The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Information Technology Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TILMICOSIN
(Extension to milk)

SUMMARY REPORT (4)

1. Tilmicosin is a macrolide antibiotic synthesised from tylosin. It has an antibacterial spectrum similar to tylosin with enhanced activity against *Pasteurella multocida* and *Pasteurella haemolitica*.

   Tilmicosin has previously been evaluated by CVMP for its use in chicken, cattle (calves only), pigs and sheep. A microbiological ADI of 0.004 mg/kg bw was established (240 µg/day for a 60 kg person), based on a NOEL of 0.4 mg/kg bw in an *in vivo* study with HFA rats, and a safety factor of 100.

   Currently, tilmicosin is included into Annex I of Council Regulation (EEC) No 2377/90 for cattle, pigs and sheep in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Tilmicosin	Tilmicosin	Bovine, Porcine, Ovine	50 µg/kg 50 µg/kg 1000 µg/kg 1000 µg/kg	Muscle Fat Liver Kidney	
		Ovine	50 µg/kg	Milk	
		Chicken	75 µg/kg 75 µg/kg 1000 µg/kg 250 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

   Tilmicosin is currently entered into Annex III of Council Regulation (EEC) No 2377/90 for bovine milk in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Tilmicosin	Tilmicosin	Bovine	40 µg/kg	Milk	Provisional MRL expires on 1.1.2001

2. Further data were provided to support an entry into Annex I of Council Regulation (EEC) No 2377/90 for bovine milk.

   For the use of tilmicosin in bovine, tilmicosin is indicated for the treatment of bacterial pneumonia in young cattle by a single subcutaneous injection of 10 mg tilmicosin/kg bw and to provide protection against mastitis in dry cows by intramammary administration via the teat duct at drying off, at a dose of 10 ml (1500 mg tilmicosin) per quarter.

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2. Following treatment of dairy cows after the last milking before drying off with a single subcutaneous injection of 10 mg tilmicosin/kg bw, a plasma peak level of 0.13 μg/ml was reached after approximately 2 hours. Tilmicosin moved rapidly and largely from the blood to the milk, reaching a milk peak level of 6 to 8 μg/ml after 8 to 24 hours. Tilmicosin depleted slowly from the dry udder secretion with a $t_{1/2}$ of approximately 34 hours.

4. One cow was treated with a single intramammary infusion of 1500 mg tilmicosin/quarter after the last milking before drying off and at seven days before calving. LC/ESI-MS revealed 4 detectable tilmicosin-related compounds in the milk samples of milkings 5, 7 and 9; namely tilmicosin, tilmicosin cis-8 epimer (an active isomer that is defined as "tilmicosin"), N-desmethyl tilmicosin (T-1) and a compound consistent with O-desmethyl tilmicosin (T12) but not fully characterised. Tilmicosin plus its cis-8 epimer accounted for approximately 96% of the detectable compounds and O-desmethyl tilmicosin for 4%. Metabolites T9 (4 times as active as the parent compound) and T10 (same activity as the parent compound) were not observed. The ratio between the parent compound and the total amount of residues cannot be determined from this study because data on only 1 cow were provided, because of the short drying-off period of 7 days, because it is not shown whether all possible metabolites can be detected using this method and because the milk was stored over a long period without showing the stability of tilmicosin and its metabolites over this period.

5. After treatment of dairy cows after the last milking before drying off with a single subcutaneous injection of 10 mg ^{14}C-tilmicosin/kg bw, a tilmicosin to total residue ratio of 87% was determined in the milk from representative samples from the 1st to 6th milking after calving. The tilmicosin concentration in milk depleted quickly, reaching levels below the proposed MRL of 50 μg/kg within 2 to 6 milkings.

6. Cows were treated with a single intramammary infusion of 1500 or 2100 mg tilmicosin/quarter after the last milking before drying off. The tilmicosin levels in the 5, 7 and 9 milking after calving with a drying-off period of 38 to 72 days ranged between the stated limit of determination of 3.6 and 52 μg/l.

7. Tilmicosin is considered to be the most suitable marker residue for bovine milk, with a high ratio of 87% between tilmicosin and the total amount of residues.

8. Minimum inhibitory concentrations (MICs) of tilmicosin were determined for *Lactobacillus* and *Propionibacillus spp* using the agar plate dilution method. The MICs ranged from 8 to 64 μg/ml under anaerobic conditions and from 4 to 32 μg/ml under microaerophilic conditions. No effect on growth of both species was found at levels of less than 1 μg/ml. Effects on starter cultures are considered unlikely.

9. An HPLC/UV-method for the determination of tilmicosin residues in milk of cattle (limit of detection: 2.7 μg/l, limit of quantification: 25 μg/l). The method has been provided in an acceptable format and is fully validated.

Conclusions and recommendation

Having considered that:

- an microbiological ADI of 4 μg/kg bw (i.e. 240 μg per 60 kg person) was established,
- tilmicosin is the marker residue,
- a ratio between the marker residue and the total amount of residues in bovine milk of 87% has been determined,
- the proposed routine analytical method for the determination of tilmicosin in bovine milk is well described and fully validated;

the Committee for Veterinary Medicinal Products recommends the inclusion of tilmicosin for bovine milk 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 tissues	Other provisions
Tilmicosin	Tilmicosin	Bovine	50 μg/kg	Milk	

Based on this MRL value, the calculated theoretical daily intake will represent about 103% of the ADI. The calculated daily intake is slightly overestimated: the small excess of the microbiological ADI is considered insignificant since a fraction of the total residue will consist of microbiologically inactive compounds and compounds which are less microbiologically active than the parent compound.