



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/CVMP/454092/2016
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR) Gamithromycin (all ruminants except bovine species)

On 23 November 2016 the European Commission adopted a Regulation¹ establishing maximum residue limits for gamithromycin in all ruminants except bovine, 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.

In ovine species gamithromycin is intended for the treatment of sheep foot rot disease, to be administered by the subcutaneous route at a recommended dose of 6 mg/kg bw.

Maximum residue limits had previously been established for bovine² and porcine³ species. Merial submitted to the European Medicines Agency an application for the extension of maximum residue limits to ovine species, on 29 October 2015.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended, on 14 July 2016, the establishment of maximum residue limits for gamithromycin in ovine species and the extrapolation of these maximum residue limits to all ruminants except bovine species.

Subsequently the Commission recommended on 5 October 2016 that maximum residue limits in all ruminants except bovine species are established. This recommendation was confirmed on 26 October 2016 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 23 November 2016.

¹ Commission Implementing Regulation (EU) No 2016/2045, O.J. L 318, of 24 November 2016

² Commission Regulation (EC) No 581/2009, O.J. L 175, of 04.07.2009

³ Commission Implementing Regulation (EU) No 2015/150, O.J.L26, of 31.01.2015



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Gamithromycin
Therapeutic class:	Anti-infectious agents / Antibiotics
Procedure number:	EMA/V/MRL/003158/EXTN/0003
Applicant:	MERIAL
Target species requested:	Ovine
Intended therapeutic indication:	Treatment of sheep foot rot disease
Route(s) of administration:	Subcutaneous injection

1. Introduction

Gamithromycin is a semi-synthetic macrolide (CAS No 145435-72-9) prepared by fermentation followed by organic synthesis. The substance is a member of the azalide subclass of macrolide antibiotics consisting of a 15-membered macrocyclic lactone ring.

Gamithromycin is used in non-lactating cattle for the treatment of bovine respiratory disease (BRD), caused by the bacteria *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*, and in pigs for the treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*.

Gamithromycin is not used in human medicine.

Gamithromycin was previously assessed by the CVMP and a toxicological ADI of 10 µg/kg bw, i.e. 600/µg/person, was established.

Currently, gamithromycin is included in 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
Gamithromycin	Gamithromycin	Bovine	20 µg/kg 200 µg/kg 100 µg/kg	Fat Liver Kidney	Not for use in animals producing milk for human consumption	Anti-infectious agents / Antibiotics
		Porcine	100 µg/kg 100 µg/kg 100 µg/kg 300 µg/kg	Muscle Skin and fat in natural proportions Liver Kidney	NO ENTRY	

MERIAL submitted an application pursuant to Article 3 of Regulation (EC) No 470/2009 for the extension of maximum residue limits to ovine species to the European Medicines Agency on 29 October 2015. The substance is proposed for use in sheep at a dose of 6 mg/kg bw, administered as a single subcutaneous injection.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously assessed the consumer safety of gamithromycin and established a toxicological ADI of 10 µg/kg bw, i.e. 600 µg/person, based on the NOEL of 1 mg/kg bw/day from a 52-week repeated dose toxicity study in dogs and applying a safety factor of 100. No further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

An *in vivo* pivotal pharmacokinetic study was conducted with the final formulation containing 15% w/v gamithromycin. Sheep (5 months of age, 41-52 kg) were treated with either a single intravenous dose of 6 mg/kg bw gamithromycin or with a single subcutaneous dose of 3, 6 or 12 mg/kg bw in a parallel design. Each group comprised 6 animals. Plasma samples were collected before treatment and at predetermined time points up to 12 days after treatment. The resulting plasma samples were analysed using a validated method with a limit of quantitation (LOQ) of 2 ng/ml. Pharmacokinetic parameters were determined using a non-compartmental model.

Following a single intravenous injection of 6 mg/kg bw, the mean area under the curve extrapolated to infinity (AUC_{inf}) was 10.0 ± 0.70 µg*hour/ml, and the mean terminal plasma half-life ($t_{1/2}$) was 34.6 ± 6.86 hours. The volume of distribution at steady state (V_{ss}) and observed clearance (Cl_{obs}) were 19.0 ± 4.30 l/kg and 602 ± 42.8 ml/hour/kg, respectively. For animals treated with subcutaneous injections of 6 mg/kg bw, the AUC_{inf} was 8.88 ± 2.33 µg*hour/ml which is comparable to the AUC_{inf} following the same dose given intravenously, resulting in 89% absolute bioavailability. The AUC_{inf} for subcutaneous injections of 3 and 12 mg/kg bw were 4.51 ± 0.78 and 15.9 ± 4.12 µg*hour/ml, respectively. The mean maximum plasma concentrations (C_{max}) were 147 ± 27.8 , 448 ± 180 , and 534 ± 302 ng/ml for subcutaneous doses of 3, 6, and 12 mg/kg, respectively. The time to reach the maximum plasma concentration in the three subcutaneous injection groups was 1.50 ± 0.84 , 2.30 ± 2.40 , and 4.07 ± 2.99 hours, indicating a rapid absorption. The terminal plasma half-life ($t_{1/2}$) following subcutaneous treatment was 46.8 ± 7.96 , 42.5 ± 5.25 , and 53.1 ± 8.79 hours, respectively, for doses at 3, 6, and 12 mg/kg bw, values which are comparable to the half-life following intravenous administration. The average C_{max} values for the 3, 6 and 12 mg/kg doses did not increase proportionally with dose. Dose proportionality of the AUC_{inf} was established over the range of 0.5 to 2.0 times the recommended dosage rate of 6 mg/kg bw.

2.2.2. Residue depletion studies

The depletion of residues has been examined in one pivotal study where gamithromycin in the final drug formulation was injected subcutaneously at the intended therapeutic dose of 6 mg/kg bw once to sheep. A total of 37 healthy sheep (19 male and 18 female) were included in the study. The animals were approximately 7 months old and weighed between 51.6 and 73.2 kg (day -1). The animals were allocated into 8 groups. Group 1 consisted of one male and one female sheep that were used as untreated controls and groups 2-8 consisted of 5 animals of both genders. Animals in groups 2-8 were

treated with gamithromycin 15% w/v by subcutaneous injection on the dorsal left side of the neck at 1 ml/25 kg bw (equivalent to 6 mg gamithromycin/kg bw) once on day 0. The two control animals in group 1 were euthanized on day 1. Groups 2, 3, 4, 5, 6, 7, and 8 were euthanized at 5, 9, 14, 21, 28, 35, and 47 days post administration, respectively and samples of bile and plasma, liver, kidney, muscle, skin, fat and injection site (inner and outer perimeter) were collected. Urine and faeces excreted over the whole day were collected from the male animal in group 1 on day 1, and two randomly selected male animals in group 5 on days 1, 2, 3, 4, 5, 7, 9, and 14 post administration.

Gamithromycin and declad (a metabolite formed by the loss of the dideoxy sugar moiety, cladinoste) concentrations in faeces, urine and edible tissues were determined using validated LC-MS/MS methods. A semi-quantitative metabolite profile was also determined in matrices using HPLC coupled with LC-high resolution MS (LC-HRMS) and extracted ion chromatography (XIC).

The gamithromycin residue concentrations in both urine and faeces were much higher than its metabolite declad. The cumulative amount of gamithromycin and declad residues in excreta showed that the majority of the residues were recovered in excreta over 7 days post administration. The cumulative amount of gamithromycin and declad residue at 14 days post administration amounted to approximately 25% of the dose in faeces and approximately 18% in urine. Thus total recovery of gamithromycin and declad in excreta was 43% of the dose, which appears to be low. However, low recovery was also been observed in cattle and pigs.

Depletion of gamithromycin from tissues followed first order kinetics with liver showing the slowest depletion rate among all tissues analyzed. The half-lives of gamithromycin depletion in liver, kidney, muscle, fat, injection site core, and injection site ring were calculated to be 5.48, 4.22, 2.55, 2.82, 4.43, and 2.39 days, respectively. The half-lives of declad were longer than those of gamithromycin.

The metabolite profiles obtained using LC-HRMS showed qualitatively similar metabolites in tissues and excreta. The amount of metabolites varied in different tissues and in excreta at various time points. The major biotransformation pathways included hydrolysis with a loss of the cladinose sugar moiety to form declad and N-dealkylation to form M8 (N-despropyl N-desmethyl gamithromycin) and M9 (N-despropyl gamithromycin). Gamithromycin also underwent biotransformation via a minor pathway to form the translactone derivative M5 (TDO) (ML-1,620,759) through an intra-molecular rearrangement. Other minor biotransformation pathways were mostly oxidative.

In liver, gamithromycin and declad were the major residues present. As the %XIC, gamithromycin decreased from 83.9% on day 5 to 63.2% on day 28. Declad concentrations as %XIC increased from 12.0% on day 5 to 32.4% on day 28. Declad was the only major metabolite in liver that exceeded 10% of XIC. None of the other metabolites exceeded 3% of XIC.

A similar pattern was noted in kidney where gamithromycin and declad were the major metabolites. Gamithromycin declined as %XIC from 89.7% on day 5 to 67.8% on day 28. As with liver, declad was the only metabolite that exceeded 10% of XIC, with none of the other metabolites exceeding 3% of XIC.

For injection site core muscle, gamithromycin and declad were the major residues. Gamithromycin, as %XIC, declined from 91.9% on day 5 to 78.5% on day 35. Declad increased from 3.76% on day 5 to 16.9% on day 35. As with liver and kidney, declad was the only metabolite that exceeded 10% of XIC and none of the other metabolites exceeded 3% of XIC. Residues of gamithromycin at the injection site remained above the limit of quantification (25 µg/kg) until the final sampling point (47 days).

In muscle, in addition to gamithromycin and declad, metabolites M8 and M9 were the major residues. As %XIC, gamithromycin declined from 70.2% on day 5 to 8.19% on day 21 while declad increased

from 8.94% on day 5 to 13.7% on day 14, and decreased to 8.57% by day 21. Concentrations of M8 and M9 increased from 6.91% and 5.89% on day 5 to 48.06% and 18.1% on day 21, respectively. However, the concentrations of gamithromycin and declad in muscle at day 21 were well below the limit of quantification of the assay and so the concentrations of M8 and M9 were also very low.

In fat, gamithromycin, declad and M9 were the major residues present. Gamithromycin decreased from 83.1% of XIC on day 5 to 37.9% by day 21 whereas declad and M9 increased from 7.3% and 2.79% on day 5 to 37.7% and 18.7% on day 35. No other metabolites exceeded 5% of XIC at any time point.

Selection of marker residue and ratio of marker to total residues

As noted above, metabolic profiling has been conducted on residues of gamithromycin in sheep tissues using LC-HRMS and has permitted the determination of the ratio of gamithromycin to total residues based on %XIC of gamithromycin, and the estimated gamithromycin marker to total ratio as a percentage based on gamithromycin and declad residue concentrations.

Gamithromycin is the major component in all edible tissue and is therefore selected as the marker residue.

By day 14 of the study described above, residues of gamithromycin had depleted to below the ADI suggesting that this is the optimum time to examine the ratio of the marker to total residues (M/T). However, residues of gamithromycin and declad in muscle and fat are below the limit of quantification at this point and therefore the preceding time point, day 9 may be chosen for these tissues. This results in M/T values of 0.72 for liver, 0.76 for kidney, 0.43 for muscle, 0.72 for fat, and 0.88 for injection site muscle.

2.2.3. Monitoring or exposure data

No monitoring or exposure data relevant to the use of gamithromycin in sheep were available in addition to the data presented elsewhere in the residue section of this report.

2.2.4. Analytical method for monitoring of residues

The proposed method for analysis of gamithromycin residues in sheep liver, kidney, fat and muscle including the injection site is based on homogenisation of tissues with phosphate buffer, followed by hexane partition and solid phase extraction. The sample extracts are analysed by LC-MS/MS using an internal standard (deuterated (D5) gamithromycin). The method has been validated over the concentration range of 25 - 25,000 µg/kg for liver, kidney and muscle including the injection site, and 25 - 2,500 µg/kg for fat. For all tissues, the limit of detection is 1 µg/kg and the limit of quantification is 25 µg/kg. The method is described in an internationally recognised format and is considered validated according to the requirements of Volume 8 of the Rules Governing Veterinary Medicinal Products in the EU.

The relevant European Reference Laboratory (EURL) has reviewed the proposed analytical method and is in agreement with the above conclusion but notes that, for an official residue control method, monitoring of two selected reaction monitoring (SRM) signals would be required (only one was provided).

2.2.5. Findings of EU or international scientific bodies

No relevant evaluations by other EU or international scientific bodies were 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 animals and therefore potential effects in dairy products were not investigated.

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

Conditions of use

Residue depletion data in milk were not provided. However, due to the long half-life for gamithromycin it is unrealistic to consider that the substance would be used for the treatment of lactating animals producing milk for human consumption. Consequently, the establishment of MRLs in milk is not proposed. In the absence of MRLs for milk the use of gamithromycin is restricted to animals not producing milk for human consumption.

Feasibility of controls

Residue depletion data demonstrate that residue levels in carcass tissues (i.e. fat and muscle) other than injection site muscle were low compared to levels in liver and kidney. Consequently, the CVMP recommends that, where the entire carcass is available, monitoring of residues of gamithromycin should focus on liver or kidney in preference to other tissues, as compliance with the liver or kidney MRLs can be expected to indicate that residues in other tissues will also be compliant with their respective MRLs.

No additional relevant factors were identified for consideration of the risk management recommendations.

3.3. Elaboration of MRLs

Based on the residue depletion data available and the ratios of marker to total residues at day 14 (liver and kidney) and 9 (muscle and fat), MRL values were derived as follows, with the values for muscle and fat set at twice the limit of quantification:

Tissue	MRL
Muscle	50 µg/kg
Fat	50 µg/kg
Liver	300 µg/kg
Kidney	200 µg/kg

Injection Site Residues Reference Value (ISRRV)

Residue depletion data demonstrate that gamithromycin levels in carcass tissues (i.e. fat and muscle) other than injection site muscle were low compared to levels in liver and kidney. In line with the approach described in the CVMP revised reflection paper on injection site residues: considerations for risk assessment and residue surveillance (EMA/CVMP/520190/2007-Rev.1), and in light of the slow depletion of residues from the injection site, an "Injection Site Residue Reference Value" (ISRRV), which specifies the level of residues at the injection site that can be considered as safe, of 1500 µg/kg was 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 gamithromycin containing products.

Withdrawal periods for injectable gamithromycin products should ensure that residue levels present in non-injection tissues do not exceed the MRLs for muscle, liver, kidney and skin and fat, respectively, and that residue levels present in injection site muscle do not exceed the ISRRV of 1500 µg/kg.

Calculation of theoretical daily intake of residues

Edible tissues	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of marker to total residues	Amount per edible tissue (µg)
Muscle	0.3	50	0.43	34.9
Fat	0.05	50	0.72	3.47
Liver	0.1	300	0.72	41.7
Kidney	0.05	200	0.76	13.2
Estimated total daily intake (µg/person)				93.2
Percent of ADI (600 µg/person)				16

Based on the figures above the theoretical consumer intake represents 16% of the ADI. When the calculation is performed taking into account the injection site residue reference value of 1500 µg/kg, i.e. considering maximal residues in injection site muscle, liver, kidney and skin and fat, the consumer intake would account for 95% of the ADI.

It is recognised that the MRLs and ISRRV proposed above would not allow extension of the MRLs to dairy animals producing milk for human consumption or to poultry producing eggs for human consumption. While gamithromycin is not currently intended for use in these animals, in general the CVMP considers it good practice to reserve a portion of the ADI to accommodate other possible uses, including those not currently foreseen. However, since the half-life is extremely long for gamithromycin it appears unrealistic to consider use of the drug for treatment of lactating animals that produce milk for human consumption as the withdrawal period for milk would be unacceptably long. No indication is foreseen in poultry for the drug. In addition, approximately 95% of the ADI is used up by the MRLs and ISRRV already established for other mammalian species, with the result that restricting the portion of the ADI that may be used for ovine species would be of questionable benefit in the event of an application to establish MRLs in laying hens. In light of these arguments use of 95% of the ADI in establishing tissue MRLs and an ISRRV is considered reasonable.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits for gamithromycin to other food-producing species. Taking into account the current scientific knowledge, the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
All ruminants except bovine	Yes	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle, pigs and sheep is similar and it can be expected that the parent compound would be a suitable marker residue in tissues of other ruminants.</p> <p>No data are available to demonstrate that the analytical method proposed for monitoring of residues in sheep tissues would be applicable for monitoring of residues in tissues of other ruminants but there is no reason for believing that they would not be.</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 foodstuff 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>It is also noteworthy that as the proposed MRLs and ISRRV use up 95% of the ADI, the current proposal would not allow the establishment of an MRL for milk.</p>
Poultry (including eggs)	No	<p>Metabolism can be significantly different in poultry compared to sheep. 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> <p>It is also noteworthy that as the proposed MRLs and ISRRV use up 95% of the ADI, the current proposal would not allow the establishment of an MRL for eggs.</p>
Horses	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle, pigs and sheep is similar and it can be expected that the parent compound would be a suitable marker residue in horse tissues. However, no specific pharmacokinetic or residue data were available for horses and therefore the assumption that the parent compound would be a suitable marker residue could not be confirmed.</p> <p>No data are available to demonstrate that the analytical methods used for monitoring of residues in pigs, cattle or</p>

		<p>sheep tissues are applicable for monitoring of residues in horse tissues.</p> <p>The numerical MRLs established for cattle, pigs and sheep are different and there are no data to demonstrate which values would be most appropriate for horses.</p>
Rabbits	No	<p>Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle, pigs and sheep is similar and it can be expected that the parent compound would be a suitable marker residue in rabbit tissues. However, no specific pharmacokinetic or residue data were available for rabbits and therefore the assumption that the parent compound would be a suitable marker residue could not be confirmed.</p> <p>No data are available to demonstrate that the analytical methods used for monitoring of residues in pigs, cattle or sheep tissues are applicable for monitoring of residues in rabbits.</p> <p>The numerical MRLs established for cattle, pigs and sheep are different and there are no data to demonstrate which values would be most appropriate for rabbits.</p>
Fin fish	No	<p>Metabolism is generally less complicated in fish than in mammals. Consequently, as the marker residue is the parent compound in cattle, pigs and sheep it can be assumed that gamithromycin would also be a suitable marker for fish meat. However, 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 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 methods used for monitoring of residues in pigs, cattle or sheep tissues are applicable for monitoring of residues in honey.</p>

The MRLs established in bovine species do not include an MRL for muscle. Consequently it is considered appropriate that the ovine values rather than the bovine values should be extrapolated all ruminants except bovine.

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

Whereas:

- the toxicological ADI of 10 µg/kg bw (i.e. 600 µg/person) was previously established as the overall ADI for gamithromycin,
- the parent compound was retained as the marker residue,
- the ratios of marker to total residues calculated at 14 days (liver and kidney) and 9 days (muscle and fat) were 0.42 in muscle, 0.72 in fat, 0.72 in liver, 0.76 in kidney and 0.88 in injection site muscle,
- residue concentrations were persistently low in fat and muscle; the MRLs proposed for these tissues are set at twice the limit of quantification,
- an injection site residues reference value (ISRRV) of 1500 µg/kg was established for ovine species,
- extrapolation of the maximum residue limits proposed for ovine tissues to tissues of other ruminants except bovine is considered appropriate,
- a validated analytical method for the monitoring of residues of gamithromycin in edible ovine tissues liver, kidney, fat and muscle is available,
- although it was not specifically demonstrated, the analytical method for monitoring of residues in ovine tissues is expected to be basically applicable for monitoring of residues in tissues of ruminants except bovine,
- for the purpose of monitoring of residues of gamithromycin it is recommended that, where the entire carcass is available, liver or kidney should be sampled in preference to muscle or fat as residues in liver and kidney deplete more slowly than residues in muscle and fat and so will provide a better basis for verifying compliance with the withdrawal period,

the Committee recommends the establishment of maximum residue limits for gamithromycin in ovine tissues. Furthermore, and with reference to Article 5 of Regulation (EC) No 470/2009, the conclusions can be extrapolated to all ruminants except bovine, in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Gamithromycin	Gamithromycin	All ruminants except bovine	50 µg/kg 50 µg/kg 300 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents / Antibiotics

Based on these MRLs, the total theoretical maximum daily intake (TMDI) from tissues is 93.2 µg/person, which corresponds to 16% of the overall ADI. Taking into account the Injection Site Residue Reference Value (ISRRV) of 1500 µg/kg the TMDI from a food basket containing 300g of injection site muscle represents approximately 95% of the ADI.

4. Background information on the procedure

Submission of the dossier

Steps taken for assessment of the substance	29 October 2015
Application validated:	18 November 2015
Clock started:	19 November 2015
List of questions adopted:	17 March 2016
Consolidated response to list of questions submitted:	13 May 2016
Clock restarted:	15 May 2016
CVMP opinion adopted:	14 July 2016