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Committee for Medicinal Products for Veterinary Use (CVMP)

European public MRL assessment report (EPMAR)

Tulathromycin (modification of the microbiological ADI and MRLs in bovine and porcine species)

On 18 December 2014 the European Commission adopted a Regulation¹ modifying maximum residue limits for tulathromycin in bovine and porcine species, valid throughout the European Union. This modification was based on the favourable opinion and the assessment report adopted by the CVMP.

Tulathromycin is used in bovine and porcine species for the treatment and prevention of bacterial and mycoplasmal infections administered by single subcutaneous injection in bovine species and intramuscular injection in porcine species.

Maximum residue limits had previously been established² in bovine and porcine species.

Pfizer Animal Health SA submitted the application for the modification of the microbiological ADI and maximum residue limits to the European Medicines Agency, on 2 February 2012.

Based on the original and complementary data in the dossier, the CVMP recommended on 10 October 2013 the modification of the microbiological ADI and of the maximum residue limits for tulathromycin in bovine and porcine species. The recommendation was for provisional MRLs.

Subsequently the Commission recommended on 28 October 2014 that provisional maximum residue limits in bovine and porcine species are established. This recommendation was confirmed on 4 November 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 18 December 2014.

¹ Commission Implementing Regulation (EU) No 1359/2014, O.J. L 365, of 19.12.2014

² Commission Regulation (EC) No 1101/2004, O.J. L211, of 12.06.2004



European public MRL assessment report (EPMAR)

Tulathromycin (modification of the ADI and MRLs in bovine and porcine species)

Summary of the scientific discussion for the establishment of MRLs

Substance name:	Tulathromycin
Therapeutic class:	Anti-infectious agents/Antibiotics
Procedure number:	EU/12/199/PFZ
Applicant:	Zoetis Belgium SA
Target species:	Bovine, Porcine
Intended therapeutic indication:	Treatment and prevention of bacterial and mycoplasmal infections
Route(s) of administration:	Single subcutaneous injection (bovine species); intramuscular injection (porcine species)

1. Introduction

Tulathromycin is a semi-synthetic macrolide (CAS 217500-96-4) prepared by fermentation followed by organic synthesis. The substance is a member of the triamilide subclass of macrolide antibiotics.

Tulathromycin is used in bovine and porcine species for the treatment and prevention of bacterial and mycoplasmal infections administered by single subcutaneous injection in bovine species and intramuscular injection in porcine species.

Tulathromycin was previously assessed by the Committee for Medicinal Products for Veterinary Use (CVMP) and 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; the microbiological ADI was considered the relevant ADI.

Currently, tulathromycin is included in Table 1 of Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
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-xylohexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents	Bovine	100 µg/kg 3 000 µg/kg 3 000 µg/kg	Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents/ Antibiotics
		Porcine	100 µg/kg 3 000 µg/kg 3 000 µg/kg	Skin and fat Liver Kidney		

Pfizer Animal Health submitted an application for the modification of the ADI and maximum residue limits to the European Medicines Agency, on 2 February 2012. The company subsequently changed the name to Zoetis Belgium SA.

2. Scientific risk assessment

2.1. Safety assessment

Tulathromycin was previously assessed by the CVMP and 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; the microbiological ADI was considered the relevant ADI.

New data on disruption of the colonisation barrier and increase of the population of resistant bacteria and a re-evaluation of previously submitted studies on disruption of the colonisation barrier have been submitted in support of the modification of the microbiological ADI.

The safety evaluation previously carried out by the CVMP is reported in detail in the MRL summary report EMEA/CVMP/894/04-FINAL. The report provided below concerns the evaluation of the new data provided and the re-evaluation of relevant data for the establishment of the microbiological ADI previously assessed.

2.1.1. Overview of microbiological properties of residues

Disruption of the colonisation barrier

For the evaluation of the microbiological effects of tulathromycin several studies were previously submitted, including *in vitro* testing of bacteria representing the human gut flora, adsorption/desorption studies to investigate binding/adsorption to faecal contents, gut modeling data to further evaluate the activity of tulathromycin when present in animal derived food and *in vivo* studies to provide excretion data for orally administered tulathromycin.

The minimum inhibitory concentration (MIC) of tulathromycin against 10 isolates from each of 10 genera regarded as dominant in the human faecal microbiota were previously assessed in accordance with CLSI standards and VICH GL 36. The lowest MIC₅₀ (median) values (pH 7.2) at the lowest inoculum size (10⁴ to 10⁷ cfu/ml) 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 MIC₅₀ (excluding non-susceptible *Proteus* ssp) was 6.96 µg/ml with a 90% lower confidence limit of 3.74 µg/ml. *In vitro* studies were provided to investigate the impact of differing pH values on the activity of tulathromycin. In the first study, activity against 4 strains of *E. coli*, 4 strains of *Enterococcus* and 4 strains of *Bifidobacterium* were tested; a supplementary study investigated the effects on the activity towards 10 strains of *Fusobacterium*. The lowering of pH from 7.2 to 6.5-6.6 caused a substantial decrease in activity against all *E. coli* and *Enterococcus* strains. The activity against *Bifidobacterium* and *Fusobacterium* strains was variable, strain dependent but with a tendency to decreased activity (up to three-fold). Antimicrobial activity of tulathromycin is known to be pH sensitive as the substance is ionized and, thus, less bioavailable under acidic conditions. As the majority of the colonic compartments in humans has an acidic milieu (pH between 6.5 and 6.7), the overall results suggested that the availability of the drug for uptake by bacteria is likely to be significantly reduced under physiological conditions. Another experiment investigated the impact of faecal matter on the activity of tulathromycin *in vitro*. The study was carried out with 4 *Escherichia coli*, 4 *Enterococcus* and 4 *Bifidobacterium* strains in the presence or absence of diluted-heat-sterilised human faeces. The activity of tulathromycin against all strains tested was found to be significantly decreased in the presence of faecal suspensions.

The effect of faecal material on the activity of tulathromycin in faecal slurries at different temperatures (20°C and 37°C) was also examined in two GLP compliant sorption/desorption studies using radiolabelled tulathromycin. The faeces were derived from 5 healthy human donors (one and four donors in the first and second study, respectively). In the first experiment at 20°C, tulathromycin was found to sorb to human faeces at a rate of approximately 70% in equilibrium. At 37°C, to better simulate the *in vivo* conditions in the gastrointestinal tract, sorption was calculated with 85%.

Results from both the studies for activity in the presence of faeces and the sorption/desorption experiments were indicative of a matrix effect on activity/solubility of tulathromycin in the presence of organic material/faecal matter. The relevance of these findings in (partly) sterilised and diluted faeces to the *in vivo* situation is difficult to assess, especially as the nature and reversibility of the interaction was unknown and as information to assess factors such as individual variability of composition of faeces and bacterial species was limited. The results were, therefore, interpreted with caution in the calculation of the ADI for colonisation barrier effects.

The fraction of the dose reaching the human gut following oral exposure was investigated in *in vivo* studies using either radiolabelled (rats and dogs) or unlabelled tulathromycin (pigs). In rats and dogs treated orally, excretion was incomplete and only approximately 15 to 26% of the dose was recovered over a 28 hours period. The results indicated, however, that the substance is predominately excreted in faeces. Rats appeared to eliminate approximately 4% and 95% of the collected radioactivity in urine and faeces, dogs approximately 19% and 75% respectively. 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. These results indicate a high variability of excretion of the substance after oral exposure in different species, which may partly be attributed to differences in the experimental design. The overall excretion profile suggested that most of the dose would be excreted in faeces with only negligible amounts in urine.

The activity of tulathromycin in the presence of cooked meat was assessed in two studies in an *in vitro* human gut model. Tulathromycin was added to cooked meat medium at concentrations of 0, 2, 8, 10 and 20 µ/ml (2 µg/ml approximately geometric mean MIC for the test strains). Test preparations were then successively incubated with pepsin, salts and pancreatin, pH adjusted and finally inoculated with the test bacterial culture (*Fusobacterium*, *Bifidobacterium* strains). Final viable counts obtained after 18 hours incubation of each test strain in the presence of tulathromycin were comparable with those obtained in the absence of antimicrobial compound at all concentration levels. These studies are considered supportive information reflecting the overall observation that in the presence of organic matter antimicrobial activity may be reduced. A factor for bioavailability of residues in animal derived food is, by convention, not considered in the MRL assessment.

Increase of the population of resistant bacteria

Three studies not previously assessed in the procedure for the establishment of MRLs have been provided. With these data, an increase in the population of resistant bacteria in the human colon cannot be directly assessed. Rather the data gave an overview of the state of resistance in animal derived pathogenic strains at the time when tulathromycin was placed on to the market.

No data from appropriate studies according to revised VICH Guideline 36 have been provided addressing the concern of an increase in the population of resistant bacteria in the human colon and it was not possible to determine a NOEC or to establish a numerical microbiological ADI for resistance development. However a comprehensive literature review demonstrated that the mode of action of tulathromycin is the same as for erythromycin and other macrolides. Furthermore, the known genetic determinants for resistance as the selective pressure for resistance transfer seem to be comparable to other macrolides. Literature data show that there is a low disposition of the macrolide class to cause resistance development at sub-lethal concentrations. In addition, the relatively high existing prevalence of macrolide resistance in the human intestinal flora in particular in enterococci were thought to make changes in resistance levels and the determination of a numerical no-effect-concentration inaccessible to experimental investigation. For these reasons it was concluded that the calculation of a microbiological ADI with respect to resistance development was not required. This is consistent with recent assessments of analogous macrolides.

2.1.2. Calculation of microbiological ADI

VICH GL 36 was used for the assessment of the microbiological hazard.

The new information provided was very limited and largely confirmed the overall findings previously assessed.

For the calculation of the ADI for colonisation barrier effects the revised formula according to VICH GL 36, was used:

$$\text{ADI} = \frac{\text{MIC}_{\text{calc}} \text{ (}\mu\text{g/ml)} \times \text{daily faecal bolus (220 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}} \text{ (}\mu\text{g/kg bw)}$$

Based on the above formula, the microbiological ADI for colonisation barrier effects was calculated as follows:

$$\text{ADI} = \frac{3.74 \mu\text{g/ml} \times 220 \text{ g}}{0.5 \times 0.5 \times 60 \text{ kg}} = 54.85 \mu\text{g/kg bw (i.e. 3.291 mg/60 kg person)}$$

The following assumptions were made:

- $\text{MIC}_{\text{calc}} = 3.74$: For calculation of the MIC_{calc} the lower 90 % confidence limit for the mean MIC_{50} of the most relevant genera was used;
- Fraction of the oral dose available for microorganisms in the intestinal tract = 0.25 (this factor corresponds to the previous correction factor for colonisation barrier effects). The factor was split into two factors of 0.5 each;
 - a factor of 0.5 based on results for reduced availability of the substance due to interaction with faecal matter and a factor of 0.5 to take account for the impact of acidic colonic pH on tulathromycin availability for gut bacteria
- 220 ml (g) = standard weight of the daily faecal bolus.

A microbiological ADI for disruption of colonisation barrier of 0.055 mg/kg bw (i.e. 3.29 mg/60 kg person) was therefore established.

The calculation of an ADI for resistance development was not considered necessary, based on scientific justification that no resistance development hazard is likely to exist at the level of the microbiological ADI for disruption of colonisation barrier effects. Therefore the ADI of 54.85 $\mu\text{g/kg bw}$ (i.e. 3.291 mg/60 kg person) calculated for colonisation barrier effects is established as the microbiological ADI for tulathromycin.

Findings of EU or international scientific bodies

Data on the evaluation of tulathromycin by other EU or international scientific bodies was not available.

2.1.3. Overall conclusions on the ADI

Previously the CVMP established a toxicological ADI of 0.05 mg/kg bw/day (i.e. 3 mg/person) and a microbiological ADI of 10.97 $\mu\text{g/kg bw/day}$ (i.e. 660 $\mu\text{g/person}$). The microbiological ADI was then considered the most relevant one for the assessment of the consumer safety and established as the overall ADI.

Having re-assessed the data with regard to microbiological properties of residues of tulathromycin and new data provided in line with the modified guideline VICH GL 36 a revised microbiological ADI of 0.055 mg/kg bw (i.e. 3.29 mg/person) was established.

Since the revised microbiological ADI of 3.29 mg/person is higher than the previously established toxicological ADI of 3 mg/person the toxicological ADI is now accepted as the relevant overall ADI.

2.2. Residues assessment

No new data on the depletion of residues were submitted. The data submitted for the establishment of existing MRLs and their evaluation is reported below.

Pharmacokinetics in target species

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 study was 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. When compared to intramuscular administration, plasma levels were significantly lower after oral use. Concentrations in lung tissue after oral administration were higher than plasma concentrations but did not reach the lung concentrations observed after parenteral administration. These data suggested lower availability of the drug following oral administration.

The metabolites of ¹⁴C-tulathromycin in the excreta of cattle and pigs (dosed at 2.5 mg/kg bw, subcutaneously or intramuscularly, 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 were formed by 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 and pigs. In conclusion, although metabolism was limited, the compound was found to be metabolised in a similar manner in rats, dogs, pigs and cattle.

2.2.1. Residue depletion studies

Radiometric studies

In a radiometric residue depletion study, 16 pigs were given a single intramuscular dose of 2.5 mg ¹⁴C-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 respectively. 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 and 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 respectively. 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 and 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-azacyclo-pentadecan-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 and fat, respectively.

In a radiometric residue depletion study, 24 calves were given a single subcutaneous dose of 2.5 mg ¹⁴C-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, respectively. At the same time points average total residue 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 respectively. 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 respectively. 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 (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.

Non-radiometric studies

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 and 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, respectively.

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 or 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, respectively. 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.

Ratio of marker to total residues

In pigs, ratios of marker to total residue of 0.94, 0.83, 0.86, 0.89 and 0.28 were calculated for liver, kidney, muscle, injection site and skin and fat respectively. In calves, the ratios of marker to total residue of 0.61, 0.78, 0.79, 0.91 and 0.46 were calculated for liver, kidney, muscle, injection site and fat, respectively. As marker to total residue ratios differ between species (cattle and pigs), it is not considered reasonable to use mean values for both species.

2.2.2. Analytical method for monitoring of residues

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 residues 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-azacyclopentadecan-15-one expressed as tulathromycin equivalents.

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 fat and skin. 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. The analytical method was validated in accordance with the requirements of Volume 8 of the Rules for veterinary Medicinal Products in the European Union and is acceptable for the existing MRL values but inadequate for higher MRLs.

2.2.3. Findings of EU or international scientific bodies

Data on the evaluation of tulathromycin by other EU or international scientific bodies was not available.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

The substance is not intended for use in dairy cattle and therefore potential effects in dairy products were not investigated.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

Residue depletion data demonstrate that tulathromycin levels in carcass tissues (i.e. fat and muscle) other than injection site muscle were low compared to levels in liver and kidney, in cattle as well as in pigs. Therefore during the previous assessment the CVMP concluded that muscle was not suitable for residue control and did not establish a MRL in muscle. However, the absence of an MRL for muscle represents a serious problem for residue control authorities as muscle is often the tissue selected for residue monitoring. Furthermore, meat imported into the EU often takes the form of lean cuts of muscle. The absence of an MRL for muscle means that it is not possible to control residues levels in imported meat of this type.

Having considered this issue at length the Committee notes that:

- it would be possible to calculate a muscle MRL based on either (i) residues expected in injection site muscle or (ii) residues expected in non-injection site muscle;
- a muscle MRL based on approach (i) (residue levels expected in injection site muscle) would be of little relevance for residue control authorities. This is because injection sites are scarce while non-injection site muscle is abundant and consequently, except on rare, chance occasions, non-injection site muscle will be sampled by residue control authorities. So in the vast majority of samples residues would be far below the injection site levels used to derive the MRL, even if the withdrawal period were not respected. So compliance with the muscle MRL would provide no information on whether the withdrawal period had been respected or on whether residue levels in other tissues comply with their respective MRLs;
- from a residue control point of view it would make far more sense to base the muscle MRL on approach (ii) (residue levels that can be expected in non-injection site muscle) as this will be representative of muscle sampled on all but very rare, chance occasions. From a consumer safety perspective this is also the preferred option, as non-injection site muscle is the muscle regularly consumed;
- If the muscle MRL is based on approach (ii) (residue levels that can be expected in non-injection site muscle), it follows that, at the withdrawal period residues in injection site muscle can be expected to exceed the MRL;
- Annex I of Regulation (EC) No 854/2004, in Section II, Chapter V, indicates that "*meat is to be declared unfit for human consumption if it: ... (i) contains residues or contaminants in excess of the levels laid down in community legislation*" (ie above the MRL);
- in practice, as injection sites will not always be easily identifiable, it cannot be assumed that they will always be removed from the food chain.

In light of the above the CVMP concludes that a muscle MRL should be set in such a way as to maximise both its relevance for residue control purposes and its ability to protect consumer health – i.e. it should be derived based on residue levels that can be expected in non-injection site muscle.

However, because it cannot be assumed that injection sites will always be removed from the food chain there is also a need to ensure that residues at the injection site do not represent a risk to the consumer. An additional value can therefore be derived, which corresponds to the maximum level of residues that would be expected in the injection site at the anticipated withdrawal period (hereafter referred to as the Injection Site Residue Reference Value – ISRRV). The ISRRV is derived as follows: the theoretical maximum daily exposure is calculated on the basis of recommended MRLs for liver,

kidney and fat and the resulting value is compared to the ADI. The ISRRV is then derived in a manner that would allow for residues in 300g of muscle to correspond to the remaining portion of the ADI. The withdrawal period should be derived in a manner that ensures that residues at the injection site will be below this value and that residues in non-injection site muscle, liver, kidney and fat will be below the MRLs for these tissues. In this way, the withdrawal period would not be longer than is necessary in order to ensure consumer safety.

As it is not feasible for control authorities to consider two different levels for the same tissue (muscle), the ISRRV should not be recommended for inclusion in Regulation (EU) No. 37/2010 and should not be used for routine residue surveillance. Rather, the ISRRV would provide a value to be used by competent authorities when setting withdrawal periods for injectable tulathromycin containing products. The withdrawal period should ensure that residues in non-injection site muscle, as well as in liver, kidney and fat are below the MRLs and that residues at the injection site are below the ISRRV. In this way the withdrawal period will ensure that, even if a consumer were to ingest an injection site, consumer exposure to residues would not represent a health risk.

In relation to residue monitoring, in some cases, residue control authorities would have access only to muscle tissue, in which case only muscle tissue would be available for residue monitoring (this is particularly the case for meat imported into Europe). However, where the entire carcass is available, the CVMP would recommend that for the purpose of monitoring residues of tulathromycin, liver or fat (skin and fat in swine) should be sampled in preference to muscle. This is because residues in these tissues deplete more slowly than residues in muscle and so would provide a better basis for verifying compliance with the withdrawal period.

3.3. Elaboration of MRLs

Based on the residue depletion data, distribution of marker residues between target tissues and ratios of marker to total residues and taking into account the toxicological ADI of 3000 µg/person, increased MRL values for liver, kidney, and fat of cattle and pigs can be calculated. In addition a MRL for muscle should be recommended.

MRLs for swine were calculated using mean marker to total residue ratios determined over a period of 4 to 36 days and marker concentrations at day 4, which was the first day when total residue intake from the food basket was below the ADI of 3000 µg/kg.

Taking into account species specific marker to total residue ratios and differences in the pharmacokinetic profile it was considered appropriate to establish separate sets of MRLs for cattle and swine.

MRLs for cattle were calculated using mean marker to total residue ratios determined over a time period of 5 to 48 days and marker residue concentrations at day 15 the first day when total residue intake from the food basket was below the ADI of 3000 µg/kg.

The following MRLs are therefore proposed:

Tissue	Swine	Cattle
Muscle	800 µg/kg	300 µg/kg
Liver	4000 µg/kg	4500 µg/kg
Kidney	8000 µg/kg	3000 µg/kg
Fat (Skin+Fat)	300 µg/kg	200 µg/kg

An "Injection Site Residue Reference Value" (ISRRV), which specifies the level of residues at the injection site that can be considered as safe, of 6000 µg/kg is proposed. This value is not intended for use in routine residue surveillance but provides a value to be used by competent authorities when setting withdrawal periods for tulathromycin containing products. The Injection Site Residue Reference Value (ISRRV) was derived in a manner that would allow for residues in 300 g of muscle to correspond to the unused portion of the ADI, based on the fact that the theoretical maximum daily exposure calculated on the basis of the MRLs for liver, kidney and fat correspond to only 35% – 41% of the ADI. Withdrawal periods for injectable tulathromycin products should ensure that residue levels present in non-injection tissues do not exceed the MRLs for muscle, liver, kidney and fat, respectively, and that residue levels present in injection site muscle do not exceed the ISRRV of 6000 µg/kg.

As intake calculations in Tables 1 and 2 show, residues in the food basket (injection site included) remain below the ADI.

Table 1: Theoretical daily intake calculation based on the proposed MRLs in pigs using a revised ADI of 3000 µg/person/day

Tissue	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio Marker/ Total residue*	Amount total residues (µg)
Muscle	0.30	800	0.86	279.07
Fat / Skin [#]	0.05	300	0.28	53.57
Liver	0.10	4000	0.94	425.53
Kidney	0.05	8000	0.83	481.93
Estimated total daily intake (µg/person)				1240.10
Total % ADI				41.34^{**}

[#] Fat and skin in natural proportions

* Overall ratios per tissue

** Calculation based on an Injection Site Residue Reference Value (ISRRV) of 6000 µg/kg accounting for muscle tissue and a ratio of marker to total residue of 0.89, would result in consumer intake of 2983.50 µg representing approximately 99.45% of the ADI.

Table 2: Theoretical daily intake calculation based on the proposed MRLs in cattle using a revised ADI of 3000 µg/person/day

Tissue	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio Marker/ Total residue*	Amount total residues (µg)
Muscle	0.30	300	0.79	113.92
Fat	0.05	200	0.46	21.74
Liver	0.10	4500	0.61	737.71
Kidney	0.05	3000	0.78	192.31
Estimated total daily intake (µg/person)				1065.66
Total % ADI				35.52^{**}

* Overall ratios per tissue

** Calculation based on an Injection Site Residue Reference Value (ISRRV) of 6000 µg/kg accounting for muscle tissue and a ratio of marker to total residue of 0.89, would result in consumer intake of 2929.77 µg representing approximately 97.66% of the ADI.

Based on the recommended MRLs, the theoretical maximum daily intake from tissues calculated using the recommended maximum residue limits, represents 41.34% and 35.52% of the ADI for pigs and cattle, respectively. However, when the calculation is performed taking also into account the ISRRV of 6000 µg/kg for both species, the consumer intake represents approximately 99.5% and 97.66% of the ADI for pigs and cattle, respectively.

Tulathromycin is not intended for use in dairy animals producing milk for human consumption, poultry (including those producing eggs for human consumption), or honey bees and therefore it is not considered necessary to reserve part of the ADI for other food commodities.

In view of the deficiency already highlighted in this report concerning the analytical method final MRLs cannot be recommended. However considering that there are no grounds for supposing that residues of the substance at the level proposed constitutes a hazard to human health a provisional MRL is recommended while further validation of the analytical method is carried out.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009 the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for tulathromycin on the basis of residue data in cattle and pigs to other food producing species and commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
Goats	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, and that cattle and goats are related species (ruminants) the assumption could be made that the same marker residue would be appropriate in goats. However, no specific pharmacokinetic or residue data were available for goats and therefore the assumption related to the marker residue could not be confirmed.</p> <p>No data are available to demonstrate that the analytical method for monitoring of residues is applicable for monitoring of residues in goats tissues.</p>
Milk	No	<p>No data are available that would allow conclusions to be drawn on the appropriate marker residue or marker to total residues ratio to use in milk. Milk is consumed on a regular basis and in large quantities and consequently data on residues in this commodity are considered necessary in order to allow adequate evaluation of the risk to consumer safety posed by residues in milk.</p> <p>No analytical method for monitoring of residues in milk was available for evaluation.</p> <p>In addition data available indicate that tulathromycin is not suitable for use in dairy animals as it partitions extensively into milk.</p>
Sheep	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, and that cattle and sheep are related species (ruminants) the assumption could be made</p>

		<p>that the same marker residue would be appropriate in sheep. However, no specific pharmacokinetic or residue data for sheep were available for this evaluation and therefore the assumption related to the marker residue could not be confirmed and the ratio of marker to total residues could not be derived at this stage.</p> <p>Sheep meat is consumed on a regular basis and in large quantities. Species specific data are therefore considered necessary to allow adequate evaluation of the risk to consumer safety posed by residues in sheep tissues.</p> <p>No analytical method for monitoring of residues in sheep tissues was available for this evaluation.</p>
Poultry (including eggs)	No	<p>Metabolism can be significantly different in poultry compared to cattle and pigs. Consequently species specific metabolism and residue data are considered necessary to allow adequate evaluation of the risk to consumer safety posed by residues in poultry-derived food commodities.</p> <p>No analytical method for monitoring of residues in poultry tissues (or eggs) was available for evaluation.</p>
Horses	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, the assumption could be made that the same marker residue would be appropriate in horses. However, no specific pharmacokinetic or residue data were available for horses and therefore the assumption related to the marker residue could not be confirmed.</p> <p>No data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pigs is applicable for monitoring of residues in horse tissues.</p>
Rabbits	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, the assumption could be made that the same marker residue would be appropriate in rabbits. However, no specific pharmacokinetic or residue data were available for rabbits and therefore the assumption related to the marker residue could not be confirmed.</p> <p>No data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pigs is applicable for monitoring of residues in rabbit tissues.</p>
Fin fish	No	<p>Metabolism is generally less complicated in fish than in cattle and pigs. As the marker residue in cattle and pigs is not the parent compound residue data in fish would be required.</p>

		No analytical method for monitoring of residues in fish meat was available for evaluation.
Honey	No	Residue depletion in honey does not occur through metabolism and consequently conclusions drawn from data in other food products cannot be extrapolated to honey. Honey specific data are required in order to allow adequate evaluation of the risk to consumer safety posed by residues in honey. No data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pigs tissues is applicable for monitoring of residues in honey.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Whereas:

- the toxicological ADI of 3 mg/person per day has been considered the overall ADI for tulathromycin,
- 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 was identified as the marker residue,
- marker to total residue ratios for pig tissues have been considered to be 0.86 in muscle, 0.28 in skin and fat, 0.94 in liver, 0.83 in kidney and 0.89 at the injection site. For cattle tissues marker to total residue ratios have been considered to be 0.79 for muscle, 0.61 for liver, 0.78 for kidney, 0.46 for fat and 0.91 for injection site,
- the Commission and residue control authorities consider that, in order to ensure the feasibility of residue controls, a single official residue limit for muscle must be published in Regulation (EU) No. 37/2010,
- residues at the injection site deplete slowly and should be considered for setting withdrawal periods,
- Injection Site Residue Reference Value (ISRRV) of 6000 µg/kg is established for cattle and swine – this value should be taken into account when deriving withdrawal periods,
- for the purpose monitoring of residues of tulathromycin it is recommended that, where the entire carcass is available, liver or fat (skin+fat in swine) should be sampled in preference to muscle as residues in these tissues deplete more slowly than residues in muscle and so will provide a better basis for verifying compliance with the withdrawal period,

and having considered that:

- an analytical method based on LC/MS/MS for the determination of the marker residue in edible tissues of cattle and pigs is available, however, not sufficiently validated in accordance with the requirements of Volume 8 taking into account the new MRL values proposed;

the Committee recommends the modification of the maximum residue limits for tulathromycin in accordance with the following table:

Pharmaco - logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
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-xylohexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents	Bovine	300 µg/kg 200 µg/kg 4500 µg/kg 3000 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption Provisional MRLs expire on 1 January 2015	Anti-infectious agents/ Antibiotics
		Porcine	800 µg/kg 300 µg/kg 4000 µg/kg 8000 µg/kg	Muscle Skin and fat in natural proportions Liver Kidney	Provisional MRLs expire on 1 January 2015	

Based on the MRLs for pigs (the worst case scenario), the total theoretical maximum daily intake (TMDI) from tissues was 1240.10 µg which accounts for 41.34% of the toxicological ADI. Taking into account the Injection Site Residue Reference Value (ISRRV) set at 6000 µg/kg the TMDI from a food basket containing 300 g injection sites represents approximately 99.5% of the ADI for edible tissues.

4. List of questions

1. In accordance with Volume 8 of the rules governing medicinal products in the EU, the applicant is requested to demonstrate that the proposed analytical method for residue monitoring is validated at half the MRL value, the MRL and twice the MRL for the newly recommended MRLs.

5. Background information on the procedure

Submission of the dossier	2 February 2012
Steps taken for assessment of the substance	
Application validated:	16 February 2012
Clock started:	17 February 2012
List of questions adopted:	14 June 2012
Consolidated response to list of questions submitted:	11 January 2013
Clock re-started:	12 January 2013
Oral explanation provided by applicant:	14 May 2013
List of outstanding issues adopted:	16 May 2013
Submission of responses to list of outstanding issues:	9 September 2013
CVMP opinion adopted:	10 October 2013