THIAMPHENICOL

First draft prepared by
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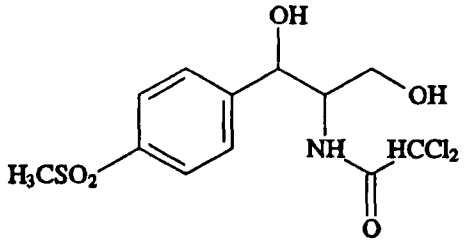
IDENTITY

Chemical names: D-d-threo-2-dichloroacetamido-1-(4-methylsulfonylphenyl)-1,3-propanediol; D(+)-threo-1-(4'-methylsulphonylphenyl)-2-dichloroacetamide propane-1,3-diol; D-threo-2,2-dichloro-N-β-hydroxy-a-(hydroxymethyl)-p-(methylsulphonyl)-phenethyl acetamide (C.A.S. name)

C.A.S number: 15318-45-3

Synonyms: Dextrosulphenidol, Thiophenicol, Win 5063-2, CV8053

Structural formula:



Molecular formula: $C_{12}H_{15}Cl_2NO_5S$

Molecular weight: 356.23

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White crystalline powder

Melting point: 164-166°C

Solubility: Water 0.5%, very soluble in dimethylacetamide (1:1), freely soluble in dimethylformamide and acetonitrile (1:10), soluble in methanol (1:20), slightly soluble in 95% ethanol (1:40) and in acetone (1:50), and barely soluble (1:1000) in ether, ethyl acetate and chloroform

pH (0.5% solution): 5.9. Changes in pH in the range 3 to 9 do not result in significant changes in solubility, but solubility is increased in strongly acid media

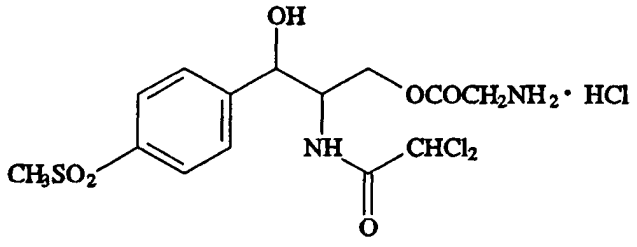
Stability: Dates of use and batch expiry dates given by contractors producing data for this assessment suggest a shelf life of 5 to 6 years, but no specific statements or recommendations are made by the sponsor, other than a statement that the product is stable if stored in closed containers, and protected from humidity and excessive heat.

Thiamphenicol glycinate hydrochloride

Thiamphenicol glycinate hydrochloride is the form in which the drug is used for parenteral administration.

C.A.S. number: 2611-61-2

Structural formula:



Molecular formula: $C_{14}H_{19}Cl_3N_2O_6S$

Molecular weight: 449.7

Thiamphenicol content: 79.2%

Solubility: Very soluble in water

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Thiamphenicol is an antimicrobial substance intended for the treatment of infectious diseases in cattle, pigs and poultry. It is used as the water soluble thiamphenicol glycine hydrochloride for parenteral therapy and as a premix composed of thiamphenicol base and corn starch, (4:1) or other mixer, for oral use.

Thiamphenicol has a similar antibacterial spectrum to chloramphenicol (Van Beers et al 1975, Sutter and Finegold, 1976). It has not been associated with aplastic anaemia in spite of extensive use in man (Yunis et al 1973).

Thiamphenicol inhibits protein synthesis in bacteria. It has a bacteriostatic action against a broad range of micro-organisms, although it may be bactericidal for some species under some conditions, and in concentrations 3 to 5 times higher than the bacteriostatic concentrations (Martindale 1971, 1973). Among the bacteria inhibited in vitro by relatively low concentrations of thiamphenicol are Clostridium, Corynebacterium diphtheriae, Diplococcus pneumoniae, Staphylococcus albus, Streptococcus pyogenes, Streptococcus viridans, Bacteroides, Fusobacterium, Bordatella, Brucella, Haemophilus, Neisseria, Pasteurella, Shigella and some vibrio strains. Some Bacilli, Erysipelothrix, Staphylococcus aureus and Streptococcus faecalis are sensitive to moderate concentrations of thiamphenicol but Listeria, Aerobacter, Escherichia, Klebsiella, Proteus and Salmonellae are sensitive only to relatively high concentrations. The compound is active against Mycoplasmas, Treponema, Rickettsias, Entamoeba and Actinomycetes, but inactive against Mycobacterium tuberculosis and Pseudomonas aeruginosa (Ravizzola et al 1984). The in vitro antimicrobial activity of the thiamphenicol glycinate ester is similar to that of thiamphenicol base.

MIC studies using standard dilution methods were carried out by the sponsor in 1989 and show MIC_{50}-values which are broadly similar to those described above, and by O'Grady et al (1980), but a few strains of

Bacteroides, Escherichia coli, Salmonellae, Staphylococci, and Pasteurellae show high MICs in vitro.

As a summary thiamphenicol is a broad-spectrum antibiotic, active against both Gram-positive and Gram-negative bacteria and especially effective against anaerobes. Thiamphenicol may be used in the treatment and control of a wide range of respiratory and alimentary tract infections of bacterial origin in calves, pigs and poultry. The oral product is not suitable for the treatment of cattle with functional rumen.

Dosage

There appears to be no firm recommendation of dosages in the dossier. Both 30 and 60 mg/kg have been used for calves, 20 - 40 mg/kg for pigs, 15 to 67 mg/kg for poultry, and 30 mg/kg in dairy cows. The oral preparations are not for use in ruminating animals. Administration for sucking calves includes 30 mg/kg of body weight daily of active ingredient, rate of addition to feed being 1000-1500 g/100 kg of milk powder, for pigs 20-30 mg/kg of body weight daily of active ingredient, rate of addition to feed 300-450 g/100 kg and for poultry, rate of addition to feed 400 g/100 kg and to water 200 g/100 l.

METABOLISM

Pharmacokinetics

General

Limited data in the sponsor's dossier show that after an intramuscular dose of 30 mg/kg, thiamphenicol occurs in cattle plasma and cows'milk within 1 to 3 hours of dosing. Similarly after intramuscular dosing the turkey and horse with 100 mg/kg, and the cow with 30 mg/kg, and orally dosing the rat, rabbit and turkey with 100 mg/kg, and the calf with 50 mg/kg, appreciable drug levels occur in plasma, as summarised in Table 1.

Rat

Intravenous administration of chloramphenicol and thiamphenicol to rats at 30 mg/kg showed that the half-life of chloramphenicol was 21.5 minutes whilst that of thiamphenicol was 46.3 minutes. When 80 mg/kg of phenobarbitone was given daily to rats for three days prior to an intravenous dose of thiamphenicol and chloramphenicol (Palmer et al 1972), the half-life of thiamphenicol was unchanged, whilst the half-life of chloramphenicol was reduced by about 50%. This demonstrates that following stimulation of the glucuronyl transferase activity of the liver, the metabolism of chloramphenicol was accelerated whilst that of thiamphenicol was little changed. Liver damage induced by surgery, slowed the metabolism of chloramphenicol but left that of thiamphenicol unchanged. Anuria immediately following anaesthesia in the rat, increased the half life of thiamphenicol, suggesting that the kidney is the main excretory route for the drug. The literature states (Walter et al 1975) that in man, renal insufficiency as measured by creatinine clearance prolonged the half-life of thiamphenicol, but hepatic insufficiency, particularly cirrhosis, did not increase it's half-life. Following oral and intramuscular administration of thiamphenicol to rats at 30 mg/kg and sequential sampling of urine and blood, the GC analysis before and after incubation of samples in the presence of beta-glucuronidase showed that thiamphenicol was excreted in the urine largely in unchanged form. Sampling was terminated after 48 hours, at which time 62% of the oral dose, and 50% of the intramuscular dose had been recovered. In similar experiments in which rats were orally dosed with ^{14}C-thiamphenicol at 30 mg/kg, 62% of the dose was recovered from urine and 35% in faeces within 48 hours after dosing. Two studies in which rats were given a single oral dose of 30 mg/kg of either thiamphenicol or ^{14}C-thiamphenicol gave similar post dose plasma concentrations as shown in Table 2. Radiolabelled thiamphenicol was determined by liquid scintillation counting and unlabelled thiamphenicol by a colorimetric method of McChesney et al, 1960.

Table 1. **Thiamphenicol levels (μg/ml) in plasma (Pl) of cattle, turkey, horse, rabbit, and rat and cow's milk (Mk) following a single dose**

Hours post dose	Cow 30 mg/kg IM		Calf 50 mg/kg Oral	Turkey 100 mg/kg IM		Horse 100 mg/kg IM	Rabbit 100 mg/kg Oral	Rat 100 mg/kg Oral
	Pl	Mk		IM	Oral			
1					40.3			32.1
2	22.2		17.1		37.0	4.5	6.8	30.6
3		5.7						
4	12.3		13.2	22.6		6.0	4.7	8.5
6	4.0	4.2	3.6		9.7	3.7	2.0	
8	1.7		1.9	8.3			0.8	
9		1.7						
10			0.4		7.7			3.7
11				5.1			0.5	
12	nil	0.6					0.3	
14					3.0			
15		0.1						
16			nil					
18					0.8			
24		nil		nil			nil	nil

Table 2. **Plasma concentrations in rat (μg/ml) of thiamphenicol following a single oral dose of 30 mg/kg of radiolabelled and unlabelled drug**

Hours post dose	^{14}C-thiamphenicol	Unlabelled drug
2	6.0	5.1 ± 0.8
4	2.3	2.8 ± 0.4
8	1.5	1.6 ± 0.3
24	0.7	0.8 ± 0.2
48	0.7	

Cannulation of the rat bile duct before thiamphenicol dosing, showed that 4% of the dose administered was excreted as unchanged drug in the bile within 4 hours of dosing, and after hydrolysis with beta-glucuronidase

around 12% was shown to be excreted in conjugated form. In other species, <5% of the dose was exreted as glucuronate, and other metabolites accounted for 1-2% of the dose. None of the metabolites were antimicrobial.

Oral dosing of rats at 30 mg/kg with ^{14}C-thiamphenicol indicated that 35% of the administered dose was recovered in faeces and 62% in urine by 48 hours after dosing. Liver, kidney and lung were the organs showing high initial and persisting drug levels.

Whole body autoradiographic studies were carried out in orally dosed rats using ^{14}C-thiamphenicol at a dose of 30 mg/kg. At 2, 4, 8, 24, 48, and 72 hours after dosing, the rats were killed, embedded in carboxymethyl cellulose, frozen, then cut with a microtome to produce 20 μ thick sections. The sections were applied to X-ray films for 20 days. The dossier states that 48 hours after dosing, the highest levels of radioactivity were present in the liver and kidney, with appreciable levels also being present in the thyroid, pancreas, lung, spleen and thymus. Gas chromatography-mass spectrometry analyses demonstrate that thiamphenicol is excreted mainly unchanged, in the urine, although some 1.5% was present as unidentified metabolites. In vitro experiments using ^{14}C-glucuronyl transferase, demonstrate that glucuronidation is not a major part of the metabolism of thiamphenicol, except in the pig, and by inference thiamphenicol does not interfere with the action of chloramphenicol. In vitro studies showed that serum binding of thiamphenicol to human and rat serum is less than 25% (compared with approaching 60% for chloramphenicol.)

Table 3. Plasma levels of thiamphenicol (μg/ml) in calves given 25 mg/kg orally, twice daily for four days

Animal	Time from last dose				
Number	6 hours	10 hours	24 hours	28 hours	34 hours
V1	8.63	6.3	1.68	0.60	0.15
V2	9.87	8.9	4.37	2.43	1.10
V4	10.8	7.9	2.04	1.18	0.51
V5	6.2	5.8	1.30	0.51	0.15
V6	8.6	8.1	1.90	1.0	0.35
V7	6.2	6.1	3.28	1.65	0.86
V8	5.6	5.4	2.40	1.0	0.55
V9	5.3	4.3	2.40	1.10	0.60
V10	6.8	5.9	2.40	1.33	0.90
V11	8.1	5.1	1.6	0.51	1.45
V12	4.3	3.3	2.5	1.06	0.23
V13	7.4	5.0	1.0	0.35	0.10
V14	3.4	2.0	0.6	0.30	0.40
V15	6.2	5.3	3.13	1.26	0.10
V16	8.96	8.5	3.65	1.37	0.70
Mean	7.1	5.86	2.25	1.04	0.54
±SD	2.1	1.9	1.05	0.55	0.40

Cattle

Sixteen calves (ages not specified) were orally dosed twice daily with thiamphenicol at 25 mg/kg for four consecutive days. Blood samples were collected for analysis at 6, 10, 24, 28 and 34 hours after the last dose. Calves were killed on the 4th, 6th, 8th, and 10th days after the last dose, and muscle, heart, liver, kidney, spleen, lung, and brain were sampled. Thiamphenicol was extracted with ethyl acetate and potassium carbonate, as described by Bories & Wal, 1983, and analysed by HPLC. Results showed that thiamphenicol concentrations above the LOQ were present in plasma at 34 hours post the last dose, Table 3. Liver, lung, and spleen showed appreciable concentrations for longer than other tissues, but all were below the LOQ eight days after cessation of dosing, Table 4. The extraction efficiency of the method is stated to be 60% and LOQ 20 μg/kg. The extraction efficiency of 67.6% in calf muscle has been determined by Nagata and Saeki (1992) using a similar method to that used by the sponsor.

Table 4. Tissue levels of thiamphenicol (μg/kg) in calves orally dosed for four days at 25 mg/kg bw per day

Days after last dose	Animal No.	Tissue						
		Lung	Liver	Kidney	Spleen	Muscle	Heart	Brain
4	2	45	65	50	100	0	130	0
4	10	61	77	115	61	0	70	0
4	15	53	65	65	90	0	0	0
6	5	70	<20	0	40	0	0	0
6	7	90	75	120	90	90	110	40
6	9	85	35	0	20	0	0	0
6	11	60	20	0	35	0	0	0
8	1	0	<20	<20	0	0	0	0
8	8	0	0	0	0	0	0	0
8	12	0	0	0	0	0	0	0
8	16	0	0	0	0	0	0	0
10	4	0	0	0	0	0	0	0
10	6	0	0	0	0	0	0	0
10	13	0	0	0	0	0	0	0
10	14	0	0	0	0	0	0	0

Eight lactating cows were given thiamphenicol by intramuscular injection twice daily for 5 consecutive days. The test substance was thiamphenicol glycinate hydrochloride, and the dose was calculated to be 15 mg/kg. Each dose was divided into two equal volumes and administered in different sites at each dosing point. Blood samples were obtained pre-dose and at 0.5, 1, 2, 4 and 6 hours after the first dose. GC with ECD was used to determine thiamphenicol concentrations. The drug appeared to be well tolerated by the animals. Plasma thiamphenicol levels reached about 18 μg/ml by 0.5 hours after the first dose, and were still quantifiable

(2.5 μg/ml) by six hours after the first dose.

Pigs

Three groups each of five pigs (average weight 30 kg) were orally dosed with thiamphenicol every 12 hours at doses of 10, 15 and 20 mg/kg for 5 days. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8, 60, 84, and 108 hours after the first dose and 24 and 48 hours after the last dose for quantification of thiamphenicol and it's glucuronide in plasma using GC with electron capture.

Table 5. Mean thiamphenicol levels (mg/kg) in pig plasma following feeding with a supplemented diet, equivalent to 30 mg/kg bw/day for five days

Study day	Time (h)	Thiamphenicol residues
1	0	ND
1	2	1.25
1	4	1.25
1	6	0.85
1	8	1.28
1	16	0.8
2	24	0.24
3	24	0.34
4	24	0.31
5	24	0.22
6	24	0.22
6	4	0.08
6	8	0.05
6	12	0.04
6	16	0.02
6	20	0.02
7	24	0.02
7	12	0.02
8	24	ND
8	12	ND
9	24	ND
9	12	0.02
10	24	ND

ND = Not detected

The maximum plasma concentration of thiamphenicol occurred 1-2 hours after dosing (1.29±0.79, 2.02±0.44 and 2.81±1.86 mg/l, respectively) and was dose related, but no such relationship was evident with thiamphenicol glucuronide. No statistical difference between the $t_{½}$ of unchanged thiamphenicol (1.2 hours) and that of it's glucuronide (1.2-1.6 hours) was found. Forty eight hours after the last 20 mg/kg treatment, the mean plasma concentration of unchanged drug was equal to the LOQ (20 μg/l) and twice the LOQ (40 μg/l) 48 hours after the last 40 mg/kg dose. The mean plasma thiamphenicol glucuronide concentrations at all time points after the last dose were higher than the concentrations of unchanged thiamphenicol. No accumulation of drug occurred in plasma during the course of the study.

A further study involved four groups each of 4 pigs with three control animals, weighing 15-22 kg. Animals were fed a cereal diet supplemented with thiamphenicol at 900 mg/kg, equivalent to 30 mg/kg/day, for five days. Blood samples to determine plasma drug concentration were collected at 0, 2, 6, 8, 16, and 24 hours from the start of the trial, at 24 hourly intervals on days 2, 3, 4, 5, and 6 of the trial and 12 hourly thereafter. Analysis was carried out by HPLC. Peak thiamphenicol levels in plasma (1280 μg/kg) were found 8 hours after the first dose, with mean concentrations of 220-800 μg/kg being found during the remainder of the dosing period. Levels declined to the LOQ (20 μg/kg) by 48 hours after the last dose. The extraction efficiency for plasma was 92.6 ± 10.2%, Table 5.

Sheep

Twelve sheep, 9-12 months of age and weighing from 30-35 kg were given 4 intramuscular doses of thiamphenicol glycine ester (20 mg/kg every 8 hours). Blood samples were taken at 10, 15 and 30 minutes, and 1, 2, 4, 6, and 8 hours after the first dose and from 15 minutes to 8 hours after the second and third injections. Two animals were killed at 2, 6, 12, 24, 48 and 72 hours after the last dose. Venous blood, bile, pericardial and peritoneal, synovial and cerebro-spinal fluids were also collected and analysed by HPLC (Abdennebi & Stowe, 1994).

Maximum drug concentrations of 20.6 mg/l in plasma occurred within the first 30 minutes after injection, and the half-life was calculated to be 1.51 ± 0.51 hours. In fluids, with the exception of cerebrospinal fluid, thiamphenicol levels were higher than in plasma, but concentrations in all fluids declined to below the LOD (10 μg/l) by 24 hours after the cessation of dosing.

Chickens

Thirty two groups each composed of six, mixed sex chickens, mean weight 1.8 kg, were dosed via their drinking water for three consecutive days, with thiamphenicol at 3 concentrations calculated to supply 15-28, 28-50, and 50-67 mg/kg bw per day. Manually filled water vessels were used to enable water intake to be measured. Blood samples were taken for the determination of plasma thiamphenicol levels at 2, 4, 6, 8, and 12 hours after the start of treatment, and also on the 2nd, 3rd, 4th, 5th, 6th, 8th and 10th day after the start of dosing. Analysis was made by using HPLC with a UV detector.

The groups of birds given the highest dose of drug showed some reduction in water intake. Plasma levels in birds on the highest dose were near to 2000 μg/l 4 hours from the start of treatment, and levels continued to rise to a mean level of 3746 ± 402 μg/l by 56 hours after the start of dosing. Birds on the lower doses showed plasma levels which rarely exceeded 1000 μg/l at any point in the trial. By 8 hours after the cessation of dosing, mean plasma levels in the birds which had received the highest dose were 385 ± 402 μg/l, but mean plasma levels in birds on the lower doses were below the LOQ. By 56 hours after the completion of dosing, the birds on the highest dose had plasma levels below the LOQ.

Radiolabelled thiamphenicol was used in further studies in 48 broiler chickens. A single oral dose of 25 mg/kg of ^{14}C-thiamphenicol (^{14}C-thiamphenicol 20%, PEG 200 55% and 2-pyrrolidone 25%) was administered by gavage into the crop, with quality control samples also taken to assess the radioactive dose administered. About 90% of the dose was excreted within 24 hours. A further 2-3% was excreted in the next 24 hours and decreasing amounts during the next 3 days. When killed 5 days after dosing, less than 1% remained in the carcase. Results indicate that during the first 120 hours after dosing, 92% of the administered dose was excreted

in faeces, a further 3% was recovered from the cage wash and feather wash, with a further 0.4% being recovered from the GI tract and carcase. Plasma levels rose rapidly reaching a mean peak of 6.6 μg equiv.ml^{-1} in females at 2 hours after the dose. Thereafter, levels decreased rapidly reaching a mean of 0.3-0.5 μg evuiv. ml^{-1} at 8 hours and approached the LOQ by 24 hours post dose. Total radioactivity under the curve indicated that the drug was extensively absorbed by the oral route.

A single dose of 5 mg/kg of ^{14}C-thiamphenicol was administered intravenously to another group of broiler chickens. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours post dose for measurement of radioactivity in plasma, and radioactivity in whole blood was determined in the samples taken at 2, 6 and 24 hours. Excreta and cage wash was collected daily for 5 days following dosing and at 5 days post dose the birds were killed. Feather washes, excreta, cage wash, gastro-intestinal tract and carcase were analysed for total radioactivity. Radioactivity levels decreased rapidly from a mean of 4.1 μg equiv.ml^{-1} at 0.25 hours post dose, to a mean of 1.4 μg equiv.ml^{-1} at one hour post dose and to 0.2 μg equiv.ml^{-1} at 4 hours post dose. By 24 hours post dose, levels in all birds were below the LOQ. Levels in blood and plasma were similar 2 hours after oral dosing, but at 6 and 24 hours after dosing, levels in whole blood contained larger amounts than plasma. At 24 hours post dose, plasma levels were at or below the LOQ. Whole blood contained a mean concentration of 0.5 and 0.2 μg equiv.ml^{-1} (oral and intravenous routes respectively). These findings indicate a much slower elimination of radiolabelled components from red blood cells than from plasma.

In another study using multiple oral dosing chickens were given ^{14}C-thiamphenicol twice daily by gavage at 25 mg/kg per day for 5.5 days (11 doses). Chickens were killed 6, 24, 72 and 120 hours after the last dose. Tissues were examined for total radioactivity. All samples were analysed using a liquid scintillation analysis, and in addition edible tissues and excreta were analysed by radiochromatographic profiling by HPLC. Thiamphenicol derived residues were measured at various time points after the last dose. At 6 hours post dose, highest concentrations of radioactivity were detected in bile with mean concentrations of 21 and 54 μg equiv.ml^{-1} being observed in male and female birds, respectively. Tissues containing the highest levels were the liver (mean of 7 and 9 μg equiv.ml^{-1}), kidney (4 and 6 μg equiv.ml^{-1}) and gizzard (5 and 7 μg equiv.ml^{-1}) in males and females, respectively. Other tissues contained concentrations similar to those in plasma, with the exception of whole blood and spleen, which contained higher levels. At subsequent time points, levels were similarly distributed but lower, and at 120 hours post dose, the highest concentrations were found in liver, kidney, spleen, and whole blood as summarised in Table 6. Liver and kidney from two birds were incubated with beta-glucuronidase for 16 hours at 37°.

Table 6. Mean chicken tissue residues (μg equiv·g^{-1}) at various time points following the cessation of twice a day oral dosing at 25 mg/kg bw per day for 5.5 days (11 doses)

Time (h)	Sex (m/f)	Liver	Kidney	Breast muscle	Fat	Plasma	Whole Blood	Spleen
6	m	7.21	4.32	0.98	0.58	0.73	3.53	1.79
6	f	8.50	5.54	1.43	0.92	1.25	4.51	2.38
24	m	3.03	1.75	0.41	0.30	0.12	2.62	1.28
24	f	4.08	1.66	0.42	0.13	0.10	2.79	1.20
72	m	1.54	0.82	0.24	0.17	0.01	2.41	0.79
72	f	2.08	1.01	0.20	0.11	0.03	3.36	1.01
120	m	1.26	0.74	0.14	0.15	0.02	1.91	0.70
120	f	1.06	0.90	0.17	0.10	0.03	2.92	0.92

Much of the radiolabelled compound was unchanged thiamphenicol, although there was an additional small (5%) peak which indicated a compound less polar than thiamphenicol. Liver had the highest residue, but extraction was poor, and profiling of the extracted residue showed that only a proportion was unchanged residue and the proportion decreased with time suggesting that the kinetics of the more polar residues was slower than that of the parent thiamphenicol. Kidney had the second highest residue, and higher proportions of unchanged thiamphenicol was found, with the amounts of bound residues lower than those in liver. Skeletal muscle showed low levels at six hours post dose (1.2-1.6 mg/kg) and most of this residue was unchanged thiamphenicol. The total residue levels in fat were similar to those in muscle, and the proportion of unchanged thiamphenicol was lower than in muscle. At three days post dose, total residues in fat were similar to those in muscle. Skin with fat showed similar levels to muscle at all time points. Enzyme deconjugation of liver and kidney did not affect the residue profiles.

This study showed that in chickens, thiamphenicol was well absorbed, and rapidly eliminated mainly as unchanged drug in the excreta through biliary and urinary mechanisms. It was metabolised to very polar materials which were poorly extractable from biological matrices and more slowly eliminated from tissues than the less polar fractions.

TISSUE RESIDUE DEPLETION STUDIES

Cattle

Sixteen calves of (ages not specified) were orally dosed twice daily with thiamphenicol at 25 mg/kg for four consecutive days. Calves were killed on the 4th, 6th, 8th, and 10th days after the last dose, and muscle, heart, liver, kidney, spleen, lung, and brain were sampled. Thiamphenicol was extracted with ethyl acetate and potassium carbonate, as described by Bories & Wal, 1983, and analysed by HPLC. Liver, lung, and spleen showed appreciable concentrations for longer than other tissues, but all were below the LOQ eight days after cessation of dosing, Table 7. The extraction efficiency of the method is stated to be 60% and LOQ 20 μg/kg. The extraction efficiency of 67.6% in calf muscle has been determined by Nagata and Saeki (1992) using a similar method to that used by the sponsor.

Pigs

In a further study in pigs, six groups each of two pigs were dosed orally twice daily with thiamphenicol at 40 mg/kg per day for five consecutive days. Two pigs were killed on the 5th day after dosing had ceased, and a further two per day on the 8th, 10th, 11th, 12th, and 15th days. Muscle, adipose tissue, liver, lung and kidney were analysed for unchanged thiamphenicol and for total thiamphenicol by HPLC after 2 hours incubation at 37°C with beta-glucuronidase. The stated LOQ and LOD for the method were 20 and 10 μg/kg, respectively.

Residues in muscle showed that levels on the 8th post dose day were higher than those on the 5th post dose day, and levels below the LOQ (20 μg/kg) were found on subsequent days. A similar situation exists with regard to levels in adipose tissue. Less variability is seen in residues in lung tissue. The results for liver show variability and increasing levels in one of the two pigs between post dose days 10 and 12, and levels above the LOQ in one pig on the 15th post dose day. Similarly, levels above the LOQ were present in the kidney of one pig at the 15th post dose day. The extraction efficiency for this trial was stated to be 64.6% for muscle, adipose tissue and lung, and 48.6% for liver and kidney. It is not stated whether the figures given are corrected for the extraction efficiency. The results are summarised in Table 8. The use of only two pigs per group, together with no post dose testing until day 5 post dose, and wide between test intervals has generated a small amount of data with wide variability. This trial is not supported by GLP documentation.

Table 7. **Tissue levels of thiamphenicol (μg/kg) in calves orally dosed for four days at 25 mg/kg bw per day (Table 7 is equal to Table 4)**

Days after last dose	Animal No.	Tissue						
		Lung	Liver	Kidney	Spleen	Muscle	Heart	Brain
4	2	45	65	50	100	0	130	0
4	10	61	77	115	61	0	70	0
4	15	53	65	65	90	0	0	0
6	5	70	<20	0	40	0	0	0
6	7	90	75	120	90	90	110	40
6	9	85	35	0	20	0	0	0
6	11	60	20	0	35	0	0	0
8	1	0	<20	<20	0	0	0	0
8	8	0	0	0	0	0	0	0
8	12	0	0	0	0	0	0	0
8	16	0	0	0	0	0	0	0
10	4	0	0	0	0	0	0	0
10	6	0	0	0	0	0	0	0
10	13	0	0	0	0	0	0	0
10	14	0	0	0	0	0	0	0

Table 8. **Thiamphenicol in pig tissues (μg/kg) after oral dosing with thiamphenicol at 40 mg/kg bw per day for 5 days**

Post dose day*	Muscle		Fat		Liver		Lung		Kidney	
5	27.5	36.5	35.8	28.6	76.5	119	154	142	439	843
8	65.8	152	40.7	<20	112	92.3	179	79.9	1122	806
10	25.1	<20	<20	<20	23.2	22.8	46.6	42.7	297	226
11	<20	<20	<20	<20	49.6	<20	<20	<20	33.2	50.6
12	<20	<20	<20	<20	60.8	NE	<20	<20	<20	42.8
15	<20	<20	<20	<20	<20	33.0	<20	<20	<20	25.7

*Two pigs per day; NE = not evaluated due to the presence of endogenous interferences; LOQ 20 μg/kg

A further study involved four groups each of 4 pigs with three control animals, weighing 15-22 kg. Animals were fed a cereal diet supplemented with thiamphenicol at 900 mg/kg, equivalent to 30 mg/kg/day, for five days. Pigs were slaughtered 4, 6, 8 and 10 days after the last dose of drug, and liver kidney, muscle, fat and lung tissue were collected at slaughter for thiamphenicol determination by HPLC. This study report notes that the supplied methodology did not prove suitable to achieve the necessary limit of detection in tissues, due to the presence of interfering co-extractives, and low recovery efficiency. Considerable work to develop a high performance liquid chromatographic or gas chromatographic method of analysis was not successful.

Chickens

Thirty two groups each composed of six, mixed sex chickens, mean weight 1.8 kg, were dosed via their drinking water for three consecutive days, with thiamphenicol at 3 concentrations calculated to supply 15-28, 28-50, and 50-67 mg/kg bw per day. Manually filled water vessels were used to enable water intake to be measured. At 8, 32, 56, 104, and 152 hours after the cessation of medication, blood, liver, kidney, lung, gizzard and muscle were collected from those birds on the highest dose for thiamphenicol determinations. Analysis was made by using HPLC with a UV detector. The groups of birds given the highest dose of drug showed some reduction in water intake. Tissue drug levels were highest in the kidney, but by 104 hours after dosing ceased, levels in all tissues were below the LOQ.

In another study using multiple oral dosing chickens were given ^{14}C-thiamphenicol twice daily by gavage at 25 mg/kg per day for 5.5 days (11 doses). Chickens were killed 6, 24, 72 and 120 hours after the last dose. Tissues were examined for total radioactivity using liquid scintillation analysis, and by radiochromatographic profiling by HPLC. Thiamphenicol derived residues were measured at various time points after the last dose. At 6 hours post dose, tissues containing the highest levels were the liver (mean of 7.86 μg equiv·ml^{-1}), and kidney (4.93 μg equiv·ml^{-1}). At subsequent time points, levels were similarly distributed but lower, and at 120 hours post dose, the highest concentrations were found in liver and kidney as summarised in Table 9. Liver and kidney from two birds were incubated with beta-glucuronidase for 16 hours at 37°.

Table 9. Mean chicken tissue residues (μg equiv·g^{-1}) at various time points following the cessation of multiple oral (twice daily by gavage) dosing at 25 mg/kg bw per day for 5.5 days (11 doses)

Time (h)	Liver	Kidney	Breast muscle	Fat
6	7.86	4.93	1.21	0.75
24	3.56	1.71	0.42	0.22
72	1.81	0.92	0.22	0.14
120	1.18	0.82	0.16	0.13

Eggs

Fifteen laying hens were used for the study. Birds were fed ad libitum, a thiamphenicol supplemented mix containing 400 mg/kg thiamphenicol for 5 consecutive days as the sole food source. Food consumption was 140 g/bird/day, which would have supplied 56 mg/day of thiamphenicol. Eggs were taken each day during the treatment period and after dosing had ceased. Thiamphenicol concentrations were determined by GC with electron capture detection using chloramphenicol as internal standard. Samples were treated with glucuronidaase in order to determine the total and glucuronated thiamphenicol.

Corrections were performed on values from eggs, based on the mean density of egg homogenate on the day before the start of dosing. During the 19 days of the trial, 275 eggs were produced by the 15 birds, reaching

an average of 14.4 eggs per day. On the first day after the cessation of dosing, the mean thiamphenicol concentration in eggs was 269 μg/kg. Seven days after the cessation of dosing, levels of thiamphenicol in the eggs from 7/15 birds were below 20 μg/kg (LOQ). The following day the drug was detected in one egg only and on the ninth day after dosing ceased, no eggs were positive for thiamphenicol residues.

Milk

Eight lactating cows were given thiamphenicol by intramuscular injection twice daily for 5 consecutive days. The test substance was thiamphenicol glycinate hydrochloride, and the dose was calculated to be 15 mg/kg. Each dose was divided into two equal volumes and administered in different sites at each dosing point. Milk samples were taken from the whole daily yield of each cow, each day during dosing and afterwards. GC with ECD was used to determine thiamphenicol concentrations. Mean levels in milk were 2400 ± 503 μg/l on day two of dosing. On the first day after cessation of treatment mean thiamphenicol concentrations were 764 ± 133 μg/l, on the next day levels in six of the eight cows were below the LOQ (20 μg/l), and on the following day levels in seven cows were below the LOQ. All samples were below the LOQ on the fourth day after dosing was completed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Colorimetric estimation of thiamphenicol following extraction with ethyl acetate, then alkaline hydrolysis, followed by oxidation and colorimetric determination at 570 nm is insensitive, as the LOQ in tissues is 5 μg/g (McChesney et al, 1960).

Gas chromatographic methods with electron capture detection have been found to have a LOQ of 0.2 μg/ml (Aoyma & Iguchi 1969). The specificity and sensitivity of gas chromatography for determining thiamphenicol in fluids was described by Gazzaniga et al (1973). They state that the LOQ for their method was 0.2-0.4 μg/ml. GC with electron capture has been found to have a LOQ for hen's eggs of 20 ng/ml, with a LOQ of 20 ng/ml for cow's milk.

Nagata & Saeki (1991) and Nagata & Saeki (1992), have used liquid chromatography to determine the thiamphenicol residues in chicken muscle, and found the LOD to be 50 μg/kg.

An HPLC method has been described by Nagata and Saeki (1992) in which the drugs were extracted from minced tissues with ethyl acetate, and the extract evaporated to dryness. The residue was dissolved in 3% NaCl and partitioned with n-hexane. The drug was then extracted with ethyl acetate and after evaporation of the solvent, the residue was cleaned up by a Florisil cartridge. HPLC analysis was carried out on a Chromatorex ODS column and thiamphenicol was quanitated by a UV detector at 225 nm. Extraction efficiency for the muscles of calves, pigs, chickens and fish was 74% or better, and LOD for muscle was 10 μg/kg.

Psomas and Iosifidoy (1993) used HPLC to recover thiamphenicol from spiked bovine muscle samples and found a recovery efficiency of 64 to 75% and an LOQ of 10 μg/kg, which is lower than other published values and lower than the limits specified in the documents supplied by the sponsor.

The methods used by the sponsor, are broadly similar to those described in the literature, and validation studies for the method used for thiamphenicol determinations in bovine milk, broiler tissues and hens eggs are presented.

APPRAISAL

Thiamphenicol differs from chloramphenicol in that it is not readily metabolized in cattle, poultry, sheep, or humans, but is predominantly excreted unchanged in the urine. In pigs, the drug is excreted both as parent drug and as thiamphenicol glucuronate.

A single oral administration of thiamphenicol to rats and rabbits at a dose of 100 mg/kg resulted in plasma

levels of 30.6 and 6.8 mg/l, respectively, within two hours of dosing. Plasma levels were below the limit of quantification (0.02 mg/l) 14 hours after dosing. A single dose of radiolabeled thiamphenicol given orally to rats at a dose of 30 mg per kg of body weight resulted in plasma concentrations of 6.0 mg/l two hours after dosing and by 48 hours after dosing 62% of the dose had been recovered in the urine and 35% from faeces.

Single doses of ^{14}C-thiamphenicol were given orally to broiler chicks at 25 mg/kg and intravenously at 5 mg/kg. Peak plasma levels after oral dosing were 6.6 mg/l two hours after dosing and 4.1 mg/l 15 minutes after intravenous administration. Plasma levels were at or below 0.02 mg/l 24 hours after dosing. Another trial in which triamphenicol was given in drinking-water for 3 days at dose rates of 15 to 67 mg/kg showed dose-related plasma levels peaking at 3.75 mg/l and being less than 0.02 mg/l 56 hours after dosing ceased. At 56 hours post-dosing, levels in liver, kidney and muscle were 0.07, 0.06 and 0.05 mg/kg, respectively, and below 0.02 mg/kg 104 hours after cessation of dosing.

In sixteen calves orally dosed with unlabelled thiamphenicol at a dose rate of 25 mg per kg of body weight per day for 4 days, HPLC analysis showed that mean plasma levels of parent drug 6, 24 and 34 hours after dosing were 7.1 ± 2.1, 2.25 ± 1.05 and 0.54 ± 0.4 mg/l, respectively.

Eight lactating cows were given intramuscularly unlabelled thiamphenicol at a dose rate of 15 mg per kg of body weight per day for five days. Mean drug levels in plasma reached 18 mg/l 30 minutes after the first dose and were 2.5 mg/l six hours after the first dose.

In another study, three groups, each of five pigs, were given unlabelled thiamphenicol orally for five days at dose rates of 20, 30 or 40 mg per kg of body weight per day. Peak plasma levels of parent drug were 1.29 ± 0.79, 2.02 ± 0.44 and 2.81 ± 1.86 mg/l for the 20, 30 and 40 mg/kg groups, respectively, reached within two hours of dosing. At all sampling times, thiamphenicol glucuronate levels were higher than those of unchanged drug. At 48 hours after the cessation of treatment the mean plasma thiamphenicol levels were 0.02 and 0.04 mg/l in the 20 and 40 mg/kg dose groups, respectively. No sampling was carried out after 48 hours post-dosing so an end-point for thiamphenicol plasma levels was not defined.

Twelve sheep were each given four intramuscular doses of thiamphenicol at 20 mg per kg of body weight at 8 hourly intervals. Peak plasma levels of 20.6 mg/l were reached within 30 minutes of dosing. Plasma drug levels decayed to less than 0.01 mg/l (the limit of detection) by 24 hours post-dosing.

Laying hens were fed a thiamphenicol-supplemented diet for five days which provided 56 mg of drug per hen per day. On the first day post-dosing, the mean thiamphenicol level in egg homogenate was 0.27 mg/kg. Seven days post-dosing the eggs from 7 of 15 hens had drug levels below 0.02 mg/kg (limit of quantification) and the remaining birds produced eggs containing 0.02-0.04 mg/kg and all eggs had residues below the limit of quantification on the 9th day post-dose.

Depletion studies following the gavage administration of ^{14}C-thiamphenicol to broilers two times a day for 5½ days at a dose rate of 25 mg per kg of body weight per day showed that tissue drug levels were higher in female than in male birds. In females at 6 hours post-dosing, bile contained the radioactive equivalent of 54 mg/l parent drug and levels in liver, kidney and breast muscle were 8.5, 5.5 and 1.4 mg/kg, respectively. At 120 hours after dosing, liver, kidney and breast muscle in females had levels of 1.06, 0.9 and 0.2 mg/kg, respectively.

In the study with 16 calves dosed orally at 25 mg per kg of body weight per day for four days, thiamphenicol concentrations in muscle were below the limit of quantification (0.02 mg/kg) 6 days post-dosing and liver and kidney levels were below the limit of quantification by the eighth day after dosing.

In the lactating cattle study (30 mg per kg of body weight dose intramuscularly for 5 days), thiamphenicol levels in milk were 2.4 mg/l on day 2 of dosing. One day post-dosing, thiamphenicol mean levels in milk were 0.76 mg/l. Milk from six of the eight cows were below 0.02 mg/l on the second day post-dosing and all milk was below the limit of quantification on the fourth day post-dosing.

Six groups, each of two pigs, were orally dosed with unlabelled thiamphenicol for five days at a dose rate of 40 mg per kg of body weight. There was considerable variation in levels of parent drug in tissue. The

concentration in fat was below 0.02 mg/kg (limit of quantification) on the tenth day post-dosing and in muscle the concentration was below the limit of quantification on the eleventh day. Liver and kidney levels were 0.03 mg/kg at 15 days post-dosing. No further samples were collected, so the end-point for kidney thiamphenicol levels could not be determined. The Committee concluded, however, that this study was deficient for assessment of residues in pigs.

Adequate analytical methods have been published usually using HPLC-UV or GLC with electron capture detection. Recoveries of over 90% have been reported, with limits of quantification and detection of 0.02 mg/kg and 0.01 mg/kg, respectively.

Maximum Residue Limits

The committee considered the following factors for recommending MRLs

- The temporary ADI 0-6 μg/kg of body weight based on a toxicological end point. This corresponds to 360 μg for a 60-kg human.

- The absence of data to determine the percentage of the marker residue to total residue in edible tissues of target species.

- The limits of quantification and detection of available analytical methods are 0.02 mg/kg and 0.01 mg/kg, respectively.

- The lack of depletion studies in target animals extending to periods beyond the withdrawal times at maximum recommended dosage.

On this bases the Committee recommended temporary MRLs of 40 μg/kg for poultry and cattle muscle, liver, kidney and fat, expressed as parent drug. These temporary MRLs are based on using twice the limit of quantification of the available analytical method.

MRLs were not recommended for eggs because of unacceptable high thiamphenicol residues. No MRLs were proposed for cattle milk or pigs, as no data were supplied on total residues in milk and insufficient residue data were supplied for pigs.

The Committee considered these temporary MRLs to be conservative values, resulting in a maximum theoretical daily intake of 20 μg per day, well below the quantity permitted by the ADI of 360 μg/day.

The following information is required for evaluation in 1999:

1. Detailed reports of the carcinogenicity study in rats on which the summary report was available at the present meeting and the range-finding study used to establish dose levels in that study.

2. Residue depletion studies with radiolabelled and unlabelled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs.

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