)	0.23(±0.10)	1.96(±0.35)	7.79(±4.31)	1337(±598)
3	0.28(±0.27)	ND	0.17(±0.03)	0.46(±0.08)	32.3(±12.3)
7	ND	ND	BQ	0.07(±0.01)	2.75(±1.25)
14	ND	NA	ND	BQ	1.43(±1.01)
21	NA	NA	NA	ND	0.29(±0.18)

BQ = Below quantitation limits
NA = Not analyzed
ND = Not detected

As expected, the results obtained by HPLC analysis resulted in lower reported values of tylosin residues than obtained by the microbiological method, although the dose in this study is about half of that used above in study 2, table IV. However, the observed depletion trend was similar with depletion occurring most slowly at the injection site. Of the other tissues, kidney appears to be the most likely target tissue with residues persisting through seven days post-treatment. At the final withdrawal time for this study, 21 days, the injection site still contained quantifiable levels of tylosin residue, although the other tissues had depleted to below 0.02 ppm.

Cattle-Injection-Milk-Microbiological Analysis

The purpose of this study was to quantitate the tylosin in milk and determine the milk withholding time necessary after treatment. Five cows (breed not given) were injected with tylosin at a dosage rate of 8.0 mg/lb body weight daily for five days. Milk was sampled shortly following the final injection (referred to as time 0 below, but exact time not specified) and also at 48, 72, 84, 96, 108, 120, 132, and 144 h after the last treatment. Analysis was by microbiological plate assay, with a sensitivity of 0.025 ppm, using *Sarcina lutea* as the test organism (Eli Lilly, 1973). The results in table VI show the depletion of tylosin in milk.

Table VI. Tylosin Residues in Milk Following Intramuscular Injection, Mean ± SD in ppm, as Determined by Microbiological Assay.

Sampling Time (hours)	Mean of Five Cows
0	0.75 ± 0.45
48	0.35 ± 0.21
72	0.14 ± 0.08
84	0.08 ± 0.08
96	0.05 ± 0.08
108-144	No Activity

At 108 hours following the last tylosin injection, residues were no longer detectable in milk.

Swine-Injection-HPLC Analysis

This study was conducted to determine the levels of tylosin residues following the administration of tylosin via intramuscular injection in growing pigs. Approximately 8 week old crossbred pigs (20 males and 20 females, breeds unspecified) weighing approximately 25 kg were injected with a dose of 10 mg/kg body weight into muscle tissue at various sites for five consecutive days. Following the final treatment, four pigs were sacrificed at practical zero (6 hours), and 3, 7, and 14 day withdrawal periods. Muscle, skin, fat, liver, kidney, and injection site tissue samples were analyzed for the presence of tylosin. Samples were analyzed by HPLC, using a method with limits of quantitation to 0.05 ppm and limits of detection to 0.02 ppm (Moran et al., 1990d).

Table VII summarizes the tissue residue results.

Table VII. Tylosin Residues in Four Pigs Per Withdrawal Time Following Intramuscular Injection, Mean (± SD) in ppm by HPLC Analysis.

Time (days)	Muscle	Fat	Skin	Liver	Kidney	Inj Site Day 5
0	0.092 ±0.033	0.067 ±0.024	0.061 ±0.012	0.355 ±0.209	0.669 ±0.235	6.38 ±2.34
3	ND	ND	ND	ND	ND	0.148 ±0.070
7	ND	ND	ND	ND	ND	ND
14	NA	NA	NA	NA	NA	ND

NA = Not analyzed
ND = Not detected

Residues were highest and persisted longest at the injection site. Of the other tissues, kidney contained the most tylosin at zero withdrawal but residues were undetectable in all but the injection site by the second sampling point at 3 days withdrawal. Using the HPLC analytical method for tylosin, following administration of an intramuscular injection of 10 mg tylosin/kg for five days, all tissue residues had depleted to below the detection limits of 0.02 ppm by seven days withdrawal.

Swine-Injection-Microbiological Analysis

In this study, tylosin residues were determined following intramuscular injections of tylosin given twice daily for three days with the objectives of measuring tylosin depletion and establishing a withdrawal time. Twenty-four pigs weighing approximately 110 kg received the injections which contained 8.8 mg tylosin per kg of body weight. An additional six animals served as controls. Groups of three pigs were slaughtered at 0, 2, 4, 7, 14, 21, 28, and 35 days after the final injection. Liver, kidney, muscle, and injection site tissues were collected and assayed by a microbiological method (Matsuoka and Johnson, Undated c). The results are summarized in table VIII.

Table VIII. Tylosin Microbiological Residues in Three Pigs Per Time Following Intramuscular Injection, Mean (± SD) in ppm.

Time (Days)	Inj Site (Muscle)	Kidney	Liver
0	2740(±3061)	30.6(±6.6)	5.2(±2.4)
7	0.92(±0.16)	0.17(±0.05)	0.53(±0.71)
14	0.87(±0.64)	<0.1	<0.1
21	0.12(±0.04)	ND	ND
28	ND		

ND = Not detected

Residues persisted longest at the injection site, but had depleted to below the quantitation limits of 0.100 ppm by 28 days following the final injection. As observed in the other studies with injectable forms of tylosin, residues in kidney at zero withdrawal were higher than in liver, although in both tissues tylosin depleted to below quantitation limits in 14 days post-treatment.

Swine-Oral-Drinking Water-HPLC Analysis

The objective of this study was to monitor tylosin in swine tissue following administration of 0.5 g tylosin/L in the drinking water for 10 days. Pigs geighing approximately 30 kg and approximately 8 weeks old were supplied the medicated water as their only source of water. Following treatment, groups of six pigs (3 males and 3 females) were sacrificed and tissues collected at practical zero (6 hour), 2, and 5 days withdrawal. Muscle, skin, fat, liver, and kidney tissue were analyzed for tylosin using an HPLC assay (Moran et al., 1990e).

Analysis of tissues revealed only one sample with detectable tissue residue, kidney tissue from one male pig at zero withdrawal which contained 20.7 ppb tylosin. In all the other tissues from groups of six pigs sacrificed at 0, 2, and 5 days of withdrawal, tylosin residues were below the detection limits of 0.020 ppm.

Swine-Feed-HPLC Analysis

This study was conducted to follow tissue residues in pigs receiving tylosin when administered in the feed at the rate of 200 g tylosin/ton for 28 days. Pigs weighing approximately 20 kg received the medicated feed and then groups of six pigs (3 males and 3 females) were sacrificed for tissue collection at practical zero (6 hours), 2, 4, and 7 days withdrawal. Muscle, skin, fat, liver, and kidney tissue samples were analyzed using a HPLC method (Moran et al., 1990f).

Tylosin residues were not detected in any tissue samples at the detection limits of 0.020 ppm at any of the withdrawal times tested, including the practical zero-time of 6 hours.

Swine-Feed-Microbiological Analysis

The objective of this study was to determine the amount of tylosin added to feed which will result in residues in swine tissue, when swine receive tylosin in the feed during the last two weeks before slaughter. Subjects used for the study were crossbred "Danish Landrace" and "Yorkshire" pigs, weighing approximately 76 to 89 kg. Treatments consisted of four groups of four pigs (3 groups of 4 hogs and 1 group of 4 sow-pigs) subjected to 0, 100, 200, or 400 ppm tylosin added to the feed. Pigs weighing approximately 90 kg were given the medicated feed for 17 days and slaughtered about 3 hours after the final feeding. Samples of kidney, liver, and tenderloin were analyzed for tylosin using a microbiological method employing *Micrococcus luteus* as the test organism (Madsen and Mortensen, 1986). The results are summarized in table IX.

Table IX. Tylosin Residue in Pig Tissue Following Consumption in the Feed During the Final Two Weeks before Slaughter, Mean (± SD) in ppm.

Ppm in feed	Kidney	Liver	Tenderloin
0	NG	NG	NG
100	NG	NG	NG
200	0.03(±0.02)	0.03(±0.01)	NG
400	0.04(±0.01)	0.05(±0.01)	NG

NG = Not given

When feed containing 200 ppm tylosin was fed to pigs for 17 days, tylosin levels in both liver and kidney were 0.03 ppm when measured by microbiological analysis (method sensitivity unspecified). The highest residue level observed was 0.05 ppm in liver after consuming 400 ppm tylosin in feed. Results of this zero withdrawal study (3 hours past the last feeding) using a microbiological analytical method do not show appreciably higher tissue residues than were reported using HPLC methodology. In the study reviewed above in which swine received 200 g tylosin/ton of feed (approx. 200 ppm) for 28 days, no residue was detected in any tissue at the HPLC method sensitivity of 0.02

ppm (Moran et al., 1990f). Using either analytical method, tylosin residues in tissue were barely detectable at zero withdrawal following consumption of up to 400 ppm tylosin in feed.

Chickens-Oral-Drinking Water-HPLC Analysis

The objective of this study was to determine tylosin residue in chicken tissues following administration of tylosin in the drinking water of broiler chickens. Chickens weighing approximately 1 kg were provided drinking water containing 0.5 g/L tylosin *ad libitum* for 8 days. Chickens were sacrificed at 0 (6 hour), 1, 5, and 10 day withdrawal periods. Muscle, skin, fat, liver, and kidney samples were collected and analyzed for tylosin using HPLC methodology with detection at 280 nm. Detection limits for the assay were 20 ppb and the limit of quantitation was 50 ppb (Moran et al., 1990b).

The analytical results showed that tylosin residues were below the limits of quantitation for all samples at all withdrawal times except for one liver sample that contained 83.4 ppb tylosin at zero (6 hours) withdrawal. Overall, the results suggest that use of 0.5g/L tylosin in drinking water could be a candidate for zero withdrawal but the current study failed to provide conclusive results. Without metabolism data in chickens, it is not possible to determine if tylosin A, which is measured by the HPLC assay, should be the marker residue and the marker to total ratio is unknown.

Chickens-Oral-Feed Premix-HPLC Analysis

This study is analogous to the one above in which tylosin was administered in drinking water. Chickens weighing approximately 1 kg were fed chicken ration containing 1000 ppm tylosin *ad libitum* for 7 days. Birds were sacrificed at the same times as the previous study and analysis was by the same method. Results showed the tylosin concentration to be detectable but below the limits of quantitation in one liver sample at practical-zero (6 hours) withdrawal. Analysis of all other tissues showed no detectable tylosin (Moran et al., 1990c).

Chickens-Oral-Drinking Water-Microbiological Analysis

In this study, the objective was to measure residual tylosin in chicken tissue after administration of 5 g tylosin tartrate/gallon drinking water for seven days. Twelve Smitherman-cross cockerels, 12 weeks old, were limited to 100 ml of medicated drinking water to ensure consumption of the treated water in a 24 hour period. Two chickens were sacrificed at 0, 24, 48, 72, 96, and 168 hours following the end of the treatment period. Liver, kidney, heart, gizzard, fat, skin, and muscle were collected and analyzed for tylosin by a microbiological method (Berkman et al., undated).

At zero withdrawal (exact number of hours unspecified), liver contained 1.03 ppm and kidney had 0.432 ppm tylosin. No residues were found above the sensitivity of the assay (0.360 ppm in kidney and 0.344 ppm in liver) after 24 hours withdrawal.

Chickens-Layers-Oral-Feed Premix-Microbiological Analysis

The objective of this study was to determine the tissue residue of tylosin phosphate (feed grade) in chickens following oral administration in feed for seven days. Seventy-four

leghorn hens, 16 months of age, received feed containing 1000 grams tylosin phosphate per ton for seven days. Fifteen hens were killed at 72, 96, 120, 144, and 168 hours after removal of medicated feed and residues were assayed by a microbiological assay (Eli Lilly, undated).

Tissue residues were detected in two livers at 72 hours and all other samples were negative for tylosin residues at the assay sensitivity of 0.3 to 0.5 ppm for liver. Apparently, no tissues were collected for analysis at zero withdrawal.

Chickens-Eggs-Microbiological Analysis

In this study, tylosin residues in eggs were measured after administration of the following treatments to laying hens: 1) five g/gallon drinking water for seven days, 2) 25 mg/kg subcutaneously in the neck, and 3) 100 mg/kg via crop intubation. Forty Heisdorf and Nelson hens (average weight 2219 g) were divided into four groups to include ten birds for each of the three treatments and another ten birds for controls. Eggs were pooled for analysis by microbiological methods (Richards and Berkman, 1960). The results are summarized in table X.

Table X. Tylosin Residues in Chicken Eggs as Determined by Microbiological Assay.

Tmt.	ppm Tylosin at Test Day Number										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	0.141	0.150	0.494	0.712	0.609	0.141	0.420	0.804	0.508	0.353	0.141
2	0.141	0.282	0.141	0.247	0.155	0.141	0.141				
3	0.141	4.794	0.353	0.240	0.522	0.141	0.141				

In the birds that received tylosin as 5 g/gallon of drinking water for 7 days (Treatment 1), residues of tylosin as high as 0.8 ppm were found in eggs. Residues decreased to the detection limit of 0.141 ppm at four days past the final treatment (Day 11 of the study). In Treatment 2, where layers received a single injection of 25 mg/kg tylosin given subcutaneously, residues above the sensitivity of the assay (0.141 ppm) persisted for 6 days post-treatment. After Treatment 3, where layers received a single treatment of 100 mg/kg by crop intubation, residues peaked at 4.8 ppm on the second day after treatment but depleted to 0.141 ppm, the sensitivity of the method, at six days post-treatment. These results show that oral or injectable administration of tylosin to layers results in significant residues in the eggs.

Turkeys

This study was for the purpose of following tylosin in turkeys after treatment with 5 g/gallon in the drinking water for seven days. Fifteen Broad Breasted Bronze turkeys, 6 months old, average weight 11.3 kg, received the medicated water. Three turkeys were euthanized for tissue collection at 0, 24, 48, 72, and 96 hours post-treatment. Samples of skin, lean muscle, heart, liver, kidney, gizzard, and fat were analyzed by a microbiological assay (Richards et al., undated).

At zero withdrawal (exact number of hours unspecified), the livers of two birds averaged 0.385 ppm tylosin, and 0.240 ppm tylosin was measured in the fat of 1 of 3 birds. All other tissues were negative except the skin of the same bird. At 24 hours, no tissues contained assayable residue except two birds with residues in skin that averaged 0.26 ppm. By 48 hours withdrawal, tylosin residues were below the assay sensitivity limits of 0.154 ppm in skin, 0.360 ppm in muscle, 0.176 ppm in heart, 0.350 ppm in liver, 0.177 ppm in gizzard, 0.178 ppm in kidney, and 0.160 ppm in fat.

Bound Residues/Bioavailability

Results from the radiolabel residue study conducted in swine, indicated that only 60% of total liver residues were extractable. No studies were presented that characterized the bound residue or indicated that attempts were made to hydrolyze the bound residue (Sieck et al., 1978a).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Residue depletion studies for tylosin have been conducted using both microbiological and chemical analytical methods. The microbiological methods measure activity and results reflect the total concentration of active anti-microbial compounds. These methods would account for tylosin A as well as the other related tylosin factors. Some studies were conducted using an HPLC method which only quantifies tylosin factor A. Results for studies using the HPLC assay are likely to show lower levels of tylosin residues.

Microbiological Method

Several revisions for the microbiological method for tylosin have occurred over the years. An agar plate method is used to determine tylosin residues in animal tissues. Samples are extracted with methanol and centrifuged. The supernatant liquid is extracted with chloroform, the extract evaporated to dryness, and then the residue redissolved in 1:1 water:methanol. This solution is placed in wells in an agar test medium. *Micrococcus luteus* , ATCC 9341 (formerly *Sarcina lutea*) is the test organism. Activity is quantified by measuring the average diameters of the zones of inhibition of the samples and comparing with standards. Total microbiological activity is expressed as tylosin equivalents. In the residue studies reviewed in this monograph, the quantitation limits were approximately 0.1 ppm (Wicker and Coleman, 1989).

HPLC Method

High performance liquid chromatography has been used to determine tylosin residues in cattle tissues and milk. Tylosin is extracted from tissues by homogenizing with a methanol/acetonitrile mixture. After centrifugation, the supernatant liquid is purified by salt precipitation, pH adjustment, and partitioning with carbon tetrachloride. Tylosin is extracted from the supernate with chloroform, followed by evaporation to dryness, and resolubilizing the residue in methanol and water. Tylosin factor A is determined by HPLC using UV detection at 280 nm. The validated limit of quantitation of this method is 0.05 ppm in all tissues including milk and the limit of detection is 0.01 to 0.02 ppm, depending on tissue type. Ruggedness of the method was tested by using different lots of HPLC

columns and two HPLC systems. Recovery from swine kidney and liver tissue ranged from 56 to 63% when spiked with 0.05 to 0.2 ppm tylosin and 62.4 to 75.8% for milk over the range tested. The precision was evaluated and a range of 6.5 to 12.3% relative standard deviation (RSD) in liver and 2.5 to 7.1% RSD in kidney is reported. The method gave a linear response over the range tested (0.25 to 20.0 g/ml). Virtually no interference was seen from the tissue matrix (information on liver and fat controls not given) and the method separates tylosin from desmycosin. However, chromatograms from tissues containing incurred tylosin residues from animals administered the drug were not reported (Moran, 1990 a).

This same HPLC method has also been adapted to swine tissues as well as chicken tissues. Validation data similar to that reported for cattle tissue are also reported for these other species. Their specific references are as follows: Swine (Moran 1990 b) and Chicken (Moran 1990 c).

APPRAISAL

A total residue study using ^{14}C tylosin in swine indicated that either liver or kidney might be the target tissue. Residue studies using unlabeled tylosin in swine and cattle showed that the target tissue may differ depending on the method of administration. For injectable forms of tylosin, other than injection site, residues were higher and depleted most slowly in kidney, indicating that it might be the target tissue. Following oral dosing, liver contained higher residues, suggesting that it should be the target tissue for oral dosage forms.

Oral dosing results in lower tissue residues than injectable forms and should require shorter withdrawal times. For production uses, where the dosage may be lower than for therapeutic applications, residue was undetectable in poultry even at zero withdrawal.

Residue studies with layers and milk cows indicate that tylosin passes into milk and eggs and would only be usable with suitable withdrawal periods.

Although tylosin is extensively metabolized, no single metabolite appears to be in a greater concentration in tissue than the parent compound, thus suggesting that it be the marker residue. Preliminary studies showed tylosin concentration in swine liver to be approximately 5% of the total extractable residue. Only 60% of total radioactivity was extractable from swine liver.

The microbiological method of analysis would be sensitive to any active metabolites such as dihydrodesmycosin that result from metabolism of tylosin in the animal. Thus, the microbiological methods should detect more tissue residue than a chemical method that determines only tylosin. However, these microbiological methods with quantitation limits at 0.100 ppm are not as sensitive as the HPLC method for tylosin which quantifies to 0.05 ppm. Either method may be suitable for regulatory purposes, but a direct comparison should be made between the two to enable meaningful comparisons between the residue studies conducted using microbiological analysis with data obtained by the HPLC method.

Until radiolabel studies to determine total residue and metabolism are conducted in all the species, it is difficult to interpret the many studies that show little or no tylosin residue. We cannot conclude that tylosin residue is absent until convincing scientific evidence shows that we are monitoring the appropriate marker residue. Even in swine, if a withdrawal time must be determined, the total residue and metabolism studies should be conducted with sampling at sufficient time intervals to follow total residue depletion to the safe concentration. As inferred above, the amount of bound residues and their steady state levels are also unknown at this time.

Following oral dosing with tylosin, residue depletion appears to be rapid. After injection of tylosin, residue tends to be higher in both kidney and liver, and it persists at the injection site.

REFERENCES

Berkman, R.N., Branscum, A & Richards, E.A. Undated. Tylosin residue studies in chickens. Experiment No. VPR-41-418. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Eli Lilly, (1973). Tylosin milk residue experiment. Experiment No. VPR-207-766. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Eli Lilly, (Undated). Tylosin chicken tissue residue study. Experiment No. VPR-192-418. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Handy, P.R. & Matsuoka, T. (1978). Tylosin tissue residues in calves following oral feeding of tylosin tartrate. Experiment No. 659-G128-45. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Madsen, A, and Mortensen, H.P. 1986. Large quantities of tylosin in the feedstuff for swine during the last two weeks before slaughter. National Institute of Animal Science, Denmark. Announcement No. 623. .

Matsuoka, T. & Johnson, W.S. (Undated a). Injection site and tissue residue in cattle following injection of tylosin. Experiment No. 659-G112-272. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Matsuoka, T. & Johnson, W.S. (Undated b). Injection site and tissue residue in cattle following injection of tylosin. Experiment No. 659-G112-295. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Matsuoka, T. & Johnson, W.S. (Undated c). Injection site and tissue residue in swine following injection of tylosin. Experiment No. 659-G112-281. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Mertz, J.L., Graper, L.K., Sieck, R.F. & Shuey, E.W. (1982). ^{14}C Tylosin Tissue Residue Study in Swine. Experiment ABC-0016. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W. (1990 a). Determination of tylosin in cattle tissues and milk using high-performance liquid chromatography. Procedure AM-AA-CA-R142-AA-791. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W. (1990 b). Determination of tylosin in swine tissues using high-performance liquid chromatography. Procedure AM-AA-CA-R138-AA-791. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W. (1990 c). Determination of tylosin in chicken tissues using high-performance liquid chromatography. Procedure AM-AA-CA-R142-AA-791. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 a). The determination of tylosin residue in tissues following administration of Tylan 200 by intramuscular injection to weaned calves. Experiment No. T1Z709002. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 b). The determination of tylosin residue in tissue following administration of Tylan soluble in drinking water to broiler chickens. Experiment No. T1Y709002. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 c). The determination of tylosin residue in tissue following administration of Tylan premix to broiler chickens. Experiment No. T1X709002. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 d). The determination of tylosin residues in tissue following administration of Tylan 200 by intramuscular injection to growing pigs. Experiment No. T1Z709001. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 e). The determination of tylosin residues in tissue following administration of Tylan soluble in drinking water of growing pigs. Experiment No. T1Y709001. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.

Moran, J.W., Coleman, M.R., Thomson, T.D. & Cochrane, R.L. (1990 f). The determination of tylosin residues in tissue following administration of Tylan premix in growing ration to pigs. Experiment No. T1X709001. Unpublished report, Lilly Research Laboratories, Greenfield, Indiana. Submitted to FAO by Eli Lilly and Co.