European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

ACETYLISOVALERYLTYLOSIN
(Extension to poultry)

SUMMARY REPORT (4)

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 authorised for pigs as an aid in the prevention and treatment of Swine Enzootic Pneumonia as a premix for medicated feed. Acetylisovaleryltylosin is also intended for use in chickens for the treatment of mycoplasmosis and in turkeys for the treatment of disease due to *Ornithobacterium rhinotracheale*. The proposed dose is 25 to 40 mg/kg bw for 3 to 5 days.

2. Acetylisovaleryltylosin was previously assessed 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 for porcine species 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	

   and in Annex III for poultry as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Acetylisovaleryl-tylosin	Sum of acetyliso-valeryltylosin 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

   Additional data have now been submitted concerning further validation of the analytical method for the monitoring of residues in poultry tissues, with regard to specificity, further to the establishment of provisional MRLs for poultry.

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

   In 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 administration 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.

4. Sixteen male and 15 female chickens were administered daily oral doses of 20 mg ^{14}C-acetylisovaleryltylosin tartrate/kg bw/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 administration. 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 plus fat from animals killed 12 hours and 3 days after the last administration 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 treatment 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 treatment, 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 plus 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 in accordance with the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Union.

   The same analytical method was validated for turkey tissues in respect to precision (repeatability and reproducibility) and linearity. The limit of quantification was 25 µg/kg for the combined marker residues in turkey tissues. Accuracy was not fully validated in accordance with the requirements of Volume 8 but 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). The method can, therefore, be used for monitoring of residues in turkeys.

Conclusions and recommendation

Having considered that:

- an ADI of 1.02 µg/kg bw (i.e. 61 µg/person) was previously established for acetylisovaleryltylosin,
- the sum of acetylisovaleryltylosin and 3-O-acetyltylosin was identified as the marker residue in chickens and turkeys,
- in chicken the ratio of the marker to total residues 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,
- residue levels in muscle and kidney are very low 3 days after treatment and therefore muscle and kidney were considered unsuitable for monitoring purposes,
- no residue studies nor analytical method were provided for eggs and therefore no MRL can be recommended for eggs,
- a validated routine analytical method for the determination of the marker residue in edible tissues of poultry, is available;

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

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Acetylisovaleryl-tylosin	Sum of acetyl-isovaleryltylosin 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

Based on these MRLs, the daily intake of total residues from the consumption of poultry meat would represent about 65% of the ADI.