European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TULATHROMYCIN

SUMMARY REPORT (1)

1. Tulathromycin is a semi-synthetic macrolide (CAS 217500-96-4) prepared by fermentation followed by organic synthesis. It is a member of the triamilide subclass of macrolide antibiotics. Tulathromycin is a mixture of two isomers: 2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-dideoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyranosyl]oxy-] 2-ethyl-3,4,10-trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one (15-membered macrocyclic ring) and (2*R*,3*R*,6*R*,8*R*,9*R*,10*S*,11*S*,12*R*)-11-[[2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-4-*C*-[(propylamino)methyl]-α-*L*-*ribo*-hexopyranosyl[oxy]-2-[(1*R*,2*R*)-1,2-dihydroxy-1-methylbutyl]-8-hydroxy-3,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-4-azacyclotridecan-13-one (translactonized 13-membered macrocyclic ring). The 13 or 15 member macrocyclic ring is referred to either as the macrocyclic ring or as the aglycone. Two carbohydrate moieties are connected to the aglycone by an ether linkage, a desosamine carbohydrate moiety and a modified cladinose carbohydrate moiety. In solution, the two isomers form a stable equilibrated mixture which is considered as the active substance. Typical lots of tulathromycin consist of 90% 2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyranosyl)oxy-2-ethyl-3,4,10-trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one and 10% (2*R*,3*R*,6*R*,8*R*,9*R*,10*S*,11*S*,12*R*)-11-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-4-*C*-[(propylamino)methyl]-α-*L*-*ribo*-hexopyranosyl)oxy] -2-[(1*R*,2*R*)-1,2-dihydroxy-1-methylbutyl]-8-hydroxy-3,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy -3-(dimethylamino)-β-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-4-azacyclotridecan-13-one.

   Tulathromycin is intended for treatment of bacterial respiratory disease in cattle and pigs. The dose is a single-dose of 2.5 mg/kg bw by subcutaneous injection to cattle and by intramuscular injection to pigs. The product is not intended for use in lactating cattle.

2. As for other macrolides, the principal mechanism of action against bacteria involves direct inhibition of essential protein biosynthesis by selective binding to bacterial 50S ribosomal subunits. Macrolides acts by stimulating the dissociation of peptidyl-tRNA from the ribosome during the translocation process. Results from oral acute and subchronic toxicology studies suggested that, apart from modest gastrointestinal disturbances, tulathromycin does not appear to exert remarkable secondary pharmacological activity on major organ systems including neurological, cardiovascular and renal effects. However, the available pharmacodynamic data were not considered sufficient to address the range of adverse reactions occasionally reported in humans treated with therapeutic doses of related macrolide antibiotics.

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3. Pharmacokinetic studies in pre-ruminant and ruminant cattle and in pigs at the recommended dosage (2.5 mg/kg bw, single subcutaneous dose in cattle, intramuscularly in pigs) indicated rapid absorption from the injection site. Absolute intramuscular bioavailability was greater than 80%. In both species, pharmacokinetics was characterised by a long plasma elimination half-life of more than 70 hours and a relatively large apparent volume of distribution of more than 10 l/kg. This is consistent with the observation of significant tissue distribution. The elimination half-life in the lung tissue, the therapeutic target tissue, was exceptionally long with 6 and 8 days in pigs and cattle, respectively. In accordance with this observation, pharmacokinetic data in the rat and dog following oral administration in the toxicology studies showed elevated levels in lung tissue. This was evident from lung/plasma concentration ratios and indicated accumulation of the drug in lung tissue which typically occurred in the earlier phases of studies and was less pronounced during the latter phases.

   An oral bioavailability studies were performed in pigs: comparing plasma concentrations and plasma AUCs for 7 days after intramuscular and oral administration of a single dose of 2.5 mg/kg bw and also describing the excretion of a single oral dose of 2.5 mg/kg bw over 14 days. As compared to intramuscular administration plasma levels were significantly lower after oral use. Concentrations in lung tissue after oral administration were higher as compared with plasma concentrations but did not reach lung concentrations observed after parenteral administration. These data suggested lower availability of the drug following oral administration.

   The metabolites of ^{14}C-tulathromycin in the excreta of cattle and pigs (dosed at 2.5 mg/kg bw, sucutaneously or intramusculary, respectively) and laboratory species dog and rat (15 mg/kg or 50 mg/kg, orally for two consecutive days, respectively) were similar with the parent compound being metabolised to a low extent and eliminated primarily as the unchanged drug. Likewise, the major component in the liver and bile of each species (also in all other edible tissues of the target species as demonstrated in the radiolabelled residue studies) was the unchanged drug. Small quantities (less than 10% each) of the metabolites in excreta and tissue samples of all four species included N-demethylation or N-oxidation of the desosamine portion of the molecule, cleavage of the modified cladinose moiety, N-depropylation of the cladinose moiety, and ester hydrolysis of the macrocyclic ring. Some minor metabolites derived from combinations of oxidation and/or N-dealkylation processes were only detectable in cattle. These metabolites were not considered to be uniquely cattle specific as all of the metabolic processes represented by these metabolites were also observed in dogs, rats or pigs. In conclusion, although metabolism was limited, the compound was found to be metabolised in a similar manner in rats, dogs, pigs, and cattle.

4. Consistent with the observations for blood pharmacokinetic parameters, excretion of radiolabelled ^{14}C-tulathromycin in cattle and pigs was relatively slow but almost complete. In pigs, faeces contained about 2/3 and urine about 1/3 of the administered dose within 23 days. In cattle, excretion was even somewhat slower (about 70% within 47 days) with the excreted dose being nearly equally divided between urine (40%) and faeces (32%). No formal radiolabelled studies on excretion balance in rats and dogs or target species following oral administration were available. Some preliminary excretion data were however collected as part of above metabolism studies in rats and dogs: Over the relatively short 28 hours period examined, excretion was incomplete and only approximately 15 to 26% of the dose was recovered. Rats appeared to eliminate approximately 4% and 95% of the collected radioactivity in urine and faeces, respectively, dogs approximately 19% and 75%. In the unlabelled oral study in pigs (2.5 mg/kg bw) 30 to 50 % parent compound was found to be excreted via faeces and only about 1% via urine. In this experiment, about 40% of the administered dose could not be accounted for.

5. The results of single dose studies in rats and dogs using oral and intravenous administration indicated low acute toxicity of tulathromycin by the oral route, and intermediate toxicity by the intravenous route. Following oral administration, the lethal doses were estimated to be greater than 1000 mg/kg bw in dogs (100, 300 and 1000 mg tulathromycin/kg bw, 2 animals per sex and dose level) and greater than 2000 mg/kg bw in rats (100, 300, 1000, 2000 mg/kg bw, 3 animals per sex and dose), i.e. higher than the maximum doses tested. However, vomiting occurred in dogs and no information on the amount of oral dose possibly lost was provided. Effects at 100 mg/kg bw were limited to mild gastrointestinal disturbances such as emesis. No drug-related effects on respiration rate, body temperature, blood pressure, heart rate, or electrocardiographic parameters were noted up to and including 1000 mg/kg bw. The highest asymptomatic dose in rats was 300 mg/kg bw based on findings as loose stools, elevated leukocytic and erythrocytic parameters and elevated hepatic enzymes (alanine aminotrasferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SHD)) at 1000 mg/kg bw and/or 2000 mg/kg bw.

   The maximum non-lethal dose by intravenous administration was 10 mg/kg bw for both dogs (1 and 10 mg/kg bw; 2 animals per sex and dose level and 30 mg/kg bw; 1 male) and male rats (0.1, 1.0, 10, 30 and 100 mg tulathromycin/kg bw, 3 males per dose). In male rats (females not tested) doses of 10 mg/kg were shown to be asymptomatic. The maximum asymptomatic dose in dogs was less than 1.0 mg/kg based on findings at 1.0 and 10 mg/kg which were indicative of gastrointestinal disturbances (loose stools). Higher intravenous doses led to deaths in case of male rats and collapse with apnoea in the single male dog tested.

   In an acute dermal toxicity study in 3 male and 3 female rabbits no mortality occurred at 24 hour exposure to 2000 mg tulathromycin/kg bw. Slight oedema and slight skin desquamation together with decreased food consumption and decreased defecation were the only observations in these animals.

6. Oral gavage repeated dose toxicity studies were carried out in rats. In the 1-month rat study (10, 50 and 200 mg tulathromycin/kg bw/day, 10 animals per sex and dose), effects included elevations in hepatic transaminase enzymes, decrease in relative liver weights, and elevations in monocyte and eosinophil counts at 200 mg/kg. Further observations were significantly decreased serum bile acids in males at all dose levels and significantly increased alanine aminotrasferase (ALT) values in females at the two higher doses. All findings were without histological correlates and the dose of 10 mg/kg bw may be established as a NOEL based on the minor serum chemistry changes recorded in males at this dose. In the 3-month rat study (5, 15 and 100 mg/kg bw/day, 20 animals per sex and dose level), changes noted at the two higher dose levels at one/two of three occasions included: slight elevations in hepatic transaminase enzymes as aspartate aminotransferase (AST) and alanine aminotramsferase (ALT), decreased gamma-glutamyltransferase (GGT) levels and decreases in serum proteins. Furthermore, at one/two of three occasions in male rats minor decreases in serum bile acids were recorded that reached statistical significance at day 91 in the lowest dose group and at two occasions at 100 mg/kg bw (day 57 and 91) but not in the mid-dose group. In female rats a very slightly but significantly increased sorbitol dehydrogenase (SHD) level was observed at one occasion (day 57) in all dose groups. No dose and/or time-relationship was apparent for these effects. All of these findings were without any histological correlates up to the highest dose tested. No overt induction of cytochrome P-450 and P-420 was observed at the end of treatment. Based on described marginal serum chemistry observations, the dose of 5 mg/kg bw may considered as a NOEL.

7. Oral gavage repeated dose toxicity studies were carried out in dogs. In the 1-month study in Beagle dogs (5, 15 and 50 mg tulathromycin/kg bw/day, 4 animals per sex and dose level) effects included elevations in AST and ALT, decreases in serum proteins, increase in absolute and relative kidney weights, and sporadic occurrence of loose stools at 50 mg/kg bw. These findings were without histological correlates. A NOEL of 15 mg/kg bw was established. Effects in the 3-month dog study (5, 15 and 50 mg tulathromycin/kg bw/day, 4 animals per sex and dose) included elevations in AST and ALT and sporadic occurrences of loose stools and emesis.

These findings (without histological correlates) were noted at 50 mg/kg with elevations in AST and ALT in one dog at 15 mg/kg (day 85). Ophthalmoscopic changes were observed in two dogs only at 15 mg/kg bw. This observation was not dose- or time-related, was unilateral, resolving in one animal and lacked a microscopic correlate. However, as retinal detachment was rarely seen in this dog breed, its relation to drug treatment is unclear but cannot be excluded. No overt induction of cytochrome P-450 and P-420 was observed at the end of the 3-month treatment. It is noted that gavage treatment in the dogs was done 1 hour post feeding, possibly to prevent vomiting in fasted animals. In both the 1- and 3-month studies in dogs, no drug-related effects on heart rate, respiration rate, body temperature, blood pressure or electrocardiographic parameters were seen at doses up to and including the highest dose tested. The NOEL was 5 mg/kg bw based on described elevation of hepatic transaminase enzymes noted in one dog and the equivocal ophthalmoscopic changes noted in two dogs at the dose of 15 mg/kg bw. In both the 1- and 3-month studies in dogs, no drug-related effects on heart rate, respiration rate, body temperature, blood pressure or electrocardiographic parameters were seen at doses up to and including the highest dose tested.

8. A two generation reproductive study in rats was performed at gavage doses of 15, 50 and 100 mg/kg bw (F0 and F1 parental, 30 animals per sex and group). There were changes in parameters such as reductions in mean liver weights at all dose levels (F0 males/females and F1 males) and increases in adrenal weights in both F0 and F1 generations at 100 mg/kg bw. Further, some serum chemistry findings in the F0 generation were noted (increases in mean AST in the 100 mg/kg bw group males, decreases in mean urea nitrogen at all dose levels in males, and decreases in total protein levels at 100 and 50 mg/kg bw). No histological correlates of any of the alterations were found. Mean F1 offspring body weight gains were increased at all dose levels but this was not considered a signal of toxicity. No adverse effects on fertility or neonatal parameters were noted. A NOEL for parental toxicity in this study may be estimated with 15 mg/kg/day based on slight liver weight changes. The NOEL for reproductive and neonatal toxicity was 100 mg/kg bw/day.

9. In a preliminary oral gavage embryo/foetal and early postnatal development study, rats were dosed with 50, 100, 200 and 500 mg tulathromycin/kg bw from gestation day 6 to 17 (8 females per group) or from gestation day 6 through lactation day 11 (8 females per group). Adverse findings noted in both the foetal development and postnatal phases at 500 mg/kg bw included high maternal mortality (25 and 63 percent in the two parts of the study, respectively), decreased maternal body weight and body weight gain and decreased food consumption. There were no adverse effects on foetal survival, foetal malformations, postnatal survival or gross examination of surviving dams at termination at any dose level. Mean pup weights were reduced throughout the postnatal phase at 500 mg/kg bw. Oral gavage doses of 15, 100 and 200 mg tulathromycin/kg bw were administered in the definitive rat embyro/foetal development study (22 animals per dose; gestation day 6 through 17). Mean daily food consumption at days 6 to 18 and net body weight change of dams were reduced at the higher doses. Compared to concurrent controls, percent of viable foetuses was lower and post-implantation loss was increased at the two higher doses. Mean foetal weights were very slightly but significantly reduced in all treated groups. No dose-relationship was apparent for this observation and it was not considered of biological relevance. There were no adverse effects on maternal reproductive parameters and foetal malformations at any dose level investigated. The NOEL for maternal toxicity and the NOEL for foetal effects in this study was 15 mg/kg bw.

10. In a preliminary oral gavage embryo/foetal development study in rabbits, tulathromycin administered at doses of 15, 50 and 75 mg/kg bw (during gestation day 7 through 20, 6 animals per dose) produced decreases in maternal body weight or body weight gain at 50 and 75 mg/kg bw. Decreased food consumption was observed in one animal at each of these dose levels. Foetal weights and gravid uterine weights were also decreased at 75 mg/kg bw.

Post-implantation loss was increased at the two higher dose levels. There were no adverse effects on foetal survival, foetal malformations or changes in results for gross examination of surviving dams at termination, at any dose level. In a definite embryo/foetal development study, doses of 5, 15 and 50 mg/kg bw (given per gavage during gestation day 7 through 20; 22 animals animals per dose) were used. There were no adverse effects on maternal reproductive parameters, foetal survival, foetal body weight, and external, visceral or skeletal findings in foetuses at any dose level. Two dams at the highest dose did not survive until the end of study. Findings regarding body weights or weight gain observed in the preliminary study at the 50 mg/kg dose were not observed. The results from both studies indicated a conservative overall NOEL of 15 mg/kg bw for maternal and foetal effects.

11. Tulathromycin was tested in a comprehensive series of mutagenicity test systems: *in vitro* with the microbial mutation assay, the mammalian cell (CHO/HGPRT) mutation assay, the chromosome aberration assay with human lymphocytes and *in vivo* with the rat micronucleus assay. Tulathromycin did not induce gene mutations, with or without metabolic activation, in either the bacterial or mammalian cell mutation assays. Tulathromycin did not demonstrate clastogenic activity, with or without metabolic activation in human lymphocytes and was negative in the rat micronucleus assay. The results of the genetic toxicology assays indicated that tulathromycin is not genotoxic.

12. Due to the absence of a chemical structural relationship to known carcinogens, the negative results of genotoxic assays, and the lack of a carcinogenic potential of other macrolide antibiotics it was assumed that tulathromycin is devoid of a carcinogenic risk.

13. No specific studies on neurotoxicity were provided. Based on results of the acute and subchronic toxicology studies in rats and dogs, target species safety studies in cattle and pigs and the available scientific literature on macrolide antimicrobials, it was concluded that tulathromycin is not likely to produce adverse neurotoxic effects.

14. Tulathromycin was evaluated for skin and ocular irritation in albino rabbits. The compound proved to be neither a corrosive material nor a skin irritant. Tulathromycin produced severe eye irritation characterised by iritis and substantial but reversible conjunctivitis and corneal opacity. The results indicated that tulathromycin is an ocular irritant in rabbits. The potential of tulathromycin to produce sensitisation following topical exposure was evaluated in guinea pigs given a combination of intradermal injections and topical applications (maximation study design). A positive reaction was produced in nearly all test animals (9 of 10) at the 24 and 48-hour scoring intervals, indicating that tulathromycin can be considered a contact sensitizer in guinea pigs. Tulathromycin can be considered an ocular irritant to rabbits and a contact sensitizer in guinea pigs.

15. Only a small number of target animal safety studies (cattle only) were available. Cardiotoxicity with degenerative multifocal damage of the myocardium was observed in individual 6-months old calves at a single subcutaneous dose of 12.5 mg/kg bw and 15 mg/kg bw. A significant increase of creatine phosphokinase and lactate dehydrogenase was observed at all dose levels tested, beginning with the lowest dose of 10 mg/kg bw. No signs of myocardial damage were observed in other studies at 7.5 mg/kg bw in 4 to 6 weeks old calves and at 12.5 mg/kg bw in 8 months old calves.

16. Data on observations in humans were not available, as tulathromycin was developed solely for veterinary use. Tulathromycin is chemically closely related to the macrolide azithromycin which is considered the parent compound of tulathromycin. Azithromycin is an antibiotic widely used in the treatment of human infections.

17. A toxicological ADI of 0.05 mg/kg bw/day (3 mg/person) was established for tulathromycin based on the NOEL of 5 mg/kg bw from the 3-month subchronic toxicity study in dogs and rats and a safety factor of 100. In the embryo/foetal development studies and the reproductive 2-generation study, no effect doses were about 300 times higher than this ADI.

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

$$\text{ADI} = (\mu g/kg\ bw) = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{CF1}\ (\mu g/ml) \times \text{daily faecal bolus (220 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

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

$$\text{ADI} = \frac{\dfrac{3.74 \times 2 \times 220\ g}{5}}{0.5 \times 60\ kg} = 10.97\ \mu g/kg\ bw \text{ (i.e. 660 } \mu g/60 \text{ kg person)}$$

The calculation of the microbiological ADI was based on MIC data generated for a range of relevant genera derived from the human gastro-intestinal tract (100 strains) using two test inocula that differed by 100-fold. The lowest MIC_{50} (median) values (pH 7.2) were found at the lowest inoculum size (10^4 to 10^7 cfu/ml) tested and ranged from *Bifidobacterium* (0.75 µg/ml), *Fusobacterium* (2 µg/ml), *Enterococcus* (3 µg/ml), *Escherichia* (4 µg/ml), *Lactobacillus* (8 µg/ml), *Clostridium* (16 µg/ml), *Eubacterium* (16 µg/ml), *Peptostreptococcus* (16 µg/ml), Bacteroides (64 µg/ml) to Proteus (higher than 128 µg/ml). The geometric mean MIC_{50} (excluding non-susceptible *Proteus* ssp) was 6.96 µg/ml with a 10% lower confidence limit of 3.74 µg/ml.

The following assumptions were made:

- MIC_{50} = 3.74: For calculation of the geometric mean MIC_{50} the lower inoculum in the susceptibility tests was used and the 10% lower confidence limit was calculated.
- CF1 = 5: tulathromycin has the same mechanism of action as other macrolides, and strains resistant to macrolides can be cross-resistant to tulathromycin. Resistance to tulathromycin may be expected to be both chromosomal and plasmid-mediated, as has been demonstrated for macrolides in general, thus the factor CF1 was set conservatively at 5.
- CF2 = 2: The correction factor CF2 includes an adjustment for differences in *in vitro* growth/MIC test conditions compared to *in vivo* conditions (e.g. bacterial density and pH in the colon). A combined factor of 2 was estimated to account for both effects (i.e. observed 2-fold MIC increase for an increase in inoculum size of 100; from10^4 to 10^7 or 10^6 to 10^9 cfu/ml) plus an observed moderate to significant increase in MIC values (approximately 3-30 fold) for selected sensitive strains when tests were conducted at pH 6.5 (pH expected in the colon).
- Fraction of the oral dose available for microorganisms in the intestinal tract = 0.5: This factor was based on combined results for *in vitro* sorption/desorption studies and MIC determinations in presence of human faeces which both suggested significant binding/inactivation of microbiological activity of at least 50% to higher than 0.75%. Though systemic absorption can also be supposed, available pharmacokinetic data on systemic availability were too limited to be included in this correction factor.
- 220 ml (g) = standard weight of the daily faecal bolus.

19. A toxicological ADI of 0.05 mg/kg bw/day (i.e. 3 mg/person) and a microbiological ADI of 10.97 µg/kg bw (i.e. 660 µg/person) were established. Specific studies were not conducted in order to establish a pharmacological ADI. However, the results of the repeated dose toxicity studies showed no evidence of the secondary pharmacodynamic effects observed with some other members of the macrolide group (cardiovascular effects, prokinetic effects, effects on microsomal enzymes or phospholipidosis) at doses at least 300 and 1500 times higher than the respective toxicological and microbiological ADIs. As the microbiological ADI is lower than toxicological ADI the microbiological ADI was considered the relevant ADI for assessing the risk for the consumer.

20. In a radiometric residue depletion study, 16 pigs were given a single intramuscular dose of 2.5 mg ^{14}C-tulathromycin/kg bw. Four pigs (2 animals per sex) were killed on days 4, 12, 24, and 36 after treatment. The average total residue concentrations in liver were 2850, 1390, 565 and 196 µg equivalents/kg on days 4, 12, 24 and 36 after treatment. At the same time points, average total residue concentrations were 6610, 2500, 793 and 266 µg equivalents/kg in kidney; 613, 124, 58 and less than 40 µg equivalents/kg in muscle; and 478, 178, 100 and less than 79 µg equivalents/kg in skin+fat. At injection sites the average total residue concentrations were 4730, 2440, 1400 and 760 µg equivalents/kg on days 4, 12, 24 and 36 after treatment. Analysis of tissues for tulathromycin showed that average ratios of unchanged drug to total residues across all time points were 0.96, 1.02, 0.96, 1.03 and 0.18 for liver, kidney, muscle, injection site and skin+fat, respectively. In each tissue, the ratios were relatively constant over time. Analysis of tissues for marker residue (the sum of residues which may be hydrolysed to (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents) yielded results comparable to those observed by the parent drug procedure. Average ratios of marker to total residues across all time points were 0.94, 0.83, 0.86, 0.89 and 0.28 for liver, kidney, muscle, injection site and skin+fat, respectively.

21. In a radiometric residue depletion study, 24 calves were given a single subcutaneous dose of 2.5 mg ^{14}C-tulathromycin/kg bw. Four calves (2 animals per sex) were killed on days 0.5, 5, 15, 25, 36 and 48 after treatment. Average total residue concentrations in liver were 6400, 13 000, 6400, 5000, 3600 and 1200 µg equivalents/kg on days 0.5, 5, 15, 25, 36 and 48 after treatment. At the same time points average total residues concentrations were 7300, 7500, 2700, 1300, 620 and 250 µg equivalents in kidney; 1800, 1120, 180, 67, less than 26 µg, and less than 26 µg equivalents/kg in muscle; and 560, 500, 210, 104, 50 and less than 50 µg equivalents/kg in fat. At injection sites the average total residue concentrations were 200 000, 13 000, 6000, 2500, 1800 and 700 µg equivalents/kg on days 0.5, 5, 15, 25, 36 and 48 after treatment. Analysis of tissues for tulathromycin showed that average ratios of unchanged drug to total residues across all time points were 0.40, 0.62, 0.71, 0.77 and 0.25 for liver, kidney, muscle, injection site and fat, respectively. Analysis of tissues for marker residue (the sum of residues which may be hydrolysed to CP-(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents) yielded results higher than those observed for the parent drug procedure. Average ratios of marker to total residues across all time points were 0.61, 0.78, 0.79, 0.91 and 0.46 for liver, kidney, muscle, injection site and fat, respectively.

22. In a non-radiometric residue depletion study, 30 pigs were give a single intramuscular dose of 2.5 mg tulathromycin/kg bw. Six pigs (3 animals per sex) were killed on days 5, 12, 18, 25 and 36 after treatment. Tissue samples were analysed for marker residue concentrations by high performance liquid chromatography with tandem-linked mass spectrometry (HPLC/MS/MS) with limits of quantification of 90, 60, 70, 6, 3 µg/kg for liver, injection site, kidney, muscle and skin+fat respectively. Average marker residue concentrations in liver were 1700, 960, 730, 280 and 150 µg/kg on days 5, 12, 18, 25 and 36 after treatment. At the same time points average marker residue concentrations were 2900, 1200, 800, 310 and 170 µg/kg in kidney; 440, 95, 70, 35 and 18 µg/kg in muscle; and 230, 110, 60, 20 and 15 µg/kg in skin+fat. At injection sites the average marker residue concentrations were 2300, 1500, 1100, 500 and 600 µg/kg on days 5, 12, 18, 25 and 36 after treatment.

23. In a non-radiometric residue depletion study, 36 ruminant calves were given a single subcutaneous dose of 2.5 mg tulathromycin/kg bw. Six calves (3 animals per sex) were killed on days 5, 12, 18, 25, 36 and 48 after treatment. Tissue samples were analysed for marker residue concentrations by HPLC/MS/MS with limits of quantification of 90, 60, 70, 6, 3 µg/kg for liver, injection site, kidney, muscle and fat respectively. Average marker residue concentrations in liver were 5600, 3900, 3200, 2400, 1200 and 650 µg/kg on days 5, 12, 18, 25, 36 and 48 after treatment. At the same time points average marker residue concentrations were 4600, 2500, 1300, 700, 400 and 210 µg/kg in kidney; 550, 170, 89, 50, 19 and 9 µg/kg in muscle; and 260, 130, 100, 42, 21 and 8 µg/kg in fat. At injection sites the average marker residue concentrations were 5100, 3200, 2300, 800, 900 and 500 µg/kg on days 5, 12, 18, 25, 36 and 48 after treatment.

24. Routine analytical methods based on HPLC/MS/MS were presented in the ISO 78/2 format. The marker residue used to determine the concentrations of tulathromycin residue in edible tissues of cattle and pigs was the sum of residues which may be hydrolysed to (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclo-pentadecan-15-one expressed as tulathromycin equivalents. These methods had not been sufficiently validated to meet the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Community with regard to 'specificity'. The limits of quantification of the method for pig tissues were 50 µg/kg for liver, 100 µg/kg for kidney and injection site and 20 µg/kg for muscle and skin + fat. The limits of quantification of the method for cattle tissues were 300 µg/kg for liver and injection site, 200 µg/kg for kidney, 30 µg/kg for muscle and 60 µg/kg for fat.

Conclusions and recommendation

Having considered that:

- an ADI of 0.011 mg/kg bw (i.e. 0.66 mg/person) was established for tulathromycin,
- total residues in standard edible tissues of cattle or pigs (excluding the injection site residues) were below the ADI at days 25 and 12 after treatment for cattle and pigs, respectively therefore, tissue residue distribution around these time points was used in the estimation of MRLs,
- the sum of residues which may be hydrolysed to (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethyl-amino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents was identified as the marker residue,
- the tissue distribution of residues and the overall ratios marker/total residues were constant over time and sufficiently similar in both species to justify setting of uniform MRLs,
- residue concentrations were persistently low in fat and muscle and fat was chosen as the target tissue representing the carcass with an MRL set at approximately twice the LOQ,
- there was a validated method based on LC/MS/MS for the determination of the marker residue in edible tissues of cattle and pigs, however specificity relative to tilmicosin, spiramycin, josamycin and acetylisovaleryltylosin was not demonstrated;

the Committee recommends the inclusion of tulathromycin 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
Tulathromycin	(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents	Bovine	100 µg/kg 3000 µg/kg 3000 µg/kg	Fat Liver Kidney	Not for use in animals producing milk for human consumption Provisonal MRLs expire on 1.7.2004
		Porcine	100 µg/kg 3000 µg/kg 3000 µg/kg	Skin + fat Liver Kidney	Provisonal MRLs expire on 1.7.2004

Based on these MRLs, it was calculated that the consumer intake of total residues from the consumption of tissues from cattle and pigs would account for approximately 95% of the ADI mentioned above

The residues at the injection site have to be taken into account when withdrawal periods are set to ensure that the residues in the total food package including the injection site do not exceed the ADI.

LIST OF QUESTIONS

1. The applicant should provide validation of the analytical method in relation to specificity in respect of the veterinary macrolides tilmicosin, spiramycin, josamycin and acetylisovaleryltylosin.