



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TILMICOSIN (Extension to bovine milk)

SUMMARY REPORT (3)

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 haemolytica*.

Tilmicosin has been evaluated by Committee for Veterinary Medicinal Products (CVMP) for its use in chicken, cattle (calves only), pigs and sheep. A microbiological ADI of 0.004 mg/kg bw was established (i.e. 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 entered 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 |

An application has now been submitted for the extension of the MRLs to bovine milk. Tilmicosin in bovine is indicated for the treatment of bacterial pneumonia in young cattle by a single subcutaneous injection of 10 mg tilmicosin/kg bw as well as for treatment of mastitis in dairy cows 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. A dose for the treatment of mastitis in dairy cows was not provided.

2. After 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 halftime of approximately 34 hours.

3. 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. Analysis by liquid chromatography with electron spray ionisation-mass spectrometry (LC/ESI-MS) revealed four detectable tilmicosin-related compounds in the milk samples of milking 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 for 4%. The ratio between the parent compound and the total amount of residues cannot be determined from this study because data on only one cow were provided, the short dryoff period of 7 days, 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.
4. After treatment of dairy cows after the last milking before drying off with a single subcutaneous injection of 10 mg ¹⁴C-tilmicosin/kg bw, the ratio of tilmicosin to total residue was determined in the milk from the 1 to 6 milking after calving as approximately 0.75, in line with the previous assessments of the active substance. A more accurate estimation was not possible due to some outstanding information regarding the analytical method used in this study. The tilmicosin concentration in milk depleted quickly, reaching levels below 40 µg/kg within 2 to 6 milkings.
5. 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 dryoff period of 38 to 72 days ranged between the stated limits of detection (LOD) of 3.6 and 52 µg/kg.
6. Tilmicosin is considered to be the most suitable marker residue for bovine milk, but only a conservative estimate of the ratio of tilmicosin and the total amount of residues in milk of 0.75 can be retained from the provided studies.
7. 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 therefore considered unlikely.
8. The applicant provided an HPLC/UV-method for the determination of tilmicosin residues in milk of cattle, which is also applicable to ovine milk. The method is described in an acceptable format. However, the method is not fully validated because the limit of detection was not determined from 20 blanks per species, the stability of tilmicosin in cow milk samples was not shown and a GLP- and a QA-statement on the validation study was absent. Furthermore, the possible interference of tylosin and spiramycin, compounds which are known to cause interference in some methods, was not studied.

Conclusions and recommendation

Having considered that:

- the microbiological ADI for tilmicosin is 4 µg/kg bw (i.e. 240 µg/person),
- tilmicosin is the marker residue,
- a conservative estimate establishes the ratio between the marker residue and the total amount of residues in bovine milk as 0.75,
- effects on starter cultures are unlikely,
- the proposed routine analytical method for the determination of tilmicosin in bovine milk is well described but not yet fully validated,

the Committee for Veterinary Medicinal Products recommends the inclusion of tilmicosin for bovine milk 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 |
|---------------------------------------|----------------|----------------|----------|----------------|-------------------------------------|
| Tilmicosin | Tilmicosin | Bovine | 40 µg/kg | Milk | Provisional MRL expires on 1.1.2001 |

Based on the above MRL and the MRL values previously established for edible tissues, the daily intake will represent about 95% of the ADI.

Before the Committee for Veterinary Medicinal Products can be consider the inclusion of a MRL for bovine milk into Annex I of Council Regulation (EEC) No. 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should explain whether tilmicosin intramammary is only intended for dry cows or also for dairy cows as mentioned in the expert report. If tilmicosin is also intended for dairy cows the recommended dosage should be stated.
2. The applicant should provide additional validation of the limit of detection in the routine analytical method for tilmicosin in milk of cows, using at least 20 blank samples and the applicant should provide the study in which the stability of tilmicosin in cow's milk was determined. Additional data are requested with respect to the possible interference of tylosin and spiramycin, compounds which are known to cause interference in some methods. A GLP- and a QA-statement on the validation study of method B05704 should be provided.
3. In order to facilitate a more precise determination of the ratio of tilmicosin to total residue, additional information regarding the validation of HPLC method AM-AA-CA-R137-AA-791, as used in the study by Donoho, A.L. and Thomson, T.D. (1990), is requested. Information on the recovery, correction for the recovery, the accuracy and the specificity of the method for bovine milk should be provided.