The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

THIAMPHENICOL

SUMMARY REPORT (2)

1. Thiamphenicol is a broad-spectrum antibiotic closely related to chloramphenicol. The chemical structure of thiamphenicol differs from that of chloramphenicol in having a sulpho-group instead of a nitro-group. It is active against both Gram-negative and Gram-positive bacteria, and is especially effective on anaerobes.

   Thiamphenicol is intended for the treatment and control of respiratory and intestinal infections in cattle and poultry; routes of administration are oral or intramuscular. However, the drug is intended for intramammary administration in both lactating and dry cows and for intrauterine administration in cows.

   Recommended dosages and durations of treatment are: up to 5000 mg/kg feed or 50 mg/kg bw to calves (oral administration) for up to 5 days; up to 50 mg/kg bw by intramuscular or intravenous injection to cattle, subdivided in two daily treatments for up to 7 days; up to 1000 mg/kg feed or 800 mg/l drinking water (up to 130 mg/kg bw) in poultry (oral administration) for up to 5 days; up to 70 mg/kg bw/day by intramuscular injection to turkeys for 3 days; up to 4000 mg (10 mg/kg bw) up to 3 times at 12-hour intervals as intramammary administration to both lactating and dry cows, and up to 6000 mg (15 mg/kg bw) as a single intrauterine administration to cows.

   Thiamphenicol is currently entered in Annex III of Council Regulation (EEC) No 2377/90 as follows :

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Thiamphenicol	Thiamphenicol	Bovine, poultry	40 µg/kg	Muscle, liver, kidney, fat	Provisional MRLs expire on 1 January 1998

2. Thiamphenicol is essentially a bacteriostatic antibiotic: it acts by binding to the 50-S subunits of the 70-S ribosomes, thus blocking peptidyl transferase and resulting in the inhibition of bacterial protein synthesis.

   No information is available on the possible secondary pharmacological effects of thiamphenicol on organ and system functions.

3. Following either oral or parenteral administration, thiamphenicol is well absorbed and rapidly and extensively distributed to the tissues in rats, guinea pigs and dogs.

   The compound is transferred through the placenta, the blood-brain barrier and into the milk.

   In a radiolabel study in the rat, 97% of a single oral 30 mg/kg bw dose was eliminated within 48 hours with approximately 65% of the dose being excreted in the urine. In the bile 3.4% of the administered dose appeared unchanged and 12% as conjugated products after 4 hours; more than 30% of the dose was excreted in the faeces within 75 hours. Thiamphenicol was excreted in urine and faeces almost entirely in the unchanged form.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel: (+44-171) 418 84 00 Fax: (+44-171) 418 84 16

In the rat the drug was widely distributed in almost all tissues, except for brain; the tissues containing the highest radiolabelled residues were the liver and kidney. The liver was the only tissue to present measurable radioactivity as long as 72 hours after administration. No significant accumulation occurred in body fat as compared to other tissues.

Unlike chloramphenicol, thiamphenicol may not be an optimal substrate for the hepatic microsomal enzyme glucuronyl transferase. In rabbits and rats, more than 95% of the administered dose is excreted unchanged. A higher level of glucuronidation occurs in guinea-pigs and pigs. About 30% of thiamphenicol undergoes glucuronidation when tested on pig hepatocytes *in vitro*.

Pharmacokinetic studies in humans show rapid absorption, distribution and elimination following oral treatment, with an elimination half-life of 2-3 hours. Diffusion into tissues is favoured by modest (less than or equal to 10%) binding to plasma proteins. Little conjugation with glucuronic acid occurs in the liver. About 55% of the administered dose is excreted in the urine as unchanged parent compound, with variable but generally low amounts of deacyl- and glucuronide conjugates; overall urinary excretion accounts for more than 50-70% of the administered dose.

The pharmacokinetic profile in plasma and urine was investigated in calves receiving a single intramuscular dose of 25 mg/kg bw of thiamphenicol. The peak plasma level occurred 1-1.5 hours after administration. The ranges of the plasma pharmacokinetic values were as follows: C_{max} 8.5-18.7 µg/ml; plasma elimination half-life 2-3 hours; volume of distribution 590-1115.4 ml/kg; clearance 346.1-375 ml/hour/kg. The total urinary recovery (0-96 hours) ranged from 22-59%.

In calves administered orally 50 mg/kg bw of thiamphenicol, subdivided into two daily administrations, for 4 days, the average plasma concentrations fell from 7.1 µg/ml 6 hours after the last treatment to 2.25 µg/ml after 24 hours down to 0.54 µg/ml after 34 hours.

The pharmacokinetic profile was investigated in dairy cows treated intramuscularly with a total daily thiamphenicol dose of 30 mg/kg bw, divided into two 15 mg/kg bw injections, for five days. The average plasma half-life of thiamphenicol was slightly more than 2 hours. The observed AUC_{0-6h} averaged 46 mg/h/ml and was 86% of the estimated total AUC (53.79 mg/h/ml). The apparent plasma clearance and the apparent volume of distribution were 290 ml/hour/kg and 860 ml/kg, respectively.

4. In a ^{14}C-radiolabelled study broiler chickens were administered an oral single thiamphenicol dose of 25 mg/kg bw. Plasma levels peaked at 1-2 hours post-dosing; the average values were 6.59 and 4.58 µg/ml in males and females respectively. The $AUC_{0-24\ h}$ was 0.97 µg/h/ml. About 90% of the dose was eliminated unmodified within 24 hours through both bile and urine; 5 days after treatment less than 1% of the dose was retained in the carcass and gastrointestinal tract.

   In a further trial 48 broiler chickens were treated orally with 50 mg/kg bw ^{14}C-thiamphenicol subdivided into two daily doses for 5« days. HPLC analysis showed that approximately 95% of the radioactivity was excreted as unmodified thiamphenicol within 48-72 hours. A two-phase depletion of tissue and bile concentrations was observed, with a rapid decrease (approximately 10-fold in bile and 2-3-fold in tissues) from 6 to 24 hours after the end of treatment, and a slower depletion rate up to 120 hours (last sampling time). Mean bile concentrations were 37.35, 3.59, 0.34 and 0.12 µg equivalent/ml after 6 hours, 1, 3 and 5 days following the last treatment, respectively.

5. Acute oral toxicity is low, the LD_{50} for thiamphenicol being higher than 5000 mg/kg in both rats and mice.

6. In a 4-week oral study on Large White Hybrid pigs, reduced weight gain and food consumption were observed at and above 50 mg/kg bw, while decreased urinary pH was detected even at the lowest dose level tested, 25 mg/kg bw.

   In a 90-day oral study on Sprague-Dawley rats, decreased erythrocyte and white blood cell counts were observed at and above 45 mg/kg bw; the changes partly reversed following an 8-week recovery period. The NOEL was 30 mg/kg bw.

   In a 13-week study, published in 1997, four groups of F344 rats (12/sex) were exposed through the drinking water to 0, 125, 250 and 500 mg/l of thiamphenicol, equal to 0, 9.0, 20.8 and 38.6 mg/kg

bw, respectively. Adverse effects were observed down to 20.8 mg/kg bw, including reduced weight gain in both sexes; decreased erythrocyte count and blood haemoglobin; increased relative liver and kidney weights and decreased relative thymus weight; reduced bone marrow erythropoiesis; testicular lesions (vacuolation of germinal epithelium, spermatogranulomas, reduced sperm in the epidydimis). No effects on organs or tissues were observed at 9.0 mg/kg bw, besides a dose-related enlargement of caecum, which is a usual side-effect in rodents following prolonged oral administration of antibiotics. However, slight but dose-related haematological (increased mean red cell volume, reduced platelet concentration) and biochemical (reduced serum total protein and cholesterol concentrations in females) changes were observed even at 9.0 mg/kg bw.

In a 6-month oral study on beagle dogs dosed with 0, 15, 30 or 60 mg/kg bw/day, tremors, lethargy and reduced weight gain were observed at the top dose level. Increased relative liver weight, decreased erythrocyte count and haematocrit values, reduced cellularity of bone marrow and focal testicular atrophy were observed down to 30 mg/kg bw. No effects, including bone marrow alterations, were observed at the end of a 2-month recovery period. The NOEL was 15 mg/kg bw.

7. No studies are available to evaluate tolerance in target species.

8. A battery of genotoxicity tests were performed, including *in vitro* bacterial mutagenicity assay, *in vitro* assay for gene mutation in mammalian cells, *in vitro* assay for chromosomal aberrations on human lymphocytes, *in vitro* assay for DNA repair on rat hepatocytes, and *in vivo* oral micronucleus test in the mouse.

All tests gave negative results; therefore thiamphenicol was considered non-genotoxic.

10. The summary report of a GLP carcinogenicity study in Fischer 344 rats has been made available to JECFA. No tumour increases were observed; however, the lack of detailed data does not allow for an independent assessment.

There is no evidence from the genotoxicity data that thiamphenicol may possess a carcinogenic potential.

11. In a pre-GLP one-generation reproduction study on rats, reduced spermatogenesis was observed down to the lowest dose level tested, 120 mg/kg bw.

Thiamphenicol did not elicit teratogenic effects in the rat; increased resorption rate and peri- and postnatal mortality were observed with concurrent maternal toxicity. There is experimental *in vitro* evidence that thiamphenicol may impair mitochondrial respiration in rat embryos. The NOEL for both maternal and developmental toxicity in rats was 40 mg/kg bw.

In an embryotoxicity/foetotoxicity study on the rabbit, maternal toxicity was observed down to the lowest level tested (1.25 mg/kg bw). No teratogenic effects were induced up to the top dose level of 5.0 mg/kg bw. Reduced foetal weight was observed with a NOEL for foetal effects of 1.25 mg/kg bw.

12. No original studies on the effects on the intestinal gut flora were provided.

A review of the available published literature shows that for most aerobic strains the MIC_{50} was more than or equal to 32 µg/ml.

On the other hand, gram-positive anaerobe bacteria are especially sensitive to thiamphenicol. The most sensitive species were *Actinomyces*, *Propionibacterium*, and *Fusobacterium*, with a MIC_{50} (most sensitive value) of 0.5 µg/ml . Since more than 10 strains were tested for such species, and *Fusobacterium* is considered as one of the most relevant microorganisms for the human gut flora, this value was considered as an appropriate basis for the microbiological ADI.

Cross-resistance has been reported between thiamphenicol and chloramphenicol. However, some chloramphenicol-resistant strains are susceptible to thiamphenicol.

No increase of drug-resistant strains was observed in mice following a 35-day dietary exposure equivalent to 4 mg/kg bw of thiamphenicol.

13. Limited data show that the NOELs for microorganisms utilised in food industry were as follows: *Lactobacillus acidophilus* more than or equal to 16 µg/ml; *Lactobacillus delbrueckii (bulgaricus)* 4 µg/ml; *Bacillus stearotermophilus (calidolactis)* 1 µg/ml.

14. The assessment made by the JECFA reports a study on the effects of thiamphenicol lifetime oral exposure on the spontaneous immunocomplex nephritis in NZW x OUW mouse strain. A reduction in immunocomplex deposition was observed at more than or equal to 50 mg/kg bw, indicating an immunosuppressive action. No effect was observed at 25 mg/kg bw.

15. Thiamphenicol has been widely used against human infections. The usual adult oral dose is 1500 mg daily (25 mg/kg bw in a 60 kg bw individual) for up to 15 days; dosages above 30 mg/kg bw are suggested for children.

    Adequate epidemiological data do not show any increased risk for aplastic anaemia in thiamphenicol-treated patients. This might be explained by the absence of the very reactive nitro-group which is characteristic of chloramphenicol, but experimental proof of this hypothesis is lacking.

    Bone marrow depression was the main side-effect observed in human patients, with a frequency of approximately 0.5%; the effect was dose-dependent and rapidly reversible following cessation of treatment. Inhibition of mitochondrial synthesis has been hypothesised as the underlying mechanism. However, a detailed appraisal of human adverse effects has not been provided; thus, a level without adverse effects in humans was not determined.

16. The compound was evaluated by JECFA in 1996.

    The JECFA considered that 1.25 mg/kg bw was the NOEL for maternal toxicity in the rabbit embryotoxicity/foetotoxicity study, and that a temporary toxicological ADI of 6 µg/kg bw should be set on that basis with a safety factor of 200, while awaiting the final report of the carcinogenicity study.

    The CVMP considers the study not appropriate for ADI determination, due to the well recognised high sensitivity of the rabbit to orally administered antimicrobial chemicals.

    There is no evidence that thiamphenicol is a genotoxic or carcinogenic compound; moreover, extensive human data show that, unlike chloramphenicol, thiamphenicol does not elicit any increase of aplastic anaemia, or of any other irreversible effects.

    A toxicological ADI can be set on the basis of the 13-week rat study. The study was of adequate quality and duration; no effects on tissues or organs were observed at the lowest dose level tested: 9.0 mg/kg bw. However, minor haematological and biochemical changes were observed down to such a dose level, which justifies a conservative safety factor of 200.

    Based on the NOEL of 9 mg/kg bw, applying a safety factor of 200, a toxicological ADI of 0.045 mg/kg bw (i.e. 2.7 mg/person) can be established.

    This ADI of 0.045 mg/kg bw will ensure an approximate safety margin of 900 as compared to the 40 mg/kg bw NOEL for post-implantation loss and maternal toxicity in the rat.

    However, a lower ADI can be derived from microbiological effects on human gut flora.

17. A MIC_{50} of 0.5 µg/ml is selected as the basis for a microbiological ADI, assessed on the most sensitive species identified among those considered most relevant to human gut flora, i.e. *Fusobacterium* sp.

For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI (µg/kg bw)} = \frac{\frac{\text{geometric mean } MIC_{50} \text{ x CF2}}{\text{CF1}} \text{ (µg/ml) x daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \text{ x weight of human (60 kg)}}$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\frac{0.50 \text{ x } 1}{1 \text{ x } 150}}{0.50 \text{ x } 60} = 2.5 \text{ µg/kg bw i.e. } 150 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1, because the most sensitive MIC_{50} were used, and because there are no data on the induction of microbial resistance to justify the adoption of a different CF;
- CF2 = 1, because there are no data on the effects of inoculum density or pH to justify the adoption of a different CF;
- 150 g is the weight of the daily faecal bolus;
- 0.50 is a conservative estimate of the fraction of the oral dose available in the human intestinal tract assuming that more than or equal to 50 % of a dose assumed orally is excreted through the urine and that faecal excretion will account for the balance.

The microbiological ADI is in a $1:10^5$ ratio with the usual oral therapeutic dosage of thiamphenicol, thus ensuring an ample safety margin for any adverse human side-effect.

18. A residue depletion study was performed in sixteen beef calves administered orally 50 mg/kg bw of thiamphenicol, subdivided in two daily administrations for 4 days. Thiamphenicol residues were analysed by HPLC (limit of detection 20 µg/kg). Liver residues ranged from 65-77 µg/kg 4 days after the end of treatment, 20-75 µg/kg after 6 days, while only one out of 4 samples showed residues equal to the limit of detection after 8 days. Kidney residues ranged from 50-115 µg/kg after 4 days; one out of 4 samples showed residue concentrations of 120 and 20 µg/kg after 6 and 8 days, respectively. No residues were observed in muscle, besides a single sample with residue concentrations of 90 µg/kg after 6 days. All samples examined at day 10 after treatment showed no detectable residues.

    No examination was performed as regards residues in body fat.

19. Four each high-yield cows and low-yield cows were treated intramuscularly with a total daily thiamphenicol dose of 30 mg/kg bw, divided into two 15 mg/kg bw injections for five days. Residues more than or equal to 800 µg/kg were detected in the milk one day after the cessation of treatment. After 2 days, the residues in milk of 6/8 cows were below the quantification limit of the gas-liquid cromatography-electron capture detector method utilised (20 µg/kg), whereas the residues in the milk of 2/8 cows were 124.5 and 34.1 µg/kg, respectively. No detectable residues were present 72 hours after the cessation of treatment.

20. A milk residue study by intramammary route was conducted to compare two different formulations with 5% and 10% thiamphenicol glycinate acetylcysteinate. Six Friesian dairy cows underwent two treatment cycles, during which 500 mg of thiamphenicol in each quarter were administered 3 times at 12-hour intervals. A 12-day interval separated the treatment cycles with the two formulations.

In each treatment cycle, milk samples were collected at the milking immediately before the first administration and for the following six days.

With the 5% formulation, mean milk residues were 85 µg/kg (0.034-0.172) at the 2nd milking after the cessation of treatment, 35 µg/kg in 2/6 cows at the 3rd milking (4/6 cows below the 20 µg/kg limit of quantification) and below the limit of quantification for all cows at the 4th milking.

With the 10% formulation, mean milk residues were 264 µg/kg (36-600) at the 2nd milking after the cessation of treatment, 28-93 µg/kg in 4/6 cows (2/6 below the limit of quantification) at the 3rd milking, 21-54 µg/kg in 2/6 cows (4/6 below limit of quantification) at the 4th milking, 26 µg/kg in a single animal at the 5th milking; all animals showed residues below the limit of quantification at the 6th milking after the cessation of treatment.

The study was not a standard residue depletion study; thus it has a limited value in assessing the residue depletion curve in milk after intramammary administration.

21. Since no tissue depletion residue studies were performed in cattle following intramuscular administration, there is no information on the depletion of thiamphenicol residues at the injection site.

22. Total radioactive residues were investigated in 48 broiler chickens treated orally with 50 mg/kg bw ^{14}C-thiamphenicol subdivided into two daily doses for 5½ days.

Liver concentrations averaged 7.80, 3.55, 1.80 and 1.16 mg equivalents/kg after 6 hours, 1, 3 and 5 days following the last treatment, respectively. Mean kidney concentrations at the same sampling times were 4.90, 1.70, 0.91 and 0.82 mg equivalents/kg. In muscle, radioactivity fell from 1.12 mg equivalents/kg at 6 hours to 0.34, 0.20 and 0.16 mg equivalents/kg at 24, 96 and 72 hours, respectively. Abdominal fat concentrations averaged 0.50, 0.21, 0.14 and 0.12 mg equivalents/kg at 6 hours, 1, 3 and 4 days; at the corresponding sampling times skin plus fat concentrations were 1.35, 0.52, 0.54 and 0.38 mg equivalents/kg, respectively.

The tissue residue depletion profile of thiamphenicol was investigated in ninety-six broiler chickens following treatment with a dose of 840 mg/l in drinking water for 5 days, equal to an average intake of 134 mg/kg bw/day. Animals were slaughtered at 2, 4, 6, 8 hours, 1, 2, 4 and 17 days after the last treatment day.

Mean liver residues were (range) 310.2 (62.9-651.7), 257.4 (46.6-452.1) and 146.6 (58.1-302.6) µg/kg after 1, 2 and 4 days, respectively. After a 17-day withdrawal, residues ranged from 7-21 µg/kg in liver (2/6 samples below limit of detection). Mean kidney residues were 382.6 (46.5-741.2), 64.4 (28.8-132.4), and 23.9 (1/6 sample below limit of detection) µg/kg after 1, 2 and 4 days, respectively; no detectable residues were present after 17 days. Mean breast muscle residues were 852.4 (112.2-3500.9), 285.3 (139.5-398.7) and 78.6 (29.3-156.3) µg/kg after 1, 2 and 4 days, respectively. After 17 days, residues ranged from 4.6-57.8 µg/kg in breast muscle (1/6 samples below limit of detection). Skin with adhering fat showed high residue concentrations at all sampling times, namely (average values): 20100 µg/kg at 2 hours after the cessation of treatment, 19500 µg/kg at 4 hours, 5900 µg/kg at 6 hours, 18600 µg/kg at 8 hours, 28000 µg/kg after 1 day, 9300 µg/kg after 2 days, 4200 µg/kg after 4 days and 5,100 µg/kg after 17 days. Average thiamphenicol concentrations in abdominal fat were (range): 4900 (1000-14900) µg/kg at 2 hours after the cessation of treatment, 4000 (800-9300) µg/kg at 4 hours, 5200 (1000- 7900) µg/kg at 6 hours, 1100 (500-1700) µg/kg at 8 hours, 3800 (600 - 8400) µg/kg after 1 day, 960 (770-1320) µg/kg after 2 days, 200 (80-490) µg/kg after 4 days, 150 (40-490) µg/kg after 17 days.

All residue concentrations above 1000 µg/kg are estimated, because they are above the concentrations for which the HPLC method is validated. Therefore, no firm conclusions can be derived about most quantitative residue values in skin plus fat and abdominal fat, especially at the earlier sampling time points.

23. Fifteen laying hens were exposed through the diet to thiamphenicol at a dose level of 400 mg/kg feed, equal to 30 mg/kg bw, for 5 days. The whole eggs (yolk plus albumen) were examined for the presence of thiamphenicol residues from 1 up to 14 days following cessation of the treatment.

Residues of unchanged thiamphenicol ranged from 72-190 µg/kg and 20-43 µg/kg (with 50% of hens showing no detectable residues in their eggs), respectively, 4 and 7 days after the end of treatment. A single hen, out of 13, showed detectable residues (23 µg/kg) 8 days after the end of treatment. Residues below the limit of quantification (less than 20 µg/kg) residues were observed after 9 days. The study shows some potential for thiamphenicol accumulation and persistence in the eggs; thus, no MRLs for eggs are proposed.

24. No residue studies are available in the turkey.

25. While the available data do not allow a detailed quantitative assessment of the marker residue : total residue ratio, on the basis of the results of pharmacokinetic and residue studies, thiamphenicol does not undergo major biotransformation processes in the target species as well as in humans.

    Unchanged thiamphenicol can thus be considered as the marker residue.

26. Two analytical methods have been validated:

    - a gas-liquid chromatography method with electron capture detection (GLC-ECD) for the determination of thiamphenicol in cow's milk: the method uses chloramphenicol as the internal standard; the peak height ratios of thiamphenicol/chloramphenicol of the standard concentrations of the calibration curve are used for the quantification; the limit of quantification is 20 µg/kg;
    - an HPLC method for the determination of thiamphenicol in bovine and poultry tissues: the limit of quantification for all relevant bovine and poultry tissues is 20 µg/kg; the limit of detection for bovine tissues is 2.5 µg/kg for muscle, 2.6 µg/kg for liver and fat, and 5.1 µg/kg for kidney. As regards chicken, the limit of detection is 2.6 µg/kg for kidney and skin, 2.9 µg/kg for muscle, 5.1 µg/kg for fat, and 5.8 µg/kg for liver.

    Both methods are presented in ISO 78/2 format.

Conclusion and recommendations

Considering that:

- a microbiological ADI of 2.5 µg/kg bw has been established,
- sufficient pharmacokinetic and residue data in target species have been provided,
- the parent compound is identified as the marker residue,
- validated analytical methods for residue monitoring purposes are available;

the Committee recommends the inclusion of thiamphenicol into Annex 1 of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Thiamphenicol	Thiamphenicol	Bovine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney Milk	
		Chicken	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

Based on these MRLs it was estimated that the daily consumer intake of thiamphenicol residues from either edible bovine tissues (300 g muscle, 100 g liver, 50 g kidney, 50 g fat) or edible poultry tissues (300 g muscle, 100 g liver, 10 g kidney, 90 g fat+skin) and 1.5 l milk will amount to 0.1 mg/day, representing about 67% of the ADI.