European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

THIAMPHENICOL
(Extension to pigs)

SUMMARY REPORT (5)

1. Thiamphenicol (CAS: 15318-45-3) 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. Thiamphenicol is active against both Gram-negative and Gram-positive bacteria, and is especially active on anaerobes.

   Thiamphenicol is used for the treatment and control of respiratory and intestinal infections in cattle and poultry by oral or intramuscular administration. The substance is also used for intramammary administration in both lactating and dry cows and for intrauterine administration in cows.

   A microbiological ADI of 2.5 µg/kg bw i.e. 150 µg/person was previously established for thiamphenicol based on microbiological effects on human gut flora. Currently, thiamphenicol is included in Annex I 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	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

   Provisional MRLs had also been previously established for pigs, ovines and fin fish, with a value of 50 µg/kg for all relevant tissues, however final MRLs could not be recommended for these species due to the inadequate information on the ratio marker to total microbiologically active residues and the absence of a fully validated analytical method for monitoring residues in edible tissues.

   A new application has now been submitted for the extension of the current MRLs for thiamphenicol to pigs, including new data to establish the ratio of marker to total residues as well as a new analytical method. New data on the metabolism of thiamphenicol in pigs were also included.

   In pigs, thiamphenicol is intended for the treatment of respiratory and intestinal infections with a recommended dose of 30 mg/kg bw orally, intramuscularly or intravenously for up to 5 days.

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2. Fifteen pigs were treated orally twice a day for 5 days with 10, 15 or 20 mg/kg bw thiamphenicol. Blood samples were analysed by gas chromatography with electron capture detector (limit of quantification 20 µg/l). For unchanged thiamphenicol, the samples were extracted in organic phase without enzyme hydrolysis, for the total thiamphenicol the samples were incubated with beta-glucurase before analysis. Thiamphenicol was rapidly absorbed and the area under the curve and C_{max} of unchanged thiamphenicol were linearly correlated with the dose. No accumulation of thiamphenicol or thiamphenicol glucuronide was evident in swine plasma after oral administration at doses of 10, 15 and 20 mg thiamphenicol/kg bw twice daily for 5 consecutive days.

   In a GLP study, 16 pigs were fed twice daily with 900 mg thiamphenicol/kg feed (approximately 30 mg/kg bw/day) for 5 days; 3 pigs were maintained as controls. Thiamphenicol was measured in plasma following solvent extraction, using a solid-phase liquid-liquid partitioning technique and HPLC (limit of detection 0.01 µg/ml). The maximum mean level of thiamphenicol in plasma (1.28 µg/ml), was found at 8 hours after the first dose administration.

   Six healthy male pigs were treated with medicated water containing 2.5 ml of 10% thiamphenicol/l of water, ad libitum, for 5 days. Peak plasma concentration (mean plasma concentration at the steady state 1.01 ± 0.44 µg/ml) was reached at 36 hours after the start of treatment and the levels remained similar until the end of the treatment period (120 hours). Elimination half live (2.9 ± 0.3 hours) was determined by linear regression of the terminal phase, when treatment was stopped. Administration of thiamphenicol in water resulted in an increase in plasma levels until the peak and steady state concentration was reached at approximately 36 hours after start of administration. Elimination of thiamphenicol from plasma was rapid upon cessation of treatment.

   Pharmacokinetic parameters of thiamphenicol were determined after single intravenous or intramuscular administration of 30 mg/kg bw to 5 male pigs. Three weeks later, the same animals were administered a second dose via the other route. Plasma thiamphenicol concentrations were determined by HPLC (limit of quantification 20 ng/ml). Intravenous thiamphenicol kinetics were fitted to a bi-exponential equation, with a first rapid disposition phase followed by a slower disposition phase.

   The thiamphenicol elimination half-life following intramuscular administration was statistically greater than following intravenous administration, probably due to the slow rate of absorption from the muscle.

   Six Large White male pigs (26.6 ± 4.1 kg) were administered a single dose of 30 mg/kg bw/day thiamphenicol intravenously. A validated HPLC analytical method with UV detection was used to determine thiamphenicol concentration in plasma. The limit of quantification of the method was 0.02 µg/ml. The linearity of the method was determined between 0.02 µg/ml and 15 µg/ml; precision and accuracy were below 15 %. Elimination half life was short (1.2 ± 0.5 h) and there was a high rate of plasma clearance (356.4 ± 119.7 ml/min).

   Three female and 3 male pigs (body weight 27 to 34 kg) were administered either 10 mg/kg bw (as solution by intravenous route) or 30 mg/kg bw (as suspension by oral route). Three weeks later the animals were administered thiamphenicol by the alternative route. Serum concentrations of thiamphenicol were determined by a validated HPLC analytical method (limit of quantification 0.05 µg/ml). The short elimination half-life and mean residence time values following intravenous administration confirms the rapid elimination of thiamphenicol in pigs compared with oral administration.

Pharmacokinetics of unchanged thiamphenicol and thiamphenicol glucuronate were determined in 4 pigs administered thiamphenicol orally at 30 mg/kg bw in the feed and thiamphenicol intravenously at 10 mg/kg bw as glycinate, following a cross-over design. Both free and total thiamphenicol were assayed in plasma and urine samples, according to GLP requirements, using a validated HPLC-UV method (limit of quantification 0.021 µg/ml for plasma and 0.21 µg/ml for urine). Concentrations of glucuronate thiamphenicol were determined by subtracting the quantity of free thiamphenicol from that of total thiamphenicol. In urine, highest concentrations of total thiamphenicol occurred 4 hours after oral administration and ranged between 610 and 723 mg/l. After intravenous administration, maximum urine concentrations also occurred after 4 hours with a range of 363 to 1136 mg/l. The area under the curve after oral administration for total thiamphenicol is about 4 times that of free thiamphenicol. Following intravenous administration, this ratio is only 2 times, suggesting that glucuronidation after oral administration is much higher than after intravenous administration.

For total thiamphenicol, the average elimination ratio, half life and distribution volume are similar in both routes of administration. Elimination parameters of free thiamphenicol are similar for the 2 routes of administration.

In a GLP study, thiamphenicol was administered to 2 groups of 4 pigs each. One group was treated orally at a dose rate of 30 mg/kg bw of thiamphenicol, once daily for 3 consecutive days. The other group was administered thiamphenicol intramuscularly at a dose rate of 30 mg/kg bw once daily for 3 consecutive days. After a washout period of 11 days animals in the first group were administered thiamphenicol intramuscularly and those in the other group received thiamphenicol in the feed. After intramuscular administration, blood samples were taken at 0, 0.25, 0.5, 1, 2, 6 and 12 hours after the first administration, 0, 1 and 12 hours after the second administration and 0, 1, 12, 24, 36 and 48 hours after the third administration. Following oral administration, blood samples were taken at 0, 0.5, 1.5, 3, 5, 7 and 12 hours after the first administration, 0, 1.5 and 12 hours after the second administration and 0, 1.5, 12, 24, 36 and 48 hours after the third administration. Determination of thiamphenicol in plasma was carried out using an HPLC/MS/MS analytical method fully validated in the range 0.20 to 20.00 µg/ml; the limit of quantification and limit of detection were 0.20 and 0.05 µg/ml, respectively. The results show a rapid absorption following both routes of administration though intramuscular administration leads to a higher plasma concentration and a higher bioavailability following intramuscular administration. The relative bioavailability of the oral route to the intramuscular route was approximately 48%. The elimination half lives were comparable for the two routes of administration. The absence of difference in plasma concentrations achieved after the first, second or third day demonstrated absence of accumulation for each of the routes of administration tested.

3. In a GLP study 32 pigs were administered daily 30 mg thiamphenicol/kg bw intramuscularly for 5 days. Groups of 2 males and 2 females were slaughtered at either 8 hours, 1, 4, 8, 15, 21 or 28 days after treatment. Administration was made into the left side of the neck on days 1 to 4 and into the right side of the neck on day 5. Tissue samples were taken for determination of residues of free thiamphenicol by HPLC method (the limit of detection and limit of quantification were 5 µg/kg and 21 µg/kg, respectively, for all matrices). The kidney showed the highest concentration of thiamphenicol 24 hours after the last treatment (6675 µg/kg), 1 648 µg/kg at 8 hours, 55 µg/kg at 4 days and 24 µg/kg after 8 days. In muscle the mean concentration of thiamphenicol was 389 µg/kg at 24 hours after the last administration and decreased rapidly to an estimated mean value of 11 µg/kg after a 4 days. Traces of thiamphenicol were detected up to 21 days. In liver 24 hours after the last treatment, the mean thiamphenicol residue value was 111 µg/kg and depleted rapidly, 4 days after the last treatment all samples showed values below the limit of quantification. The mean concentration of thiamphenicol in skin+fat was 32 µg/kg 4 days after the last treatment and 17 µg/kg after 8 days (below the limit of quantification in 3 samples out of 4). In the injection site after 8 days of treatment the mean residue concentrations were 35 µg/kg and 14 µg/kg at 15 days after the last treatment. Traces of thiamphenicol were detected up to 21 days after the last treatment.

In a GLP study, 9 male pigs were administered daily for 5 or 6 days at 50 mg/kg bw of thiamphenicol by intramuscular route. Groups of 3 animals were slaughtered at either 2 hours, 8 hours or 4 days after the last treatment. Tissue samples were assayed for residues of thiamphenicol according to a microbiological method and a HPLC method. The limit of detection and limit of quantification of the HPLC method were, respectively, 15 and 25 µg/kg for all tissues. Results of determination of residues of free thiamphenicol and total thiamphenicol by the HPLC method showed that 4 days after the end of treatment free thiamphenicol was not detectable in kidney, liver and muscle and total thiamphenicol was not detectable in liver and muscle. Eight hours after the last administration high concentrations of thiamphenicol glucuronate were found in tissues, representing more than 30% of the total thiamphenicol residues in kidney, liver and skin + fat, and near 5% in muscle. Four days after the end of treatment the concentration in kidney of total thiamphenicol was 25.0 µg/kg and the concentration of free thiamphenicol was below the limit of quantification (25.0 µg/kg), in skin+fat the concentration of total thiamphenicol was 54.0 µg/kg and the concentration of free thiamphenicol was 47.2 µg/kg. As 4 days after the end of thiamphenicol administration tissue residues of total thiamphenicol were near or below the limit of detection (apart from those for skin + fat), at 8 hours free thiamphenicol was only a portion of total thiamphenicol for kidney, liver and skin + fat and no results were provided between 8 hours and 4 days, therefore no conclusion could be drawn from this study on the ratio between free thiamphenicol and total thiamphenicol in pigs.

In a GLP study 2 female pigs were intramuscularly administered 0.2 ml of an aqueous solution containing thiamphenicol (equivalent to 50 mg thiamphenicol/kg bw) per day for 5 days,. The animals were sacrificed at 4 and 8 hours after the last administration, respectively. Free thiamphenicol levels were determined simultaneously in the tissue samples using validated HPLC/UV and microbiological (diffusion assay, using *Bacillus stearothermophilus*) method. Both methods were validated in the range 1000 to 6000 µg/kg with a limit of quantification of 1000 µg/kg for all tissues. The injection site showed the highest concentrations of free thiamphenicol, 1323215 and 1175000 µg/kg by HPLC and microbiological method respectively in one pig sacrificed 4 hours after the last administration and 149635 and 138100 µg/kg in the pig sacrificed at 8 hours. High tissue concentrations were obtained by HPLC and microbiological method in kidneys: 35 920 and 33 995 µg/kg, respectively, at 4 hours and 14150 and 15535 µg/kg at 8 hours. In muscle the concentrations resulted in 6351 and 5805 µg/kg respectively at 4 hours and 2738 and 2678 at 8 hours after the last administration. In liver the values by HPLC and microbiological method were 4700 and 4910 µg/kg at 4 hours, 2510 and 2110 µg/kg at 8 hours. The lowest residues of thiamphenicol were obtained in skin + fat samples, 2095 and 2040 µg/kg at 4 hours by HPLC and microbiological method, 548 µg/kg by HPLC at 8 hours (below the sensitivity limit of the microbiological method, 1000 µg/kg). The correlation between the results of the two assay methods was approximately 1.

In a GLP study, 21 pigs were assigned to five groups of four animals (2 male and 2 female) each and 1 control female animal. The 5 treatment groups received thiamphenicol by the intramuscular route in the neck once a day for 5 consecutive days at a dose of 0.2 ml/kg bw/day, equivalent to 50 mg thiamphenicol/kg bw/day. Animals in groups 1 to 5 were sacrificed at 8, 12, 24, 48 or 96 hours respectively after the last administration. The determination of thiamphenicol in muscle, liver, kidney and skin with adhering fat and muscle injection site was carried out simultaneously, on the same samples, using validated chemical and microbiological methods. The chemical determination of free thiamphenicol was performed by HPLC connected to a UV or MS/MS detector depending on the range concentration. The HPLC/UV method was validated in the range 1000 to 6000 µg/kg. One HPLC/MS/MS method was validated in the range 25 to 100 µg/kg for all tissues and the other HPLC/MS/MS method in the range 100 to 400 µg/kg (muscle, skin + fat) and 100 to 600 µg/kg (liver, kidney). The microbiological method was validated in the range 1000 to 6000 µg/kg for all tissues (diffusion assay, using *Bacillus stearothermophilus*) and between 100 and 600 µg/kg for liver, 100 and 400 µg/kg for muscle and skin + fat and between 200 and 600 µg/kg for kidney (turbidimetric assay, using *Haemophilus influenzae*).

Results in the same order of magnitude in all edible tissues were obtained by HPLC and microbiological methods. The HPLC values of free thiamphenicol in different tissues were; 34578 µg/kg at 8 hours, 15 528 µg/kg at 12 hours, 3 778 µg/kg at 24 hours, 594 µg/kg at 48 hours and 329 µg/kg at 96 hours after the last treatment in kidney; 4 399 µg/kg at 8 hours, 2389 µg/kg at 12 hours, 274 µg/kg at 24 hours, 65 µg/kg at 48 hours and 35 µg/kg at 96 hours in muscle. The injection site showed high concentrations of thiamphenicol, 1 554 189 and 1 139 128 µg/kg at 8 and 12 hours, 5 581 µg/kg at 24 hours, 71 µg/kg at 48 hours and 39 µg/kg at 96 hours. The lowest values were found in liver (1 816 µg/kg, 902 µg/kg, 130 µg/kg, 89 µg/kg and 59 µg/kg as mean residue respectively from 8 to 96 hours after the last treatment) and in skin + fat (1 034, 531, 101, 113 and 82 µg/kg respectively from 8 to 96 hours). For all tissues the individual and mean results obtained by microbiological method were similar to the values obtained by HPLC at all sampling times until 48 hours after the last treatment. Since the ratio is approximately 1 (ranging from 0.84 to 1.15), this suggested that glucuronated thiamphenicol, or any other metabolite of thiamphenicol present in edible pig tissues, was not microbiologically active *in vitro*.

Twelve pigs were administered thiamphenicol orally daily for 5 days at 40 mg/kg bw, divided into 2 daily administrations; 2 animals were slaughtered at 5, 8, 10, 11, 12 and 15 days after the last administration. Tissue samples were taken for determination of residues of free thiamphenicol according to a validated HPLC method (limit of quantification: 20 µg/kg).

The maximum level of residues were always observed at 5 and 8 days after treatment; the most persistent residues were in liver and kidney. Free thiamphenicol mean levels in muscle were 32 µg/kg, 108.9 µg/kg and below the limit of quantification from 5 to 15 days after the last treatment; in skin + fat the results were 32.2 µg/kg and below the limit of quantification respectively from 5 to 15 days after the last treatment; in liver 97 µg/kg, 102 µg/kg and 23 µg/kg and below the limit of quantification respectively from 5 to 15 days after the last treatment; in kidney 641 µg/kg, 964 µg/kg 261,5 µg/kg, 41,9 µg/kg and below the limit of quantification respectively from 5 to 15 days after the last treatment.

In a GLP study, 20 pigs, 2 month-old, 35 to 42 kg bw were allocated in 4 groups of 5 animals and administered thiamphenicol at 30 mg/kg bw per day (divided into 2 daily administrations) for 5 days in the feed. The animals were sacrificed at 5, 10, 16 or 18 days after treatment. Tissues were assayed for residues of free thiamphenicol, using a validated HPLC-UV method (limit of detection 5 µg/kg and limit of quantification 21 µg/kg). Residues of free thiamphenicol were not detected at any time in muscle, except in 2 animals at the fifth and tenth day after the last administration (535 and 128 µg/kg, respectively), while subsequently the values decreased to 84 µg/kg and 39 µg/kg after 16 and 18 days. In liver residues were 56 µg/kg 5 days after treatment and 38 µg/kg 10 days after treatment, while they fall to 22 and 27 µg/kg at day 16 and 18, respectively. Skin showed elevated residual concentrations of free thiamphenicol with a mean value of 95 µg/kg even at the final slaughter time, fat showed limited concentrations at all time periods, with mean value of 7, 11, 9 and 6 µg/kg, at 5, 10, 16 and 18 days after the last treatment respectively.

4. In a newly provided GLP study twenty male and twenty female pigs were treated with thiamphenicol, by intramuscular injection, at a dose rate of 50 mg/kg/day. Thiamphenicol was administered, alternatively, to both sides of the neck. Samples of liver, kidney, skin + fat, muscle and both injection sites were obtained at 1, 2, 4, and 7 days after administration (10 animals per sampling time). The tissue samples collected were analysed before and after deconjugation of glucuronoconjugated thiamphenicol with ß-glucuronidase. The analytical method was HPLC/MS/MS based. The limit of quantification of the analytical technique was 20 µg/kg for all the tissues. The highest free thiamphenicol tissue concentrations were found in the injection sites and decline from more than 5 000 µg/kg in the left injection site at 1 day withdrawal to 521 µg/kg in the right injection site at 7 days after administration. The tissue with less free thiamphenicol residue was the non injected muscle with 136 µg/kg at 1 day after administration to less than the limit of quantification at 2 days after administration.

The ratio of free thiamphenicol concentrations to total thiamphenicol concentration in the muscle samples was close to 1 at all sampling times, indicating that in this tissue no conjugated thiamphenicol residues were found. From this new study the ratios of free thiamphenicol concentrations to total thiamphenicol concentration were calculated as between 0.63 (day 1 after administration) and 1.00 (day 4) in liver, between 0.44 (day 2) and 1.00 (day 7) in kidney and between 0.52 (day 1) and 0.62 (day 7) in skin + fat, the kidney being the tissue with higher concentrations until 7 days after administration.

The ratios of free thiamphenicol to total thiamphenicol were calculated as 0.8, 0.4, 0.6 and 1 for liver, kidney, skin + fat and muscle respectively 2 days after administration.

In the same experiment, the animals were housed in individual metabolic cages to obtain samples of faeces and urine during and after thiamphenicol administration. For the urine and faeces samples a complete validation of the HPLC/MS/MS method was provided with a limit of quantification of 100 µg/kg. The faecal and urinary depletion of free thiamphenicol and conjugated thiamphenicol was very fast. While in faces the ratio of free thiamphenicol to total thiamphenicol was close to 1 in all the samples obtained after last product administration, in the urine samples this ratio increased from 0.350 at 1 day after admistration to 1 at 4 days after administration.

5. A new HPLC/MS/MS method for monitoring of thiamphenicol residues for muscle, kidney, and skin + fat was provided. No data were provided for this method in liver. The method was presented in ISO 78/2 format, and validated for kidney and skin + fat according to Volume 8 of the Rules Governing Medicinal Products in the European Union. For this method the limit of quantification for muscle, kidney, and skin + fat were 50 µg/kg, 25 µg/kg and 25 µg/kg respectively. The specificity of the analytical method in respect to chloramphenicol and florfenicol was verified. The method was not fully validated for muscle at half the proposed MRL (50 µg/kg), with regard to accuracy, precision and limit of quantification.

   An additional method used for the residue depletion studies was also available with a limit of quantification of 20 µg/kg for muscle, kidney, skin + fat and liver but not fully validated according to Volume 8. This method is essentially similar to the monitoring method.

6. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) previously established an ADI of 2.5 µg/kg bw and temporary MRLs in muscle, liver, kidney and fat for pigs. The temporary MRLs were 0.05 mg/kg for muscle, liver, kidney and fat (pigs) expressed as the sum of thiamphenicol and its conjugates, measured as thiamphenicol. However, these were not extended in 1999 because the information requested by JECFA was not provided.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 2.5 µg/kg bw (150 µg/person) was previously established for thiamphenicol based on microbiological effects on human gut flora,
- from the new data provided it could be concluded that the parent substance was the only microbiologicaly active residue found in pig tissues after thiamphenicol administration so it was retained as the marker residue,
- after the administration of thiamphenicol to pigs the main metabolite found in tissues was glucuronoconjugated thiamphenicol.
- the ratio free thiamphenicol to total thiamphenicol concentrations in all the target tissues was calculated as 0.8, 0.4, 0.6 and 1 for liver, kidney, skin + fat and muscle respectively,
- the same values of bovine and chicken MRL tissues were retained for porcine species,
- a new validated analytical method is available for monitoring of residues is available but not fully validated with regard to muscle and liver;

The Committee for Medicinal Product for Veterinary Use recommends the inclusion of thiamphenicol in Annex III 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	Porcine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2007

Based on these MRLs values, the daily intake taking into account porcine tissues plus bovine milk will represent about 71% of the ADI.

Before the Committee can consider the inclusion of thiamphenicol for porcine tissues in Annex I of Council Regulation (EEC) No. 2377/90 the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should provide a validated analytical method for the determination of thiamphenicol residues in pig liver. For pig muscle the method should be validated in respect to the accuracy, precision and limit of quantification according the established MRL. The validation should be carried out according to Volume 8 of the Rules Governing Medicinal Product in the European Union.