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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

THIAMPHENICOL (extension to pigs, sheep and fin fish)

SUMMARY REPORT (3)

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 active on anaerobes.

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

2. A microbiological ADI of 2.5 µg/kg bw i.e. 150 µg for a 60 kg was previously established for thiamphenicol based on microbiological effects on human gut flora. Currently, thiamphenicol is included in Annex I of Council Regulation (EEC) 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

3. Thiamphenicol is proposed for the treatment of pigs against respiratory and intestinal disease, for the treatment of sheep against respiratory and intestinal diseases, mastitis and foot infections, and for the treatment of fin fish against vibriosis and pasteurellosis. Routes of administration are oral and intramuscular. Recommended dosages and duration of treatment are 30 mg/kg bw to swine (oral administration) for up to 5 days, up to 30 mg/kg bw by intramuscular or intravenous injection to swine, ovine, and up to 40 mg/kg bw in fin fish for up to 5 days (oral administration).

4. Sixteen pigs were treated by oral route with thiamphenicol twice a day for 5 days with 30 mg/kg bw/day. Blood samples were collected for 5 days after last treatment and analysed for the presence of thiamphenicol by HPLC (limit of detection: 20 µg/l, limit of quantification: 10µg/l). Maximum mean plasma level of thiamphenicol (1280 µg/l) was reached at 8 hours after the first dose administration. Mean concentration are in the range 220 to 800 µg/l; mean levels declined to give concentrations below or close to the limit of detection (20 µg/l) by 48 hours withdrawal.

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Fifteen pigs, divided in three groups, were treated by oral route twice a day for 5 days with three different doses of thiamphenicol (10, 15 and 20 mg/kg bw/day). Blood samples were collected until 48 hours after the last treatment and analysed by gas chromatography with electron capture detector, limit of quantification: 20 µg/l, in order to evaluate the unchanged thiamphenicol and total thiamphenicol. Thiamphenicol was rapidly absorbed after oral administration and the area under the blood concentration-time curve (AUC) and C_{max} of unchanged thiamphenicol were linearly correlated with the dose. The glucuronidation, in pigs, was found to be an important route of elimination and a partial saturation of enzymatic system for glucuronidation may occur at high dose of thiamphenicol. No accumulation in plasma was evident after oral administration at the doses of 10, 15 and 20 mg/kg twice daily for five consecutive days.

The pharmacokinetics of thiamphenicol and of the glucuronide were determined in plasma and urine of four pigs after single treatment with thiamphenicol at 30 mg/kg bw by oral route and 10 mg/kg bw of thiamphenicol glycinate. Blood samples were collected until 24 hours after the last treatment and urine until 48 hours. The determination of thiamphenicol and thiamphenicol glucuronide were performed by HPLC (limits of quantification: 20 µg/l for plasma and 200 µg/l for urine). After oral administration, the mean pharmacokinetic parameters for free and total thiamphenicol were as follows: ke: 0.1911 h^{-1} (free), 0.1693 h^{-1} (total); $t_{1/2}$: 3.88 hours (free), 4.57 hours (total); AUC: 23961 µg·h/l (free) and 91042 µg·/l (total). In the urine the highest concentration is reached 4 hours after administration with a range from 610 µg/ml to 723 µg/ml of total thiamphenicol. After intravenous administration, the mean pharmacokinetic parameters for free and total thiamphenicol were as follows: ke: 0.2029 h^{-1} (free), 0.1569 h^{-1} (total); $t_{1/2:}$ 3.43 hours (free), 4.64 hours (total); AUC: 12643 µg·h/l (free) and 23292 µg·h/l (total). In the urine the highest concentration is reached 4 hours after administration with a range from 363 µg/ml to 1136 µg/ml of total thiamphenicol.

5. Kinetic and distribution of thiamphenicol in sheep were studied in two experiments. In the first study, 18 sheep were treated by intravenous route (9 animals) and by intramuscular route (9 animals) with thiamphenicol at 20 mg/kg bw. Blood samples were collected until 10.5 hours after drug administration and analysed by HPLC (limit of detection: 100 µg/l) for the determination of thiamphenicol. After intravenous injection of 20 mg/kg, $t_{1/2\alpha}$ was 0.18 hours and $t_{1/2\beta}$ was 1.53 hours. The apparent volume of distribution, steady state volume of distribution and distribution volume during the elimination phase are about 0.36, 0.80 and 1.09 1/kg respectively. The median values of K_{12}, K_{21} and K_{el} were 1.59, 1.26 and 1.26 h^{-1} respectively. Following intramuscular administration, peak plasma concentration is reached within 10 to 20 minnutes, with bioavailability of 87.5%. In the second study 10 sheep were treated by oral route and by intravenous route with 60 mg thiamphenicol/kg bw. Blood samples were collected until 24 hours after drug administration and analysed for the level of thiamphenicol by HPLC (limit of detection: 0.1µg/ml). After intravenous and oral administration of 60 mg/kg, the drug is detected in plasma 10 minutes after treatment, with maximum concentrations of about 2.5 µg/ml after 6 hours post-treatment. These very low concentrations were maintained for 24 hours. The AUC oral was 29.98 µg·h/ml (only 30% of oral dose is systemically available).

   Twelve sheep were treated by intramuscular route with 20 mg/kg bw of thiamphenicol every 8 hours for 4 times. Blood samples were collected until 8 hours after the third drug injection. Samples of biological fluids (blood, bile, pericardial, peritoneal, synovial and cerebrospinal fluids) were collected at 2, 6, 12, 24, 48 and 72 hours after last treatment and analysed for the determination of thiamphenicol by HPLC (limit of detection: 10 µg/l). Thiamphenicol is rapidly absorbed from injection sites yielding peak plasma concentrations of about 20 µg/ml within 30 minutes Following, plasma concentrations declined with an elimination half life of 1.51±0.51 hours and a mean residence time of 2.05±0.51 hours. In biological fluids, the mean concentrations of the antibiotic became undetectable after 24 hours (with the exception of the cerebrospinal fluid) with mean values comprised in the range from 9.75 to 31.25 µg/ml after 2 hours and from 0.5 to 1.25 µg/ml at 24 hours. These values are higher than the corresponding plasma concentrations. In the cerebrospinal fluid the drug is detected only at 2 and 6 hours.

6. Fish were treated by oral route with two different doses of thiamphenicol (15 and 30 mg/kg bw/day) in single and repeated treatments (5 days). Water temperature: 15±2°C. Blood samples (6 animals at each sampling time) were collected up to 72 hours after dosing in the single-dose experiment and over 14 days after the end of treatments in the repeated-dose study and analysed for the presence of thiamphenicol by HPLC (limit of quantification: 50 µg/ml). The absorption of thiamphenicol from medicated feed was slow and limited in comparison with the absorption with the force fed administration. Thiamphenicol concentrations in blood declined quickly and were under the limit of quantification 50 µg/ml 7 days after the end of treatment.

   Some pharmacokinetic studies were performed in young yellowtail fish. Thiamphenicol was administered as a single dose mixed in feed at the dose of 100 mg/kg bw. Blood samples were collected 3, 6, 12, 24 and 48 hours after administration from 7 fish per timepoint. The results obtained by colorimetric method indicated a peak-concentration 3 to 6 hours after administration (C_{max}: 9.4 to 12.1 µg/ml) and disappearance from the blood in 48 hours. In fish, high levels of thiamphenicol were measured in bile 24 hours after oral administration.

7. In a study performed in accordance with GLP, twenty pigs were divided into four groups and fed for 5 days with a diet containing thiamphenicol in a concentration sufficient to guarantee the dosage of 30 mg/kg bw/day. The groups were treated at different times in order to sacrifice all the animals on the same days. Samples of muscle, liver, kidney, fat and skin were taken. All the samples were stored at −25°C until assay. The withdrawal time adopted were 5, 10, 16 and 18 days. The analyses were conducted using an HPLC method (limit of detection: 5 µg/kg, limit of quantification: 20µg/kg). The results obtained showed that thiamphenicol was not present in muscle at any times, except for two samples at 10 days; for liver the mean values of residues were: 56, 38, 27 and 22 µg/kg at 5, 10, 16 and 18 days after the end of treatment, respectively. For kidney: 535, 128, 84 and 39 µg/kg at 5, 10, 16 and 18 days after the end of treatment, respectively. For fat: 7, 11, 9 and 6 µg/kg at 5, 10, 16 and 18 days after the end of treatment, respectively. For skin 228, 120, 153 and 95 µg/kg at 5, 10, 16 and 18 days after the end of treatment, respectively.

   Thirty-two pigs were divided into 8 groups; 7 groups were treated with thiamphenicol administered by intramuscular injection at the dose of 30 mg/kg (0.12 ml/kg bw per day of a commercial product) for 5 consecutive days. One group was used as negative control. The groups were slaughtered at 8 and 24 hours and 4, 8, 15, 21 and 28 days after the last administration. Samples of muscle, injection site, liver, kidney, visceral fat, skin and fat, lung were taken and analysed by HPLC (limit of quantification: 21 µg/kg, limit of detection: 5 µg/kg). The kidneys showed the highest concentration 8 hours (6675 µg/kg) and 24 hours (1648 µg/kg) after the last treatment. The mean value decreased to 55 µg/kg after 4 days and to 24 µg/kg after 8 days. The compound was no longer detected in any of the samples collected 28 days after the suspension of the treatment. Lungs showed mean residue values of 2240 µg/kg at 8 hours and 529 µg/kg at 24 hours after the last treatment. In muscle the mean concentration of thiamphenicol was 390 µg/kg at 24 hours and rapidly decrease below the limit of quantification after 4 days. In liver samples, collected 24 hours after the last treatment, the mean thiamphenicol residue value was 111 µg/kg. Thiamphenicol depleted rapidly with values below the limit of quantification at 4 days after the last treatment. In the skin and subcutaneous fat the mean concentration was 32 µg/kg at 4 days and below the limit of quantification in 3 samples out of 4 after 8 day withdrawal period. The injection site showed traces of thiamphenicol still 28 days after the last treatment.

8. Sixteen sheep divided into four groups were treated once a day for 5 consecutive days by intramuscular administration with 30 mg/kg bw/day of thiamphenicol (0.12 ml/kg/day of a commercial product). Samples of kidneys, liver, visceral fat muscle and injection site were collected at 4, 8, 12 ad 16 days after the end of treatment and analysed by HPLC (limit of quantification: 21 µg/kg). Thiamphenicol was detected in 3 kidney samples out of 4 collected after 4 day withdrawal period. The mean thiamphenicol concentration was 40 µg/kg (individual values ranging from 22 to 117 µg/kg). Thiamphenicol was no longer detected in the kidney at the subsequent withdrawal periods. Liver samples did not show detectable levels of thiamphenicol. The mean concentration of residues in the abdominal fat was 342 µg/kg after 4 day withdrawal period. Only thiamphenicol traces were found in 2 fat samples collected 8 and 12 days after the last treatment. After 4 days concentration of 50 µg/kg of thiamphenicol were found in the neck muscle, where the test compound was injected. Quantifiable residues were detected up to 16 days after the end of treatment. In the muscle, thiamphenicol was already not quantifiable after a 4 days withdrawal period.

9. Thiamphenicol was administered mixed with feed to sea bass (*Dicentrarchus labrax*) at the dose of 40 mg/kg bw/day for 5 days. Samples of muscle, liver, skin and vertebrae from 10 fish per timepoint were collected on day 2 and 4 of treatment and on days 1, 2, 3, 5, 7 and 10 after the end of treatment and analysed by HPLC (limit of quantification: 20 µg/kg and 50 µg/kg for skin). Three days after the end of treatment the levels of thiamphenicol ranged between 28 and 30 µg/kg (only three samples out of ten were positive) in muscle and were below the limit of quantification of the HPLC method (50 µg/kg) in skin.

   Residue depletion of thiamphenicol was also studied in sea-bream (*Sparus aurata*) administered with the feed at the dose dose of 40 mg/kg bw/day for 5 days. Samples of muscle, liver, skin and vertebrae from 10 fish per timepoint were collected on day 2 and 4 of treatment and on days 1, 2, 3 and 5 after the end of treatment and analysed by HPLC (limit of quantification: 20 µg/kg for muscle and 50 µg/kg for skin). Three days after the end of treatment the levels of thiamphenicol were 30 µg/kg in muscle (only three samples out of ten were positive) and were below the limit of quantification of the HPLC method in skin.

   Thiamphenicol was administered mixed with feed to young yellowtails at the dose of 45 mg/kg bw/day and 90 mg/kg bw/day for 14 days. Muscle, liver kidney and skin with fat were sampled on the 10^{th} day of administration and 0, 3, 7, 10, 14, 21 and 28 days after the end of treatment. The highest residual levels were found in liver, followed by kidney, skin and muscle and fell below the limit of quantification (20 µg/kg by gas-chromatographic method) in all tissues examined 3 days after the end of treatment.

10. While the available data do not allow a detailed quantitative assessment of the ratio of marker residue to total residue, on the basis of the results of pharmacokinetic and residue studies, thiamphenicol does not undergo major phase I biotransformation processes in the target species as well as in humans. Unchanged thiamphenicol can thus be considered as the marker residue.

11. An HPLC method for the detection of thiamphenicol in plasma and urine of pigs and tissues of pigs and sheep was provided. For all swine matrices, in a range of concentration of 20 to 1000 µg/kg (for urine 200 to 10000 µg/l), the precision was with a coefficient of variation lower than 15% and the accuracy between 89 and 105%. The limit of quantification was 21 µg/kg for all the matrices with the exception of the urine where the limit was 210 µg/l. The limit of detection for the different matrices were the following: muscle, liver, kidney, fat and skin 5 µg/kg; plasma 5µg/l; urine 26 µg/l. For all ovine matrices, in the range 20 to 1000 µg/kg, the precision was with a coefficient of variation lower than 15% and the accuracy between 74 and 107%. The limit of quantification was 21 µg/kg for all the matrices. The limit of detection for the different matrices was the following: muscle and fat 2.6 µg/kg, liver 2.4 µg/kg, kidney 5.2 µg/kg. The parameters as linearity, selectivity, limit of detection, accuracy and precision, limit of quantification and robustness are calculated according Volume VI of the Rules Governing Medicinal Products in the European Community. The specificity of the method has not been validated with regard to florfenicol and chloramphenicol. Moreover, Volume VI requests for swine the quantification of residues in skin plus fat in natural proportion, but for this method the validation was carried out in fat and skin as separate tissues, for this reason the amount of residues in skin plus fat in natural proportion has to be calculated. Moreover, a non-validated HPLC method, with liquid/liquid extraction, for the detection of thiamphenicol in muscle and skin of sea-bream *(Sparus aurata)* with quantification limits of 20 µg/kg and 50 µg/kg, respectively, was provided. The limit of detection was not reported in the study report.

Conclusion and recommendation

Having considered that:

- and ADI of 2.5 µg/kg bw, i.e. 150 µg for a 60 kg person was established,
- thiamphenicol is the marker residue,
- in pigs and sheep, 8 days after the end of the treatment the mean concentrations of residues were below the limit of quantification of the analytical method (21 µg/kg) in almost all of the samples analysed, and in fin fish residues in skin and muscle were below the limit of quantification of the analytical method (20 µg/kg in muscle and 50 µg/kg in skin) already 3 days after the last treatment,
- an analytical method is available for monitoring residues in all edible porcine, ovine and fin fish tissues, but it has not been fully validated;

the Committee 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 1.1.2001
		Ovine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	
		Fin Fish	50 µg/kg	Muscle and skin in natural proportions	

Based on these MRLs it was estimated that the daily consumer intake of thiamphenicol residues from either edible tissues and 1.5 l milk will amount to 0.1 mg/day, representing about 67% of the ADI.

Before the Committee can consider the inclusion of thiamphenicol in Annex I to 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 data allowing to establish the ratio of marker towards microbiologically active residues in relevant target tissues of target species (muscle, skin+fat, liver and kidney for pigs; muscle and skin in natural proportion for fin fish).

2. The Applicant should re-submit the routine analytical method for porcine and ovine tissues providing the validation data for the specificity of the method with regard to florfenicol and chloramphenicol and the validation data for skin plus fat in natural proportion, in pigs.

3. The Applicant should provide a validated method for the monitoring of residues in fin fish tissues, considering that residues in fish have to be quantified in skin and muscle in natural proportion.