CHLORTETRACYCLINE

First draft prepared by
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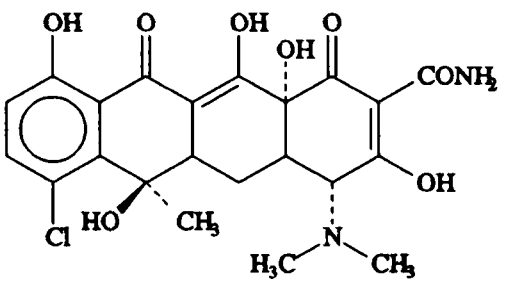
IDENTITY

Chemical names: 7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Synonyms: AC 13555; CL 13555; Chlortetracycline; W 4565; Aureomycin; Aureomycin 1000; AUROFAC; Duromycin; Emyrenil; Nidantin; Ossian; Ozoboi; Pietil; Prodoxol; Urinox; Uritrate; Uro-Alvar; Urotrate; Uroxin; Von Boch; Uroxol; Utibid.

Structural formula:



Chlortetracycline

Molecular formula: $C_{22}H_{23}ClN_2O_8$

Molecular weight: 478.88

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Chlortetracycline, chlortetracycline hydrochloride or the calcium salt of chlortetracycline

Major Impurities: Epichlortetracycline (8% max.)

Appearance: A finely divided yellow powder

Melting point: 168-169°C

Optical rotation: $[\alpha]_D^{23} = -275^0$ (MeOH)

Solubility: Water - 8.6 g/L, MeOH - 17.4 g/L, EtOH - 1.7 g/L

Stability: Chlortetracycline hydrochloride and the calcium salt of chlortetracycline are stable 2 and 3 years, respectively, at room temperature.

Manufacture: Chlortetracycline is an antibacterial agent obtained by aerobic fermentation of a strain of *Streptomyces aureofaciens*. It is obtained commercially by large scale fermentation.

Mode of Action: All tetracyclines act by the inhibiting attachment of aminoacyl-t RNA to the A site on the 30S ribosome to prevent protein synthesis. Translation is inhibited by 1 molecule of the tetracycline per ribosome. It is postulated that a tetracycline-magnesium complex is formed at the ribosome, making it less flexible and therefore unable to bind aminoacyl-t RNA. It has been found that binding of tetracyclines to the 30S ribosome is dependent on proteins S7, S14 and S19 (Sande and Mandell, 1990).

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

The major agricultural use of the substance is in intensive poultry and swine husbandry and also in beef and cattle production, both as a therapeutic and prophylactic agent. Chlortetracycline is usually administered mixed in the feed at doses of 10-500 g/ton for poultry, sheep and swine or between 2 and 10 mg/kg bw per day for calves, cattle and sheep. Lower doses are generally prophylactic whereas higher doses are used therapeutically. Chlortetracycline is also used in combination with other drugs such as amprolium, clopidol, decoquinate, ethopabate, hygromycin B, monensin, penicillin, robenidine, roxarsone, sulfamethiazole and zoalene.

Chickens and Turkeys

Promote growth and improve feed efficiency; prevention and treatment of chronic respiratory disease; protection against, and treatment of infection by chlortetracycline sensitive organisms; control of sinovitis, prevention and control of coccidiosis; treatment of blue comb, infectious enteritis and sinusitis, mud fever and hexamitiasis.

Swine

Promote growth and improve feed efficiency; prevention and treatment of bacterical swine enteritis; maintenance of weight gain in the presence of atrophic rhinitis; reduce spread of leptospirosis; reduction of abortion rate.

Cattle

Promote growth and improve feed efficiency; prevention of foot rot; reduction of bacterial diarrhea and bacterial pneumonia; prevention of anaplasmosis, reduction of losses due to respiratory infections.

Sheep

Promote growth and improve feed efficiency; aid in reduction of losses due to enterotoxemia and vibronic abortion.

Horses

Promote growth and improve feed efficiency.

METABOLISM AND PHARMACOKINETICS

Metabolism

Chlortetracycline is moderately well absorbed from the intestinal tract and there is considerable evidence that it is not significantly metabolised. It is excreted from the animal body, in both feces and urine, either in the unchanged form or in a form with much reduced antimicrobial activity. Epichlortetracycline, to which the parent is readily converted, is the main identifiable 'metabolite' which is probably largely an artefact formed during isolation from biological material. In laboratory animals, the kidney and the liver are of major importance in the excretion of chlortetracycline.

Quantitative studies in which ^{14}C-labeled chlortetracycline hydrochloride was administered by oral, intraperitoneal (IP) and intravenous (IV) routes to adult male Wistar rats and by the IV route to male beagle dogs have been reported (Wulf and Eisner, 1961; Eisner and Wulf, 1962). Orally administered chlortetracycline was excreted primarily in the faeces, whereas after IP dosing the amounts recovered from feces and urine were approximately the same. Urinary excretion predominated after IV administration of chlortetracycline at 15 mg/kg but as the dose was raised, the ratio of urinary to fecal excretion shifted to favor fecal excretion at an IV dose of 60 mg/kg. Potential metabolites were identified by paper chromatography, preceded where necessary by separation using column chromatography. Quantification was based on radiometric analysis. By all routes of administration and excretion, the recovery of *in vitro* antimicrobial activity was significantly lower than the recovery of radioactivity. The major 'metabolite', was identified as 4-epichlortetracycline, which accounted for 23 to 35% of the radioactivity recovered in the urine in rat studies and 32 to 60% in the dog study. This epimer is essentially inactive in the microbiological assay. The extent to which epimerisation occurs *in vivo*, if at all, is not clear, since this epimerisation is known to occur readily chemically and it is possible that epimerisation occurred under the conditions used for extraction of fecal samples. Indeed, in an *in vitro* experiment, approximately 20% of ^{14}C-labeled chlortetracycline added to normal dog urine was recovered as the 4-epichlortetracycline after 24 hours. Very small amounts of isochlortetracycline in the urine and feces of some of the animals were also detected. This compound is readily formed when chlortetracycline is subjected to a range of conditions, from neutral to alkaline. When ^{14}C-labeled or unlabeled 4-epichlortetracycline was administered IV to rats, there was little or no conversion of this epimer to a microbiologically active material (chlortetracycline). The authors concluded that "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" (Wulf and Eisner,1961).

Pharmacokinetics in Laboratory Animals

Oral doses of chlortetracycline ranging from 6 to 800 mg/kg bw were administered to female white rats and to guinea pigs (Eisner et al, 1953). In general, there was no direct proportional correlation of serum chlortetracycline concentration with dose. Administration of the same dose daily for nine days gave higher serum levels than those found with the single doses. Simultaneous administration of various organic acids, such as citric acid, increased serum chlortetracycline leves in doses ranging from 6 to 800 mg/kg.

Rats given single oral doses of 75 mg/kg bw of chlortetracycline attained plasma levels averaging 2.1 mg/L one hour post dosing. Plasma concentrations declined to 0.8 mg/L by six hours after dosing (Berte and Vandoni, 1962)

Beagle dogs given single oral chlortetracycline doses of 25 mg/kg bw showed peak serum levels ranging from 0.40 to 1.9 mg/L two hours after dosing which declined to an average of 0.21 mg/L after 24 h (Kandis, 1958). When the dogs were given a single IV dose of 10 mg/kg chlortetracycline, serum levels averaged 6.6 mg/L at one hour post dosing; declining to 2.4 mg/L at 8 h, 0.29 mg/L at 24 h and 0.06 mg/L at 48 h.

Adult white rabbits were given single oral doses of two different formulations of chlortetracycline at 20 mg/kg by stomach tube (Neuschl, 1991). Serum levels of chlortetracycline averaged 2.3 mg/L three hours after dosing; declining to 0.82 mg/L after 12 h and 0.09 mg/L after 24 h.

Pharmacokinetics in Turkeys

Single doses of 10, 15, and 20 mg/kg bw of chlortetracycline hydrochloride in aqueous solution were administered orally by gavage directly into the crops of small Beltsville White turkeys following a 17-hour fasting period (Pollet et al, 1984). The resulting plasma levels of chlortetracycline suggest that plasma concentrations were linearly related to dose. Peak plasma levels were reached 2 to 6 hours post-dosing, with the peak time being latest for the largest dose.

Investigations in several species have shown that divalent cations, such as calcium, have a suppressant effect on the absorption of tetracycline antibiotics. The co-administration of organic acids such as citric acid with the tetracycline antibiotic enhances blood serum levels of the tetracycline, probably because such acids form complexes with and sequester divalent cations thereby negating their depressant effect. Additional single oral dose studies in turkeys confirmed that the presence of calcium and magnesium in the birds' drinking water produced significantly lower blood chlortetracycline levels than were achieved when citric acid was added to the dosing solution. When minerals present in the gastrointestinal tract were kept to a minimum, the addition of citric acid to the dosing solution had little effect on the resulting plasma chlortetracycline levels (Pollet et al, 1984).

A study in which turkeys were fed a diet containing 440 mg/kg of chlortetracycline with and without 1.5% sodium sulfate after an overnight fast failed to show any effect of sodium sulfate on chlortetracycline levels in blood taken three hours after initiation of the medicated feed (Tindall and Berger, 1966a).

A study in turkeys where chlortetracycline was administered intravenously at a dose of 0.9 mg/kg b.w. demonstrated that chlortetracycline was rapidly removed from the blood and excreted via the bile (Dyer, 1988). The ratio of the peak bile/plasma concentrations was 254:1 at 2 h and was greater than 1:1 between 10 and 240 min. Over the period 4 hours after dosing, about 8.5% of the administered dose was accounted for in the bile.

Pharmacokinetics in Chickens

The oral time-course data for chickens dosed with chlortetracycline at 25 mg/kg followed by determination of chlortetracycline serum levels over a 24-hour period fitted a 2-compartment model adequately. Addition of citric acid to the oral chlortetracycline dose made little difference to the absorption rate constant obtained for chlortetracycline alone. The uptake of orally administered chlortetracycline was rapid but only a small fraction was absorbed. Co-administration of citric acid not only increased the mean serum concentration significantly but also produced distinctly higher elimination and distribution rates for chlortetracycline. After 4 h, the serum levels of birds dosed with either chlortetracycline alone or a chlortetracycline-citric acid combination were approximately the same (Pollet et al., 1983).

In another study, broiler chickens given chlortetracycline in water at 400, 800 and 100 mg/gallon, either alone or co-administered with citric acid, gave blood levels of chlortetracycline which failed to show any consistent correlation with the presence or absence of citric acid in the dose (Berger, 1982c). The effect of aflatoxin on the chlortetracycline blood levels of broiler chicks administed in combination has also been studied. There was a significant decrease in half-life levels of chlortetracycline and a significant increase in the systemic clearance in birds receiving IV chlortetracycline together with 2.5 mg/kg aflatoxin in the feed over control birds receiving chlortetracycline alone (Miller and Wyatt, 1985).

Two different drinking water formulations on chlortetracycline given to broiler chickens by oral gavage (10.8 mg/kg bw) gave chlortetracycline blood level averaging 0.13 mg/mL at 2 h and 0.20 mg/mL at 6 h. After 24 h the blood chlortetracycline levels in the majority of birds was below 0.015 mg/L, the limit of detection for the assay method (Schumacher, 1968).

In another study, chlortetracycline was administered via the drinking water to chickens at 120 mg/L for 7 days. The chlortetracycline blood concentrations, shown in Table 1, peaked at 0.14 mg/L 8 h after start of treatment

and declined somewhat thereafter. The average daily intake of chlortetracycline ranged from 17.4 to 23.7 mg/kg bw over the 7 days of the treatment.

Table 1. Average Chlortetracycline Intake and Blood Levels in Chickens Given 120 mg/L of Chlortetracycline in Drinking Water for 7 Days (Gingher, 1989b).

Hours of Medication	Bird Average Weight (kg)	Chlortetracycline Intake (mg/kg)	Blood Chlortetracycline (mg/L)
0	1.218		ND
2	NM	NM	0.067 (0.049-0.107)
4	NM	NM	0.064 (0.049-0.074)
8	NM	NM	0.140 (0.086-0.225)
24	1.413	23.7	0.067 (0.051-0.085)
72	1.462	18.5	0.084 (0.055-0.131)
120	1.642	19.4	0.090 (0.044-0.129)
168	1.734	17.4	0.043 (0.024-0.067)

NM = Not Measured; ND = Not detected

Two studies were conducted in which different formulations of chlortetracycline were administered via the drinking water at a concentration of 264 mg/L (Berger, 1971; Schumacher, 1968). The resulting blood levels of chlortetracycline are shown in Table 2. It is interesting to note that, while the younger (smaller) birds consumed somewhat more chlortetracycline on a bw basis, the resulting blood concentrations were lower. Chlortetracycline levels in the blood of most birds were below the limit of detection of the assay by 24 hours after withdrawal of medicated water.

Table 2. Average Blood Chlortetracycline (CTC) Levels (in mg/L) in Chickens Given 264 mg Chlortetracycline/L of Drinking Water for 3 Days.

Reference	Berger, (1971)			Schumacher, (1968)	
Average Body Weight, kg	1.77	1.82	2.09	0.95	0.93
Av. Daily CTC Intake, mg/kg	31.9	28.8	25.1	36.8	36.6
Time on Medication	Average Blood Chlortetracycline Levels (mg/L)				
2-3 h	0.32	ND-0.33	0.23	ND-0.03	ND-0.08
6 h		ND-0.23	ND-0.23	0.11	ND-0.18
24 h	0.4	0.24	0.14	0.15	0.2
72 h	0.4	0.27	0.17	NM	NM
Withdrawal Time					
24 h	ND-0.03	ND-0.02	NM	NM	NM
48 h	ND-0.02	ND	ND	NM	NM
72 h	ND-0.02	ND-0.012	ND	NM	NM

ND = Not Detected; NM = Not Measured

Several studies have been conducted where chlortetracycline was given in the feed at 300 mg/kg for seven days (Gingher, 1989e, 1990b, 1990f). The resulting levels of chlortetracycline in the blood, presented in Table 3, show considerable differences in blood chlortetracycline concentrations between studies. The differences

between data is probably due to differences in calcium levels in the basal diets. In these studies, blood chlortetracycline levels were inversely proportional to the level of calcium in the basal diets. This is not unexpected as many investigators have shown that calcium will depress absorption of chlortetracycline. The studies also show that medication of chickens via the feed is a reliable method of providing therapeutic levels of chlortetracycline which are well maintained throughout the period of administration of medicated feed.

Table 3. Average Blood Chlortetracycline (CTC) Levels (mg/L) in Chickens Fed 300 mg/kg CTC in the Diet for 7 Days.

Reference	Gingher (1989e)	Gingher (1990b)	Gingher (1990b)	Gingher (1990f)
Calcium in feed %	1.28	0.88	0.88	0.79
Average Body Weight, kg	1.52	1.03	1.06	0.88
Av. CTC Daily Intake, mg/kg	28.9	34.7	33.2	35.7
Hours medication	Blood Chlortetracycline (mg/L)			
0	ND	ND	ND	ND
2	0.081	0.118	0.136	0.411
4	0.108	0.184	0.203	0.413
6	NM	0.173	0.246	0.444
8	0.218	0.216	0.234	0.504
24	0.105	0.157	0.191	0.411
72	0.112	0.148	0.123	0.317
120	0.086	0.112	0.122	0.283
168	0.056	0.118	0.112	0.256

ND = Not Detected; NM = Not Measured

Accumulation of Residues in Eggs

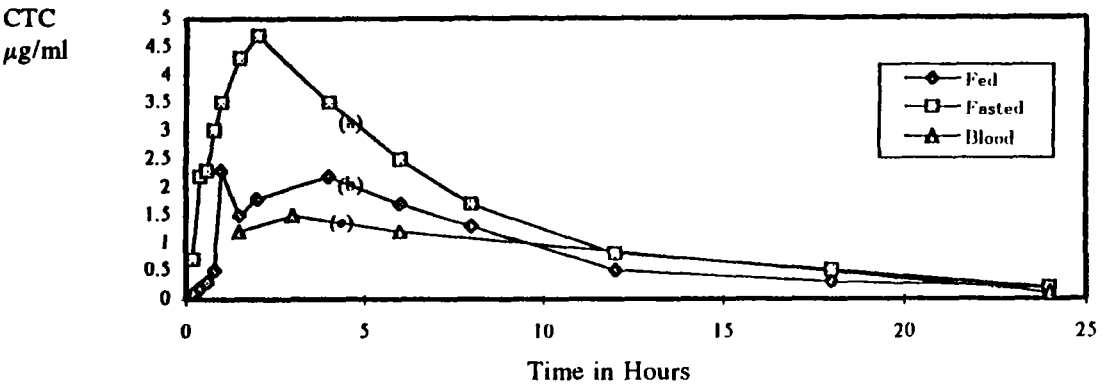
In an early study, Broquist and Kohler (1954) found no residues of chlortetracycline in eggs from hens fed 220 mg/kg chlortetracycline in the feed. When the level in feed was raised to 2200 mg/kg, eggs had chlortetracycline residues ranging from 0.15 to 3.1 mg/kg in whole egg.

Meredith et al (1965) dosed laying hens with 1000 mg/kg chlortetracycline in the diet, showed residues averaging 0.05 mg/kg of whole egg. Cooking by poaching or scrambling reduced the antibiotic activity significantly, but failed to destroy all residual antibiotic activity.

Pharmacokinetics in Swine

Chlortetracycline hydrochloride was administered intra-arterially (11 mg/kg) and as an oral drench (33 mg/kg) to ten 21.0 to 31.5-kg pigs. Five of the pigs were fasted 18 hours prior to dosing and five of the pigs were fed *ad libitum* prior to dosing. The average plasma chlortetracycline concentration data for the orally dosed pigs are shown in Figure 1 (a,b). The mean volume of distribution, determined by area-under-the-curve calculations, for the fasted pigs (0.967 ± 0.210 L/kg) was significantly less ($P < 0.05$) than the mean volume of distribution for the fed pigs (1.39 ± 0.31 L/kg). Mean total body clearance of the drug was also significantly less ($P < 0.05$) in the fasted pigs (0.165 ± 0.055 L/kg/h) as compared with the fed pigs (0.307 ± 0.053 L/kg/h). The elimination constants were not found to be statistically different [($P < 0.05$): 0.1811 L/kg$\pm$0.0057 for the fasted pigs; 0.2260 ± 0.0461 for the fed pigs]. The bioavailability for both groups was similar; $19.12\% \pm 8.3\%$ for the fasted pigs and $17.88\% \pm 5.3\%$ for the fed pig (Kilroy et al, 1990).

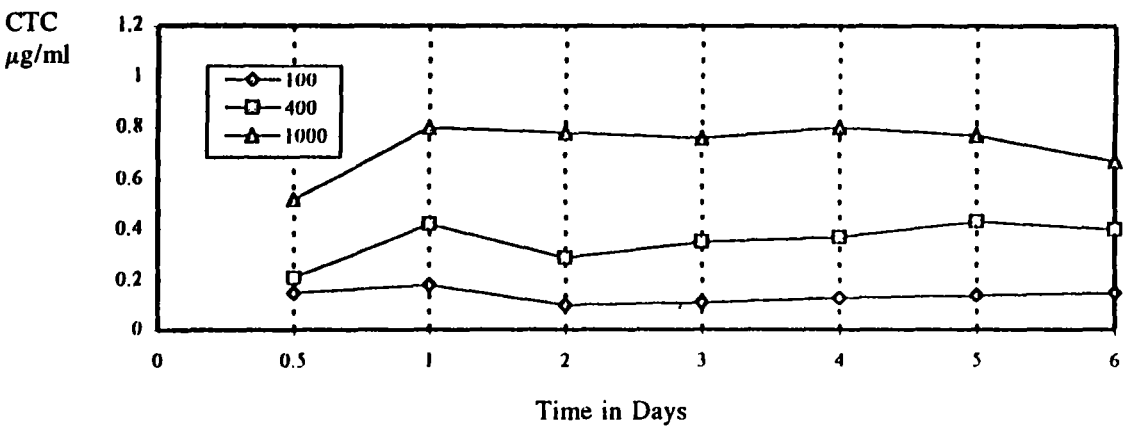
Figure 1. **Chlortetracycline (CTC) Plasma Concentrations Following Single Oral Dose of 33 mg/kg Body Weight by Oral Gavage to Fed and Fasted Pigs**



Ten pigs, weighing 22.2 to 25.0 kg and not fasted prior to treatment, were orally-gavage-dosed with two different soluble powder formulations of chlortetracycline. Blood chlortetracycline concentrations for the first 24 hours following dosing are shown in Figure 1(c). These data suggest that peak blood levels were achieved somewhat later than was seen in the work Kilroy et al (1990). Exept for one sample from the 6-h bleeding, which was much higher than any of the other 6-h samples, these results agree well with the results of Kilroy et al for nonfasted pigs (Berger, 1967b).

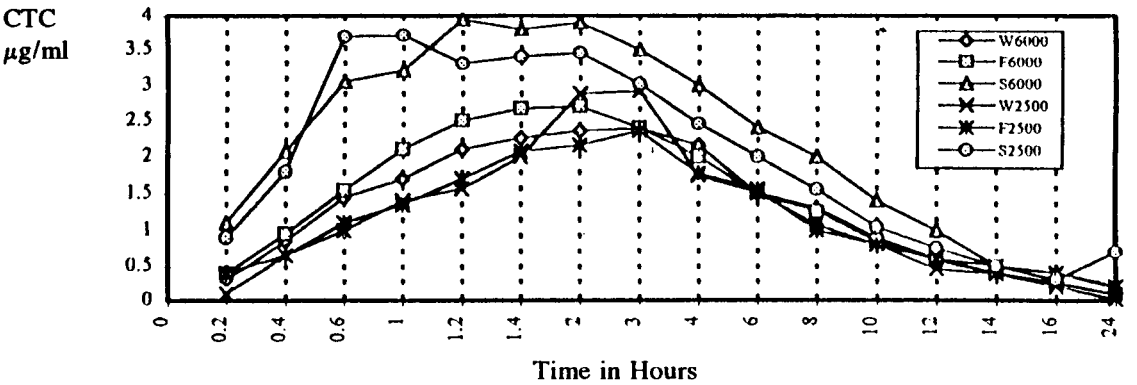
Three groups of six pigs which weighed 34.5 to 44.1 kg were fed corn-soy diets *ad libitum* which were fortified with chlortetracycline HCl at 100, 400 or 1000 mg/kg. Chlortetracycline concentrations were determined in plasma samples collected over a 6-day period. Plasma chlortetracycline levels reached a plateau within 24 hours after initial access to the feed (Figure 2) and were proportional to the dose of drug consumed (r^2=0 97) (Kilroy et al, 1990).

Figure 2. **Chlortetracycline Concentrations in Plasma of Pigs Fed Corn-Soy Diets *ad libitum*, Fortified with CTC HCl at 100, 400 or 1000 mg/kg.**



The influence of feed preparation on the pharmacokinetics of orally administered chlortetracycline was demonstrated by Sutter and Wanner (1990). Pigs were divided into 3 groups which received a dry, a moist, or a very wet, soupy diet each containing chlortetracycline concentrations of 6000 and 2500 mg/kg, respectively. The results shown in Figure 3, demonstrate a significantly higher chlortetracycline bioavailability in animals fed the very wet diet compared with the moist or dry diets although the amount fed furnished a dose of 40 mg chlortetracycline/kg bw in each case.

Figure 3. **CTC Serum Concentrations after Oral Dose of 40 mg/kg of Body Weight. W = Dry, F = Moist and S = Very Wet**

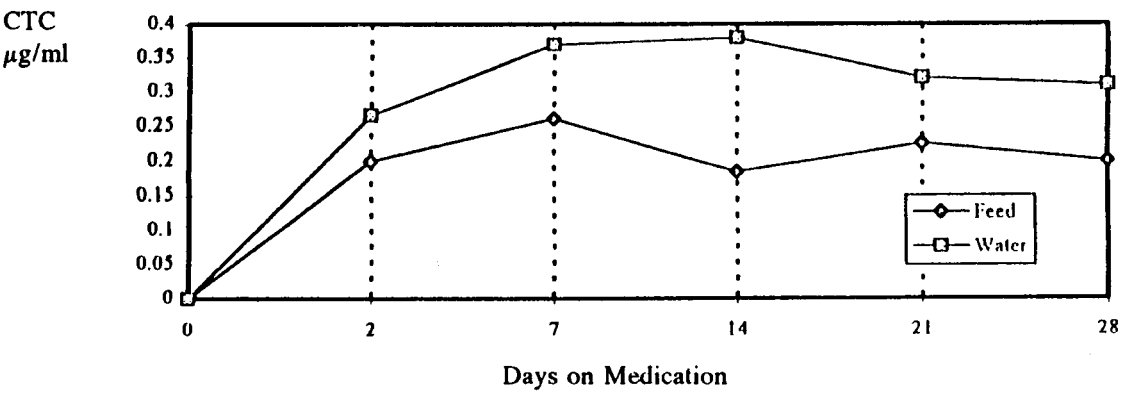


A study conducted at South Dakota State University indicated that metallic cations have an effect on chlortetracycline absorption (Wahlstrom et al, 1982). Calcium sulfate was shown to be a satisfactory calcium source, but it did not influence chlortetracycline absorption. This study also showed that dietary levels of sodium sulfate up to 2.13% increased serum chlortetracycline levels. Increase in dietary phosphate levels also had the same effect.

Blood chlortetracycline concentrations of pigs given access to drinking water containing 66 mg/kg chlortetracycline with an equal concentration of sulfamethazine over an eight day period reached 0.135 mg/L of blood after one day, and remained at about that level for the remaining eight-days. Blood chlortetracycline levels dropped to values around the LOD of the assay method (0.015 mg/L) by 24 hours after withdrawal of the drug (Wang, 1971b).

Blood chlortetracycline concentrations in pigs given chlortetracycline in feed or in drinking water for a 28-day period are shown in Figure 4. After correction for differences of intake by each administration method, water medication resulted in approximately 25% higher blood chlortetracycline blood concentrations at all sampling points than those observed with medicated feed. (Berger, 1967c).

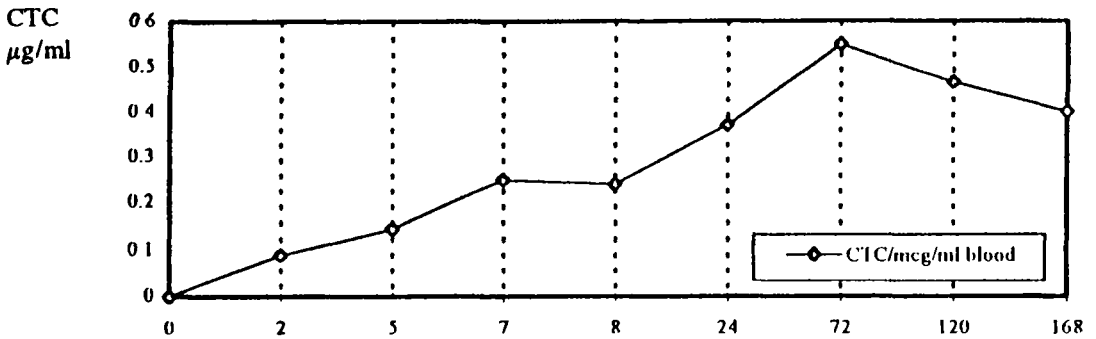
Figure 4. **Chlortetracycline (CTC) Blood Concentrations of Pigs Given 66 ppm CTC in the Drinking Water Versus 100 ppm in the Feed for 28 Days.**



Blood chlortetracycline concentrations from pigs given free choice access to drinking water containing 120 mg/kg chlortetracycline for 7 days is shown in Figure 5. Average blood chlortetracycline levels rose rapidly for the first 6 hours, and then more slowly until day 3. Individual variations in blood concentrations make it doubtful if any real differences can be expected after the first day on medication. Average

concentration of chlortetracycline in lung tissue on day 7 was 0.264 mg/kg. Average daily intake of chlortetracycline was 14.96 mg/kg bw (Berger, 1989d).

Figure 5. Chlortetracycline (CTC) Blood Concentrations of Pigs Given 120 ppm CTC in Drinking Water for 7 Days.



In another study pigs were given drinking water containing 147 mg/kg chlortetracycline for seven consecutive days. Blood and lung concentrations of chlortetracycline were measured after 1, 3, and 7 days on the treatment. Concentrations of chlortetracycline in the lung tissue averaged 60 to 85% higher than those found in the blood (Rooney, 1990).

There were no significant differences in chlortetracycline levels in blood of pigs receiving 110 mg/kg chlortetracycline in the feed for 28 days, supplied by different formulations (Berger, 1984, Garces, 1981). Within each study the blood concentrations averaged in a range of 0.11-0.43 mg/L over the dosing period.

The average values of data from studies in which pigs received 165 mg/kg chlortetracycline in feed for 7 consecutive days showed that blood concentrations of chlortetracycline rose steadily for the first 24 hours on medicated feed, after which average levels declined slightly by 7 days. The premixes used to prepare these feeds also contained sulfamethazine, and in three cases, penicillin (Rooney, 1989c). Pigs receiving 300 mg/kg chlortetracycline in feed for 7 consecutive days (Berger, 1989c; Berger, 1990; Gingher, 1990a) gave blood levels of the drug in two of the three comparisons which were very similar to those obtained with the 165 mg/kg results above (Gingher, 1990a).

Pharmacokinetics in Cattle

The plasma and tissue concentration and pharmacokinetics of chlortetracycline were determined in milk-fed and conventionally fed Holstein calves by Bradley et al (1982). The pharmacokinetics of chlortetracycline after a single intravenous dose of 11 mg chlortetracycline/kg bw were similar for both milk-fed and conventionally-fed calves (Figure 6). The drug was rapidly distributed from plasma into the peripheral compartment but was slowly eliminated, with detectable levels of chlortetracycline continuing for 72 hours after dosing. Milk-fed calves had a larger area under the plasma level curve following a single oral dose of 22 mg chlortetracycline/kg bw. Futhermore, a larger fraction of the dose was absorbed together with a smaller volume of distribution and a smaller overall body clearance rate. The mean plasma concentrations are shown in Figure 7. The chlortetracycline concentration in tissues following an oral dose was greatest in the kidney, followed by liver, heart, skeletal muscle, spleen and brain. Tissue depletion closely paralleled the decline in plasma concentration. These investigators suggest that the effects seen in milk-fed calves would probably be enhanced by administration of the drug in the milk replacer, as practiced in veal calf management. A study by Luthman and Jacobsson (1983), showed that when calves were given a dose of 50 mg chlortetracycline/kg bw in water, milk replacer or milk, the serum concentrations of chlortetracycline were lower when administered in milk replacer or milk (Figure 8). However, the serum chlortetracycline concentrations obtained by all three modes of administration can be considered quite satisfactory.

Figure 6

Mean Plasma Concentrations of CTC in Calves after Intravenous Administration of a Single Dose of 11 mg CTC/kg Body Weight

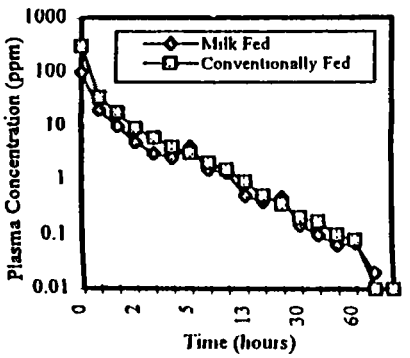


Figure 7

Mean Plasma Concentrations of CTC in Calves after Oral Administration of a Single Dose of 22 mg CTC/kg Body Weight

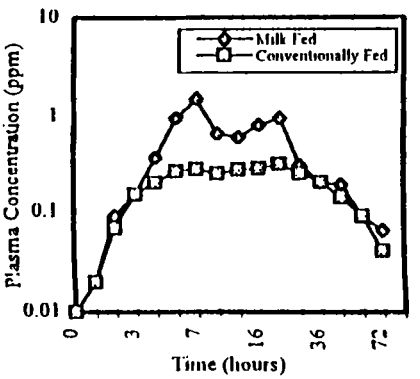
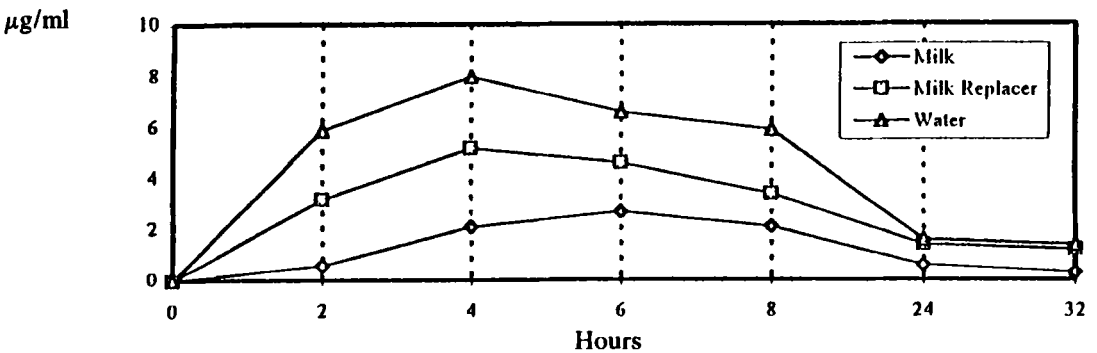


Figure 8. **Serum Concentrations of Chlortetracycline in Calves after Oral Administration of 50 mg/kg of Body Weight in Water, Milk Replacer and Cows Milk.**



A more recent study measured blood concentrations of chlortetracycline following administration of 700 mg chlortetracycline in a milk replacer to young Holstein calves twice daily over a 7-day period (Rooney, 1988a). On the first day of treatment average chlortetracycline blood levels peaked at 0.76 mg/L four hours after the first feeding of medicated milk replacer. On days 1, 3, 5, and 7 of treatment, average blood levels from samples taken four hours after the morning feeding were between 0.88 and 1.01 mg/L. Lung samples collected at euthanasia, four hours after the last feeding of medicated milk replacer were 1.21 to 1.90 mg/kg of tissue.

Similar data has been obtained following administration for seven consecutive days of either a soluble powder formulation of chlortetracycline in milk replacer at the dose of 13.3 mg/kg bw administered once daily (Rooney, 1989a) or with one soluble bolus of chlortetracycline twice daily, providing approximately 22 mg chlortetracycline/kg bw per day (Goodale, 1988). Blood chlortetracycline concentrations averaged 1.48 mg/L eight hours after the initial feeding of medicated milk replacer. On days 2, 3, 6 and 7 of treatment blood chlortetracycline levels from samples taken four hours after medication averaged between 1.47 and 1.61 mg /L. Chlortetracycline levels of lung samples collected at euthanasia, 4 hours after the last medicated feeding, were 2.05 to 2.93 mg/kg of tissue. On the first day of the bolus treatment, average blood chlortetracycline levels peaked at 0.204 mg/mL eight hours after the first treatment. Average blood levels of chlortetracycline from samples taken on days 3, 5, and 7 of treatment were between 0.71 and 0.92 mg/L. Lung samples collected at euthanasia contained a mean of 2.12 mg/kg.

In a much earlier study with somewhat older calves medicated once daily by bolus at approximately 5.5 mg/kg bw, blood chlortetracycline levels ranged from 0.13 to 0.69 mg/L during a three day treatment period (Shor, 1961b). Blood chlortetracycline levels gradually declined to an average of 0.06 mg/L four days after the last treatment.

Experiments conducted in young calves, compared the depletion of chlortetracycline from the blood following cessation of treatment with various oral dosing forms of chlortetracycline at a total daily dose of 22 mg/kg bw (Berger, 1974; Berger and Garces, 1981). The results of these studies, summarized in Table 4, show good agreement within each study regarding the magnitude and depletion of blood chlortetracycline for the various formulations. However, much higher blood chlortetracycline levels were seen in one study than the other, yet the bolus formulation tested was exactly the same for both studies. The calves showing the higher blood chlortetracycline levels were younger and received the medication divided between 2 daily doses, while the calves exhibiting the lower blood chlortetracycline levels received the medication once daily. The younger calves were fed a milk replacer diet only, while the older calves received a calf starter grain mix in addition to the milk replacer diet. It is probable that these older calves had experienced some rumen development, which according to the work of Bradley et al. (1982) would be expected to cause a lower absorption of chlortetracycline and a more rapid elimination of the drug from the body. This hypothesis is supportes by data shown in Table 4.

Table 4. Depletion of Blood Chlortetracycline Levels in Calves Following Cessation of Treatment with Soluble Boluses, Soluble Powder Formulations or Tablets for 5 or 10 Days at 22 mg Chlortetracycline/kg Body Weight Daily

Reference	Berger, 1974	Berger, 1974	Berger, 1974	Berger and	Berger and
Formulation[1]	B	SP	SP	B	T
Administered	Once Daily	Once Daily	Once Daily	Twice Daily	Twice Daily
Average Weight, kg	80.4	86.7	87.2	48.4	47.4
Days on CTC	10	10	10	5	5
Withdrawal Time	**Chlortetracycline, mg/kg of tissue**				
1 hour	0.41	0.72	0.42	1.83	1.93
2 hours	0.4	1.32	0.47	1.79	2.06
4 hours	0.42	1.22	0.57	2.03	1.97
8 hours	0.62	0.64	0.73	2.48	2.15
12 hours	0.58	0.48	0.68	NM	NM
1 day	0.33	0.19	0.35	1.42	1.46
2 days	0.14	0.07	0.13	0.72	0.82
3 days	0.07	0.04	0.07	0.42	0.55
4 days	0.04	0.03	0.04	0.32	0.39
5 days	0.02	0.02	0.03	0.15	0.23

[1]Formulation: B = soluble bolus; SP = soluble powder; T = tablet; NM = Not Measured

Studies have also been conducted in which older calves averaging 172-193 kg were given 22 or 11 mg/kg bw chlortetracycline in the feed for 4 or 5 days. The animals receiving the 22 mg/kg dose showed peak chlortetracycline levels of 0.454 and 0.458 mg/L at 12 and 16 h post feeding which declined to an average of 0.346 mg/L by 24 h after feeding (Craig, 1992). Lung tissue obtained 12 hours after the fifth daily medication contained an average of 1.166 mg/kg chlortetracycline. Cattle receiving 11 mg/kg daily had peak chlortetracycline blood levels of about 0.210 mg/L 12 and 16 hours after consuming the medicated feed which declined to an average of 0.148 mg/L by 24 hours post dosing (Biroc, 1992b). These levels agree very well

with the earlier work of Alford (1970) who found that cattle receiving 10 to 12 mg chlortetracycline/kg bw in the feed for 3 days averaged blood levels of 0.235 mg chlortetracycline/L on the second and third days of receiving the medicated rations.

Tissue Distribution

Tissue distribution data for chlortetracycline is predominantly confined to experiments in laboratory animals with less evidence available from food animals.

In a study of the intestinal absorption in rats, tissue levels of chlortetracycline at 1 to 5 hours after dosing are shown in Table 5. Tissue levels were highest in liver and kidney at all times (Berte and Vandoni, 1962). In a study in which adult white rabbits were given single oral doses of two different formulations of chlortetracycline by stomach tube at 20 mg/kg, levels of the drug twenty-four hours after dosing were highest in liver, averaging 1.84 mg/kg, followed by kidney, lung and heart. No measurable chlortetracycline was found in muscle tissue in this experiment (Neuschl, 1991). Studies in rats (Buyske et al, 1960), mice and rabbits (Miller and Wyatt, 1985) have shown that chlortetracycline has a great affinity for bones and teeth, and remains in these tissues and structures for long periods of time.

Table 5. Chlortetracycline Levels in Tissues and Plasma of Rats Following a Single Oral Dose of 75 mg/kg bw (Berte and Vandoni, 1962).

	Chlortetracycline (mg/kg)				
Time After Dosing, min	Plasma	Lungs	Brain	Liver	Kidney
60	2.1±0.56	5.2±0.13	0.11±0.04	16.2±0.6	21.8±6.4
120	1.1±0.2	3.8±2.1	0.09±0.03	21.4±0.9	20.1±4.9
180	0.8±0.4	2.3±1.0	0.02±0.01	15.2±1.2	14.8±3.2
240	0.7±0.6	2.2±0.9	0.03±0.02	10.0±0.7	11.2±1.0
360	0.8±0.3	2.1±0.45	0.03±0.01	5.3±1.0	8.7±0.66

^{14}C-labeled chlortetracycline was administered IV to two beagle dogs to study the distribution throughout the body. Four hours after dosing the liver contained most radioactivity, followed in decreasing order by the kidney, ileum, jejunum, heart and duodenum. A large proportion of the recovered radioactivity was found in the urine, intestinal contents and bile. With the exception of subcutaneous fat, radioactivity was found throughout all tissues and fluids examined (Kelly, 1964). A summary of the findings is presented in Table 6.

Table 6. Total Micrograms of Chlortetracycline in the Whole Tissues of Dogs Four Hours After an Intravenous Dose of 10 mg/kg (Kelly, 1964).

Tissue	Dog A	Dog B	Tissue	Dog A	Dog B
Liver	5638	7782	Jejunum	559	723
Kidney	805	718	Ileum	755	790
Heart	634	529	Cecum	168	40
Lungs	366	426	Colon	255	156
Brain	69	60	Rectum	101	154
Bile	2750	3240	Stomach Contents	39	31
Spleen	123	102	Duodenal Contents	146	238
Pancreas	173	164	Jejunal Contents	845	876
Uterus	50	80	Ileal Contents	2146	2128
Trachea	36	44	Colon Contents	2481	1293
Esophagus	70	92	Rectal Contents	21	749
Stomach	230	347	Diaphragm	150	181
Duodenum	538	540	Urine	20711	N.M.

Dog A Body Weight 6.2 kg and Dog B 8.0 kg; NM = Not Measured

TISSUE RESIDUE DEPLETION STUDIES

Turkeys

Chlortetracycline residues in turkeys were measured in body fluids and tissues at 2, 8 and 24 hours following a single oral dose of of 15 mg/kg bw (Table 7). This study clearly shows that chlortetracycline levels are highest in the kidneys and/or liver at all three times, suggesting that either kidney or liver should be considered the target tissue (Pollet et al, 1984). These findings are supported by a study in which turkeys were given chlortetracycline bisulfate via the drinking water at the rate equivalent to 264 mg/kg of chlortetracycline HCl for 14 days (Berger, 1972). The results show conclusively that concentrations of chlortetracycline are highest and most persistent in the kidney, followed by the liver (Table 8), as would be expected since these are the two organs directly involved in the excretion of chlortetracycline.

Table 7. Concentration of Chlortetracycline in Tissues and Plasma Samples Following Oral Administration of 15 mg Chlortetracycline/kg Body Weight to Turkeys (Pollet et al, 1984)

	Hours After Chlortetracycline Administration		
Tissue	2	8	24
	Chlortetracycline, mg/kg		
Liver	3.05 ± 2.01	2.32 ± 1.37	0.61 ± 0.44
Kidney	8.76 ± 7.24	10.84 ± 8.19	2.57 ± 0.35
Red Muscle	0.23 ± 0.16	0.28 ± 0.17	0.05 ± 0.04
White Muscle	0.09 ± 0.06	0.15 ± 0.15	0.05 ± 0.02
Brain	0	0.5 ± 0.01	0.07 ± 0.05
Plasma	0.6 ± 0.44	0.44 ± 0.25	0.11 ± 0.05

A number of tissue residue experiments have been conducted where therapeutic doses of chlortetracycline were administered to turkeys via the drinking water or feed. A summary of the residue depletion data from kidney

and liver tissues of turkeys medicated via the drinking water or feed are presented in Table 9.

Table 8. Summary of Chlortetracycline Residues in Tissues of Turkeys Following Medication with 264 mg/kg Chlortetracycline in Drinking Water for 14 Days (Berger, 1972)

	Chlortetracycline, mg/kg of Tissue			
Withdrawal Day	Muscle	Liver	Kidney	Skin/Fat
0	0.27	1.04	3.82	0.22
1	ND - 0.04	0.15	0.56	0.16
2	ND - 0.03	0.08	0.34	0.12
3	ND - 0.04	0.17	0.46	0.12
4	ND	ND - 0.08	0.3	0.08
5	ND	0.07	0.26	0.05

ND = Not Detected

Table 9. Concentrations of Chlortetracycline in Liver and Kidney Tissues of Turkeys Receiving Chlortetracycline in the Drinking Water

Reference	Berger, (1972)	Berger, (1982b)	Shor, (1962)	Shor, (1962)	Shor and Gale (1965)	Gutzman, (1990c)
Conc. in Water, (mg/kg)	264	106	528	528	528	528
Days on Medication	14	14	3	3	14	14
Chlortetracycline Average Daily Intake, (mg/kg)	17.4	16	63.3	71.7	19.8	26.4
Average Bird weight, (kg)	4.3	1.85	1.06	1.06	6.95	6.81
Withdrawal Day	Chlortetracycline, mg/kg of Liver					
0	1.04	0.26	1.00	1.56	0.36	0.38
1	0.15	0.06	0.12	0.14	ND-0.04	ND-0.22
2	0.08	0.04	0.10	0.10	ND-0.04	ND-0.22
3	0.17	ND	NM	NM	NM	NM
4	ND-0.08	NM	ND-0.05	ND-0.06	NM	NM
5	0.07	NM	NM	NM	ND-0.03	ND
Withdrawal Day	Chlortetracycline, mg/kg of Kidney					
0	3.82	1.24	3.73	11.52	1.57	2.20
1	0.56	0.24	0.80	0.91	0.13	0.89
2	0.34	0.17	0.60	0.73	0.12	0.13
3	0.46	ND	NM	NM	NM	NM
4	0.30	NM	0.26	0.40	NM	NM
5	0.26	NM	NM	NM	ND-0.08	0.09

NM = Not Measured; ND = Not Detected

Chickens

In common with other species, residues of chlortetracycline are the greatest and persist the longest in liver and kidney tissues of chickens. This is demonstrated in Table 10, with data from chickens which received 110 mg/kg of chlortetracycline in feed for 51 days followed by a 5-day feeding of a diet containing 550 mg/kg chlortetracycline (Gingher, 1979). At zero-day withdrawal from medicated feed, residues of chlortetracycline averaged 3.9 mg/kg in kidney, 0.79 mg/kg in liver, 0.27 mg/kg in muscle, less than 0.04 mg/kg in fat and less than 0.06 mg/kg in skin. By day 4, chlortetracycline could not be detected in muscle, fat and skin, while liver averaged less than 0.05 mg/kg and kidney averaged less than 0.25 mg/kg. It can be concluded that when chlortetracycline is not detectable in liver and kidney, residues will also be absent from all other edible tissues. Therefore, depletion of chlortetracycline residues in these two marker tissues has been emphasised in this review.

Table 10. Residue Depletion in Tissues from Chickens which Received 110 mg/kg Chlortetracycline in Diet for 51 Days Followed by 550 mg/kg in the Diet for 5 Additional Days (Gingher, 1979).

		Chlortetracycline mg/kg of tissue				
Withdrawal Day		**Muscle**	**Liver**	**Kidney**	**Fat**	**Skin**
0	Average	0.27	0.79	3.9	ND-0.04	ND-0.06
	Range	0.18-0.36	0.54-1.08	2.65-7.10	ND-0.09	ND-0.10
3	Average	ND-0.026	ND-0.06	0.38	ND	ND-0.12
	Range	ND-0.028	ND-0.08	0.31-0.47	----	ND-0.12
4	Average	ND	ND-0.05	0.25	ND	ND
	Range	----	ND-0.05	0.20-0.32	----	----

ND = Not Detected

A summary of residue data from chickens treated via the drinking water at levels of 120 to 528 mg/kg for periods ranging from 3 to 14 days is shown in Table 11 (Berger, 1982a; Gingher, 1989a; Stoner, 1983). As would be expected, the magnitude of the residues are in direct proportion to the concentration of chlortetracycline in the drinking water. Liver is essentially free of chlortetracycline residues two days after withdrawal, while measurable amounts of chlortetracycline (0.04 to 0.15 mg/kg) persist in kidney four days after withdrawal.

Four studies were conducted in which broiler chicks received 220 mg/kg chlortetracycline in the diet for the first three weeks of life. In each experiment the basal ration contained approximately 0.8% calcium and 1.5% of sodium sulfate to provide conditions for maximum absorption of the chlortetracycline. The average residue depletion data from liver and kidney tissues are shown in Table 12. Residues averaged from 1.45 to 2.18 mg/kg in kidney and 0.42 to 0.75 mg/kg in liver at zero-day withdrawal. Residues were below 0.1 mg/kg in liver by 3 to 5-days withdrawal. Residues in kidneys ranged from 0.05 to 0.11 mg/kg chlortetracycline at the 7-day withdrawal point (Berger, 1967e; Tindall and Berger, 1966b,c,d).

Table 11. Residues in Liver and Kidney Tissues From Chickens Receiving Chlortetracycline in the Drinking Water

Reference	Gingher, 1989a		Berger, 1982a		Stoner, 1983	
Chlortetracycline in Water, mg/kg	120		264		528	
Days on Medication	7		14		3	
	Chlortetracycline, mg/kg					
Withdrawal Day	Liver	Kidney	Liver	Kidney	Liver	Kidney
0	0.276	NM	0.36	>2.87	0.87	3.78
1	ND-0.049	NM	ND-0.08	0.37	0.07	0.36
2	ND-0.03	NM	ND-0.06	0.32	ND-0.03	0.23
3	ND	NM	NM	NM	NM	NM
4	ND	NM	NM	NM	ND	0.09

ND = Not Detected; NM = Not Measured

Table 12. Residues of Chlortetracycline in Liver and Kidney Tissues from Chickens Receiving 220 mg/kg Chlortetracycline in Feed for First Three Weeks

Reference	Berger,1967e		Tindall and Berger, 1966b		Tindall and Berger, 1966c		Tindall and Berger, 1966d	
Ca in diet, %	0.8		0.81		0.81		0.81	
Na_2SO_4 in diet %	1.5		1.5		1.5		1.5	
Average bw, kg[1]	0.379		0.372		0.413		0.4	
Average Daily CTC Intake, mg/kg[2]	22		24.2		19.8		22.4	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	0.53	2.01	0.42	1.45	0.71	1.91	0.75	2.18
3	NM	NM	NM	NM	0.04	0.27	NM	NM
4	NM	NM	NM	NM	0.04	0.24	NM	NM
5	ND-0.04	0.21	ND	0.17	ND-0.05	0.24	ND-0.03	0.15
7	ND	0.09	ND	0.08	NM	NM	ND	0.11

[1]Average weight at end of medication period; [2]Average daily chlortetracycline intake last week of medication; NM = Not Measured; ND = Not Detected

Two separate studies (Drain, 1962a ; Gingher, 1980) in older chickens administered 220 mg/kg chlortetracycline in the feed showed residue levels below 0.05 and 0.5 mg/kg in liver and kidney, respectively, 3 days after withdrawal of medication. Results are shown in Table 13.

Table 13. Residues of Chlortetracycline in Liver and Kidney Tissues from Older Chickens Receiving 220 mg/kg Chlortetracycline in Feed

Reference	Drain, 1962a		Gingher, 1980	
Calcium in diet, %	0.47		0.83	
Average Body Weight, kg	approx. 1.8		2.23	
Days on Medication	5		55	
Withdrawal Day	Chlortetracycline, mg/kg of Tissue			
	Liver	Kidney	Liver	Kidney
0	0.66	0.42	0.71	0.75
1	ND-0.06	0.25	NM	NM
3	ND	0.07	ND-0.03	0.3
4	NM	NM	ND-0.04	0.28
6	ND	0.05	NM	NM

NM = Not Measured; ND = Not Detected

Residue depletion data from a study in which 10-week old broiler chickens received diets containing 0.4% calcium and levels of chlortetracycline ranging from 880 to 2200 mg/kg for a 5-day treatment period is presented in Table 14 (Drain, 1962b). Chlortetracycline levels found were related but not directly proportional to the quantity of the drug consumed. Residues in the liver were all at or below 0.1 mg/kg after 1 day withdrawal of medicated feed. Kidney tissues were all below 1 mg/kg chlortetracycline after one-day withdrawal, but measurable residues (0.04 to 0.40 mg/kg) were still found in kidney tissue 10 days after withdrawal.

Table 14. Residues in Liver and Kidney Tissues From 10-week old Broiler Chickens which Received High Levels of Chlortetracycline in the Diet for 5 Consecutive Days (Drain, 1962b)[1]

	Chlortetracycline; mg/kg Liver (L) and Kidney (K) Tissue							
Dose	880 mg/kg		1320 mg/kg		1760 mg/kg		2200 mg/kg	
WT, days	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	0.9	6.44	1.3	6.93	1.27	10.44	1.75	11.82
1	ND-0.02	0.16	0.04	0.25	0.07	0.46	0.07	0.49
3	ND-0.035	0.12	ND-0.04	0.31	0.04	0.24	ND-0.03	0.2
6	ND	0.08	ND-0.02	0.09	ND-0.025	0.17	ND	0.11
8	ND	0.07	ND-0.025	0.18	ND-0.03	0.11	ND-0.03	0.17
10	ND-0.02	ND-0.05	ND	0.1	ND-0.03	0.19	ND-0.03	0.15

[1]Diet contained 0.4% Calcium; ND = Not Detected

The results of two more recent studies conducted with 300 mg/kg chlortetracycline in feed for a 7-day treatment period are shown in Table 15 (Gingher, 1988b, Gingher, 1990f). Liver tissues contained 0.228 to 1.10 mg/kg of chlortetracycline at the zero-day withdrawal point, while kidney tissues contained 2.45 to 3.05 mg/kg chlortetracycline. Residues in liver were all below 0.4 mg/kg at one day withdrawal. Very small amounts of chlortetracycline were still apparent in some kidney samples after 20 days withdrawal.

Table 15. Residues of Chlortetracycline (mg/kg) in Liver and Kidney Tissues of Chickens Receiving 330 mg/kg Chlortetracycline in Feed for 7 Days

Reference	Gingher, 1988b	Gingher, 1990f	
Calcium, % in diet	1.28	0.79	
Average Body Weight	1.479	1.04	
Average Daily Intake, CTC, mg/kg	28.6	31	
Withdrawal Day	Liver	Liver	Kidney
0	0.328	0.99	2.72
1	ND-0.031	NM	NM
2	ND-0.026	NM	NM
3	ND	NM	NM
6	NM	ND-0.027	0.211
8	NM	NM	0.144
10	NM	NM	0.091
13	NM	NM	0.079
15	NM	NM	0.053
17	NM	NM	0.041
20	NM	NM	ND-0.038

ND = Not Detected (<0.025 mg/kg); NM= Not Measured

Chicken Eggs

Two recent studies have been conducted in which chlortetracycline was administered to White Leghorn laying hens at 300 mg/kg in feed (Gingher, 1989c) and at 120 mg/kg in the drinking water (Gingher, 1989d). In both studies the treatment periods were for seven consecutive days. Eggs were collected during and following the treatment period. In the feed study, average daily chlortetracycline intakes ranged from 27.7 to 47.4 mg chlortetracycline/bird and averaged 35.7 mg chlortetracycline/bird/day during the 7-day medication period. Residues ranging from 0.037 to 0.044 mg/kg of whole egg were found in 4 of 10 eggs assayed at the zero-day withdrawal. At one day withdrawal, residues ranging from 0.035 to 0.042 mg/kg of whole egg were found in 3 of 10 eggs assayed. No chlortetracycline residues were found in the eggs at subsequent withdrawal days. In the water medication study, average daily chlortetracycline intake ranged from 21.8 to 36.3 mg chlortetracycline/bird and averaged 26.9 mg chlortetracycline/bird/day over the 7-day treatment period. Residues ranging from 0.040 to 0.043 were found in 4 of 10 eggs assayed at zero day withdrawal. At one-day, withdrawal residues of 0.040 and 0.042 mg/kg chlortetracycline were found in 2 of 10 eggs assayed. No chlortetracycline residues were found in the eggs at subsequent withdrawal days. In these studies, the whole egg was assayed and the limit of detection of the method was 0.0375 mg/kg chlortetracycline of whole egg.

Katz et al. (1972) studied continuous feeding at levels of chlortetracycline of 55, 110, 165 and 220 mg/kg in the feed. Egg production and chlortetracycline residues in eggs were studied over a 4 month period. Their data showed that 11.1, 83.8, 94.8 and 100% of eggs had measurable residues of chlortetracycline from hens fed 55, 110, 165 and 220 mg/kg in the feed, respectively. The assay procedure used was more sensitive than that used by the Gingher (1989), measuring chlortetracycline concentrations as low as 0.021 mg/kg. Measurable residues at the 165 and 220 mg/kg feed levels were no longer detectable 3 days after removal of the medication.

Roudaut et al. (1989), treated laying hens with feed containing 600 mg/kg chlortetracycline for a seven day period. Residues were measured separately in the yolk and albumin fractions of the egg. A summary of the residue data obtained in this study is presented in Table 16. Measurable residues were found in the albumin

for 5 days and in the yolk for 9 days after withdrawal of the medication from the feed. The LOD of the method was 0.01 mg/kg for albumin and 0.06 mg/kg for yolk. About 70%, of the chlortetracycline excreted via the egg, was found in the yolk and about 30% in the albumin.

Table 16. Chlortetracycline Residues in Albumin and Yolk Fractions of the Egg and the Whole Eggs Following a 7 Day Treatment with 600 mg/kg in the Feed (Roudaut et al., 1989)

	Chlortetracycline, mg/kg (Mean ± SD)		
Days of Treatment	**Albumin**	**Yolk**	**Whole Egg**
7	0.1 ± 0.03	0.41 ± 0.11	0.19 ± 0.05
Withdrawal Day			
1	0.07 ± 0.02	0.48 ± 0.12	0.19 ± 0.05
2	0.03 ± 0.009	0.50 ± 0.15	0.17 ± 0.05
3	0.02 ± 0.004	0.37 ± 0.10	0.12 ± 0.03
4	0.02 ± 0.003	0.3 ± 0.08	0.1 ± 0.02
5	0.01 ± 0.003	0.23 ± 0.07	0.08 ± 0.02
6	<0.01	0.14 ± 0.03	0.05 ± 0.01
7	NM	0.11 ± 0.02	0.04 ± 0.008
8	NM	0.08 ± 0.02	0.03 ± 0.007
9	NM	0.06 ± 0.01	0.02 ± 0.004
10	NM	<0.06	

NM = Not Measured

Swine

Residue depletion data for edible tissues of pigs fed 440 mg/kg chlortetracycline in feed for 14 days is presented in Table 17. These data demonstrate that swine are similar to other species in that the highest and most persistent residues occur in kidney and liver tissue (Berger, 1983). Korsrud and MacNeil (1987), using an HPLC procedure for measuring chlortetracycline in tissues, have concluded that the kidney should be used as the target tissue for the detection of chlortetracycline residues in swine. Therefore, as for the other species, major emphasis in this summary have been placed on the depletion of residues from liver and kidney tissues.

Table 17. Chlortetracycline Residue Depletion in Tissues from Pigs Which Received 440 mg/kg Chlortetracycline in Feed for 14 Days (Berger, 1983).

	Chlortetracycline, mg/kg of Tissue			
Withdrawal Day	**Muscle**	**Liver**	**Kidney**	**Fat**
0	0.75	1.88	>3.78	0.2
1	0.28	0.65	1.69	0.06
2	0.23	0.68	1.5	0.06
3	0.14	0.53	0.8	0.04

Residue depletion data for pigs which received 66 to 198 mg/kg chlortetracycline in the drinking water for 5 to 28 consecutive days is summarized is Table 18 (Berger, 1968, 1989e; Drain, 1961a). When 66 mg/kg chlortetracycline was given for 28 consecutive days, residues in both liver and kidney were higher and more

persistent than when the same dose was given for only 5 days. As would be expected, the greatest residues were obtained for the pigs receiving the highest dose of chlortetracycline in the water. Over 90 percent of the chlortetracycline was removed from the liver and kidney during the first 24 hours after withdrawal of the medicated water. The rate of depletion was much lower in subsequent days.

Table 18. Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Receiving CTC 66 to 198 mg/kg in Drinking Water.

Reference	Berger, 1968		Berger, 1968		Berger, 1989e		Drain, 1961a	
Chlortetracycline, mg/kg in water	66		66		120		198	
Days on medication	28		5		7		5	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	2.04	2.05	1.13	2.05	0.93	2.05	2.72	2.05
1	NM	NM	NM	NM	NM	NM	0.12	0.43
2	NM	NM	NM	NM	NM	NM	0.05	0.31
4-5	0.07	0.16	NM	NM	NM	NM	0.12	0.26
7	0.05	0.14	ND-0.03	0.06	NM	NM	0.06	0.17
10	0.07	0.12	ND-0.04	ND-0.04	ND-0.036	0.43	NM	
14-15	ND-0.04	0.07	ND	ND-0.03	ND	0.042	ND-0.04	NM
20-21	0.03	0.09	ND	ND-0.025	ND-0.034	ND-0.036	ND-0.03	0.08
28-30	ND	ND-0.03	ND	ND	ND	ND-0.029	ND	0.06

NM = Not Measured; ND = Not Detected

Data from studies in which pigs received 110 or 330 mg/kg chlortetracycline in feed for periods ranging from 31 to 98 consecutive days are summarised in Table 19. Although, in most cases, the premixes furnishing the chlortetracycline also contained sulfamethazine and penicillin or nitrovin, only chlortetracycline residue data appears here. When 110 mg/kg chlortetracycline was fed for 31 days and for 98 days, the concentration of chlortetracycline in liver and kidney was over twice as high for the 31-day feeding compared to that for the 98-day feeding period, and depletion time was correspondingly longer. This is, in part, probably due to the size of the pigs at the time of drug withdrawal. The average weight at drug withdrawal time was 33.6 kg for the 31-day feeding period as compared to 83.8 kg for the 98-day feeding period. The younger, smaller pigs consumed more feed than the older pigs, therefore receiving a higher chlortetracycline dosage on a bw basis. When 330 mg/kg chlortetracycline was fed for a 98-day period, residue levels of chlortetracycline were about twice those for pigs fed 110 mg/kg chlortetracycline for the same time (Alford, 1967; Berger, 1966b; Sass and Messersmith, 1964; Stoner, 1962b).

In recent studies when 165 mg/kg chlortetracycline in feed was given for 7 consecutive days, average chlortetracycline concentration was 0.95 mg/kg in liver and 1.38 mg/kg in kidney at 0-day withdrawal (Table 20). By the 10-day withdrawal, all residues in both liver and kidney were below 0.05 mg/kg of tissue (Gingher, 1990e; Rooney, 1989d).

Table 19. Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Which Received 110 or 330 mg/kg Chlortetracycline in Feed

Reference	Stoner, 1962b		Sass and Messersmith, 1964		Berger, 1966b		Alford,1967	
Days on Medication	31		98		98		63	
Average Weight, kg	33.6		83.8		89.9		49.5	
Drug in feed, mg/kg	110		110		330		110	
	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)							
Withdrawal Day	(L)	(K)	(L)	(K)	(L)	(K)	(L)	(K)
0	0.85	1.01	0.35	0.39	0.71	0.83	0.74	1.02
3	0.09	0.15	NM	NM	NM	NM	NM	NM
5	0.08	0.15	ND-0.04	0.06	0.11	0.16	NM	NM
7	0.08	0.14	0.04	0.1	0.13	0.14	ND-0.08	0.12
10	NM	NM	ND-0.04	0.04	0.07	0.09	NM	NM

NM = Not Measured; ND = Not Detected

Table 20. Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Pigs Which Received 165 mg/kg Chlortetracycline in Feed for 7 Days

Reference	Gingher, 1990e		Rooney, 1989d	
Tissue	Liver	Kidney	Liver	Kidney
Withdrawal Day	Chlortetracycline, mg/kg of Tissue			
0	0.943	1.38	0.96	1.38
10	ND-0.033	0.041	NM	NM
12	ND-0.027	ND-0.033	NM	NM
15	ND	0.031	ND	ND-0.037
20	ND	ND-0.025	ND	ND-0.030
25	NM	NM	ND	ND

NM = Not Measured; ND = Not Detected (<0.025 mg/kg)

Additional studies have been conducted in which 300 and 400 mg/kg chlortetracycline in feed were given to pigs for 7 consecutive days (Berger, 1989a ; Gingher, 1990d). Chlortetracycline residues averaged 1.45 mg/kg in liver and 1.93 mg/kg in kidney at zero-day withdrawal of the 300 mg/kg of chlortetracycline. For the pigs receiving the 400 mg/kg chlortetracycline dose in feed, zero-day withdrawal residue levels averaged 1.32 and 2.69 mg/kg for liver and kidney, respectively. Residue levels drop to less than 10% of these values by three days after withdrawal of the medicated feed, but the depletion of the residues is more gradual thereafter, for these tissues (Table 21). Both the liver and the kidneys are directly involved in the removal of chlortetracycline from the body via the bile and the urine.

Table 21. Chlortetracycline Residues Depletion in Liver and Kidney Tissues of Pigs which Received 300 to 400 mg/kg Chlortetracycline in Feed for 7 Days

Reference	Berger, 1989a		Ginger, 1990d		Gingher, 1990d	
Chlotetracycline, mg/kg in Feed	300		300		400	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)					
	(L)	(K)	(L)	(K)	(L)	(K)
0	1.67	1.57	1.23	2.29	1.32	2.69
3	NM	NM	0.129	0.121	0.111	0.148
5	NM	NM	0.102	0.087	0.083	0.107
7	NM	NM	0.069	0.08	0.067	0.069
10	NM	NM	0.058	0.06	0.034	0.047
12	NM	NM	ND-0.067	0.041	ND-0.049	0.047
15	0.044	0.051	0.036	0.038	0.046	0.048
20	ND	ND-0.029	ND-0.034	ND-0.035	ND-0.037	0.035
25	ND	ND	NM	NM	NM	NM
30	ND	ND	NM	NM	NM	NM

NM = Not Measured; ND = Not Detected (<0.025 mg/kg)

Cattle

The depletion of chlortetracycline from edible tissues of calves following a 10-day treatment at a dose of 22 mg/kg bw daily is presented in Table 22 (DeLay, 1973). These were young calves, averaging 42 kg bw, receiving a milk replacer diet with medication supplied by soluble boluses once daily. Residues at zero-day withdrawal were highest in kidney, followed by liver, muscle and fat. After ten days withdrawal, residues of 0.06 to 0.15 mg/kg and 0.14 to 0.16 mg/kg remained in liver and kidney tissue, respectively. As has been shown in other species, the kidney and liver can be considered the target tissues.

A summary of recent chlortetracycline depletion studies from liver and kidney of young calves following therapeutic doses of the drug from various dosage formulations for 7 consecutive days is shown in Table 23. The calves in two of the studies received a diet of whole milk (Berger, 1989b; Goodale, 1988c), while in the other two studies the calves received a diet of reconstituted milk replacer (Rooney, 1988b; 1989b). The daily doses of chlortetracycline ranged from 13.3 to 30.2 mg/kg bw. Residues of chlortetracycline at zero-day withdrawal were not directly proportional to the administered dose. The comparative results at day zero withdrawal between bolus and powder formulations, where the average daily dose of 21.7 mg/kg via the bolus formulation exceeded the average of 13.3 mg/kg given in the soluble powder formulation, are particularly intriguing. The soluble powder gave liver and kidney residue values of 13.7 and 19.2 mg/kg, well in excess of those from the bolus formulation, 1.82 and 2.18 mg/kg in liver and kidney, respectively. No ready explanation could be advanced for such disparate results. Residues from liver and kidney samples did not exceed 0.05 mg/kg after the 25-day withdrawal or the 45-day withdrawal, respectively. However, detectable residues were still found in kidney samples at the last withdrawal point for each of the studies. Although not shown in Table 23, no detectable chlortetracycline residues were found in fat samples after the zero-day withdrawal, while residues were not detected in muscle samples by withdrawal day 25 to 35, depending on the study. An earlier study in which young calves were given chlortetracycline soluble boluses at the rate of 22 mg/kg body weight per day, residues averaged 4.57 mg/kg in kidney, 3.22 mg/kg in liver, 1.26 mg/kg in muscle, and 0.49 mg/kg in fat at zero-day withdrawal. At 10 days withdrawal, chlortetracycline residues in kidney samples ranged from 0.14 to 0.16 mg/kg and in liver samples from 0.06

to 0.10 mg/kg (DeLay, 1973).

Table 22. Depletion of Chlortetracycline Levels from Tissues of Calves Following Oral Treatment at 22 mg/kg bw Daily for 10 Days (DeLay, 1973)

Withdrawal Day		Chlortetracycline, mg/kg of tissue			
		Muscle	Liver	Kidney	Fat
0	Average	1.26	3.22	4.57	0.49
	Range	1.08-1.55	2.70-3.65	4.30-4.90	0.31-0.63
3	Average	0.47	1.39	1.26	0.15
	Range	0.38-0.59	1.11-1.80	1.00-1.55	0.10-0.20
7	Average	0.14	0.27	0.45	0.04
	Range	0.07-0.21	0.12-0.46	0.24-0.70	0.03-0.06
10	Average	0.03	0.09	0.15	Neg-0.03
	Range	0.02-0.04	0.06-0.10	0.14-0.16	Neg-0.04

Neg = Below the LOD of the assay

Residue depletion data from an earlier study are available for calves following a 5-day medication with chlortetracycline soluble powder given by oral drench (Berger, 1967d). These calves averaged 67 kg body weight and were receiving alfalfa hay and a calf fitting ration ad libitum. So, rumen development was probably well advanced. Average residues of 1.44 and 2.18 mg/kg chlortetracycline were seen in liver and kidney, respectively, at zero-day withdrawal. After 3 days withdrawal, liver samples contained no detectable chlortetracycline and kidney samples averaged 0.05 mg/kg.

Results of three studies in which cattle were dosed daily with chlortetracycline via the feed at average rates of 22, 11 and 4.4 mg/kg body weight are summarized in Table 24. In the first study, cattle averaging 208 kg bw were given 22 mg/kg bw of chlortetracycline for 14 consecutive days (Gingher, 1981). Residues were greater than 5.0 mg/kg in kidney and averaged 1.9 mg/kg in liver at zero-day withdrawal. No residues were detected in liver by day 10, while kidney decreased to 0.05 mg/kg at this time. Kidney samples continued to average 0-0.05 mg/kg at 14 and 21 days withdrawal. In the second study, two-year old cattle received 11 mg chlortetracycline/kg bw daily for 61 consecutive days (Berger, 1965). Zero-day residue concentrations in this study were roughly one-half of those seen in the first in which twice the daily dose of chlortetracycline was fed. By ten days withdrawal, residues were not detected in liver while kidney samples averaged 0.05 mg/kg chlortetracycline. In the third experiment, cattle were fed 4.4 mg chlortetracycline/kg bw daily, together with an equal amount of sulfamethazine, for 28 days (Munger, 1978). Chlortetracycline residues averaged 1.37 and 0.52 mg/kg in kidney and liver, respectively, at the zero withdrawal sampling. No detectable residues of chlortetracycline were found in any tissues at the 10-day or subsequent withdrawals (Table 24).

Table 23. **Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Calves Following Various Oral Dosing Forms for 7 Days.**

Reference	Berger, 1989b	Goodale, 1988c	Rooney, 1988b	Rooney, 1989b
Formulation	A-20	B	MA-200	SP
Average Calf Weight	38.4	46.1	43	41.3
Dose, mg/kg/d	30.2	21.7	16.3	13.3
Withdrawal Day	Chlortetracycline, mg/kg of Liver Tissue			
0	16.7	1.82	6.5	13.7
15	NM	NM	0.073	NM
20	0.125	NM	0.075	NM
25	0.069	0.038	NM	ND-0.043
30	NM	ND-0.029	NM	NM
35	NM	ND-0.024	0.034	ND-0.036
40	NM	ND-0.029	NM	NM
45	ND-0.034	NM	NM	ND-0.025
55	NM	NM	NM	ND
60	NM	NM	ND-0.023	ND
65	ND-0.028	NM	ND	NM
75	ND	NM	NM	NM
	Chlortetracycline, mg/kg of Kidney Tissue			
0	25.3	2.18	9.7	19.2
15	NM	NM	1.09	NM
20	0.232	NM	0.092	NM
25	0.101	0.058	ND	0.059
30	NM	ND-0.039	NM	NM
35	NM	ND-0.030	0.069	0.058
40	NM	0.036	NM	NM
45	0.035	NM	NM	0.043
55	NM	NM	NM	0.031
60	NM	NM	ND-0.028	NM
65	0.033	NM	ND-0.028	ND-0.032
75	0.028	NM	NM	NM

Formulation: A-20 = AUROFAC 20 with neomycin and electrolytes in milk; B = CTC soluble boluses; MA-200 = AUROFAC 200-MA in milk replacer; SP = CTC soluble powder in milk replacer; NM = Not Measured; ND = Not Detected

Table 24. Depletion of Chlortetracycline (CTC) Residue From Liver and Kidney Tissues From Cattle Following Treatment with CTC in Feed

Reference	Gingher, 1981		Berger, 1965		Munger, 1978	
Average Weight, kg	208		458		318	
Days on CTC	14		61		28	
Dose, mg CTC/kg/d	22		11		4.4	
Withdrawal Day	Chlortetracycline, mg/kg of Liver (L) and Kidney (K)					
	L	K	L	K	L	K
0	1.9	>5.0	0.99	2.53	0.52	1.37
3	NM	NM	0.18	0.56	NM	NM
5	0.05	0.17	NM	NM	NM	NM
10	ND	0.05	ND	0.05	ND	ND
14	0.05	0.05	NM	NM	ND	ND
21	ND	0.05	NM	NM	ND	ND

NM = Not Measured; ND = Not Detected (<0.025mg/kg)

Table 25. Depletion of Chlortetracycline (CTC) residues From Liver and Kidney Tissues from Cattle Following Treatment with AUREO S 700 in Feed

Reference	Berger, 1970	Colavita, 1967	Drain, 1966a	Drain, 1966b	Langner, 1976
Average Weight, kg	240	174	160	126	345
Days on CTC	94	29	33	30	28
mg CTC/kg/d	1.46	2.01	2.19	2.78	1.01
Withdrawal Day	Chlortetracycline, mg/kg of Liver				
0	ND-0.05	0.16	0.16	0.09	0.06
1	NM	NM	0.16	0.06	NM
2	NM	NM	0.05	NM	NM
4	NM	NM	ND	0.05	NM
7	ND	ND	ND	0.06	ND
10	ND	NM	NM	NM	ND
Withdrawal Day	Chlortetracycline, mg/kg of Kidney				
0	0.12	0.17	0.37	0.16	0.14
1	NM	NM	0.32	0.1	NM
2	NM	NM	0.1	NM	NM
4	NM	NM	0.04	0.08[1]	NM
7	ND	0.05	ND	0.10[1]	ND
10	ND	NM	NM	NM	ND

NM = Not Measured; ND = Not Detected (<0.025 mg/kg of tissue; [1]Contamination of withdrawal feed suspected, as control cattle tissues were initially negative, and then became positive at 4-7 day withdrawal points.

A series of experiments were conducted in which AUREO S 700(R) (a feed premix containing 77 g/kg of chlortetracycline and sulfamethazine) was fed to cattle for periods ranging from 28 to 94 days. Average daily dosages of chlortetracycline ranged from 1.01 to 2.78 mg/kg body weight in these studies (Table 25). Residue levels in liver and kidney were either less than the detection limit or were very low after seven days withdrawal of medicated feed (Berger, 1970; Colavita, 1967; Drain, 1966a,b; Langner, 1976).

Milk

Soluble bolus formulations of chlortetracycline are used for vaginal or intrauterine administration in cows for reproductive infections. A study was conducted in which four lactating Holstein cows received intrauterine administration of four chlortetracycline soluble boluses (2 grams chlortetracycline) as a single treatment 1 to 3 days postpartum. Average blood concentrations of chlortetracycline peaked at 0.149 mg/kg four hours after treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detected at 5 and 7 days post-treatment. Average levels of chlortetracycline in milk peaked at 0.146 mg/kg on day 1 post-treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detectable at 5, 6 and 7 days after treatment (Goodale, 1988a).

Residue data are available for two intramammary infusion products used for treatment of mastitis. The first study was conducted using an infusion product containing 426 mg of chlortetracycline per 6 mL syringe. One syringe was infused in each of the four quarters of the udder, and milk samples were assayed at 12 hour intervals until 120 hours post-medication. The 12-hour postmedication milk showed the highest activity, averaging 70 mg/kg chlortetracycline at that time. All milk samples were still positive at 96 hours post-treatment (average 0.07 mg/kg chlortetracycline). Four of the six cows still showed low activity (0.012 to 0.03 mg/kg) at the final sampling 120 hours post-treatment (Hewell, 1967). The second study was conducted with TARGOT(R) mastitis suspension containing 200 mg of chlortetracycline, 100 mg neomycin sulfate and 100 mg of dihydrostreptomycin sulfate (the latter two measured as base) per 6 mL syringe. One syringe was infused in each quarter of the udders of 10 clinically normal Dairy Friesians yielding approximately two gallons of milk daily. Individual cow milk samples were taken at 12-hour intervals for 144 hours after treatment. All milk samples contained less than 0.03 mg/L chlortetracycline at 120 hours after infusion and less than 0.125 mg/L dihydrostreptomycin sulfate-neomycin sulfate (combined assay) at 72 hours after treatment (Nelson, 1968).

Studies have shown that milk from cows receiving 0.22 mg chlortetracycline/kg bw daily by feed medication has no detectable chlortetracycline residues (Henderson, 1953; Shor et al, 1959). When the feeding level of chlortetracycline was increased to 1.1 or 2.2 mg/kg bw daily, small amounts (up to 0.23 μg/mL) were found in the milk. After 48 hours withdrawal of medicated rations, all milk samples were again negative. The LOD of the assay was 0.01 μg/mL.

Ducks

One study was reported in which ducks were fed diets containing 400 mg/kg chlortetracycline for 3 weeks. At zero-day withdrawal of the medicated feed, average chlortetracycline residues of 0.39, 0.64, 1.95 and 0.04 mg/kg of muscle, liver, kidney and fat with skin, respectively, were found. After 5 days withdrawal, drug was not detected in fat with skin, but average chlortetracycline residues of 0.03, 0.06 and 0.24 mg/kg were still present in muscle, liver and kidney, respectively (Ferguson, 1972). Results are shown in Table 26.

Table 26. Depletion of Chlortetracycline Residues in Ducks Receiving 400 ppm Chlortetracycline in Feed for 3 Weeks, (Ferguson, 1972)

Withdrawal Day	Chlortetracycline, mg/kg of Tissue			
	Muscle	Liver	Kidney	Fat/Skin
0	0.39	0.64	1.95	0.04
1	0.1	0.18	0.6	ND
2	0.08	0.14	0.49	ND-0.011
3	0.04	0.1	0.35	ND-0.012
5	0.03	0.06	0.24	ND

ND = Not Detected

Sheep

Lambs were fed a fattening ration containing 50 mg/kg chlortetracycline (Kohler and Abbey, 1971) or 50 mg/kg chlortetracycline plus 50 mg/kg sulfamethazine (Wang, 1972a). A summary of the residue depletion data are shown in Table 27. Liver had no detectable residues of chlortetracycline after 4 days and kidney had no detectable residues of chlortetracycline after 8 days withdrawal from the medicated diets.

Table 27. Depletion of Chlortetracycline (CTC) Residues in Liver and Kidney Tissues from Sheep After Having Received 50 ppm of CTC With and Without 50 ppm of Sulfamethazine (SMZ) in the Feed for 42 Days

Reference	Kohler Abbey 1971				Wang 1971a			
CTC, ppm in Feed	50				50			
SMZ, ppm in Feed	0				50			
Withdrawal Day	CTC mg/kg							
	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat
0	0.027	0.11	0.33	ND	0.04	0.21	0.39	ND
2	ND	ND	ND-0.06	ND	NM	NM	NM	NM
4	ND	ND	ND	ND	ND	ND	0.04	ND
6	NM	NM	NM	NM	ND	ND	0.05	ND
8	NM	NM	NM	NM	ND	ND	ND	ND

NM = Not Measured; ND = Not Detected (<0.027, <0.03, <0.028 and <0.025 mg/kg in muscle, liver, kidney and fat, respectively)

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Because of the wide spread use of chlortetracycline and related tetracyclines there is a large body of literature detailing analytical procedures to quantitate residues. Most published methods in food matrices measure either the total antimicrobial activity or the concentration of free chlortetracycline. The predominant methodology routinely used to determine tetracycline antibiotics is high-performance liquid chromatography (HPLC). The majority of HPLC methods which have been validated for chlortetracycline determination are equally valid for both tetracycline and oxytetracycline.

A comprehensive review of analytical methods for tetracyclines has recently appeared (Oka and Paterson, 1995). A brief summary of more widely used procedures appears here.

Pharmaceutical preparation of tetracycline antibiotics frequently contain small amounts of impurities such as 4-epitetracyclines, anhydrotetracyclines and 4-epianhydrotetracyclines. Although these impurities are microbiologically inactive, 4-epianhydrotetracycline has been identified as the cause of Fanconi-syndrom (Flimter, 1973; Fluop and Drapkin, 1965) and it is therefore preferable to precisely quantitate tetracycline impurities during analysis.

The validated limit of chlortetracycline quantification (LOQ) in residue work reported above has been established at 0.020 mg/kg (20 ppb) in a variety of tissues. No interferences are experienced from typical veterinary products used in animal production.

High Performance Liquid Chromatography (HPLC)

Most of the successful chemical methods for reliable tetracycline analysis reported during the last 25 years are complex. Such complexity is imposed by the ease with which tetracyclines form chelate complexes with metal ions and the propensity to bind strongly with both proteins in the analytical matrix and silanol groups of the separation columns used during analytical determination. These problems were minimised by the use of either ethylenediaminetetracetic acid and oxalic acid at each analytical step (Oka et al, 1985 and references therein). The majority of liquid chromatographic methods presently used for determination of residues of tetracycline antibiotics espouse the principles of the Oka method and details of variations on the method have been exhaustively reviewed (Oka and Paterson, 1995). A collaborative study on the determination of chlortetracycline, oxytetracycline and tetracycline in edible animal tissues has recently been conducted (MacNeil et al, 1995).

A number of HPLC procedures for the analysis of tetracycline antibiotics take advantage of their metal-chelating capacity as a method to clean up tissue extracts prior to HPLC determination. Thus, much of the data for the residue studies reported above were gathered using an HPLC procedure which incorporated a copper-treated column during the isolation of analyte from the tissue matrix (Guzman, 1990, 1991, 1993, Farrington et al, 1991). However, the major principles of the Oka methodology were retained. In this procedure, chlortetracycline residues were extracted from the porcine muscle, liver, kidney, abdominal fat and skin with adhering fat tissues with an aqueous succinate buffer solution at pH 4. The filtered tissue extract was then applied to a copper (II)-loaded chelating sepharose column. Chlortetracycline was eluted from the copper (II)-loaded column with EDTA/succinate buffer solution at pH 4 onto an Amberlite XAD-2 column. Chlortetracycline was eluted from this column with methanol and quantitated by high performance liquid chromatography (HPLC). Chromatographic separations are carried out on a LC-8 reverse phase column with detection by UV at 350 nm with quantification against an external chlortetracycline standard.

A similar HPLC analytical procedure has been developed and validated for other animal tissues including milk and eggs by laboratories in Germany. This analytical methodology is described in Bestimmung von Rückständen, prepared by the Department of Animal Health of Bavaria, Germany (R127). Based on to the similarity of this method to the methods developed by Cyanamid (M 2023 and M 2328), the use of the HPLC procedures in all animal tissues is supported.

A different approach to tetracycline analysis has been reported by Agasoster and Rasmussen (1992) using a combination of on-line dialysis and solid phase extraction for clean-up, followed by HPLC with post-column photochemical derivatisation which results in the quantitative convertion of the tetracycline into a highly fluorescent product.

Liquid Chromatography - Mass Spectrometry (LC-MS)

In order to obtain definite confirmation of the identity in tetracycline residue analysis the use of a LC-MS confirmation is advisable. The use of a volatile LC-eluent is manditory in such separations. The use of either polystyrene-divinyl benzene co-polymer LC packing materials or well end-capped alkyl bonded chromatographic packing manufactured from ultra-pure silica gel has been recommended (Oka et al, 1993). The volatile eluent was comprised of methanol-acetonitrile-0.001M trifluoracetic acid mixtures. The use of different ionisation techniques in LC-MS of tetracyclines has been recently reviewed but no single method suitable for routine confirmatory work appears superior to all others at present. Electrospray ionisation would be ideally suitable for tetrayclines and may well be the future ionisation technique of choice for LC-MS measurement for this and a number of other antibiotic classes.

Microbiological Determination of Chlortetracycline

Microbiological procedures have been developed for the determination of chlortetracycline residue in tissues of swine, cattle, poultry, as well as for milk and eggs (Mariano, 1989, 1991).

In general, antibiotic residues are detected by modified US FDA cylinder plate agar diffusion microbioassays. Data summarized in Table 28 have been generated using tissue samples from treated animals to compare the results obtained using the microbiological assay and the HPLC assay techniques. These data clearly support the comparability of the two techniques.

Although microbiological and chemical assays have been shown to be comparable for a particular tetracycline antibiotic, it is not possible quantitatively to utilise microbiological assays for tetracyclines because there is a significant microbiological activity difference between different antibiotics of this class. Chemical methods are required for positive identification and quantification. However, microbiological assays remain valuable for screening purposes.

Table 28. Comparison of Microbiological Assay and HPLC Analysis for Chlortetracycline (CTC) Residues in Kidneys from Pigs which Received 300 to 400 mg/kg in Feed for 7 Days (Gingher, 1990d)

CTC, mg/kg in feed		300	300	400	400
Assay Method		MB	HPLC	MB	HPLC
Withdrawal Day		Chlortetracycline, mg/kg of Kidney			
0	Average	2.29	1.925	2.69	2.255
	Range	1.45-3.35	1.029-3.023	1.64-3.15	1.362-2.773
3	Average	0.121	0.101	0.148	0.124
	Range	0.108-0.129	0.095-0.114	0.074-0.245	0.062-0.221
5	Average	0.087	0.068	0.107	0.082
	Range	0.072-0.124	0.051-0.097	0.077-0.153	0.055-0.111
7	Average	0.08	0.054	0.069	0.049
	Range	0.067-0.100	0.042-0.074	0.058-.087	0.039-0.060
10	Average	0.06	0.04	0.047	0.029
	Range	0.050-0.070	0.034-0.044	0.039-0.053	0.023-0.034
12	Average	0.041	0.024	0.047	0.031
	Range	0.029-0.070	<0.02-0.033	0.030-0.062	0.020-0.043
15	Average	0.038	0.023	0.048	0.03
	Range	0.032-0.050	<0.02-0.03	0.037-0.060	0.022-0.039
20	Average	ND-0.035	<0.02	0.035	0.023
	Range	ND-0.046	<0.02-0.029	0.031-0.040	<0.02-0.034

MB = Microbiological assay; HPLC = High Performance Liquid Chromatography; ND = Not Detected

APPRAISAL

Chlortetracycline was last evaluated by JEFCA at the 12th Meeting in 1968, together with oxytetracycline and tetracycline. At that time, maximum residue levels were recommended of 0.05 mg/kg in meat and eggs and 0.02 mg/kg in milk.

The predominant use of chlortetracycline is as a prophylactic added to animal feed. It is rapidly but only moderately well absorbed from the GI tract and is eliminated in both urine and faeces either unchanged or in a microbiologically inactive form. There is no evidence that chlortetracycline is significantly metabolised *in vivo* although some *in vitro* chemiccal isomerisation of the drug can occur, either in the animal and/or during isolation. Microbiological assay is therefore a satisfactory method to acquire depletion of chlortetracycline from tissues. Tissue depletion measurements conducted both by microbiological and chemical assay give very similar results. Differencies in microbiological potency of individual tetracyclines, however, requires a preference for chemical determination of residues for regulatory purposes. Since chlortetracycline undergoes minimal metabolism, it is the appropriate marker compound for determination of residues in tissues.

Serum level and residue studies indicate that tetracycline is both rapidly absorbed and quickly cleared from edible tissues following oral administration. Kidney and liver tissues in all species show the largest concentration

of chlortetracycline both at the withdrawal from the medication, and at any time point during the withdrawal period. These are also the last tissues to clear chlortetracycline residues. When liver and kidney tissues are in compliance with any designated MRL, residues in muscle will be less than 10% those found in kidney and fat should not cause any problem. Either liver or kidney (or both) could be considered as target tissue. It is recommended that kidney is the preferred target tissue.

In pigs receiving prophylactic chlortetracycline at the dose of 400 mg/kg in feed for 7 days mean residue levels were at 3 and 5 days 0.15 and 0.11 mg/kg in kidney and 0.11 and 0.08 mg/kg in liver after withdrawal of medication. Alternative administration of 198 mg/kg chlortetracycline in drinking water for 5 days gave residue levels of 0.31 and 0.05 mg/kg in kidney and liver, respectively, 2 days after withdrawal of drug.

Calves on feed containing 20 mg/kg for 7 days attain average chlortetracycline levels of 0.12 and 0.04 mg/kg in kidney and liver, respectively, 15 days after withdrawal of medication. Alternative dosing of calves with 22 mg/kg chlortetracycline, either as a soluble bolus or in drinking water, for 2-10 days gave residue levels of 0.45 and 0.27 mg/kg, respectively in kidney and liver, 7 days after drug withdrawal.

Cattle given 22 mg/kg of CTC in feed had kidney and liver residue levels of 0.20 and 0.10 mg/kg, respectively, 5 days after withdrawal of medication. Lactating cows given a single intrauterine dose of chlortetracycline gave residue levels in milk which were less than 0.05 mg/kg 3 days after dosing. Milk contained average residues of 0.07 mg/kg 4.5 days after cessation of treatment with mastitis formulations given at 426 mg/kg for 5 days. A dose of 3 g given to a lactating cow by intrauterine infusion leads to residues of less than 0.15 mg/kg 84 hours post dosing.

Broilers chickens receiving 528 mg/L of chlortetracycline in water together with feed containing 200 mg CTC/kg for 3 days had average residue levels in kidney and liver of 0.5 and 0.09 mg/kg, respectively, 2 days after withdrawal of drug. Residues levels averaging 0.3 and 0.05 mg/kg respectively were found in kidney and liver of chickens, given 200 ppm chlortetracycline continously in feed, 1 day after withdrawal from drug. Residues in eggs were below 0.05 mg/kg immediately after withdrawal of drug administered at the rate of 120 mg/L in drinking water for 7 days but doses of 600 mg/kg in feed over the same period gave residue levels of 0.19 mg/kg at day 1 of withdrawal. Turkeys given feed containing 600 mg/kg of chlortetracycline gave residue levels averaging 0.4 and 0.1 mg/kg in kidney and liver, respectively, 4 days after withdrawal. Four days after cessation of treatment, residue levels averaging 0.4 and 0.1 mg/kg, respectively, were found in kidney and liver of turkeys dosed with 528 mg/L chlortetracycline in drinking water for 3 days.

Two limited residue depletion studies were reported in lambs fed continuously with a fattening ration containing 50 mg/kg of chlortetracycline. At zero withdrawal time, the kidney, liver, muscle and fat contained 0.33, 0.11, 0.027 and <0.025 mg/kg of chlortetracycline residues, respectively. No residues were detected in these tissues at 4 days withdrawal.

Maximum Residue Limits

In reaching its decision on MRLs for chlortetracycline (and tetracycline) the Committee considered the following:

- MRLs were recommended for oxytetracycline at the 36th meeting of the Committee for all species of 600 μg/kg in kidney, 300 μg/kg in liver, 100 μg/kg in muscle, 100 μg/kg in milk, 200 μg/kg in eggs, and 10 μg/kg in fat. These levels were the lowest detectable by validated antimicrobial methods;

- chlortetracycline and tetracycline have been allocated a group ADI of 0-3 μg per kg of body weight with oxytetracycline;

- modern analytical techniques allow much more sensitive and specific assays than those provided by antimicrobial assays;

- the recommended target tissues for residue analysis in cattle, pigs and poultry are kidney and muscle. Based on limited data, the kidney is the recommended target tissue for sheep; and

- the marker residue for all three substances is parent drug.

The Committee recommended the following temporary MRLs for (both) chlortetracycline (and tetracycline) in cattle, pigs and poultry, expressed as parent drug:

Muscle - 100 μg/kg
Liver - 300 μg/kg
Kidney - 600 μg/kg
Eggs (poultry) - 200 μg/kg

The Committee also recommended temporary MRLs for sheep liver and kidney of 300 μg/kg and 600 μg/kg, respectively, expressed as parent drug.

The following information is required for evaluation in 1996:

- The results of residue depletion studies in cattle, sheep, pigs and poultry to determine the rate of depletion of residues in milk (cows), fat (all species) and in muscle, liver, kidney and fat (sheep), treated in accordance with approved uses of these substances; and

- new and validated methods of analysis for chlortetracycline, oxytetracycline and tetracycline residues in tissues and milk.

ADI and MRLs allocated to chlortetracycline, and tetracycline are the same as those previously allocated to oxytetracycline at the 36th meeting for the given tissues and species. Although the Committee realized that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to tetracyclines were defined as applying both to residues of individual tetracyclines and to the sum of combined tetracycline residues, including chlortetracycline, oxytetracycline and tetracycline.

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