CHLORTETRACYCLINE AND TETRACYLINE

First draft prepared by
Dr. R.J. Wells
Australian Government Analytical Laboratories
Pymble, Australia

ADDENDUM
to the chlortetracycline and tetracycline monographs prepared by the 45th meeting of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996

Introduction

The 36th Joint FAO/WHO Expert Committee on Food Additives meeting in 1990 established MRLs for oxytetracycline 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[1] for all species for which residue depletion data were provided (cattle, swine, sheep, chickens, turkeys and fish). These MRLs were approved through the CODEX Alimentarius Commission in 1994.

An ADI of 0-3 μg/kg of body weight was assigned to oxytetracycline. The MRLs assigned by the Committee were based on the lowest values which could be monitored with the microbiological methods of analysis available at that time. Consequently, the 36th JECFA panel concluded that "the estimated maximum daily intake of oxytetracycline is 150 μg in milk, 30 μg in muscle, 0.5 μg in fat, 20 μg in eggs, 30 μg in liver, and 30 μg in kidney taking into account the food intake data as stated on page 9 of the 36th report of the Committee, WHO Technical Report Series 799, Geneva 1990, yielding a total of approximately 260 μg. This value slightly exceeds the ADI of 200 μg per person (one-tenth of the no-observed-effect-level of 2 mg per day). Since the ADI was derived from a conservative value of 2 mg per person per day and the consumption data are at the upper limit of the range for individual intake of animal products, the Committee concluded that the recommended MRLs do not present a risk for the consumer.

The MRL of 100 μg/kg recommended for milk contributed 150 μg to the theoretical food basket (daily consumption 1.5 l) and was the major factor in assuring that the ADI was exceeded by 30%.

The 45th Joint FAO/WHO Expert Committee on Food Additives meeting in 1995 allocated the same ADIs and MRLs, except milk, to chlortetracycline and tetracycline as those previously allocated to oxytetracycline at the 36th meeting, 100 μg/kg for muscle (cattle, pigs, poultry), 300 μg/kg for liver (cattle, pigs, sheep, poultry), 600 μg/kg for kidney (cattle, pigs, sheep, poultry), and 200 μg/kg for eggs (poultry). The MRLs were temporary pending further information as indicated below. Although the Committee realised that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to the tetracyclines were defined as applying to both individual tetracyclines or the sum of the combined tetracycline residues. The ADI of 0-3 μg/kg of body weight previously assigned to oxytetracycline was converted to a group ADI with chlortetracycline and tetracycline at that meeting.

In arriving at its determination of MRLs, the 45th JECFA considered the recommendations of the 36th JECFA for oxytetracycline outlined above in combination with the decision to allocate a group ADI to CTC, OTC and TC. Target tissues for the analysis of all three tetracyclines were kidney and muscle in cattle, pigs and poultry and, based on limited data, kidney was the target tissue in sheep.

The following information was required for evaluation by the 47th JECFA in 1996:

1. The results of residue depletion studies in milk (cattle), in fat of cattle, pigs and poultry and in muscle, liver, kidney and fat of sheep in accordance with approved uses of these substances.

---

[1] Note, the FAO Food and Nutrition Paper 41/3, incorrectly reports the MRL for fat as 100 μg/kg.

2. New and validated methods of analysis of chlortetracycline, oxytetracycline and tetracycline.

TISSUE RESIDUE DEPLETION STUDIES

General

It was inferred during discussions at the 45th JECFA that possibly specific formulations were both registered and used on a regular enough basis perhaps to warrant demands for extra residue data.

The report of the 45th JECFA reflected these discussions by requiring results of residue depletion studies in milk (cattle), in fat of cattle, pigs and poultry and in muscle, liver, kidney and fat of sheep in accordance with approved uses of these substances. The problems raised by this requirement is that the various formulations, particularly those used in milk production, were not specifically identified. Indeed, it was not certain if many of the formulations mentioned by several Committee members during discussion of chlortetracycline and tetracycline were registered or even currently available.

The information supplied is almost entirely derived by a re-culling of the initial extensive dossier supplied by Cyanamid. Furthermore, it deals exclusively with chlortetracycline data with no mention whatever of tetracycline residue data. Most of the information given below was included in the FAO Food and Nutrition Paper 41/8, but is reiterated here for the reader's easy reference.

Sheep

Two studies detail work on the depletion of chlortetracycline residues in liver, kidney and muscle tissues and in fat from sheep following dosing with 50 mg/kg, of chlortetracycline with and without 50 mg/kg of sulfamethazine (SMZ) in the feed for 42 Days.

Table 1. Depletion of Chlortetracycline Residues in Liver, Kidney, Muscle and Fat from Sheep Receiving 50 ppm of CTC with and without 50 ppm of Sulfamethazine (SMZ) in the Feed for 42 Days

Reference	Kohler and Abbey, 1971				Wang, 1971a			
CTC ppm in feed	50				50			
SMZ ppm in feed	0				50			
Withdrawal day	CTC mg/kg of Tissue				CTC mg/kg of Tissue			
	Liver	Kidney	Muscle	Fat	Liver	Kidney	Muscle	Fat
0	0.11	0.33	0.03	ND	0.21	0.39	0.04	ND
2	ND	ND-0.06	ND	ND	NM	NM	NM	NM
4	ND	ND	ND	ND	ND	0.04	ND	ND
6	NM	NM	NM	NM	ND	0.05	ND	ND
8	NM	NM	NM	NM	ND	ND	ND	ND

ND = Not Detected, below the sensitivity of the assay; NM = Not Measured

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 postpaltum. 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 post-medication 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/kg chlortetracycline/mL at 120 hours after infusion and less than 0.125 mg/kg dihydrostreptomycin sulfate-neomycin sulfate (combined assay) at 72 hours after treatment (Nelson, 1968). A summary of the mean milk chlortetracycline levels are shown in Table 2.

Table 2. Mean Milk Chlortetracycline Levels from 10 Cows Dosed with TARGOT(R) Mastitis Suspension Containing 200 mg of Chlortetracycline, 100 mg Neomycin Sulfate and 100 mg of Dihydrostreptomycin Sulfate

Hours post infusion	Mean chlortetracycline level (mg/L)	Assay limit (mg/L)
0	<0.03	0.03
12	34.01	1.25
24	16.78	0.75
36	5.0	0.48
48	1.1	0.06
60	0.49	0.06
72	0.19	0.06
84	0.05	0.03
96	0.04	0.03
108	0.035	0.03
120	<0.03	0.03

Studies have shown that milk from cows receiving 0.22 mg chlortetracycline/kg b.w. 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 b.w. daily, small amounts (up to 0.23 mg/L) were found in the milk. After 48 hours withdrawal of medicated rations, all milk samples were again negative. The sensitivity of the assay was 0.01 mg/L.

Cattle

Drain (1966a) report results of feeding 2.78 mg of CTC/kg to cattle for 30 days. Chlortetracycline residue levels in kidney and liver reached 0.37 and 0.16 mg/kg respectively whereas fat levels never exceeded the assay reporting level of 0.025 mg/kg. Similar negative fat results were obtained by Langner (1976) by feeding 1.01 mg of CTC/kg to cattle for 28 days and Colavita (1967) by feeding 2.01 mg of CTC/kg to cattle for 29 days.

The depletion of chlortetracycline from edible tissues of calves following a 10-day treatment at a dose of 22 mg/kg b.w. daily is presented in Table 3. These were young calves, averaging 42 kg b.w., 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.

Table 3. Depletion of Chlortetracycline Residues 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
0	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
3	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
7	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
10	Range	0.02-0.04	0.06-0.10	0.14-0.16	Neg-0.04

Neg = Negative, below the sensitivity of the assay.

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 4.

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 formulation, where the average daily dose of 21.7 mg/kg exceeded the average of 13.3 mg/kg given in a 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. Although not shown in Table 4, no detectable chlortetracycline residues were found in fat samples after the zero-day withdrawal.

Table 4. 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
Calf weight, kg	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
Withdrawal day	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

Formulation: A-20 = AUROFAC 20 with neomycin and electrolytes in milk; B = CTC soluble boluses; MA200 = AUROFAC 200 MA in milk replacer; SP = CTC soluble powder in milk replacer; NM = Not Measured; ND = Not Detected

In summary, it should be noted that it is only at very high chlortetracycline dosing levels that fat residues are found and that these residue are 10-fold lower that the residues found in kidney and liver. No residues have been detected in fat at a withdrawal time where kidney tissue meets the assigned group MRL for tetracyclines.

Pigs

Data from studies in which pigs received 110 mg/kg chlortetracycline in feed for periods of 31 and 98 consecutive days are summarised in Table 5. When 330 mg/kg chlortetracycline was fed for a 98-day period (Berger, 1966b - see FNP 41/8, p. 51), residue levels of chlortetracycline were about twice those for pigs fed 110 mg/kg chlortetracycline for the same time (Sass and Messersmith, 1964; Stoner, 1962b). Drug levels in fat were more than 10 times lower than found in kidney and liver at early stages of withdrawal and chlortetracycline could not be detected after 5 days following withdrawal of treatment.

Table 5. **Chlortetracycline Residue Depletion in Liver, Kidney and Fat Tissues of Pigs Which Received 110 mg/kg chlortetracycline in Feed for 31 and 98 days**

Reference	Stoner, 1962b			Sass and Messersmith, 1964		
Days on Medication	31			98		
Weight of Pig, kg	33.6			83.8		
Drug in Feed, mg/kg	110			110		
Withdrawal day	Chlortetracycline, mg/kg					
	Liver	Kidney	Fat	Liver	Kidney	Fat
0	0.85	1.01	0.05	0.35	0.39	ND
3	0.09	0.15	0.01	NM	NM	ND
5	0.08	0.15	ND	ND-0.04	0.06	ND
7	0.08	0.14	ND	0.04	0.1	ND
10	NM	NM	NM	ND-0.04	0.04	ND

NM = Not Measured; ND = Not Detected, below the sensitivity of the assay.

Residue depletion data for edible tissues of pigs fed 440 mg/kg chlortetracycline in feed for 14 days is presented in Table 6. These data demonstrate that swine are similar to other species in that the highest and most persistent residues occur in kidney and liver tissue but are more than 10-fold lower in fat (Berger,1983).

Table 6. **Chlortetracycline Residue Depletion in Tissues from Pigs Which Received 440 mg/kg Chlortetracycline in Feed for 14 Days (Berger,1983)**

Withdrawal day	Chlortetracycline, mg/kg of Tissue			
	Muscle	Liver	Kidney	Fat
0	0.75	1.88	>3.78	0.2
1	0.28	0.65	1.69	0.06
3	0.23	0.68	1.5	0.06
4	0.14	0.53	0.8	0.04

Additional studies have been conducted in which 300 and 400 mg/kg chlortetracycline in feed were given to pigs for 7 consecutive days (Gingher, 1990d). As shown in Table 7, levels of chlortetracycline in fat were very much less than those in liver and kidney and were not detected 5 days after withdrawal.

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

Reference	Gingher, 1990d					
CTC, mg/kg Feed	300			400		
Withdrawal day	Chlortetracycline, mg/kg					
	Liver	Kidney	Fat	Liver	Kidney	Fat
0	1.23	2.29	0.08	1.32	2.69	0.10
3	0.109	0.121	ND	0.111	1.48	ND-0.02
5	0.102	0.087	ND	0.083	0.107	ND
7	0.069	0.08	ND	0.067	0.069	ND
10	0.058	0.06	ND	0.034	0.047	ND
12	ND-0.067	0.041	ND	ND-0.049	0.047	ND
15	0.036	0.038	ND	0.046	0.048	ND
20	ND-0.034	ND-0.035	ND	ND-0.037	0.035	ND
25	NM	NM	ND	NM	NM	ND
30	NM	NM	ND	NM	NM	ND

NM = Not Measured; ND = Not Detected, less than 0.025 mg/kg of tissue

Poultry

Chickens

Two separate studies (Drain, 1962a ; Gingher, 1980) in older chickens adminstered 220 mg/kg chlortetracycline in the feed showed residue levels of 0.66 and 0.71 mg/kg in liver and 0.42 and 0.75 mg/kg in kidney, respectively, at day 0 after withdrawal of medication compared to levels of 0.02 and 0.04 mg/kg in skin with adhering fat. At day 1 withdrawal no residues of chlortetracycline were detected in fat. Similarly, Gingher (1979) found no chlortetracycline residues in fat of chickens, fed medicated diets of 110 ppm chlortetracycline with added monensin for 51 days, 1 day after withdrawal of medication.

A more recent study conducted with 300 mg/kg chlortetracycline in feed for a 7-day treatment period (Gingher, 1988b) to chickens followed a similar trend. Liver tissues contained 0.328 mg/kg of chlortetracycline at the zero-day withdrawal point, while kidney tissues contained 2.45 mg/kg chlortetracycline. Residues in skin with adhering fat were 0.078 mg/kg at 0 day withdrawal and were below 0.025 mg/kg at one day withdrawal.

A summary of residue data from chickens treated via the drinking water at level of 120 mg/kg for a period of 7 days is shown in Table 8 (Gingher, 1989a). Liver is essentially free of chlortetracycline residues two days after withdrawal, while no measurable amounts of chlortetracycline persist in fat one day after withdrawal.

Table 8. Residues in Liver and Fat Tissues From Chickens Receiving Chlortetracycline in the Drinking Water

Reference	Gingher, 1989a	
CTC in Water, mg/kg	120	
Days on Medication	7	
	Chlortetracycline, mg/kg of Tissue	
Withdrawal day	Liver	Fat
0	0.276	0.1
1	ND-0.049	ND
2	ND-0.03	ND
3	ND	ND
4	ND	ND

ND = Not Detected, less than sensitivity of method

Turkeys

Turkey poults were fed medicated feed at a concentration of 0 and 400 g/ton chlortetracycline in a low calcium diet from one day old to 21 days of age. Tissues and blood was collected from 0 to 5 days after withdrawal of medication. The limit of detection of the microbiological assay was 0.05 mg/kg for liver and 0.025 for muscle, fat and kidney (Drain, 1961).

Average residue levels in fat were:

Withdrawal day	Average residue level (mg/kg)
0	0.47
1	0.17
2	0.09
3	0.085
4	0.075
5	0.057

Fifteen week-old turkeys were medicated with chlortetracycline as a soluble powder in the drinking water to provide 55 mg CTC/kg for 14 days. Tissues were measured at 0, 6, 12, 24 and 36 hours after withdrawal using a microbiological assay. Average fat levels at zero hour withdrawal were 0.047 mg/kg in males and 0.025 mg/kg in females. Levels fell below the limit of quantification after 6 hours.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Microbiological Methods

There has been no general improvement in sensitivity from any reported validated microbiological method since the review of oxytetracycline at the 36th JECFA where quantitation levels of 100 μg/kg were established. In establishing these levels of quantification, the 36th JECFA allowed a safety margin of twice the levels attainable, thus levels of 50 μg/kg were achieved in the validated antimicrobial assay submitted to the Committee (J. Boisseau, comment made at the 45th JECFA). Table 28 of the monograph on Chlortetracycline in the *FAO Food and Nutrition Paper 41/8* is reproduced here as Table 9 to show the comparative results from the analysis of porcine kidneys for chlortetracycline using both chemical (HPLC) and antimicrobial assay methods (MB).

It should be noted that the results for both methods are comparable and that the limit of quantification is 20 μg/kg. However, such limits of quantification by microbiological assay are not achievable for either oxytetracycline or tetracycline.

The different levels to which the tetracyclines are able to be detected and quantified in a microbiological assay raises another problem when bioassay is used as the sole regulatory method of residue analysis. In a routine laboratory assay in muscle, the Australian Government Analytical Laboratories attains quantification limits (LOQ) of 100 μg/kg for oxytetracycline and tetracycline in both muscle and milk whereas chlortetracycline is at least 3 times more sensitive in the same tests. Limits of Detection (LOD) are 3 times lower than LOQs and would allow detection of residues of all three antibiotics in the target tissues of kidney and muscle as well as in milk at levels of 50 μg/kg.

Microbiological assays, despite being a cost effective method to monitor antibiotic residues, are not able to yield positive identification of the residue(s) detected. The allocation of a group ADI to three tetracyclines required that the MRLs assigned were defined as applying to both individual tetracyclines or the sum of the combined tetracycline residues. Under these circumstances, residue methods are required which identify individual tetracyclines but no microbiological assay will meet this criterion.

Therefore it becomes mandatory to employ a chemical method for regulation of tetracyclines usage. However, a general microbial inhibition procedure will prove a useful and cost effective preliminary screen prior to identification and quantification by chemical analysis.

Chemical Methods

Modern chemical methods of tetracycline antibiotic analysis individually identify and quantify all three tetracyclines discussed here at levels at or below the MRLs allocated to the tetracyclines. Indeed, some methods published in the last few years reach levels of detection and quantification in milk which would readily allow a lowering of the MRL of OTC in milk to 50 μg/kg and establishment of MRLs for chlortetracycline and tetracycline at the same level. Methodology has been reviewed in the monograph on Chlortetracycline in the *FAO Food and Nutrition Paper 41/8.*

In general, residues of oxytetracycline and tetracycline are more readily recovered and quantified by HPLC than are residues of chlortetracycline. Recoveries of oxytetracycline and tetracycline in both muscle and milk are typically 10-20% higher than for chlortetracycline. However, CTC can be detected at concentrations 3 times lower than can either OTC and TC in the microbiological inhibition methods.

A collaborative study of the Farrington-Carson method for tetracycline analysis has been reported for milk (Carson et al, 1996). Eight laboratories analysed known control and fortified milk sample for 7 tetracyclines including chlortetracycline , oxytetracycline and tetracycline. At fortification levels of 15 μg/l, mean recoveries (%RSD) were 61.7% (25.4) for CTC, 75.2% (12.5) for OTC and 73.6% (12.6) for TC. At fortification levels of 30 μg/l, mean recoveries (%RSD) were 64.2% (7.4) for CTC, 77.5% (8.2) for OTC and 74.8%(8.7) for TC. Results from these studies meet the Center for Veterinary Medicine (CVM, US FDA) guidelines for

accuracy and precision for residue analysis at this concentration target level. The method is free from analytical interferences and is able to accommodate large sample numbers in routine use.

Table 9. Comparison of Microbiological Assay and HPLC Analysis for Chlortetracycline Residues in Kidneys from Pigs which Received 300 to 400 mg/kg in Feed for 7 Days (Gingher, 1990d)

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

More recent work includes a collaborative study (MacNeil et al, 1996) between 13 laboratories using the Oka method, first published in 1985. This study was conducted both on fortified (50 μg/kg) and incurred samples of porcine and bovine muscle and kidney. In general oxytetracycline and tetracycline could be more readily quantified at lower levels than could chlortetracycline. It was concluded that tetracycline residues could be successfully analysed in the animal tissues tested at levels of 100-600 μg/kg depending on the analyte-tissue combination. Although the equipment required is available in most standard analytical laboratories, the methodology is not exceptionally robust and is dependent on the availability of disposable cartridges from analytical suppliers which meet necessary performance criteria which are not always achieved. It also appears that more experienced laboratories attain detection limits and analyte recoveries well ahead of less adept laboratories. Some problems in this study arose from the manipulations required in the fortification of samples and results reported must represent a poorer outcome than would be attained by any one laboratory working on incurred samples.

JECFA Requirement for New and Validated Methods of Tetracycline Analysis

The 45th JECFA required new and validated methods of tetracycline analysis to be submitted for evaluation by the 47th JECFA in 1996.

It appears that, in setting this requirement, the 45th JECFA Committee was focussing on the need to readily monitor milk and milk products to lower levels than presently possible by microbiological methods. Certainly published methods allow quantification of tetracyclines in other tissues at levels consistent with the assigned MRLs. A more sensitive new microbiological assay or the introduction of an immunochemical method for milk might therefore be a sufficient requirement of the sponsor.

However, a review of published methods for tetracycline analysis suggests that allocated MRLs for tetracylines can be satisfactorily monitored by a combination of the microbiological (screening for antibiotic residues) and chemical (identification and quantification) analyses presently available.

Although it is not the function of JECFA to advise on the methodology to be pursued, the introduction of an immunochemical method might be appropriate in this case. Immunochemical methods such as ELISA could well be an easily applicable alternative to or could be used in conjunction with a microbiological method and might overcome the difference in microbiological response for different tetracyclines in antimicrobial assays. Such methods are presently commercially available and tend to be substance rather than class specific. One such immunochemical method which has had its performance claims validated by the Association of Official Analytical Chemists (AOAC International) is discussed below.

The results of this validation study meet the JECFA requirements for a sufficiently sensitive new validated method to be presented to the 47th JECFA in 1996 and are therefore presented in some detail.

Validation of a Commercial Test Kit for Tetracyclines in Milk

The AOAC Research Institute has performed validation studies of a commercially available test kit for chlortetracycline, oxytetracycline and tetracycline (Charm Sciences Inc. Test Kit for Tetracyclines in Milk: AOAC Research Institute Report, 1996). The assay is based on a competitive radioimmuno-assay between the target tetracycline and ^{3}H-tetracycline using antibodies bound to microbial receptors which are specific only for tetracyclines.

The test kits results were compared with those of chemical analysis conducted by an experienced independent laboratory for both selectivity and sensitivity. The kit gave no false positives for any of 60 negative control samples, easily meeting the criterion that a test kit should be at least 90% selective with 95% confidence. The kit also met the AOAC criterion of at least 90% sensitivity with a 95% confidence level at the claimed detection level for each of the tetracyclines tested as shown in Table 10.

Table 10. Charm II Tetracycline Drug Test Kit: 90% Sensitivity Level

Antimicrobial agent	90% Sensitivity level (μg/l)	US FDA Safe level (μg/l)
Chlortetracycline	28	30
Oxytetracycline	19	30
Tetracycline	5	80

Table 11 shows the results of a large series of analyses of milk for tetracyclines conducted with the test kit and performed by the independent testing laboratory. The results show that the ability of the test kit to detect residues of all three tetracyclines in milk exceeds that of the chemical analytical technique used by the independent testing laboratory to confirm these positive findings. It is noteworthy that in these studies both the test kit and the chemical method used to confirm test kit results have the ability to detect residues of chlortetracycline, oxytetracycline, and tetracycline at levels at or below 30 μg/L.

Table 11. **Comparison of Results from a Series of Analyses of Milk for Tetracycline Residues Conducted by Both the Charm II Tetracycline Drug Test Kit and an Independent Laboratory**

Substance	Concentration (μg/l)	Commercial Kit[a]		Independent Laboratory[b]	
		Positive (out of 30)	Positive (%)	Positive (out of 30)	Positive (%)
CTC	5	2	7		
	6	3	10		
	9			9	30
	12	15	50	12	40
	16			15	50
	18	28	93		
	23			29	97
	24	30	100		
	30	30	100	29	97
OTC	3	1	3		
	6	4	13	2	7
	7			0	0
	10			16	53
	12	24	80		
	15			28	93
	18	28	93		
	24	30	100		
	30	30	100	30	100
TC	2			1	3
	3			15	50
	4	28	93	26	87
	6	30	100	30	100
	8	30	100		
	16	30	100		
	80	30	100	30	100

[a] Data resubmitted by kit manufacturer and reviewed by FDA's Center for Veterinary Medicine and the AOAC Research Institute; [b] Data collected by the University of New Hampshire

The accepted practice of herd administration of both chlortetracycline and oxytetracycline led to an additional sensitivity criterion in the US-FDAs 'Protocol for Evaluation of Milk Residue Screening for Drugs other than β-Lactams and Sulfonamides'. This specification requires that approved test kits produce no more than 10% positive results at a level that could be incurred in a farm bulk from a herd in which medicated feeds had been widely used. These levels were experimentally determined at 3 and 5 μg/L for oxytetracycline and chlortetracycline residues respectively. The Charm II test kit met these specifications with 3% and 7% positives for oxytetracycline and chlortetracycline respectively at the levels determined above.

The AOAC concluded that, used in accordance with agreed testing procedures, the Charm II Tetracycline Test Kit would 'be expected to produce significantly less than 1% false violative results for milk with low levels of oxytetracycline and chlortetracycline residues (sic).

The Committee recognised that the validated analytical methodology available for tetracyclines is of sufficient sensitivity to accommodate JECFA-allocated MRLs in tissues for all three tetracyclines. Moreover, a new interlaboratory study in milk and the availability of a commercial tetracycline test kit which has undergone rigorous comparison with a validated chemical method allows the reliable monitoring of tetracyclines in milk at levels well below 50 μg/kg.

APPRAISAL

A detailed comparison of chlortetracycline levels in fat and kidney of cattle, pigs, sheep and poultry at various times after withdrawal of medication indicated that residues of chlortetracycline in fat were at least 9 times lower than levels in kidney and depleted far more rapidly. Cattle fed 22 mg per kg of body weight chlortetracycline for 10 days had mean fat levels of 40 μg/kg 7 days after dosing, whereas mean values in kidney, liver and muscle were 450, 270 and 140 μg/kg, respectively. Pigs fed 400 mg per kg of body weight chlortetracycline for 7 days contained mean fat levels of 100 μg/kg at zero day withdrawal and mean residue values in kidney and liver of 2690 and 1320 μg/kg respectively. Two separate studies in chickens administered 220 mg/kg chlortetracycline in the feed showed residue levels of 660 and 710 μg/kg in liver and 420 and 750 μg/kg in kidney, respectively, at day 0 after withdrawal of medication compared to levels of 20 and 40 μg/kg in skin with adhering fat. At day 1 withdrawal no residues of chlortetracycline were detected in fat. Another study found no chlortetracycline residues in fat of chickens, fed medicated diets of 110 ppm chlortetracycline with added monensin for 51 days, 1 day after withdrawal of medication.

Due to the rapid depletion of tetracyclines in fat, the Committee concluded that fat is not an appropriate target tissue for this class of drug and recommend that the assignment of an MRL for fat is not required.

Recent HPLC chemical methods of tetracycline antibiotic analysis individually identify and quantify all three tetracyclines at levels at or well below the MRLs allocated to the tetracyclines. Two validation trials have been published in 1996 which clearly attain levels of detection and quantification in all tissues which allow regulation of assigned MRLs. Furthermore, a published validation study in milk demonstrates that current methodology would readily permit a lowering of the MRL of tetracyclines in milk to 50 μg/kg. This validated quantitative method is also supported by both microbiological and immunochemical screening methods with the requisite detection levels and performance characteristics.

Notwithstanding the capability of analytical methods to identify and quantify residues at a lower MRL in milk, the Committee retained an MRL of 100 μg/l for oxytetracycline and recommended this same milk MRL for chlortetracycline and tetracycline. In maintaining this milk MRL, the Committee considered data showing that for oxytetracycline, milk levels fell below 100 μg/l only after 6-8 milkings following intramammary infusion or 10-14 milkings following administration of long acting formulations. Data for chlortetracycline also show that at least 6-10 milkings would be necessary following administration of intramammary infusion formulations to ensure that no violative milk levels were encountered if an MRL of 100 μg/l were adopted. A lowering of the milk MRL to 50 μg/l would result in unacceptable withdrawal times for milk.

The Committee also reaffirmed the opinion of the thirty-sixth Committee that no risk to human health would result from the ADI of 180 μg/day being exceeded by 30%, if these MRLs previously established for oxytetracycline were also recommended for chlortetracycline and tetracycline.

Maximum Residue Limits

The Committee recommended that the MRLs for oxytetracycline of 600 μg/kg in kidney, 300 μg/kg in liver and 100 μg/kg in muscle of cattle, pigs, sheep, and poultry and of 100 μg/l in milk of cattle and sheep, and 200 μg/kg in eggs of poultry, be extended to chlortetracycline and tetracycline.

The Committee recommended that the MRL of 10 μg/kg for oxytetracycline in fat be withdrawn and that MRLs in fat for chlortetracycline and tetracycline are not required.

Based on the food basket used by the Committee, the theoretical maximum daily intake of chlortetracycline, oxytetracycline and tetracycline, used alone or in combination, would be 260 μg/day.

REFERENCES

Agososter, R.M.L. and Rasmussen, K.E. (1992). *J. Pharm Biomed Anal*, **10**, 349-354.

Alford, B.T. (1967). American Cyanamid Company Report **FD 15:** 1143-1235. Experiment P-66-21-FT.

Alford, B.T. (1970). American Cyanamid Company Report **FD 18:** 1143-1163, Experiment BB-66-7-FT.

AOAC Research Institute Report, (1996). Charm Sciences Inc. Test Kit for Tetracyclines in Milk, *J Assoc Off. Anal. Chem. International*, 19, 36A-39A.

Berger, H. (1965). American Cyanamid Company Report **FD 13:** 1-29. Experiment BB-64-4-FT.

Berger, H. (1966b). American Cyanamid Company Report **FD 14:** 634-68O. Experiment P-64-14-FT.

Berger, H. (1967). American Cyanamid Company Report **FD 15:** (b) 466-492. Experiment P-66-14-FT, (c) 671-715. Experiment P-66-17-FT, (d) 319-369. Experiment BB-67-1-FT, (e) 1382-1447. Experiment A65-36-FT.

Berger, H. (1970). American Cyanamid Company Report **FD 18:** 2015-2126k. Experiment BB-66-10-FT.

Berger, H. (1971). American Cyanamid Company Report **FD 19:** 29-44. Experiment A-70-24-FT-I.

Berger, H. (1972). American Cyanamid Company Report **FD 20**: 87-140. Experiment A-67-21-FT.

Berger, H. (1974). American Cyanamid Company Report **FD 22:** 293-341. Experiment B-73-25FT.

Berger, H. (1982). American Cyanamid Company Report **FD 29:** (a) 1745-1781. Experiment A-8 1-1, (b) 1782-1814. Experiment A-81-2, (c) 1709-1744. Experiment L-1852.

Berger, H. (1983). American Cyanamid Company Report **FD 31**: 705-731 . Experiment P-82-16.

Berger, H. (1984). American Cyanamid Company Report **FD 32:** 1157-1255 . Experiment P-82-12.

Berger, H. (1989). American Cyanamid Company Report **FD 37**: (a) Report No. 7. Experiment P-88-7, (b) Report No. 5. Experiment B-88-16, (c) Report No. 6. Experiment P-88-7A, (d) Report No. 8. Experiment P-88-8A, (e) Report No. 9. Experiment P-88-8.

Berger, H. (1990). American Cyanamid Company Report **FD 38:** Report No. 68. Experiment P-89-27.

Berger, H. and T.R. Garces. (1981). American Cyanamid Company Report **FD 28**: 1347-1386. Experiment B-8O-8.

Berte, F. and Vandoni, G. (1962). *Chemotherapia*, **5**, 219-230.

Biroc, S.E. (1992b). American Cyanamid Company Report **FD 40:** Report No. 16.00. Experiment L-2437.

Bradley, B.D., Allen, E.H., Showalter, D.H. and Colaianne, J.J. (1982). *J Vet Pharmacol Therap*, **5**, 267-278.

Broquist, H.P., and Kohler, A.R. (1954). *Antibiotics Annual* 1953-1954, 409-415.

Buyske, D.A., Eisner H.J. and Kelly, R.G. (1960). *J Pharm Exper Therapy*, **130**, 150-156.

Carson, M.C., and Breslyn, W., (1996). Simultaneous determination of multiple tetracycline residues in milk by metal chelate affinity chromatography. *J Assoc Off Anal Chem International*, **19**, 29-42.

Craig, S.L. (1992). American Cyanamid Company Report **FD 40:** Report No. 12.00. Experiment L-2453.

Colavita, J. (1967). American Cyanamid Company Report **FD 15:** 246-303. Experiment BB-66-1-FT.

DeLay, R.L. (1973). American Cyanamid Company Report **FD 21:** 732-77O. Experiment B-72-44-FT.

Drain, J.J. (1961). American Cyanamid Company Report **C.F. 6:** 17-22D, Experiment A-61-9-LT.

Drain, J.J. (1962). American Cyanamid Company Report **FD 10:** (a) 400-508. Experiment A-62-5-FT, (b) 510-699, Experiment A-62-5-FT, Phase II.

Drain, J.J. (1966). American Cyanamid Company Report **FD 14:** (a) 272-309. Experiment BB-65-8-FT, (b) 310-349. Experiment BB-65- 12

Eisner, H.J. and Wulf, R.J. (1963). *J Pharmacol Exp Ther*, **142**, 122-131.

Eisner, H.J., Stern, K.E., Dornbush, A.C. and Oleson, J.J. (1953). *J Pharmacol Exp Ther*, 442-449

Elliott, R.F. and Whitehill, A.R. (1957). *Proc Soc Exp Biol Med*, **94**,119-122

Farrington, W.H.H., Tarbin, J.A., Bygrave, J.and Shearer, G. (1991). *Food Additives and Contaminants*, **8**, 55-64.

Ferguson, E.F.. (1972). American Cyanamid Company Report, **B 539:** Experiment A-724-54-FT.

Flinter, G.F. (1963). *Journal of the American Medical Association*, **184**, 111.

Fluop, M and Drapkin, A. (1965). *New England J Med*, **9**, 986.

Garces, T.R. (1979). American Cyanamid Company Report **FD 29:** 1-58, Experiment P-80-11.

Gingher, P.E. (1979). American Cyanamid Company Report **FD 27:** 600-670, Experiment A-78-9b

Gingher, P.E. (1980). American Cyanamid Company Report **FD 28:** 131-197, Experiment B-809-15

Gingher, P.E. (1981). American Cyanamid Company Report **FD 29:** 150-186, Experiment A-79-4b

Gingher, P.E. (1988). American Cyanamid Company Report **FD 36:** (a) Report No. 106. Experiment A-87-39, (b)American Cyanamid Company Report **FD 36:** (a) Report No. 123. Experiment A-88-7.

Gingher, P.E. (1989). American Cyanamid Company Report **FD 37:** (a) Report No. 3. Experiment A-88-8, (b) Report No. I0. Experiment A-88-8A, (c) Report No. 12. Experiment A-88-11, Report No. 13. Experiment A-88-12, (e) Report No. 4. Experiment A-88-7A,

Gingher, P.E. (l990). American Cyanamid Company Report **FD 38:** (a) Report No. 28. Experiment P-89-26, (b) Report No. 56. Experiment A-89-10, (c) Report No. 73. Experiment P-89-28A, (d) Report No. 75. Experiment P-90-4, (e) Report No. 80. Experiment P-89-28, (f) Report No. 83,. Experiment A-88-20.

Goodale, W.S. (1988). American Cyanamid Company Report **FD 36::** (a) Report No. 122 . Experiment B-88-12A, (b) Report No. 124,. Experiment B-88-14A, (c) Report **FD 36:** Report No. 125. Experiment B-88-14.

Guzman, B. (1990). American Cyanamid Company Method M 2023; "Chlortetracycline (CL 13,555).

Guzman, B (1990c). American Cyanamid Company Report C 3455; "Chlortetracycline (CL 13,555):

Guzman, B. (1993). American Cyanamid Company Method M 2328; "CL 13,555 (Chlortetracycline).

Guzman, B. (1991). American Cyanamid Company Report C 3474; "Chlortetracycline" (CL 13,555).

Henderson Jr, B.W., J.W. Cobble and H.L. Easterbrooks. (1953). *J Dairy Sci*, **40**, 617.

Hewell, D.B. (1967). American Cyanamid Company Report **B-428.** Experiment L-1232.

Kanegis, L. (1958). Antibiotic A-VIII. The comparative pharmacology of the tetracyclines, American Cyanamid Company Report **P.R. 4:** 884-921.

Katz, S.E., Fassbender, C. A. and Dowling, J. J. Jr. (1972). *J Assoc Off Anal Chem*, **55**, 128-133.

Kelly, R.G. (1964). American Cyanamid Company Report **P.R. 9:** 485-492. (Pearl River).

Kilroy, C.R., Hall, W.F., Bane, D.P., Bevill, R.F. and Koritz, G.D. (1990). *J vet Pharmacol Therap*, **13**,: 49-58.

Korsrud, G.O. and J. D. MacNeil. (1987). *Food Additives and Contaminants*, **5**, 149-153.

Langner, P.H. (1976). American Cyanamid Company Report **FD 24:** 41-129. Experiment B-75-17-FT.

Luthman, J. and Jacobsson, S-O. (1983). *Nord Vet-Med*, 35,: 292-299.

MacNeil, J.D., Martz, V.K., Korsrud, G.O., Salisbury, C.D.C., Oka, H., Epstein, R.L., and Barnes, C.J., (1996). Chlortetracycline, oxytetracycline and tetracycline in edible animal tissues, liquid chromatographic method: Collaborative study. *J Assoc Off Anal Chem International*, **19**, 405-417.

Mariano, M. (1991). American Cyanamid Company Method M 1870.02;

Mariano, M. (1989). American Cyanamid Company (a) Method M 1868.1; (b) Method M 1869.1.

Meredith, W.E., H.H. Weiser and A.R. Winter. (1965). *Applied Microbiology*, **13:** 86-88.

Miller, B.L. and Wyatt, R.D. (1985). *Poultry Sci*, **64**, 1637-1643.

Munger, L.L. (1978). American Cyanamid Company Report **FD 26:** I066-1108. Experiment B-78-1.

Nelson, J. (1968). Cyanamid International Report.III: Report No. 32.

Neuschl, J. (1991). *Arch Exp Veterinarmed*, **45**, 105-112.

Oka, H., Matsumoto, H., Uno, K., Harada, K.-I., Kadowaki, S. and Suzuki, M. (1985). Journal of Chromatography. **325**, 265-

Oka, H. and Paterson, J. (1995). Chemical Analysis of Tetracycline Antibiotics. in 'Chemical Analysis of Antibiotics Used in Agriculture' Oka, H., Nakagawa, H., Harada, K.I., MacNeil, J. D. (eds), AOAC International, Arlington, VA.

Oka, M., Ikai, Y., Mayakawa, J., Harada, K.-I., Suzuki, M., Himei, R., Horie, M., Nakazawa, K.-I., Suzuki, M., Humei, R., Horie, M., Nakazawa, H. and MacNeil, J.D. (1993). *J Agric Food Chemistry*, submitted for publication.

Pollet, R.A., Glatz, C.E., Dyer, D.C. and Barnes, H.J. (1983). *Am J Vet Res*, **44**, 1718-1721.

Pollet, R.A., Glatz, C.E. and Dyer D.C. (1984). *Poultry Sci*, **63**, 1110-1114.

Rooney, K.A. (1988). American Cyanamid Company Report **FD 36:** (a) Report No. 120. Experiment B-88-l0A, (b)Report No. 121. Experiment B-88-10.

Rooney, K.A. (1989). American Cyanamid Company Report **FD 37**: (a) Report No. 2. Experiment B-88-l5A, (b) Report No. 11. Experiment B-88-I5, (c) Report No. 14. Experiment P-88-14A., (d) Report No. 19 Experiment P-88-14

Rooney, K.A. (l990). American Cyanamid Company Report **FD 38:** Report No. 22. Experiment P-897.

Roudaut, B., Moretain, J.P. and Boisseau J. (1989). *Food Additives and Contaminants*, **6:**, 71-78.

Sande, M.A., and Mandell, G.L. (1990). Antimicrobial Agents. Tetracyclines, Chloramphenicol, Erythromycine and Miscellaneous Antibacterial Agents. in Goodman and Gillmans's The Pharmacological Basis of Therapeutics, 8th Edition, Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor. P. *eds*.p 1117-1145.

Sass, B., and Messersmith, R. (1964). American Cyanamid Company Report **FD 12**: 790-815. Experiment P-64-14-FT.

Schumacher, W.E. (1968). American Cyanamid Company Report **FD 16:** 352-378. Experiment A-67-36-FT.

Shor, A.L. (1961b). American Cyanamid Company Report **C.F. 5**: 115-120. Experiment BD-61-1-LT.

Shor, A.L. (1963). American Cyanamid Company Report **CD II**: 281-289. Experiment A-63-4-FT.

Shor. A.L. and G.O. Gale. (1965). American Cyanamid Company Report **FD 13:** 387-405. Experiment A-64-4-FT.

Shor, A.L., Johnson, W.P. and Abbey A. (1959). *J Dairy Sci*, **42**, 1203-1208.

Stoner, J. (1962b). American Cyanamid Company Report **CD I**: 137-150. Experiment P-61-1-LT.

Stoner, J.C. (1963). American Cyanamid Company Report **CD II**: 1-64. Experiment A-62-14-LT

Sutter, H.M. and Wanner, M. (1990). *Tierarztl Prax*, **18**, 27-32.

Tindall, E.E. and Berger, H. (1966a). American Cyanamid Company Report **FD 14**: (a) 976-1001. Experiment A-66-4-FT, (b) 1170-1213. Experiment A-65-30-FT, (c) 1214-1261. Experiment A-65-38-FT, (d) 1323-1381. Experiment A-65-37-FT.

Wahlstrom, R.C., Balios, J.P., Baker, R. and Libal, G.W. (1982). *Nutr Rep Int*, **26**, 873-878.

Wang, G.T. (1971). American Cyanamid Company Report **FD 19:** (a): 1309-1371.

Wulf, R.J. and Eisner, H.J. (1961). American Cyanamid Company Report **C.P. 1;** 626-683. (Pearl River).