European Medicines Agency
Veterinary Medicines and Inspections

London, 28 November 2007
Doc. Ref: EMEA/CVMP/469245/2007-Final

COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

EUROPEAN PUBLIC MRL ASSESSMENT REPORT (EPMAR)

TYLVALOSIN (ACETYLISOVALERYLTYLOSIN) (Modification of the acceptable daily intake, ADI)

INTRODUCTION

On 21 November 2007 the European Commission adopted a Regulation[1] modifying the name of the substance formerly known as acetylisovaleryltylosin for which MRLs are established in Annex I of Council Regulation (EEC) No 2377/90, to tylvalosin (INN).

These maximum residue limits were based on the previous favourable opinions and the assessment reports adopted by the Committee for Medicinal Products for Veterinary Use.

Tylvalosin had maximum residue limits already established for porcine and poultry species.

In addition, ECO Animal Health submitted an application for the modification the ADI for tylvalosin to the European Medicines Agency (EMEA), on 6 July 2005.

Tylvalosin is used in pigs in the prevention and treatment of swine enzootic pneumonia, treatment of porcine proliferative enteropathy (ileitis) and swine dysentery orally. Tylvalosin is also intended to be used in chickens for the treatment of mycoplasmosis and in turkeys for the treatment of disease due to *Ornithobacterium rhinotracheale*.

The recommendation to modify the name of acetylisovaleryltylosin to its INN name, tylvalosin, including the modification of the name of its marker residue, was confirmed on 28 September 2007 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 20 November 2007.

Based on the original and complementary data in the dossier, the Committee for Medicines for Veterinary Use concluded that the final microbiological ADI and overall ADI for tylvalosin is modified to 2.07 µg/kg bw (124.2 µg/person).

EXPLANATORY NOTE

The European Public MRL Assessment Report is a public document giving the overview of the assessment carried out by the Committtee 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: Tylvalosin, acetylisovaleryltylosin, 3-O-acetyltylosin, porcine, poultry

[1] Commission Regulation (EC) No 1353/2007, O.J. L 303 of 21.11.2007

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

Substance name: **Tylvalosin**

Procedure number: **EU/05/149/ECO**

Applicant: **ECO Animal Health**

Target species: **Porcine, poultry**

1. Introduction

Tylvalosin (INN), formerly known as acetylisovaleryltylosin, used as the tartrate salt, is a macrolide antibiotic which is active against Gram-positive bacteria. Tylvalosin has a similar chemical structure to tylosin. The substance is manufactured from tylosin by a bioconversion process. The drug substance contains at least 80% tylvalosin and also contains some impurities derived from substances present in the starting material. The substance is used in pigs in the prevention and treatment of swine enzootic pneumonia, treatment of porcine proliferative enteropathy (ileitis) and swine dysentery orally. Tylvalosin is also intended to be used in chickens for the treatment of mycoplasmosis and in turkeys for the treatment of disease due to *Ornithobacterium rhinotracheale*.

Tylvalosin was previously assessed by the CVMP and a microbiological ADI of 1.02 µg/kg bw (i.e. 61 µg/person) and a toxicological ADI of 220 µg/kg bw were established. The microbiological ADI was considered the overall ADI.

Currently, acetylisovaleryltylosin (tylvalosin) is included in Annex I of Council Regulation (EEC) No 2377/90 for porcine and poultry species in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Tylvalosin	Sum of tylvalosin and 3-O-acetyltylosin	Porcine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	
		Poultry	50 µg/kg 50 µg/kg	Skin + fat Liver	Not for use in animals from which eggs are produced for human consumption.

An application was submitted to modify the microbiological ADI, in accordance with the current CVMP-VICH guideline 36: General Approach to Establish a Microbiological ADI (CVMP/VICH/467/03-FINAL).

Further to the assessment of the application, a CVMP opinion on the modification of the ADI for tylvalosin was adopted in June 2007. The CVMP agreed to modify the acceptable daily intake (ADI) for tylvalosin (acetylisovaleryltylosin) to 1.04 µg/kg bw i.e. 62.4 µg/person but did not agree with the applicant's proposal to further increase the ADI.

An intention to appeal against the opinion and grounds for appeal were subsequently submitted to the EMEA. The applicant also provided oral explanations to the Committee.

2. Safety assessment

2.1 *Overview of microbiological effects*

Potential effects on human gut flora

A new GLP-compliant study to determine MIC data for both tylvalosin and its major metabolite, 3-acetyltylosin, was performed in accordance with the current CVMP/VICH guideline, using an appropriate range and number of relevant bacterial strains. MIC_{50} values were determined for ten strains from each of the groups of bacteria listed in the guideline, giving a total of 100 individual strains. All of the tested strains were isolated from faecal samples obtained from healthy human volunteers (male and female) who had experienced no symptoms of diarrhoea within the 4 weeks prior to sample collection and had no antibiotic treatment in the 3 months prior to collection. Within each group, the individual strains were from different individuals. The test system used was standardised agar dilution MIC methodology in accordance with NCCLS standards.

The results for tylvalosin were as follows: Bacteroides fragilis 0.5 µg/ml, Bacteroides non-fragilis spp. 0.5 µg/ml, Bifidobacterium 0.062 µg/ml, Clostridium 0.25 µg/ml, Enterococcus 1.0 µg/ml, Escherichia coli more than 128 µg/ml, Eubacterium 0.5 µg/ml, Fusobacterium 4.0 µg/ml, Lactobacillus 2.0 µg/ml, Peptostreptococcus 0.5 µg/ml.

The results for 3-acetyltylosin were as follows: Bacteroides fragilis 1.0 µg/ml, Bacteroides non-fragilis spp. 1.0 µg/ml, Bifidobacterium 0.062 µg/ml, Clostridium 0.0625 µg/ml, Enterococcus 2.0 µg/ml, Escherichia coli more than 128 µg/ml, Eubacterium 0.25 µg/ml, Fusobacterium 0.5 µg/ml, Lactobacillus 4.0 µg/ml, Peptostreptococcus 0.5 µg/ml. In the study to determine MIC values for 3-acetyltylosin, the purity of the batch of 3-acetyltylosin was reported as 87.46%.

MICcalc values for tylvalosin and 3-acetyltylosin were determined using the MIC50 values and calculated to be 0.335 µg/ml and 0.226 µg/ml, respectively.

A faecal binding study was conducted with tylvalosin and 3-acetyltylosin. Faecal samples from three healthy human volunteers (male and female) which were diluted using Mueller Hinton Broth were used in the study. Faecal concentrations of 0, 10, 25 and 50% were incubated at 37 ± 1°C and samples (1.0 to 1.3 ml) removed at 0 (immediately after preparation), 0.5, 1, 2, 6 and 8 hours. Samples were then centrifuged and duplicate aliquots of the supernatant pipetted into wells in two separate microtitre plates, one of which served as a sterility control and received no further treatment, while the other had E. faecalis suspension added to each well giving a final bacterial density of approximately 5 x 105 cfu/ml. Plates were read after 24 ± 2 hours.

For the 10 and 25% faecal concentrations, 50% binding was observed immediately in 2 of 3 samples for both the parent drug and the metabolite 3-acetyltylosin. Apparent binding increased to up to 80% after 6 or 8 hours in 3 of the 6 samples. In one of the 3 samples no apparent binding was observed until after 6 hours. Apparent binding was highest in the 50% faecal concentration reaching more than 98% after 6 hours for both substances.

The CVMP, during its initial assessment, raised concerns regarding the validation of the protocols for this type of microbiological investigation and identified inherent limitations of the model. However, during the appeal procedure the applicant provided detailed clarifications on the method used for the microbiological assay. Following these clarifications the CVMP considered that overall, the *in vitro* data do indicate that tylvalosin is inactivated by faeces in significant amounts. The Committee considered that 50% of the active substance reaching the gut could safely be considered to be inactivated by faeces. The data were not sufficiently robust to support the applicant's conclusion that 98% of the drug was inactivated.

Therefore, the previously accepted fraction of the oral dose available (0.8) multiplied by a binding factor of 0.5 was used in the calculation of the microbiological ADI.

There are generally considered to be three mechanisms responsible for resistance to macrolides, often referred to as MLSB (macrolides, lincosamides and streptogramin B) resistance as they also affect lincosamides and streptogramins. The first involves alteration of the ribosomal target site, due to the presence of erm gene-encoded methyltransferases. The second involves utilisation of active efflux mechanisms effected by a membrane protein encoded by a number of different genes and was initially considered to be specific to macrolides. A number of genes (mefA, mefE, msrA, msrC and mreA) have been associated with macrolide efflux in Gram-positive bacteria. Whilst mef and mreA genes have clearly been associated with macrolide resistance, the msrA gene has also been associated with streptogramin resistance. These genes are transmissible, and are considered to be transmitted via transposons. The third mechanism of resistance is less common and involves production of inactivating enzymes.

Via the MLSB resistance mechanism, macrolide resistance is linked to lincosamide and streptogramin resistance. Many of these macrolide resistance genes are borne on transmissible genetic elements with a broad host-range and are capable of being transmitted from Gram-positive to Gram-negative bacteria and vice versa. Many of these macrolide resistance genes have also become physically linked to other antibiotic resistance genes. Macrolide resistance is most commonly acquired by enterococci from the ermB gene. These genes encode a group of rRNA methyltransferases that dimethylate adenine residues in the 23S rRNA that correspond to position 2058 in E. coli. This results in an MLSB resistant phenotype. ErmB genes also confer MLSB resistance to Streptococcus.

Risk assessment that considered the use of tylvalosin and other macrolides and the risk of resistance development was considered. Enterococcus spp. have in recent years emerged as important bacterial pathogens in nosocomial infections which have acquired resistance to different antibiotics. Determinative risk assessment addressing public health consequences of macrolide use in food animals within the US population concluded that the risk of acquiring enterococci resistant to the agents of choice for the treatment of human enterococcal infections via the food chain to be limited. Furthermore, it was argued that the probability of using a macrolide for therapy of a serious enterococcal infection in human medicine would be remote.

A study was conducted using a mixture of three Enterococcus strains, with differing erythromycin susceptibility (MIC of erythromycin from 0.062 to 1 µg/ml), all isolated from the faeces of healthy unmedicated humans. Broth cultures of the three strains were mixed (approximately equal cell density of each strain) and this mixed culture was used to inoculate two portions of sterile 20% w/v pooled human faeces in Mueller Hinton Broth. The two faecal slurries contained either no antimicrobial agent or erythromycin at a concentration of 0.016 µg/ml. These inoculated slurries were incubated at 37°C for 24 hours and appropriate dilutions were sub-cultured onto Kanamycin Aesculin Azide Agar (KAAA), for enumeration of Enterococcus species. Each slurry was sub-cultured onto KAAA containing no additional antimicrobial agents and onto the same agar containing erythromycin concentrations of 0.5, 2 and 8 µg/ml, to identify development of any Enterococcus population with increasing levels of erythromycin resistance. In the erythromycin treated faecal slurry, viable counts obtained on KAAA containing 8 µg erythromycin per ml fell below the limit of detection (20 cfu/ml; log10 = 1.3) on all but one occasion during the 31 day experiment. The isolated increase in erythromycin resistance was considered transient and could not be clearly attributed to the presence of erythromycin in the faecal slurry. This study demonstrated that continuous exposure of Enterococcus spp. to a sub-MIC residue-like concentration of erythromycin (0.016 µg/ml) did not produce any measurable increase in erythromycin resistance within the Enterococcus population. Considering cautiously the above arguments, it was deemed not to be necessary to determine a microbiological ADI with respect to resistance development.

2.2 Calculation of microbiological ADI

For the purposes of calculating the microbiological ADI, it was considered that the worst case would be to consider that all the material reaching the colon is the metabolite 3-acetyltylosin (3-AT) and 80% of the fraction of the oral dose is available to microorganisms.

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.

And therefore the microbiological ADI was calculated as indicated below:

$$\text{ADI} = \frac{0.226 \text{ µg/ml} \times 220 \text{ g}}{0.8 \times 0.5 \times 60 \text{ kg}} = 2.07 \text{ µg/kg bw µg/kg bw} = 124.2 \text{ µg/person}$$

The following assumptions were made:

- MIC_{calc} 3-acetyltylosin = 0.226 µg/ml (the MIC_{calc} values were calculated for both tylvalosin and 3-acetyltylosin and found to be 0.335 µg/ml and 0.226 µg/ml respectively)
- 220 g was the weight of the daily faecal bolus,
- 0.8 was the portion of an oral dose that enters the colon (70 to 88% of the total radiolabelled oral dose was excreted in the faeces of rats and pigs, only 1 to 7% was identified as parent compound but in the absence of data to show otherwise, the metabolites were assumed to have the same antimicrobial activity as the parent compound).
- 0.5 was the portion of the substance reaching the colon that remains available to to microorganisms

2.3. ***Overall conclusions on the ADI***

The Committee for Medicinal Products for Veterinary Use concluded that the microbiological ADI for tylvalosin is modified to 2.07 µg/kg bw (124.2 µg/person).

Considering that the previously established toxicological ADI is 220 µg/kg bw, the microbiological ADI remains the overall ADI for tylvalosin.

3. Conclusions and recommendation

Having considered that:

- the MIC_{calc} values were calculated for both tylvalosin and 3-acetyltylosin and found to be 0.335 µg/ml and 0.226 µg/ml respectively,
- the fraction of the oral dose available for microorganisms was found to be 40% (80% x 50%)
- the microbiological ADI was based on the MIC_{calc} for the metabolite (3-acetyltylosin), which is lower than that of the parent tylvalosin,
- the microbiological ADI was confirmed as the overall ADI,
- existing MRLs for tylvalosin remain unchanged;

The CVMP, further to the consideration of the grounds for appeal and the argumentation presented during the oral explanation, recommends that the final microbiological ADI and overall ADI for tylvalosin is modified to 2.07 µg/kg bw (124.2 µg/person).

Background information on the procedure

Submission of the dossier	27 May 2005
Steps taken for assessment of the substance	
Application validated:	7 July 2005
Clock started:	8 July 2005
List of questions adopted:	5 October 2005
Consolidated response to list of questions submitted:	14 April 2007
Clock re-started:	15 May 2007
CVMP opinion adopted:	13 June 2007
Notification of intention to appeal submitted:	2 July 2007
Grounds for appeal submitted:	13 August 2007
Clock started:	14 August 2007
Oral explanation provided by applicant:	9 October 2007
CVMP opinion adopted:	10 October 2007