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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Tulathromycin (modification of the microbiological ADI and MRLs in bovine and porcine species) – after provisional maximum residue limits (MRLs)

On 10 March 2015 the European Commission adopted a Regulation¹ establishing maximum residue limits for tulathromycin in bovine and porcine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Tulathromycin is used in bovine and porcine species for the treatment and prevention of bacterial and mycoplasmal infections and is 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 an application for the modification of the microbiological ADI and maximum residue limits to the European Medicines Agency, on 2 February 2012. The CVMP subsequently agreed to the modification of the microbiological ADI and recommended provisional MRLs in bovine and porcine species, which were adopted by the European Commission on 18 December 2014³.

Pfizer Animal Health SA submitted responses to the list of outstanding issues after provisional MRLs on 4 April 2014.

Based on the available data the CVMP recommended, on 10 July 2014, the removal of the provisional status of the MRLs.

Subsequently the Commission recommended, on 15 November 2014, that maximum residue limits in bovine and porcine species are established. This recommendation was confirmed on 6 December 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 10 March 2015.

¹ Commission Implementing Regulation (EU) No 2015/394, O.J. L66, of 11.03.2015

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

³ Commission Implementing Regulation (EU) No 1359/2014, O.J. L 365, of 19.12.2014



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:	Pfizer Animal Health SA
Target species:	Bovine and 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. The substance is administered by single injection of 2.5 mg/kg bw applied subcutaneously in bovine species, and intramuscularly in porcine species.

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/day (i.e. 660 µg/person) were established (2002). The microbiological ADI was considered the most relevant for the assessment of the consumer safety and was established as the overall ADI.

Currently, tulathromycin is included in Table 1 of the Annex to 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		

Subsequent to the establishment of the above MRLs the CVMP assessed an application for the modification of the ADI for tulathromycin (2013) and established a revised microbiological ADI of 0.055 mg/kg bw (i.e. 3.29 mg/person).

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 has been accepted as the relevant overall ADI.

Considering the new overall ADI the CVMP recommended the revision of MRLs established for tulathromycin in bovine and porcine species in accordance with the following table (these MRLs have yet to be adopted by the European Commission):

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-	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	Muscle		

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
	15-one expressed as tulathromycin equivalents		300 µg/kg 4000 µg/kg 8000 µg/kg	Skin and fat in natural proportions Liver Kidney	MRLs expire on 1 January 2015	

In June 2014 the CVMP adopted a recommendation for the establishment of maximum residue limits in ovine and caprine species in accordance with the following table (these MRLs have yet to be adopted by the European Commission):

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-tetra-hydroxy-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	Ovine, caprine	450 µg/kg 250 µg/kg 5400 µg/kg 1800 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents/ Antibiotics

Further to the establishment of provisional MRLs for tulathromycin in bovine and porcine species, the applicant responded to the request for additional data concerning the validation of the analytical method in both species, in order to allow for the establishment of final MRLs in bovine and porcine species. The scientific assessment previously carried out by the Committee leading to the recommendation for the establishment of provisional MRLs in bovine and porcine species is reported in the paragraphs below. Section 2.2.4 on the analytical method for monitoring of residues has been updated to take account of the response provided by the applicant following the recommendation for the establishment of provisional MRLs. Considerations, conclusions and recommendations presented in section 3 have been modified accordingly.

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 overall ADI.

New data were on disruption of the colonisation barrier and the increase of the population of resistant bacteria were subsequently submitted along with a re-evaluation of previously submitted studies on disruption of the colonisation barrier with a view to supporting a modification of the microbiological ADI.

The safety evaluation previously carried out by the CVMP is reported in detail in the 2004 summary report for tulathromycin (EMA/CVMP/894/04-FINAL). The safety section in this document provides only a review of the microbiological ADI, based on evaluation of the new data submitted, and the re-evaluation of the data previously provided for the establishment of the microbiological ADI. Consideration of the impact on the overall ADI is also provided (in section 2.1.3).

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 representative of 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 on the general approach to establish a microbiological ADI. 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 have 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 obtained 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 as 85%.

Results from both the studies for activity in the presence of faecal material 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% of 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 µg/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 as 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 were 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 to address 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 those for 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 on the general approach to establish a microbiological ADI was used for the assessment of the microbiological hazard.

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

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

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 a scientific justification indicating 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 µg/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

No information on evaluation of tulathromycin by other EU or international scientific bodies was 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 µg/kg bw/day (i.e. 660 µg/person). The microbiological ADI was then considered the most relevant for the assessment of the consumer safety and was established as the overall ADI.

Having re-assessed the data with regard to microbiological properties of residues of tulathromycin and having considered the new data provided in line with the revised 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 accepted as the relevant overall ADI for tulathromycin.

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 were 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 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 given 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 $\mu\text{g}/\text{kg}$ for liver, injection site, kidney, muscle and skin and fat respectively. Average marker residue concentrations in liver were 1700, 960, 730, 280 and 150 $\mu\text{g}/\text{kg}$ 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 $\mu\text{g}/\text{kg}$ in kidney; 440, 95, 70, 35 and 18 $\mu\text{g}/\text{kg}$ in muscle; and 230, 110, 60, 20 and 15 $\mu\text{g}/\text{kg}$ in skin+fat. At injection sites the average marker residue concentrations were 2300, 1500, 1100, 500 and 600 $\mu\text{g}/\text{kg}$ 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 $\mu\text{g}/\text{kg}$ for liver, injection site, kidney, muscle and fat respectively. Average marker residue concentrations in liver were 5600, 3900, 3200, 2400, 1200 and 650 $\mu\text{g}/\text{kg}$ 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 $\mu\text{g}/\text{kg}$ in kidney; 550, 170, 89, 50, 19 and 9 $\mu\text{g}/\text{kg}$ in muscle; and 260, 130, 100, 42, 21 and 8 $\mu\text{g}/\text{kg}$ in fat. At injection sites the average marker residue concentrations were 5100, 3200, 2300, 800, 900 and 500 $\mu\text{g}/\text{kg}$ 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

At the time of the recommendation for the establishment of provisional MRLs in bovine and porcine species confirmation was sought that the existing analytical methods were appropriate for use in monitoring residues at levels of up to twice the MRLs, as is required by Volume 8 of The rules governing medicinal products in the European Union. Clarification on this issue was subsequently provided.

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 $\mu\text{g}/\text{kg}$ for liver, 100 $\mu\text{g}/\text{kg}$ for kidney and injection site and 20 $\mu\text{g}/\text{kg}$ for muscle and fat and skin. The limits of quantification of the method for cattle tissues were 300 $\mu\text{g}/\text{kg}$ for liver and injection site, 200 $\mu\text{g}/\text{kg}$ for kidney, 30 $\mu\text{g}/\text{kg}$ for muscle and 60 $\mu\text{g}/\text{kg}$ for fat.

The validation data show that the analytical method is suitable to measure concentrations of 0.5 x MRL, 1 x MRL and 2 x MRL in liver, kidney, muscle and fat (or skin and fat in porcine species). The much higher concentrations in injection sites are also covered by the validated range of the analytical method (although the ISRRV is not an MRL and consequently the method range is not formally required to encompass this value).

The relevant European Reference Laboratory has reviewed the analytical methods and is in agreement with the above conclusions.

2.2.3. Findings of EU or international scientific bodies

No information on the evaluation of tulathromycin by other EU or international scientific bodies was 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 muscle other than injection site muscle were very low compared to levels in injection site muscle, in cattle as well as in pigs and therefore muscle is not considered a target tissue. However, in order to enable residues control of meat when only lean cuts of muscle are available a MRL for muscle is required. For this substance the approach described in the CVMP revised reflection paper on injection site residues: consideration for risk assessment and residue surveillance (EMA/CVMP/520190/2007-Rev.1) was considered appropriate for derivation of the muscle MRL.

No other risk management considerations were considered relevant.

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 as well as a MRL for muscle were calculated as described in the paragraphs below.

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 also established. 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.

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, pigs and sheep 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
Milk	No	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

		<p>adequate evaluation of the risk to consumer safety posed by residues in milk.</p> <p>The use of tulathromycin in dairy cows has been investigated and tulathromycin was shown to partition extensively into milk. Based on the required 1.5 l consumption factor, the use of the substance in dairy animals would not be practicable.</p> <p>No analytical method for monitoring of residues in milk was available for 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> <p>No analytical method for monitoring of residues in fish meat was available for evaluation.</p>
Honey	No	<p>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</p>

		<p>specific data are required in order to allow adequate evaluation of the risk to consumer safety posed by residues in honey.</p> <p>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.</p>
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3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the toxicological ADI of 3 mg/person per day is 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,
- an Injection Site Residue Reference Value (ISRRV) of 6000 μ g/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,
- an analytical method based on LC/MS/MS for the determination of the marker residue in edible tissues of cattle and pigs is available;

the CVMP confirms the maximum residue limits established for tulathromycin in bovine and porcine species and recommends the removal of the provisional status of these 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-	Bovine	300 μ g/kg 200 μ g/kg 4500 μ g/kg 3000 μ g/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents/ Antibiotics

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
	[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylohexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one expressed as tulathromycin equivalents	Porcine	800 µg/kg 300 µg/kg 4000 µg/kg 8000 µg/kg	Muscle Skin and fat in natural proportions Liver Kidney		

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. 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 (provisional MRLs):	10 October 2013
Submission of responses to list of questions after provisional MRLs	4 April 2014
CVMP opinion adopted (after provisional MRLs)	10 July 2014