European Medicines Agency
Veterinary Medicines and Inspections

16 July 2009
Doc. Ref: EMEA/CVMP/567075/2008

COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

EUROPEAN PUBLIC MRL ASSESSMENT REPORT (EPMAR)
GAMITHROMYCIN
Bovine species

INTRODUCTION

On 3 July 2009 the European Commission adopted a Regulation[1] establishing maximum residue limits for gamithromycin in bovine 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.

Gamithromycin is intended for use in bovine species for the treatment of respiratory diseases by single subcutaneous injection.

Gamithromycin had provisional maximum residue limits previously established for bovine species with an expiry date of 1 July 2009[2].

Mérial SAS submitted the application for the establishment of maximum residue limits to the European Medicines Agency (EMEA), on 1 August 2006.

Based on the original and complementary data in the dossier, the Committee for Medicines for Veterinary Use concluded on 14 January 2009 that the substance should be included in Annex I of Council Regulation (EEC) 2377/90, in accordance with Article 2 of the Regulation.

Subsequently, the Commission recommended on 5 June 2009 that maximum residue limits in bovine species are established. This recommendation was confirmed on 14 June 2009 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 3 July 2009.

EXPLANATORY NOTE

The European Public MRL Assessment Report is a public document giving the overview of the assessment carried out by the Committee for Medicinal Products for Veterinary Use (CVMP) of an application submitted for the establishment of maximum residue limits (MRLs). The document is based on the CVMP assessment report of the application from which confidential information has been deleted.

KEY WORDS: *gamithromycin, bovine, MRLs*

[1] Commission Regulation (EC) No 581/2009, O.J. L 175, of 04.07.2009
[2] Commission Regulation (EC) No 203/2008, O.J.L 60, of 05.03.2008

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SUMMARY OF THE SCIENTIFIC DISCUSSION FOR THE ESTABLISHMENT OF MRLs

Substance name: **Gamithromycin**

Therapeutic class: **Antimicrobial (macrolide)**

Procedure number: **EU/06/158/MER**

Applicant: **Mérial SAS**

Target species: **Bovine (non-lactating)**

Intended therapeutic indication: **Treatment of bacterial respiratory disease**

Route (s) of administration: **Subcutaneous**

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 was developed for non-lactating cattle for the treatment and prevention of bovine respiratory disease (BRD), caused by the bacteria *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*.

The product is administered by single-dose subcutaneous administration of 6 mg gamithromycin/kg body weight.

Gamithromycin is not used in human medicine.

2. Safety assessment

2.1 *Overview of pharmacological properties*

Pharcodynamic properties including mode of action

As for other macrolides, the principal mechanism of action against bacteria involves direct inhibition of essential protein biosynthesis by selective binding to bacterial 50S ribosomal subunits. Gamithromycin acts by stimulating the dissociation of peptidyl-tRNA from the ribosome during the translocation process. Results from oral acute and subchronic toxicology studies suggested that, apart from modest gastrointestinal disturbances, gamithromycin does not appear to exert remarkable secondary pharmacological activity on major organ systems including neurological, cardiovascular and renal effects.

Pharmacokinetic properties (mainly in laboratory animals)

Gamithromycin orally administered in rats and dogs was mainly excreted in faeces up to 90% and 74% respectively.

Pharmacokinetic information provided indicates that although the fraction of various metabolites may vary among species, gamithromycin is metabolised in a similar manner in rats, dogs and cattle.

2.2 *Calculation of pharmacological ADI*

Specific studies to establish a pharmacological ADI were not conducted. However, results from oral acute and subchronic toxicology studies suggested that, apart from modest gastrointestinal disturbances, gamithromycin does not appear to exert remarkable secondary pharmacological activity on major organ systems including neurological, cardiovascular and renal effects. Therefore, the establishment of a pharmacological ADI was not considered relevant for the safety assessment of the substance.

2.3 *Overview of toxicology*

Acute toxicity

The results of single oral dose studies in rats indicated low acute toxicity of gamithromycin. Following oral administration, the lethal dose was greater than the limit dose of 2000 mg/kg bw in rats. No substance-related effects were identified on respiration rate, body temperature, blood pressure or heart rate. However in some animals hypotonia, low body temperature, discoloured paws, alopecia, salivation, redness around the nose, discoloration of inguinal fur and diarrhoea were noted. Gamithromycin was considered to possess low acute toxicity.

Tolerance in target species

Target animal safety studies were conducted in cattle weighing about 200 kg at 0, 6 , 18 and 30 mg/kg bw/day (3 times recommended treatment at days 0, 5, 10). Local reactions were detected (site-swelling with heat). Pain was observed in the 18 and 30 mg/kg bw/day groups on day 1, but disappeared on day 4. At the end of the study, only mild thickened skin could be observed. Injection at the therapeutic dose was well tolerated. No other clinical effects could be detected during the course of the study. Gross lesions included skin thickening at the site of injection (6 mg/kg bw/day), discoloration in two animals and oedema on one shoulder. Microscopic alterations were minor. A moderate increase in platelet count occurred on days 10 and 15 in all treated groups. These alterations were considered secondary to the local inflammation process at the site of injection.

Repeated dose toxicity in rats

Three repeated-dose toxicity studies have been carried out in rats. The first study was developed over 13 weeks and the animals (20 per sex per dose) were exposed by gavage (0, 1, 3, 10, or 100 mg gamithromycin/kg bw/day). Effects included decrease in body weight and food consumption, higher absolute neutrophil count in females, moderately lower total protein and albumin for females, mildly higher cholesterol and mildly lower triglycerides for males, mildly increase aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) for males and females, mildly elevated alkaline phosphatase and 5'nucleotidase for males, mildly lower 5'nucleotidase for females, mildly higher inorganic phosphorus for males and females and mildly lower urine pH for females. Further observations were an increase in the adrenal weights for males (100 mg/kg bw/day), increase in mean ovary weight for females (100 mg/kg bw/day) and increased mean absolute and relative liver weight in males (100 mg/kg bw/day). There were no treatment-related macroscopic alterations. Hypertrophy of the bile duct epithelium, hepatocyte necrosis, multinucleated hepatocytes and centrilobular hepatocellular hypertrophy were observed in males and females in the high dose. Hypertrophy of medullary epithelium was observed in the kidney of males and females in the same group. All of these findings were without any histological correlates up to the highest dose tested. Based on described clinical and pathological observations, the dose of 10 mg/kg bw was considered as the NOEL for this study.

A second 13-week study was conducted in rats. Gamithromycin was administered in the diet at 0, 300, 600 or 1000 µg/kg feed corresponding to 0, 30, 60 or 100 mg/kg bw/day (10 animals per sex per dose). No clinical signs were observed and no difference in body weight was detected, although a trend towards a decrease in the high dose group was noted. Clinical pathology observations did not reveal any consistent pattern of alterations. No evidence of target-organ toxicity was found. Bile duct epithelial alterations were observed in the high dose group (mild cytoplasmic vacuolation). A NOEL of 60 mg/kg bw was set on the basis of change in body weight and bile duct alterations.

The third study in rats was conducted in order to elucidate the toxic mechanism underlying the toxic effects and lesions observed in the 13-week studies. Animals were exposed to 300 mg/kg bw/day by

gavage for 21 days. Body weight decreased was observed but no specific gross lesions could be detected. Histopathological findings include vacuolization in liver and kidney cells.

Repeated dose toxicity in mice

Two repeated-dose toxicity studies were carried out in mice. In the first study mice were exposed to gamithromycin in the feed at 0, 210, 420 or 2100 µg/kg feed, corresponding to 0, 50, 100 or 500 mg/kg bw/day, respectively (10 animals per sex per dose). No treatment-related clinical sign of toxicity was observed and no significant differences in body weight, food consumption, clinical chemistry or haematology data were observed. Some alterations were observed in the liver at 500 mg/kg bw/day consisting of finely granular, pale-staining cytoplasm around the nucleus of the hepatocytes. The NOEL in this study was 100 mg/kg bw/day.

In the second study, mice (10 per sex per dose) were exposed to a diet containing 0, 1470 or 1890 µg/kg feed of gamithromycin, corresponding to 0, 350 or 450 mg/kg bw/day. Body weight was decreased by 13 to 15% in the high dose group. Liver lesions of centrilobular vacuolation and necrosis/inflammation were recorded in this group as well. A NOEL of 350 mg/kg bw/day was established.

Repeated dose toxicity in dogs

Two repeated-dose toxicity studies have been carried out in dogs. In the first study animals were dosed 0, 1, 3, 10 or 30 mg/kg bw/day by oral gavage for 13 weeks (4 animals per sex per group). There were no treatment-related alterations with respect to body weight, food consumption, clinical signs or electrocardiographic evaluations. Significant effects on body weight of the 1 mg/kg dose group were recorded but there were no change in body weight in the higher dose groups. Gamithromycin did not induce any change in clinical pathology test results up to 10 mg/kg bw/day. Mildly increased AST and ALT were recorded at 30 mg/kg bw/day. Treatment-related microscopic alterations occurred in the liver of 2 males and 4 females at 30 mg/kg bw/day. Minimal retinal degeneration was detected at 30 mg/kg bw/day. A NOEL of 1 mg/kg was established.

In the second study, dogs received gamithromycin orally via gel-capsules daily for 52 weeks at 0, 0.3, 1 or 3 mg/kg bw/day (6 dogs per sex per group). No treatment-related signs of toxicity were noted and none of the clinical pathology alterations observed were consistent or dose-dependent. Treatment-related alterations included microscopic vacuolization in the epididymis of males and ocular non-pigmented epithelium in both genders at 3 mg/kg bw/day. A NOEL of 1 mg/kg bw/day was established.

Effects on reproduction including developmental effects

A two generation reproductive study in rats was performed at doses of 0, 100, 300 or 1000 µg/kg feed equivalent to 0, 10, 30 or 100 mg/kg bw (F0 and F1 parental, 26 rats per sex per group). Significant increases in pituitary weight were recorded in the high dose group (females, P, F1). However, there were no histopathological alterations. Bile duct vacuolation in the liver was detected at the high dose in adults (P, F1). Offspring survival was not affected. Litter size, however, was significantly reduced at 100 mg/kg bw/day. Consequently, increased offspring body weight was noted in this group. A treatment-related reduction in litter size was seen in the high dose group, while a slight and not significant reduction in litter size was seen in the low and intermediate groups. A NOEL of 30 mg/kg bw/day was retained.

In oral gavage embryo/foetal development study in rats, gamithromycin administered at doses of 0, 150, 300 and 450 mg/kg bw/day (during gestation day 7 through 18, with 25 animals per dose) caused a significantly decrease in body weight gains in the 150, 300 and 450 g/kg bw/day groups. Mortality was noted at 300 mg/kg bw/day (1 female) and at 450 mg/kg bw/day (17 females). In the 450-dose group, chromorhinorhea, signs of dehydration, soft or liquid faeces, red perioral substance, cold to touch and pale extremities, laboured breathing, decreased motor activity, prostration, tremors, rales, dyspnea were recorded. Chromorhinorhea was also detected in 2 and 8 females of the 150 and 300 mg/kg bw/day groups respectively. Dehydration, soft or liquid faeces, cold to touch, red perioral substance also occurred in dams in the 300 mg/kg bw/day group. Uterine weight at day 21 was significantly reduced in the high dose group. Body weight on day 21 corrected for uterine weight was significantly reduced in the 300 and 450 mg/kg bw/day groups. A NOEL for maternal toxicity could

not be set because effects were observed at the lowest dose (150 mg/kg bw/day). Caesarean-section observations were performed on 24, 22, 23 and 7 dams of groups 0, 150, 300, 450 mg/kg bw/day, respectively. Foetal body weight was reduced in the 300 and 450 mg/kg bw/day groups.

No other litter parameters were affected by the treatment. Delayed skeletal development, in relation to significantly reduced body weight was detected in the 2 high dose groups. Litter and foetal incidence of wavy ribs were increased in the 300 and 450 mg/kg bw/day groups. Foetal and litter incidence of incompletely ossified arch of lumbar vertebra, sternal centra, ischia and/or pubes were significantly increased in the high dose group. Significant reduction in the number of ossified caudal vertebrae and hind limb phalanges occurred in the 300 mg/kg bw/day group, while significant reduction in the number of ossified caudal vertebrae, xiphoid, forelimb metacarpals and phalanges and hind limb metatarsal and phalanges occurred in the high dose group. A NOEL of 150 mg/kg bw/day was established for foetal effects.

An oral gavage embryo/foetal development study was performed in mice administering doses of 0, 100, 300 and 1000 mg/kg bw/day (during gestation day 7 through 17, with 25 animals per dose). Four dams in the high dose group died. Clinical signs of relevance included decreased motor activity, ungroomed coat, ptosis, soft/liquid faeces. Maternal body weight and body weight gains were reduced in the high dose group. Gravid uterine weights were reduced in the 300 and 1000 mg/kg bw/day groups. Early resorptions were noted at 1000 mg/kg bw/day (2 litters being completely resorbed). They were 1, 2, and 1 dead foetuses in the 0, 300, 1000 mg/kg bw/day groups. Foetal body weights were significantly reduced in the high dose group. Foetal alterations included cleft palate, malpositioned testes, enlarged frontal sutures, incomplete ossified palate, incomplete ossified ribs and delays in sternal ossification and the number of ossification sites per litter in the hyoid, caudal vertebra, sternal centra, metacarpals, tarsals, metatarsals and phalanges of the fore- and hind limbs at 1000 mg/kg bw/day. Some reduced forelimb phalanges ossification sites were noted at 300 mg/kg bw/day. Based on these results, the NOEL for maternal and developmental toxicity was 300 mg/kg bw/day.

Mutagenicity

Gamithromycin was tested in a comprehensive series of mutagenicity test systems. Negative results were obtained in the Ames' assay, in the mouse lymphoma cell assay, in the *in vivo* micronucleus assay and in the *in vivo* unscheduled DNA synthesis assay. Positive results were detected at the highest doses (1200 µg/ml) in the *in vitro* chromosomal aberration assay with and without metabolic activation. Based on the mutagenicity tests it can be concluded that gamithromycin possesses a genotoxic potential *in vitro* which cannot be confirmed *in vivo*.

Carcinogenicity

Two carcinogenicity studies have been conducted in rats and mice. In rat, gamithromycin was administered in the diet at 0, 135, 490 or 1430 µg/kg feed (males) and 150, 570 or 1670 µg/kg feed (females) corresponding to 0, 30 to 40, 110 to 150 and 325 to 440 mg/kg bw/day (70 rats per sex per group). No specific clinical sign could be observed and linked with the treatment. Various clinical observations were reported in both the control and treated groups, but not found to be biologically significant. Mortality occurred in several instances, but the mortality rates and mean survival times were not significantly different between groups, except for the high dose group (males). Mean body weight gain was significantly decreased in the high-dose groups (5 to 23% in males, 4 to 20% in females) together with a decrease in mean food consumption. Clinical pathology did not reveal any specific and treatment-associated effect. Neoplastic lesions in males and females were not considered to be treatment-related, and no specific histopathological lesion could be detected. All neoplastic and non-neoplastic findings were incidental and within normal historical values for this species and strain, and without any dose-response relationship. Gamithromycin was not considered to be carcinogenic in rats.

In mice, an 18-month carcinogenicity study was conducted. Gamithromycin was administered in the diet at 0, 100, 300 or 1000 µg/kg corresponding to 0, 10, 30 or 100 mg/kg bw/day (70 mice per sex per group). No specific clinical sign could be observed and linked with the treatment. Mean body weight gain was significantly decreased in the high-dose group females (minus 11%), together with a

decrease in mean food consumption (minus 5%). A 5% decrease in body weight gain was noted across all treated groups in males. Clinical pathology did not reveal any specific and treatment-associated effect. Neoplastic lesions in males and females were not considered to be treatment-related, and no specific histopathological lesion could be detected. Gamithromycin was not considered to be carcinogenic.

Neurotoxicity

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

2.4 *Calculation of the toxicological ADI*

The lowest NOEL in the toxicological evaluation was identified in the 52-week repeated dose toxicity study in dogs at 1 mg/kg bw/day. Based on this NOEL and applying the standard uncertainty factor of 100 to account for inter and intra-species variability, a toxicological ADI of 0.01 mg/kg bw (0.6 mg/person) was established.

2.5 *Overview of microbiological effects*

Potential effects on human gut flora

MIC_{50} values for 10 genera representing the normal human intestinal microbiota were determined. The lowest MIC_{50} values ranged from Bifidobacterium (0.125 µg/ml), Clostridium (0.25 µg/ml), Fusobacterium (0.5 µg/ml), Eubacterium (0.5 µg/ml), Enterococcus (2 µg/ml), Escherichia (2 µg/ml), Bacteroides (4 µg/ml), other Bacteroides (4 µg/ml), Peptostreptococcus (4 µg/ml) to Lactobacillus (32 µg/ml). The MIC_{calc} was 0.74 µg/ml.

The fraction of an oral dose available for colonic microorganisms was calculated using the percentage of total radioactivity of gamithromycin found in the colon of dogs at 6 hours after administration in combination with *in vitro* binding studies on human faeces. The amount of gamithromycin residue entering the dog colon 6 hours after two oral doses of approximately 10.7 mg/kg bw is about 44% of the daily oral dose.

The loss of antimicrobial activity of gamithromycin after interaction with faecal slurries was examined in a GLP compliant study including faeces from seven human donors. A number of variables were addressed including the impact of faecal slurry concentration and contact time between slurry and residue concentration. Incubations with gamithromycin/faeces combinations were performed for time periods of up to 12 hours, after which faecal solids were removed by centrifugation and the antibacterial activity in the supernatant was examined by use of *Enterococcus gallinarum* as test organism. The results showed considerable variability between concentrations of the same sample and also between samples from different individuals.

Apparent binding was highest in the 50% faecal concentration reaching more than 83% at all time points in one of three experiments, with lower levels of apparent binding seen following incubation with a 25% faecal slurry. There are presently no accepted and validated protocols for this type of microbiological investigation and due to model inherent limitations, the nature and reversibility of the interaction seen remains unknown. Additionally, use of sterilised faecal material (in which enzyme activity is destroyed) makes interaction via covalent enzyme-catalysed binding highly unlikely, and confirmatory data based on chemical methods or adsorption/desorption type studies were not used in order to determine the nature of the binding.

Due to the concerns raised above, the CVMP concluded, in its original assessment, that the fraction of the oral dose considered to be available to microorganisms in the gut should not be lowered based on the binding/inactivation studies provided. Therefore, the accepted fraction of the oral dose available (44%) was used in the calculation of the microbiological ADI.

In the appeal the applicant requested the CVMP to reconsider its decision not to take the *in vitro* binding/inactivation studies into account. In the ensuing assessment the Committee upheld its original concerns but considered that, despite these, the data do demonstrate that, to some extent, gamithromycin is inactivated by faecal material. However, the CVMP considered that the data were not sufficiently robust to support the applicant's conclusion that 83% of the drug was inactivated.

Due to its reservations over the method and the interpretation of the results, the Committee concluded that it would be appropriate to accept that the degree of inactivation corresponds to the lowest degree of inactivation seen following incubation of drug with a 50% faecal slurry. In this case the lowest level of inactivation seen with a 50% slurry was 50%.

The overall fraction of an oral dose available to colonic microorganisms was concluded to be 0.22 (0.44 x 0.50).

Increase of resistance

On the basis of the data from azithromycin suggesting that the sub-lethal concentrations of the substances of the azalide class of antibiotics including gamithromycin present a low potential hazard for inducing resistance development in commensal bacteria, the Committee agreed that it was not necessary to determine a microbiological ADI with respect to resistance development.

Potential effects on microorganisms used in industrial processing of foodstuffs

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

2.6 Calculation of microbiological ADI

For the establishment of the microbiological ADI the following formula was used:

$$\text{ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{mass of colonic contents}}{\text{Fraction oral dose available} \times \text{weight of human}}$$

Where:

- MIC_{calc} is defined as MIC_{calc} is defined as the lower 90% confidence limit for the mean MIC_{50} of the most relevant genera;
- Mass of colon contents is 220g;
- Weight of human is 60kg;
- Fraction of oral dose available is defined as total dose available for colonic microorganisms based on *in vivo* measurements for the drug administered orally.

Using the input data of MIC_{calc} (0.74µg/ml) and the value obtained from the reported assay on the fraction of oral dose available (0.22) the microbiological ADI with respect to the colonisation barrier is calculated as follows:

$$\text{ADI} = \frac{0.74 \times 220}{0.44 \times 0.5 \times 60} = 12.33 \text{ µg/kg bw (i.e. 740 µg/person)}$$

2.7. Overall conclusions on the ADI

The establishment of a pharmacological ADI was not considered necessary for the safety assessment of gamithromycin. A toxicological ADI of 0.01 mg/kg bw/day (i.e. 0.6 mg/person) was established based on the lowest NOEL identified in the toxicological evaluation of 1 mg/kg bw/day observed in the 52-week repeated dose toxicity study in dogs and using an uncertainty factor of 100 to account for inter and intra-species variability. A microbiological ADI of 12.33 µg/kg bw (i.e. 740 µg/person) with respect to the colonisation barrier was established.

As the toxicological ADI is lower than the microbiological ADI, the toxicological ADI of 10 µg/kg bw (i.e. 600 µg/person) was retained as the overall ADI for gamithromycin.

3. Residues assessment

3.1 *Pharmacokinetics in target species*

Pharmacokinetic studies in pre-ruminant and ruminant cattle at the recommended dose (6 mg/kg bw, as a single subcutaneous injection) indicated rapid absorption from the injection site. Absolute intramuscular bioavailability was almost 100%. The pharmacokinetics of gamithromycin were characterised by a long plasma elimination half-life of about 50 hours and a large apparent volume of distribution of approximately 25 l/kg. This is consistent with the observation of significant distribution in various tissues including the respiratory tract.

3.2 *Residue depletion studies*

The metabolites of tritiated-gamithromycin in the excreta of cattle (dosed at 6 mg/kg bw, subcutaneously) and laboratory species rat and dog (100 mg/kg or 10 mg/kg, orally, respectively) were similar, with the parent compound being metabolised and eliminated primarily as the unchanged substance in faeces. Recovery of total radioactivity in faeces was up to 90%, 35% and 58% of the dose in rat, dog and cattle, respectively. Likewise, the major component in tissues was the unchanged substance. Small quantities of the metabolites in excreta and tissue samples of all three species included declad (loss of a cladinose), N-dealkylated-declad and translactone derivative. Some minor metabolites derived from combinations of oxidation and/or N-dealkylation processes were detectable as well.

Gamithromycin was identified as the marker residue. Extractability of radioactive residues in liver decrease with time from about 85% at 21 days to about 60% at 70 days. The ratio of marker to total residues in liver was 0.1 to 0.21. In kidney the figures for total residue were 741, 51 and 10 µg equivalents/kg and for parent compound 350, 137 and less than 10 µg/kg leading to marker to total ratios between 0.27 to 0.47. In muscle tissue, total residues were 38 equivalents/kg at 21 days and below limit of quantification thereafter. No parent compound could be detected in muscle at any time point. Similar figures occurred in the fat tissue. Total injection site residues were 16048, 649 and 80 µg equivalents/kg on days 21, 49 and 70 respectively. It was reported that only 65%, 29% and 27% of the total drug in the injection site was parent compound.

In a non-radiometric study gamithromycin was administered subcutaneously at a dose of 6 mg/kg. Six cattle were slaughtered at days 10, 21, 35, 49 and 70 after treatment. Analyses were performed by LC-MS/MS. The concentration of gamithromycin in liver declined from 2790 µg/kg at day 10 to 175 µg/kg at day 35 after which the concentration was below limit of quantification (100 µg/kg). In kidney gamithromycin concentration declined from 2470 µg/kg at 10 days to 17.4 µg/kg at 49 days (limit of quantification equal to 10 µg/kg). In muscle tissue no residue could be found (limit of detection equal to 10 µg/kg) at any time point. In fat residues could only be detected at 10 days at a concentration of 30 µg/kg. Injection site residues were high and declined from 25563 µg/kg at 10 days to 372 µg/kg at 49 day. At day 70 one out of 6 animals still had residue at the injection site (81 µg/kg).

3.3 *Selection of marker residue and target tissues*

From the results of the radiolabelled study it was concluded that gamithromycin should be retained as marker residue.

Based on the total residue study, a long withdrawal period is expected when the residue level at the injection site is included in the food basket. Therefore ratios of marker to total residues were determined on basis of the residue data from day 49 (as ratios from later time points were not available).

The ratio of marker to total residues at day 49 was 0.10 in liver, 0.27 in kidney, 0.71 in fat and 0.29 in injection site muscle.

Considering that very low residues were found in fat and muscle and that at least one MRL should be established in a carcass tissue, fat was considered the most appropriate target tissue for monitoring purposes.

3.4 *Elaboration of MRLs*

Based on the residue depletion data MRL values of 200 µg/kg and 100 µg/kg can be recommended for liver and kidney respectively. Very low residue levels were detected in fat and muscle and were not quantifiable in muscle at day 10 and in fat at day 21. Considering that at least one MRL should be established in a carcass tissue an MRL value at twice the limit of quantification of the analytical method, i.e. 20 µg/kg should be recommended for fat.

Gamithromycin is not intended for use in dairy cattle and therefore residue depletion data in milk were not provided. As a consequence no MRL is recommended for milk and the use of the substance shall be restricted to animals that do not produce milk for human consumption.

3.5 *Calculation of theoretical daily intake of residues*

Detailed calculation of theoretical daily intake of residues

Edible tissue or products	***Daily consumption (kg)***	***MRL proposal (µg/kg)***	***Ratio of the marker/total residue***	***Residue amount per edible tissue or product (µg/kg)***
Muscle	*0.30*	---	---	---
Fat	*0.05*	*20*	*0.71*	*1.41*
Liver	*0.10*	*200*	*0.10*	*200*
Kidney	*0.05*	*100*	*0.27*	*18.52*
Estimated total daily intake (µg/person)				*219.93*
ADI (µg/person)				*600*

The theoretical consumer intake of total residues represents 36.6% of the ADI.

3.6 Analytical method for monitoring of residues

A HPLC/MS/MS method for determination of the marker residue, gamithromycin, in bovine tissues (fat, liver and kidney) validated according to the requirements of Volume 8 of The Rules Governing Medicinal Products in the European Union, is available. The limits of detection and quantification in fat, liver, and kidney are 1 µg/kg and 10 µg/kg, respectively.

4. Conclusions and recommendation

Having considered that:

- the toxicological ADI of 10 µg/kg bw (i.e. 600 µg/person) was 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 49 days were 0.10 in liver, 0.27 in kidney, 0.71 in fat and 0.29 in injection site muscle,
- residue concentrations were persistently low in fat and muscle and fat was chosen as the target tissue for monitoring of residues in the carcass with an MRL set at twice the limit of quantification,
- a validated analytical method for the monitoring of residues of gamithromycin in edible bovine tissues (liver, kidney and fat) is available;

The Committee recommends the inclusion of gamithromycin into Annex I of Council Regulation (EEC) No 2377/90 for bovine species in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
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

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

Glossary

ADI – Acceptable daily intake
HPLC/MS/MS – High performance liquid chromatography with tandem mass spectrometry
MRL – Maximum residue limit
NOEL – No observed effect level

Background information on the procedure

Submission of the dossier:	1 August 2006
Steps taken for assessment of the substance:	
Application validated:	10 August 2006
Clock started:	11 August 2006
List of questions adopted:	8 November 2006
Consolidated response to list of questions submitted:	11 June 2007
Clock re-started:	12 June 2007
CVMP opinion adopted:	11 July 2007
Notification of intention to appeal submitted:	23 July 2007
Grounds for appeal submitted:	14 August 2007
Clock started:	15 August 2007
Oral explanation provided by applicant:	9 October 2007
CVMP opinion adopted:	10 October 2007
Establishment of provisional MRLs by European Commission	*4 March 2008*
Submission of the responses to the list of questions to EMEA after provisional MRLs	17 October 2008
Submission of the responses to the list of questions to Rapporteurs after provisional MRLs	24 October 2008
Clock re-started:	24 October 2008
CVMP opinion adopted:	14 January 2009