European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

ACETYLISOVALERYLTYLOSIN
(Extension to poultry)

SUMMARY REPORT (3)

1. Acetylisovaleryltylosin, used as the tartrate salt, is a macrolide antibiotic which is active against Gram-positive bacteria. Acetylisovaleryltylosin has a similar chemical structure to tylosin. The substance is manufactured from tylosin by a bioconversion process. The drug substance contains at least 80 % acetylisovaleryltylosin and also contains some impurities derived from substances present in the starting material. It is used as an aid in the prevention and treatment of Swine (Mycoplasma) Enzootic Pneumonia (Swine Epidemic Pneumonia) caused by sensitive organisms. The product is a 5% w/w premix which is used incorporated into pig feed, for up to 5 days, at a rate of 0.4 to 1 kg product per tonne of finished feed, equivalent to 20 to 50 mg/kg feed acetylisovaleryltylosin activity.

   Acetylisovaleryltylosin has been evaluated previously by the CVMP and a microbiological ADI of 1.02 µg/kg bw (i.e. 61 µg/person) was established. Currently, acetylisovaleryltylosin is included in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Acetylisovaleryl-tylosin	Sum of acetyliso-valeryltylosin and 3-O-acetyltylosin	Porcine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	

2. An application has now been submitted for an extension of the MRLs for acetylisovaleryltylosin to poultry. The proposed indication for chicken is the treatment of mycoplasmosis due to *M. gallisepticum* and in turkey treatment of disease due to *Ornithobacterium rhinotracheale*. The recommended dose is 25 to 40 mg/kg bw for 3 to 5 days.

3. A series of *in vivo* and *in vitro* studies indicated that acetylisovaleryltylosin had no important pharmacological effects apart from the antimicrobial activity.

   In old non-GLP *in vivo* and *in vitro* absorption, distribution, metabolism and excretion studies acetylisovaleryltylosin was rapidly absorbed after oral administration to rats, pigs and chickens. In pigs, peak blood concentrations were attained 2 hours after oral dosing and in under an hour in chickens. The substance and a metabolite 3-O-acetyltylosin were widely distributed to the tissues. In rats, pigs and chickens residues of 3-O-acetyltylosin were higher than residues of the unchanged substance within 2 hours of administration. Highest concentrations were found in the liver, bile and kidneys.

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4. Sixteen male and 15 female chickens were administered daily oral doses of 20 mg ^{14}C-acetyliso-valeryltylosin tartrate/kg body weight/day for 3 consecutive days orally in the crop. Excreta and cage wash were examined and the major route of elimination was via excreta which accounted for approximately 90 % of the administered dose. The total radioactivity recovered in liver and kidneys following repeat oral administration of [^{14}C] acetylisovaleryltylosin varied between 0 and 0.03 % of the total administered dose. The relative distribution of radioactivity between the various tissues generally remained unchanged with time.

5. In the same study as above, chicken were slaughtered (in groups of 3 males and 3 females) at 12 hours, 3, 5 and 7 days after the last dose. Total residues in tissues were determined by combustion and liquid scintillation counting. Total residues were highest in liver and depleted from mean values of 467 µg equivalents/kg at 12 hours to 107 and 83 µg equivalents/kg at 3 and 5 days. Mean total residues in kidney depleted from 271 µg equivalents/kg at 12 hours to 39 and 21 µg equivalents/kg at 3 and 5 days respectively. Mean total residues in muscle depleted from 24 µg equivalents/kg at 12 hours to undetectable levels after 3 days. Mean total residues in abdominal fat pad fat samples were 119 µg equivalents/kg at 12 hours, 92 µg equivalents/kg at 3 days and 44 µg equivalents/kg at 5 days. Mean total residues in samples of skin plus fat were 686 µg equivalents/kg at 12 hours, 298 µg equivalents/kg at 3 days and 99 µg equivalents/kg at 5 days. Samples of liver, kidney and skin/fat from animals killed 12 hours and 3 days after the last dose were extracted with methanol and re-analysed by liquid scintillation counting. The samples were then analysed by HPLC-radioprofiling. In the chickens killed 12 hours after dosing, 3-O-acetyltylosin accounted for approximately 1.9 to 16.3 % and 46.5 to 77.6 % of the total residues in liver and kidney respectively. Acetylisovaleryltylosin accounted for approximately 1.5 % in liver and was not detected in kidneys. In the chickens killed 12 hours after dosing, 3-O-acetyltylosin accounted for 3.5 to 21.8 % and acetylisovaleryltylosin accounted for 12.8 to 29.6 % of the total residues in skin/fat respectively. Up to 5 unidentified components were found in the liver and kidney samples, each accounting for 1 to 39 % of the total residues and up to 9 unidentified components were found in skin/fat samples, each accounting for 1 to 24.7 % of the total residues.

6. Six turkeys were administered daily oral doses of 50 mg acetylisovaleryltylosin/kg bw/day for 5 days. The presence of the proposed marker residues of acetylisovaleryltylosin in edible tissues was confirmed.

7. The proposed routine analytical method for chicken tissues was based on HPLC with mass spectrometric detection and was described in the ISO 78/2 format. The limit of quantification was 25 µg/kg for the combined marker residue in all chicken tissues. The method had been validated with respect to accuracy, precision (reproducibility and repeatability) and linearity but specificity and stability in a number of freeze thaw cycles has not been demonstrated, therefore the method is not validated according to Volume 8 of the Rules Governing Medicinal Products in the European Union.

   The same analytical method was validated for turkey tissues in respect to accuracy, precision (repeatability and reproducibility) and linearity. The limit of quantification was 25 µg/kg for the combined marker residues in turkey tissues. However the accuracy was not fully in accordance with Volume 8. The method can be used in turkeys and fulfils the requirements of the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL).

Conclusions and recommendation

Having considered:

- an ADI of 1.02 µg/kg bw (i.e. 61 µg/person) was established for acetylisovaleryltylosin,
- the sum of acetyliso-valeryltylosin and 3-O-acetyltylosin was identified as the marker residue in chickens and turkeys,
- in chickens the mean total residue in all tissues was rapidly depleted so that at 12 hours, 3, 5 and 7 days after treatment the amount of residues likely to be ingested by consumers represents 193.7, 63.6, 28.5 and 28.4 % of the ADI respectively,
- in chicken the ratio of the marker to total residue in skin plus fat was 0.2 after 3 days but marker residue levels were below the limit of quantification in all other tissues and residue intake was calculated from the total residue levels,
- no MRLs should be set for muscle and kidney tissues as the residue levels from day 3 onwards are very low,
- no residue studies nor analytical method were provided for eggs, so the substance will not be allowed to be used in birds from which eggs are produced for human consumption,
- a validated routine analytical method for the determination of the marker residue in edible tissues of chickens is available that can provisionally be used for monitoring purposes until the outstanding issues relating to validation have been completed,
- an analytical method for the determination of residues in tissues of poultry is available;

the Committee recommends the inclusion of acetylisovaleryltylosin in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Acetylisovaleryl-tylosin	Sum of acetylisovaleryl tylosin and 3-O-acetyltylosin	Poultry	50 µg/kg 50 µg/kg	Skin + fat Liver	Not for use in animals from which eggs are produced for human consumption. Provisional MRLs expire on 1.7.2006

Based on these MRLs, the daily intake of total residues from the consumption of poultry meat would represent about 64.9 % of the ADI.

Before the Committee for Medicinal Products for Veterinary Use can consider the inclusion of acetylisovaleryltylosin in Annex I of Council Regulation (EEC) No. 2377/90 for poultry, the points listed in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should complete the validation of the analytical method for liver and skin plus fat tissues of chickens in accordance with the requirements of Volume 8. Specificity and stability in a number of freeze thaw cycles should be demonstrated.