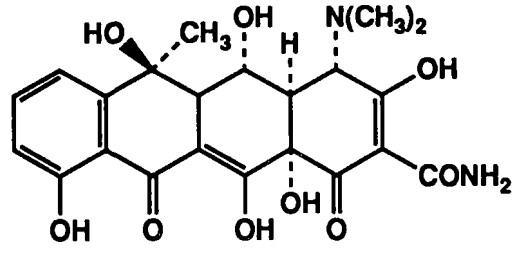
OXYTETRACYCLINE

IDENTITY

Chemical Name: 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Structural formula:



Molecular Formula: $C_{22}H_{24}N_2O_9$

Molecular Weight: 460.44

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Oxytetracycline is a highly active, broad-spectrum antibiotic which is produced by a fermentation process involving the actinomycete, Streptomyces rimosus. Oxytetracycline is present as either the amphoteric base compound, the hydrochloride salt or as a quaternary ammonium salt complex.

The solubility of the base salt varies widely with pH, at 23° it has a minimum solubility at pH 5 (500 μg/ml), a solubility of 31.4 mg/ml at pH 1.2 and 38.0 mg/ml at a pH of 9.0. Oxytetracycline crystals show no loss in potency on heating for 4 days at 100°. The amphoteric base forms salts with acids and bases.

The hydrochloride salt is the most common form in parenteral and water soluble animal health products. It is a yellow crystalline compound that is odorless and slightly bitter in taste. It is very soluble in water (1 g/ml maximum solubility) and organic solvents (e.g., 33 mg/ml in 95% ethanol). In pure state hydrochloride crystals show <5% inactiviation after 4 months storage at 56°. Aqueous solutions of the hydrochloride salt at pH 1.0 to 2.5 are stable for at least 30 days at 25° and solutions held at pH 3.0 to 9.0 show no detectable loss in storage at 5° for 30 days.

The quaternary ammonium salt complex is the most common form in feed premixes. Preparation of this complex results from a reaction of the fermentation broth containing oxytetracycline with a monotallow trimethyl-ammonium chloride. This reaction facilitates removal of oxytetracycline from the fermentation broth, and the resulting complex, which is relatively water insoluble, typically contains 50% oxytetracycline hydrochloride activity and not more than 15% quaternary ammonium compound with remaining components made up of ash, fermentation solubles and moisture. Feed premix products are mixed to their desired potency by adding carriers such as mineral oil, calcium carbonate, roughage by-products and other components to attain the desired physical characteristics.

The stability of oxytetracycline in feed premixes has been extensively evaluated and is well established at more than 90% retained potency after 24 months storage under ambient conditions. Other oral dosage forms (tablets and water soluble powders) retain more than 90% of their original potency under ambient conditions for at least 24 and 48 months, respectively. Injectable oxytetracycline products are also very stable as shown by the retention of more than 90% potency for at least 24 months storage.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Oxytetracycline animal health products are available in four major dosage form product groupings: (1) feed premixes (2) injectables (3) soluble powders and (4) tablets. These products are used in farm animal species which include chickens, turkeys, calves, beef cattle, dairy cattle, swine and sheep primarily for the treatment and control of infectious animal diseases caused by bacterial pathogens. In addition, certain feeding levels of oxytetracycline are used for the improvement of weight gains and feed efficiency in turkeys, calves, beef cattle, swine and sheep, increasing milk production in dairy cows and increasing egg production in chickens and turkeys.

From the maximum use levels listed in Table I, certain maximum dosage regimens are considered most likely to yield the highest residues in food producing animals. A summary of these maximum dosage regimens is presented in Table II. In preparing this summary, all indications for use for the disease and/or production claims for each product were reviewed. From these indications for use in the various target animal species, maximum dosage regimens were selected primarily on the basis of their level and duration of dosage.

Table I. Oxytetracycline Animal Health Products With Listings of Active Forms, Concentrations, Species Use, Maximum Use Levels and Withdrawal Times

Active Form Concentration of Oxytetracycline	Indications For Use - Species	Maximum Use Levels	U.S. Reg. Withdrawal Time Before Slaughter
FEED PREMIXES:			
Quarternary Salt, 10 g/lb(22 g/kg)	Chickens	550 g/metric ton of feed	24 hours
Quarternary Salt, 50 g/lb(110 g/kg)	Turkeys	220 g/metric ton	3 days
Quarternary Salt, 100 g/lb(220 g/kg)	Swine	550 g/metric ton	5 days
	Calves	11 mg/kg body wieght daily	5 days
	Beef Cattle	11 mg/kg	5 days
	Dairy Cattle	100 mg/head daily	none
	Sheep	110 g/metric ton	none
Quaternary Salt, 50 g/lb (110 g/kg)	Calves	11 mg/kg	5 days
	Beef Cattle	11 mg/kg	5 days
	Dairy Cattle	100 mg/head daily	none
Quaternary Salt, 100 g/lb(220 g/kg)	Catfish, Salmonids	8.25 mg/kg daily	21 days

Active Form Concentration of Oxytetracycline	Indications For Use - Species	Maximum Use Levels	U.S. Reg. Withdrawal Time Before Slaughter
INJECTABLES:			
Amphoteric Base, 200 mg/ml	Beef/Non-Lactating Dairy Cattle	20 mg/kg body wieght daily	28 days
	Swine	20 mg/kg	28 days
Hydrochloride Salt, 100 mg/ml	Beef/Non-Lactating Dairy Cattle	11 mg/kg	19 days
Hydrochloride Salt, 50 mg/ml	Chickens	55 mg/kg	5 days
	Turkeys	55 mg/kg	5 days
	Beef/Non-Lactating Dairy Cattle	11 mg/kg	22 days
	Swine	11 mg/kg	22 days
Hydrochloride Salt, 100 mg/ml	Beef/Non-Lactating Dairy Cattle	11 mg/kg	15 days
	Swine	11 mg/kg	22 days
SOLUBLE POWDERS:			
Hydrochloride Salt, 55 mg/g	Laying Hens, Chicken/ Turkey Breeders	26.5 mg/L	5 days
Hydrochloride Salt, 55 mg/g	Chickens	106 mg/L	None
	Turkeys	106 mg/L	5 days
	Cattle	22 mg/kg body weight daily	5 days

Active Form Concentration of Oxytetracycline	Indications For Use - Species	Maximum Use Levels	U.S. Reg. Withdrawal Time Before Slaughter
SOLUBLE POWDERS: (continued)			
Hydrochloride Salt, 55 mg/g	Dairy Cattle	22 mg/kg	5 days/60 hours (5 milkings) for milk
	Swine	265 mg/L	5 days
	Sheep	53 mg/L	None
Hydrochloride Salt, 225 mg/g	Chickens	106 mg/L	None
	Turkeys	106 mg/L	5 days
Hydrochloride Salt, 756 mg/kg	Chickens	212 mg/L	None
	Turkeys	106 mg/L	5 days
	Swine	22 mg/kg	5 days
	Cattle	22 mg/kg	5 days
	Sheep	22 mg/kg	5 days
TABLETS:			
Hydrochloride Salt, 250 mg/ tablet	Calves-Beef/ Dairy Cattle	22 mg/kg body weight daily	7 days

Table II. Oxytetracycline Use Conditions Yielding The Highest Residues in Food Producing Animals

Dosage Forms/Products	Species	Disease Claims	Maximum Dosage
Feed Premixes:	Chickens	Air-Sacculitis	550 g/metric ton in feed for 5 days
	Turkeys	Hexamitis, infect. sinusitus, infect. synovitis	220 g/metric ton in feed for 7-14 days
	Calves/ Beef Cattle	Bacterial diarrhea	11 mg/kg body weight daily for 7-14 days
	Dairy Cattle	Bacterial diarrhea	100 mg/head/day fed continuously
	Swine	Leptospirosis	550 g/metric ton in feed for 7-14 days
	Sheep	Bacterial diarrhea	110 g/metric ton in feed continously
	Catfish/ Salmonids	Bacterial hemorrhagic septicemia, ulcer disease	8.25 mg/kg body weight for 10 days
Injectables:	Cattle	Pneumonia/shipping fever complex, etc.	20 mg/kg for one dosage; 11 mg/kg daily for 4 days
	Swine	Bacterial enteritis, pneumonia,	20 mg/kg for one dosage; 11 mg/kg daily for 4 days
	Cattle	Pneumonia/shipping fever complex, etc.	11 mg/kg daily for 4 days

Dosage Forms/Products	Species	Disease Claims	Maximum Dosage
Injectables: (continued)	Chickens/ turkeys	Air saccultis, CRD, fowl cholera, infec. sinusitis	55 mg/kg daily for 4 days
	Beef/Non-lactating dairy cattle	Pneumonia/shipping fever complex etc.	11 mg/kg daily for 4 days
	Swine	Bacterial enteritis pneumonia, leptospirosis	11 mg/kg daily for 4 days
Soluble powders:	Chickens	CRD, fowl cholera	212 mg/L for 7-14 days
	Turkeys	Hexamiltiasis, infec. synovitis	106 mg/L for 7-14 days
	Cattle	Bacterial enteritis, pneumonia	22 mg/kg body weight daily for 5 days
	Swine	Bacterial enteritis, pneumonia, leptospirosis	22 mg/kg body weight daily for 5 days
	Sheep	Bacterial enteritis, pneumonia	22 mg/kg body weight daily for 5 days
Tablets:	Beef/Dairy Cattle	Bacterial enteritis, pneumonia	22 mg/kg (divided doses) daily for 4 days

METABOLISM STUDIES

Extensive literature exists on the absorption and excretion of the various important tetracyclines in man, the dog and the rat. Typical publications are referenced (Schach von Wittenau et al, 1963, 1965, 1972; Steigigel et al, 1961; Kunin et al, 1961). These closely related compounds are all absorbed after oral administration and excreted via the urine and feces. The degree of oral absorption of the various tetracyclines is a function of the lipophilicity of the compound. The least lipophilic, oxytetracycline, is the

least well absorbed and the most lipophilic doxyxyline, is the best absorbed. The absorption of the other tetracyclines fall between these two extremes.

Metabolism studies have been conducted with many of the tetracyclines. Kelly and Buyske determined the metabolic fate of tetracycline in dogs and rats using radioisotopically labeled tetracycline (Kelly et al, 1960). The urine and feces of rats dosed orally with tetracycline were subjected to countercurrent distribution. The major peak in the distribution accounted for over 95% of the excreted radioactivity and showed a distribution pattern identical to that of tetracycline. Microbiological activity in the individual tubes in the distribution showed an exact correlation with radiological measurements. Similar results were obtained with dog urine indicating no chemical transformation occurred in the body of the dog. Small amounts of 4-epitetracycline detected in the rat excreta were ascribed by the authors to epimerization of tetracycline during countercurrent distribution. The authors concluded: "These data show that with the exception of metal chelate formation, tetracycline is chemically unaltered by the rat. The urines from the dog also contained unchanged drug thus indicating that in this species no metabolic transformation of tetracycline has occurred."

The results indicating the lack of metabolism of tetracycline in rats were confirmed by Bocker and Estler (1979). Rats were dosed with tetracycline (or with a-6-deoxyoxytetracycline), the animals were killed and the antibiotic was extracted from various organs. Extraction was performed under conditions which did not allow in vitro epimerization of tetracycline to 4-epitetracycline. In previous in vitro experiments in which the antibiotic was extracted from spiked tissue, the extraction was found to be 90% - 100% complete. The organ extracts from the dosed animals were examined by high pressure liquid chromatography and by microbiological assay. Both methods yielded identical results, so no tetracycline metabolic products were present in the organ extracts.

In a similar study, Kelly, et al. (1961) determined the metabolism and tissue distribution of 6-demethylchlortetracycline in dogs following intravenous dosing. Seventy-five percent of the administered dose was recovered in the urine and fifteen percent in the feces by seven days post-medication. Of the total radioactivity excreted, approximately 93% was identified as unmetabolized demethylchlortetracycline. Thus, essentially no demethylchlortetracycline is metabolized in dogs.

The metabolic fate of chlortetracycline in rats and dogs was determined by Eisner and Wulf (1963) following oral, intravenous or intraperitoneal radiolabeled drug administration. Chlortetracycline related compounds were separated from excreta by column and by paper chromatography and measured by radiometric and microbiological assays. Excreta from both species showed two main components, chlortetracycline and 4-epichlortetracycline, and a very small amount of isochlortetracycline. Four epichlortetracycline has essentially no microbiological activity. When this component was subtracted from the total radioactive material, the radiometric and microbiological assays were in close agreement. Chlortetracycline was shown to be partially converted to 4-epichlortetracycline and isochlortetracycline in vitro in urine and feces, and the authors ascribed the presence of these compounds in the excreta of dosed animals to this artifact. The authors concluded: "Like tetracycline and demethylchlortetracycline, chlortetracycline appears not to be metabolized to any

significant degree by rats or dogs in spite of the finding of 4-epichlortetracycline and isochlortetracycline in the excreta of these animals. ...It would appear that the changes seen in chlortetracycline after administration to the animals were due more to chemical instability at physiological conditions than to metabolic transformation."

Kelly, and Kanegis (1967) investigated the metabolism of radiolabeled 7-dimethylamino-6-demethyl-6-deoxytetracycline in rats and dogs. The isolation techniques used were similar to those in previous metabolism experiments with the tetracyclines. The results were summarized as follows: "Of the radioactivity excreted by each of three rats, over 80% was in the form of unchanged minocycline or the carbon-4 epimer of minocycline. (Minocycline rapidly epimerizes under conditions of isolation and is in equilibrium with its 4-epimer.) A single uncharacterized metabolite accounted for the remaining excreted radioactivity. Of the radioactivity excreted by the dog, over 95% was in the form of minocycline or its carbon-4-epimer. Neither the rat nor the dog expired significant quantities of radiolabeled carbon dioxide."

Schach von Wittenau et al. determined the metabolic fate of a-6-deoxytetracycline in the rat (1972) and in man and the dog (1971) using radiolabeled drug. Primarily doxycycline along with some degradation products were found in rat urine, bile and feces. A similar degradation of doxycycline was observed in vitro in rat urine. On the basis of the data obtained, the authors stated: "The present information suggests that radioactivity recovered as compounds other than unchanged drug may have resulted from chemical, not enzymatic degradation. Doxycycline at 37° degrades in alkaline solutions. It appears probable that most of the degradation products found in bile and urine are formed after excretion by liver or kidney. In accordance with the higher alkalinity of rat urine compared to human urine, the recovery of unchanged drug is lower in rats."

In the dog, following an intravenous dose, radioactivity in plasma appeared to be unchanged doxycycline only. Urinary radioactivity was mostly unchanged drug, but about 5% of the dose was eliminated by this route in some other form. Fecal radioactivity was recovered as unchanged doxycycline. Doxycycline in the dog appears to resist enzymatic degradation. Similar results were obtained when doxycycline metabolism was examined in man. The authors concluded: "The disposition of doxycycline by man and dog was investigated with the aid of labeled drug. .....More than 90% of dose was recovered as undegraded drug from urine and feces of both species, but some labeled doxycycline was excreted by dogs in antibacterially inactive form."

Only two publications are available in which drug distribution studies were conducted using radiolabeled oxytetracycline, and neither sheds light on the metabolism of the drug. Snell et al. (1957-1958) determined the distribution of C14-labeled oxytetracycline in the rat. Leevy et al. (1958-1959) determined the distribution of C14-labeled oxytetracycline in man. Neither publication can be interpreted by contemporary standards, since the oxytetracycline was prepared by fermentation and the purity was not adequately determined. No attempt was made to isolate the radioactive compounds and determine the identity.

All available evidence suggests minimal, if any, metabolism of the tetracycline antibiotics in rats, dogs or man. Although no useful oxytetracycline metabolism papers have been published, the data available for other tetracyclines allows the conclusion to be reached that no metabolism occurs in animals. Tetracycline differs from oxytetracycline only in that an R4 hydroxyl group present in oxytetracycline is absent from tetracycline. Tetracycline is not metabolized by either rats or dogs (Kelly et al, 1960). That the R4 hydroxyl group in the tetracycline is not subject to enzymatic attack in vivo has been demonstrated by the lack of metabolism of a-6-deoxyoxytetracycline, which contains a R4 hydroxyl group, in rats, dogs and man (Schach von Wittenau et al, 1971, 1972). Therefore, that oxytetracycline would be metabolized in animals is exceedingly unlikely. Hence a microbiological assay would be expected to detect all residues of oxytetracycline in tissues from animals.

RESIDUE DEPLETION STUDIES

A summary is presented in Table III of the depletion data for oxytetracycline tissue residues following withdrawal from products administered to the various species at maximum dosage regimen levels. Comments presented in this section are related to the depletion data for products within each dosage form category when administered at maximum dosage regimens for each target animal species.

Table III. Summary of Oxytetracycline Tissue Residue Data Following Withdrawal From Products Used For Various Species/Dosage Regimens

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)				
FEED PREMIXES:							
			Day 0	Day 1		Day 2	
Chickens	550 g/metric ton for five days	Liver	0.93 (6/6)	NR		NR	
		Muscle	0.35 (6/6)	NR		NR	
		Kidney	1.70 (2/2)	0.28 (2/2)		<0.16 (1/2)	
		Fat/Skin	NR	NR		NR	
			Day 1	Day 2	Day 3	Day 4	Day 5
Chickens	600 ppm (j) for 7 days	Whole egg	0.24	0.21	0.14	0.11	----
		Egg yolk	0.50	0.49	0.33	0.25	<0.20
			Day 1		Day 2		Day 3
Chickens	300 ppm (j) for 7 days	Whole egg	0.12		0.10		----
		Egg yolk	0.22		0.20		<0.20

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)		
FEED PREMIXES:					
			Day 0	Day 1	Day 3
Cattle	22 mg/kg body-weight daily (divided doses) for 4 days	Liver	1.02 (3/3)	<1.07 (2/3)	<0.23 (2/6)
		Muscle	0.54 (3/3)	<0.45 (2/3)	<0.28 (2/6)
		Kidney	1.02 (3/3)	1.70 (3/3)	0.36 (6/6)
		Fat	<0.23 (1/3)	NR	<0.64 (2/6)
(Note: No residue was detected for all tissues for days 5(c) and 7)					
			Day 5		
Cattle	22 mg/kg body-weight for 21 days	Liver	NR		
		Muscle	NR		
		Kidney	NR		
		Fat	NR		
			Day 1	Day 3	
Swine	550 g/metric ton for 21 days	Liver	<0.31 (2/3)	NR	
		Muscle	<0.25 (1/3)	NR	
		Kidney	0.89 (3/3)	<0.25 (2/3)	
		Fat	NR	NR	
(Note: No residue was detected for all tissues for days 5, 7 and 16)					
			Day 1	Day 15	
Sheep	33 mg/kg body-weight daily for	Liver	0.47 (3/3)	NR	
		Muscle	<0.26 (2/3)	(d)	
		Kidney	1.15 (3/3)	NR	
		Fat	0.20 (3/3)	(d)	
(Note: No residue was detected for all tissues for days 3, 5 and 8)					

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)			
FEED PREMIXES:						
			Day 1	Day 2	Day 3	Day 5
Fish	10 mg/kg body-weight daily for 10 days	Liver	<0.21 (8/16)	<0.16 (5/16)	<0.15 (6/16)	<0.14 (2/16)
		Muscle	<0.21 (1/16)	NR	NR	NR
			Day 7	Day 10	Day 14	
		Liver	<0.16 (7/12)	<0.13 (1/12)	<0.14 (2/12)	
		Muscle	NR	NR	NR	
(Note: No residue was detected for all tissues for day 21)						
INJECTABLES:						
			Day 7	Day 14	Day 17	
Cattle	20 mg/kg body-weight for one dosage	Liver	0.40 (3/3)	NR	NR	
		Muscle	<0.96 (2/3)	NR	NR	
		Kidney	0.90 (3/3)	<0.25 (1/3)	<0.25 (3/3)	
		Fat	<0.16 (1/3)	NR	<0.15 (1/3)	
		Injection Site	51.03 (3/3)	1.00 (3/3)	<0.21 (2/3)	
(Note: No residue was detected for all tissues for days 21 and 28)						
			Day 7	Day 14	Day 21	
Cattle	11 mg/kg body-weight daily for 4 days IM	Liver	1.38 (3/3)	0.48 (3/3)	NR	
		Muscle	NR	NR	NR	
		Kidney	4.42 (3/3)	1.54 (3/3)	1.40 (3/3)	
		Fat	<0.26 (2/3)	NR	NR	
		Injection Site	>341 (3/3)	>246 (3/3)	NR	
(Note: No residue was detected for all tissues for days 28 and 35)						

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)		
INJECTABLES:					
			Day 7	Day 14	
Swine	20 mg/kg body-weight daily for 1 dosage, IM	Liver	0.49(3/3)	NR	
		Muscle	<0.31(1/3)	NR	
		Kidney	1.23(3/3)	<0.38(1/3)	
		Fat	NR	NR	
		Injection Site	1731(3.3)	<0.38(1/3)	
(Note: No residue was detected for all tissues for days 21 and 28)					
			Day 7	Day 14	Day 21
Swine	11 mg/kg body-weight daily for 4 days, IM	Liver	0.91(3/3)	<0.25(1/3)	NR
		Muscle	0.39(3/3)	NR	NR
		Kidney	3.54(3/3)	0.41(3/3)	<0.32(1/3)
		Fat	0.36(3/3)	<0.22(1/3)	NR
		Injection Site	0.78(3/3)	<0.16(1/3)	NR
(Note: No residue was detected for all tissues for days 28 and 35)					
			Day 0	Day 19	Day 22
Cattle	11 mg/kg body-weight daily for 4 days, IM	Liver	9.18 (3/3)	0.48 (3/3)	0.14 (2/3)
		Muscle	2.99 (3/3)	0.13 (3/3)	0.10 (3/3)
		Kidney	24.97 (3/3)	0.15 (3/3)	0.08 (3/3)
		Fat	0.59 (3/3)	0.22 (3/3)	0.06 (3/3)
			Day 23	Day 26	Day 27
			0.05 (3/3)	NR	NR
			0.11 (3/3)	0.02 (1/3)	NR
			0.07 (3/3)	0.09 (3/3)	0.05(2/3)
			0.08 (3/3)	0.04 (3/3)	NR
		Day	16 18	19	20
Cattle	11 mg/kg body-weight daily for	Liver	NR NR	<0.13(1/3)	NR
		Muscle	NR NR	<0.18(2/3)	NR
		Kidney	NR 0.16(3/3)	<0.20(2/3)	NR
		Injection Site	1.67(3/3) NR	>3.65(3/3)	<0.19(1/3)
(Note: No residue was detected for all tissues for day 22)					

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)		
INJECTABLES:					
			Day 5	Day 7	Day 9
Swine	11 mg/kg body-weight daily for 4 days, IM	Liver	<0.30 (2/3)	<0.27 (1/3)	<0.25 (1/3)
		Muscle	NR	NR	NR
		Kidney	0.87 (3/3)	<0.61 (2/3)	0.52 (3/3)
		Fat	<0.25 (1/3)	NR	NR
		Injection Site	NR	<1.71 (2/3)	<0.25 (1/3)
(Note: No residue was detected in liver, kidney or injection site for days 14, 19 and 26(g))					
			Day 5	Day 10(c)	Day 12
Cattle	11 mg/kg body-weight daily for 4 days, IM	Liver	<0.16 (2/3)	NR	NR
		Muscle	NR	NR	NR
		Kidney	0.38 (3/3)	NR	NR
		Fat	NR	NR	NR
		Injection Site	<0.20 (2/3)	NR	(d)
(Note: No residue was detected for all tissues for days 14, 19, and 26(g))					
			Day 1	Day 5	Day 10
Swine	11 mg/kg body-weight daily for 4 days, IM	Liver	2.37 (3/3)	NR	NR
		Muscle	1.95 (3/3)	NR	NR
		Kidney	9.02 (3/3)	0.62 (3/3)	0.34 (3/3)
		Fat	<0.25 (2/3)	NR	NR
		Injection Site	>7.41 (3/3)	NR	NR
			Day 12	Day 14	Day 15
		Kidney	<0.32 (2/3)	<0.42 (3/3)	0.30 (3/3)
			Day 16	Day 17	Day 18
		Kidney	<0.13 (1/3)	<0.20 (1/3)	NR

(Note: No residue was detected in all tissues except kidney as of day 12. Day 14 and on, no assays of tissue other than kidney was performed)

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (µg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)			
INJECTABLES:						
			Day 1	Day 2	Day 3	Day 4
Chickens	30 mg/kg/ day for 3 days (j)	Egg Yolk	2.15	3.77	2.60	2.07
		Whole Egg	0.56	1.33	1.83	1.54
			Day 5	Day 6	Day 7	Day 8
		Egg Yolk	1.66	0.80	0.45	0.32
		Whole Egg	0.95	0.78	0.56	0.31
			Day 9	Day10	Day11	Day12
		Egg Yolk	0.29	0.22	0.20	<0.20
		Whole Egg	0.15	0.13	0.11	0.10
			Day 1	Day 2	Day 3	Day 4
Chickens	15 mg/kg/ day for 3 days (j)	Egg Yolk	0.96	1.55	1.27	1.10
		Whole Egg	0.86	0.78	0.51	0.42
			Day 5	Day 6	Day 7	
		Egg Yolk	0.74	0.47	0.25	
		Whole Egg	0.28	0.17	0.10	
SOLUBLE POWDERS:						
			Day 0	Day 1	Day 2	
Chickens	529 mg/L for 10 days	Liver	0.61 (6/6)	0.32 (6/6)	<0.16 (2/6)	
		Muscle	<0.27 (5/6)	NR	NR	
		Kidney	1.60 (2/2)	0.88 (2.2)	0.66 (2/2)	
		Fat/Skin	0.42 (6/6)	NR	NR	
			Day 3	Day 4	Day 5	
		Liver	<0.16 (1/6)	NR	NR	
		Muscle	NR	NR	NR	
		Kidney	0.36 (2/2)	0.36 (2/2)	<0.36 (1/2)	
		Fat/Skin	NR	NR	NR	

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)				
SOLUBLE POWDERS:							
			Day 0				
Chickens	211 mg/L for 14 days (h)	Liver	0.28 (3/4)				
		Muscle	0.15 (2/4)				
		Fat/Skin	0.28 (4/4)				
			Day 0	Day 1			
Turkeys	55 mg/kg body-weight for 14 days (h)	Liver	0.90	0.36			
		Muscle	0.53	0.19			
		Fat/Skin	0.37	0.22			
			Day 3	Day 5(c)	Day 7		
Calves and Cattle	22 mg/kg body-weight daily for 7 days	Liver	<0.21 (2/3)	NR	NR		
		Muscle	NR	NR	NR		
		Kidney	0.42 (3/3)	NR	NR		
		Fat	NR	NR	NR		
			Day 3	Day 5			
Swine	22 mg/kg body-weight daily for 4 days	Liver	NR	NR			
		Muscle	NR	NR			
		Kidney	NR	NR			
		Fat	NR	NR			
			Day 1	Day 2	Day 3		
Swine	22 mg/kg body-weight daily for 4 days (h)	Liver	0.193 (4/4)	0.194 (4/4)	0.105 (4/4)		
		Kidney	0.387 (4/4)	0.395 (4/4)	0.176 (4/4)		
			Day 5	Day 6			
		Liver	<0.089(2/2)	<0.089			
		Kidney	0.149 (2/2)	0.099			
			Day 1	Day 2	Day 3	Day 4	Day 5
Chicken	0.5 g/L for 5 days (j)	Egg Yolk	0.48	0.42	0.35	0.28	<0.20
		Whole Egg	0.24	0.18	0.16	0.12	(d)

Dosage Forms/ Species	Maximum Dosage Regimen Tested	Tissues Assayed(a)	Average Residue Levels Detected (μg/g) (No.Positive/No. Assayed) At Various Withdrawal Times (b)				
SOLUBLE POWDERS:							
			Day 1	Day 2	Day 3	Day 4	Day 5
Chicken	0.25 g/L for 5 days (j)	Egg Yolk	0.35	0.37	0.28	0.22	<0.20
		Whole Egg	0.17	0.16	0.13	0.10	(d)
TABLETS:							
			Day 0		Day 1		Day 3
Beef/Dairy Calves	22 mg/kg body-weight daily for 4 days	Liver	1.02 (3/3)		<1.07 (3/3)		<0.23 (4/6)
		Muscle	0.54 (3/3)		<0.45 (2/3)		<0.28 (2/6)
		Kidney	1.02 (3/3)		1.70 (3/3)		0.36 (6/6)
		Fat	<0.23 (1/3)		NR		<0.64 (2/6)

(Note: No residue was detected for all tissues for days 5(c) and 7)

FOOTNOTES:

(a) Unless otherwise noted, the number of tissues from animals at each withdrawal time were as follows: chickens - 6, except for kidney (composite of 2 samples from 3 chickens each); cattle, swine and sheep - 3; catfish - 16.

(b) Average residues includes all assays with positive values and values below assay sensitivity (which were reported as < values at the assay sensitivity limit; e.g., a <0.15 value averaged with two other values of 0.25 and 0.19 would be reported as <0.20 (2/3). NR = no residue detected within assay sensitivity in any samples (assay sensitivity at range of 0.05 to 0.25 μg/g).

(c) Assay of tissues from 6 cattle.

(d) No assays were performed on this day.

(e) Assay of tissues from 8 catfish.

(f) Average assay values included positive values and some zero values.

(g) Assay of tissues from 1 animal.

(h) See Pfizer (1989).

(i) Unless otherwise noted, all data has been obtained from Leevy et al, (1958-1959).

(j) See I.D. Russel Co. (1984 and 1986).

Feed Premixes

Medication with oxytetracycline at maximum dosage regimens in feed or other oral dosage forms for chickens, cattle, swine and sheep yielded quite low levels of drug residues which persisted for only a few days. The highest residue levels were found in the kidney and liver in all species yet these were at levels greater than 1 mcg/g for only up to two days. Residues in muscle and fat tissue were lower than levels found in kidney and liver.

A study with catfish involved dosage at 10 mg/kg of bodyweight daily for 10 days. Dosage was approxomately 1.2 times the highest use level and gave only one positive muscle tissue assay (one of 16 samples on day 1). Liver residues, although quite low and frequently less than assay sensitivity limits, did persist through Day 14. The highest single liver assay was only 0.475 μg/kg on day 1; therefore, all residues were less than 1.0 μg/g during the entire study. Data also were collected at 5 and 20 mg/kg (0.6 and 2.4 time the highest use level). These data showed similar pattern of residue depletion as the data from dosage at 10 mg/kg.

Injectables

Oxytetracycline injectable (200 mg/ml) was evaluated in cattle and swine under two maximum dosage regimens; (1) 20 mg/kg of bodyweight daily for one dosage given intramuscularly (IM) and (2) 11 mg/kg of bodyweight daily for four days IM.

Tissues were collected at 7-day intervals (except at 17 days in cattle dosed at 20 mg/kg) and assays revealed quite low residue levels for all tissues except the 7 and 14 day injection site tissues. All injection sites were cleared of residues at 21 days. Other than injection sites, kidney had the highest tissues residues, and liver was the next highest. Muscle and fat had the lowest assays; detectable residues were essentially gone at 14 days and completely gone at 21 days. The only tissues showing positive assays past 14 days withdrawal were injecion sites and fat tissue (day 17) and kidney (days 17 and 21) for cattle and kidney (day 21) for swine.

Oxytetracycline was evaluated in cattle injected IM at a dosage of 11 mg/kg bodyweight daily for four days. Tissues were collected on day 0 (no withdrawal) and on days 19, 22, 23, 26 and 27 after dosage. Tissue residues on day 0 for kidney, liver and muscle samples were approximately 25, 9 and 3 μg/g, respectively. At all other withdrawal times, none of the tissues approached 1 μg/g.

The assay methodology reported in this study was highly sensitive with many values at 0.05 mcg/g or lower being reported. As a result, extremely low levels of oxytetracycline were reported in all tissues on most of the withdrawal days. Nevertheless, assay values of "no residue" were attained for all tissues except kidney on day 27.

Studies were conducted with cattle and swine both given IM dosages of 11 mg/kg for 4 consecutive days. Withdrawal times were initiated at a later but shorter time for cattle (16 to 22 days) as compared to swine (5 to 26 days). Injection site tissues retained residues slightly longer than other tissues in cattle (day 20 vs day 19), whereas with swine, kidney, injection site and liver samples retained residues for only nine days. Residue levels less than 5 μg/g were observed on the first withdrawal day samples were taken for both cattle (day 16) and swine (day 5). Residues less than 1 μg/g were attained on day 20 in cattle and day 9 in swine.

Cattle and swine were both administered a 100 mg/ml injectable at a dosage level of 11 mg/kg for four days, and tissues were collected at withdrawal times up to 18 days. Data show that oxytetracycline was rapidly cleared from all cattle tissues with "no residues" found on day 5 for muscle and fat or on day 10 for all remaining tissues. With the exception of kidney residues, similar values were determined with swine. Conversely, swine kidney residues persisted through day 17 after dosage. All tissues were less than 1 μg/g by withdrawal day 17 after dosage. All tissues were less than 1 μg/g by withdrawal day 5 for both cattle and swine.

Soluble Powders

Tissue depletion studies have been conducted utilizing maximum dosage regimens with chickens, cattle and swine. As with other studies involving oral administration of oxytetracycline, tissue residues were quite low and persisted only a few days after drug withdrawal. Chickens, which were administered 2.5 times the maximum recommended dosage, had the most persistent tissue residues. All tissues except kidney were cleared of residues by day 4; kidney tissue showed a residue in one of two samples at day 5.

With cattle all tissues were cleared by day 5 following dosage. "No residue" levels were detected after 3 days withdrawal in swine. Levels less than 1 μg/g were observed on the first withdrawal day (day 3) sampled in cattle and swine and on day 1 in chickens.

Tablets

The results from two studies have been combined to demonstrate residue depletion of oxytetracycline in this product when administered at the maximum dosage of 22 mg/kg bodyweight daily (in divided doses) for four days. Residue depletion was studied for 7 days after the last dosage. Residues were quite low and only exceeded 1 μg/g for liver and kidney on day 0 (no withdrawal) and day 1. All tissues were cleared of residues by day 5.

METHODS OF RESIDUE ANALYSIS

Oxytetracycline has been analyzed by chemical and microbiological methods. The classical method for oxytetracycline analysis is a microbiological method as described by Kramer et al. (1968) The assay is an agar diffusion method which uses Bacillus cerus ATCC 11778 as the test organism. The assay has a variable sensitivity of 0.002 to 0.20 ppm depending on the tissue matrix being assayed.

There are a variety of chemical assays available for the analysis of oxytetracycline in tissues and milk. In a paper by Oka et al. (1985), oxytetracycline, as well as other tetracyclines, were measured in beef liver using an high performance liquid chromatography (HPLC). The detection limit for oxytetracycline was 0.05 ppm. Levels as low as 1 ppb in bovine milk and meat have been attained using a tandem mass spectrometric approach (Traidi et al. , 1985).

APPRAISAL

Due to limited metabolism of oxytetracycline, the determination of the parent compound in edible tissues by microbiological methods provides sufficient residue information for a safety assessment.

From the information in Table III, it is readily apparent that residues following dosage with oral dosage forms (feed premixes, soluble powders and tablets) are rapidly cleared from tissues. With the exception of small residues in kidney tissues, all tissues were cleared of detectable levels of oxytetracycline within 5 days following dosage.

Injectable forms of oxytetracycline yielded higher residues of oxytetracycline which persisted longer than oral dosage forms. Extended withdrawal times are frequently required for long acting formulations of oxytetracycline.

An ADI of 0-3 μg/kg of body weight does not permit recommending MRLs for edible tissues, milk and eggs that can be monitored with currently available microbiological methods of analysis.

It is recommended that MRLs be established in milk, muscle, fat and eggs at the detection level of the microbiological method: 0.1, 0.1, 0.1 and 0.2 mg/kg respectively in all species. Also, MRLs are recomended in liver and kidney of 0.3 and 0.6 mg/kg, respectively in all species. The latter two recommendations reflect the typical residue distribution of oxytetracycline in these tissues.

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Annex 1

RECOMMENDATIONS ON COMPOUNDS

Substance	Acceptable Daily Intake (ADI) for humans and other toxicological recommendations	Recommended Maximum Residue Limit (MRL)
Anthelmintic Drugs		
Closantel	0-0.03 mg per kg body weight	Edible tissues of sheep: 1.5 mg/kg Bovine tissues: muscle: 0.5 mg/kg[1] kidney: 2 mg/kg[1] liver: 1 mg/kg[1]
Ivermectin	0-0.0002 mg per kg body weight	Liver (all species): 0.015 mg/kg Fat (all species): 0.02 mg/kg
Levamisole	0-0.003 mg per kg body weight[2]	Edible tissues and milk (all species): 0.01 mg/kg[1]
Antimicrobial Agents		
Benzylpenicillin	0.03 mg per person per day[3]	Liver, kidney and muscle (all species): 0.05 mg/kg Milk: 0.004 mg/kg
Oxytetracyline	0-0.003 mg per kg body weight	All species: muscle: 0.1 mg/kg liver: 0.3 mg/kg kidney: 0.6 mg/kg fat: 0.01 mg/kg milk: 0.1 mg/kg eggs: 0.2 mg/kg