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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUMEQUINE
(Extension to bovine milk and turkey)

SUMMARY REPORT (3)

1. Flumequine is a synthetic antibiotic belonging to the second-generation quinolone group and is mainly active against Gram negative bacteria. It is used in bovine, ovine, chicken, rabbits, goats, horses and salmonidae, . Flumequine is administered either by oral route or by parenteral routes (intramuscular or subcutaneous). The recommended therapeutic regimen doses range from 6 to 18 mg/kg bw twice a day for 3 to 5 days. It is mainly used for treatment of enteric infections in domestic species.

   A microbiological ADI of 8.25 µg/kg bw, i.e. 495 µg per person, had been previously established by the Committee for Veterinary Medicinal Products (CVMP).

   Currently, flumequine is included 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
Flumequine	Flumequine	Bovine, ovine, porcine, chicken	50 µg/kg 50 µg/kg 100 µg/kg 300 µg/kg	Muscle Fat or skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2000
		Salmonidae	150 µg/kg	Muscle and skin in natural proportions	

   Further to the evaluation of the response to the list of questions related to the establishment of provisional MRLs, the Committee recommended the inclusion of flumequine in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

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Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Flumequine	Flumequine	Bovine, ovine	200 µg/kg 300 µg/kg 500 µg/kg 1500 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption
		Porcine	200 µg/kg 300 µg/kg 500 µg/kg 1500 µg/kg	Muscle Skin + fat Liver Kidney	
		Chicken	400 µg/kg 250 µg/kg 800 µg/kg 1000 µg/kg	Muscle Skin + Fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption
		Salmonidae	600 µg/kg	Muscle and skin in natural proportions	

An application has now been submitted for an extension of the MRLs to bovine milk and to turkey. The recommended therapeutic regimen doses range from 6 to 24 mg/kg bw per day for 3 to 5 days.

2. The effects of flumequine on *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. lactis biovar diacetylactis, Lactococcus lactis ssp. cremoris, Leuconostoc Mesenteroides ssp. cremoris, Leuconostoc Mesenteroides ssp. dextranicum, Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus Delbrueckii ssp. lactis, Lactobacillus Delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum* were tested. It was shown that concentrations of flumequine up to 800 µg/kg had no effect on the standard dairy starter cultures, the difference between the pH of milk inoculated with starter cultures and spiked with a range of flumequine concentrations and that of the control being less than 0.3 pH unit.

3. In a radiometric study, four lactating cows were given subcutaneously 12 mg/kg bw/day of ^{14}C-flumequine for 3 days. Milk was collected until 48 hours after the last administration.

   The total radioactivity, the microbiologically active residues and the parent compound concentrations were simultaneously measured in milk. The total radioactivity levels were measured by liquid scintillation. The concentrations of the parent compound were determined by a high performance liquid chromatography, the limit of quantification being 5 µg/kg. The concentrations of the microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification and of detection being 100 and 50 µg/kg, respectively.

   The radioactivity levels in milk declined from 808 µg flumequine equivalents/kg, 3 hours after the last injection to 433, 202, 71, 36 and 17 µg flumequine equivalents/kg, at 6, 12, 24, 36 and 48 hours after the last injection, respectively.

   The mean concentrations of flumequine were 205, 110, 50, 20, 8 µg/kg and lower than 5 µg/kg at 3, 6, 12, 24, 36 and 48 hour, respectively. The mean ratio of flumequine towards total radioactivity was 0.25.

   The concentrations of the microbiologically active residues were 288 and 130 µg microbiologically active residues/kg at 3 and 6 hours after the last injection, respectively and then declined very rapidly and the levels of microbiologically active residues were below the limit of detection (less than 50 µg/kg), 12 hours after the last administration and at later time-point. The mean ratio flumequine towards total microbiologically active residues was 0.85.

In milk, flumequine represents the main microbiologically active compound, therefore it was retained as marker residue.

4. In a non-radiometric study, eight lactating cows (high or low yielding) received by subcutaneous route, flumequine at a dose of 12 mg/kg bw/day for 5 days. Milk was collected until 204 hours after the last administration. The flumequine concentrations were determined by an HPLC method, the limits of quantification and detection being 5 and 1 µg/kg, respectively. The concentrations of flumequine declined from 107 µg/kg, 12 hours after the last administration to 28, 21, 11 µg/kg at 24, 36 and 48 hours post dosing. For later sampling time, flumequine could only be measured in a few animals and the individual values ranged from 6 to 16 µg/kg.

5. Five groups of 6 turkeys were given, via drinking water, flumequine at a dose level of 18 mg/kg bw/day for 5 days. Animals were slaughtered at 24, 36, 48, 72 and 96 hours after the end of the treatment. The concentrations of flumequine and of its metabolite (7-hydroxyflumequine) were assessed by a HPLC method. Twenty-four hours after the end of the treatment, the concentrations of flumequine were 60, 66, 56 and lower than 100 µg/kg in muscle, skin + fat, liver and kidney. At later sampling, flumequine could only be measured in skin + fat (74, 53 (n=5) and 56 (n=4)) at 48, 72 and 96 hours. At 36 hours post dosing, flumequine could not be quantified in muscle (except in one animal, 115 µg/kg), in liver and in kidney. In kidney, the concentrations of flumequine were lower than 100 µg/kg at 48 and 72 hours post dose and flumequine could not be detected at 96 hours . Flumequine could not be detected in muscle (below 13 µg/kg) , 48, 72 and 96 hours post dose. It could not be quantified in liver below (50 µg/kg) at 48 hour post dose and detected (below 6 µg/kg), 72 and 96 hours post dose.

   The metabolite 7-hydroxyflumequine could only be measured in skin + fat of some animals: two or three animals per time-point, the concentrations ranging from 74 to 25 µg/kg. In the other edible tissues, 7-hydroxyflumequine could not be detected (below 11, 12 and 11 µg/kg in muscle; liver and kidney, respectively).

6. Taking account of the CVMP Note for Guidance on the establishment of MRLs for minor animal species (document EMEA/CVMP/153a/97), turkey is considered as a minor species. As flumequine is proposed for an inclusion in Annex I to Council Regulation (EEC) No 2377/90 for chicken, a major animal species, extrapolations to the corresponding minor animal species can be made: the same marker residue (flumequine) and same MRL values were appropriate for the edible tissues of turkeys.

7. The analytical methods proposed for the determination of residues of flumequine in bovine milk and in edible tissues of turkeys, based on HPLC with fluorimetric detection, were validated in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community and described according to ISO 78/2 format. The analytical method proposed for edible turkey tissues is the same as that which had been previously developed for the edible tissues of chickens and the limits of quantification are 100 µg/kg for kidney and 25 µg/kg for liver, muscle and skin + fat. For milk, the limits of quantification and detection are 5 and 1 µg/kg, respectively.

Conclusions and recommendation

Having considered that :

- a microbiological ADI of 8.25 µg/kg bw/day, i.e. 495 µg per person has previously been established,
- flumequine was identified as marker residue in milk and considering that the marker residue represents 85 % of the microbiologically active residues,
- considered the CVMP Note for Guidance on the establishment of MRLs for minor animal species the marker residue retained for chicken and MRL values established for edible tissues of chicken were appropriate for the edible tissues of turkeys,
- analytical methods for monitoring residues in bovine milk and in edible tissues of turkey are available and are fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community;

the Committee for Veterinary Medicinal Products recommends the inclusion of flumequine for bovine milk and edible tissues of turkey 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
Flumequine	Flumequine	Bovine	50 µg/kg	Milk	
		Turkey	400 µg/kg 250 µg/kg 800 µg/kg 1000 µg/kg	Muscle Skin + fat Liver Kidney	

Based on these MRLs values, the daily intake including residues from meat and milk origins will represent a maximum of approximately 82% of the microbiological ADI.