

## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### FLUMEQUINE

#### SUMMARY REPORT (1)

1. Flumequine is a synthetic antibiotic belonging to the quinolone group and is active against Gram negative bacteria. It is administered to various animal species, orally in most cases, at a dosage of 12 mg/kg. It is mainly used for treatment of enteric infections in domestic species.
2. Flumequine is currently under the evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). An ADI has not been established at the time of writing this report.
3. Acute toxicity studies in various animal species including mice, rats, rabbits and dogs show that it has low toxicity. LD<sub>50</sub> levels exceed 1 g/kg bw. Subacute toxicity tests involving repeated administration over periods of two to three weeks confirm this low toxicity.
4. 90-day subchronic toxicity tests were carried out on rats, young dogs and mice.

In rats after administration of 200, 400 or 800 mg flumequine/kg bw/day effects, chiefly on the relative weight of the liver, were recorded. Since this effect is observed in all the animals treated, a NOEL could not be determined on the basis of this study.

In young dogs, flumequine did not induce adverse effects up to 100 mg/kg bw/day.

In another study, the twice daily oral administration of flumequine pellets 200 mg for 3 and 13 weeks at the dose level of 150 mg/kg bw/day induced few clinical signs (vomiting, low food consumption), marked reduction in bodyweight gain for females and minimal to slight arthropathies with cartilage damage. Only slight arthropathy was induced at the dose level of 60 mg/kg bw/day. No toxic signs were observed after the administration of 15 and 30 mg/kg bw/day.

In the 90-day subchronic toxicity carried out on CD-1 mice, flumequine was administered to at level doses of 0, 25, 50, 100, 400 and 800 mg/kg bw/day for males and dosages of 0, 100, 400 and 800 mg/kg bw/day for females. In the two high doses groups, the histopathological examination of the livers revealed, in both males and females, periacinar single cell necrosis and inflammation, periacinar pigment laden macrophages, increased ploidy of hepatocytes, hepatocytic intranuclear inclusions, increased periacinar hepatocytic fatty vacuolation. However, a periacinar hepatocytic hypertrophy was only observed in males : in 7 of 12 animals of the 800 and 400 mg/kg bw dose groups, in 5 of 12 animals in the 100 mg/kg bw dose group and in 1 animal in the 50 mg/kg bw dose group and these lesions were dosage-related. In addition, an inhibition of the activity of NADPH-cytochrome P450 for females of the two highest dose group and of UDP-glucuronosyltransferase for males at 50 mg/kg bw was also reported. 25 mg/kg bw/day was considered as the NOEL for hepatotoxicity in mice.

5. A one-year chronic toxicity test on dogs (0, 50, 100 and 200 mg/kg bw/day) showed that convulsions may be caused at frequencies related to the dose. No effect was observed at a daily dose of 50 mg/kg.
6. Teratology studies were conducted in rats (0, 100, 200 or 400 mg/kg bw), mice (50, 100, 200, and 400 mg/kg bw) and rabbits (100, 200 or 400 mg/kg bw). None of these tests showed flumequine to be teratogenic or embryotoxic, but at doses exceeding 100 mg/kg per day it does have an effect on bone formation. The NOELs for the most sensitive species, rats and mice, were 100 mg/kg bw.
7. The mutagenic potential of flumequine was explored in *in vitro* tests such as the Ames test, HGRPT test, and mammalian gene mutation assay on Chinese hamster ovary cells, and an *in vivo* mammalian

chromosome aberration test on rat marrow bone. Negative results obtained enabled the conclusion that flumequine was not genotoxic.

8. In addition, published data on compounds of the same family showed that the inhibitory action of quinolones against mammalian topoisomerases is very low when compared to their action against prokaryote DNA-gyrase. The same conclusion was advanced for flumequine although no experimental data to support this were provided.
9. In the two-year carcinogenicity study in rats dosed with flumequine at 200, 400 or 800 mg/kg bw/day, no carcinogenic effects were observed.
10. In a 18-month carcinogenicity study in mice, flumequine was administered in the feed at 0, 400 or 800 mg/kg bw. The combined incidence of benign and malignant liver tumours was dose related : 37 % in the 400 mg/kg bw dose group, 88 % in the high dose group vs. 9 % in the control group for males and 13 % in the high dose females vs. 0 % for the control and the low dose groups. Dose related changes in the hepatocytes which paralleled the liver tumour incidence occurred in the low dose males and in the high dose males and females.

A further dietary study of flumequine was carried out on male mice receiving 0 (control), 800 mg/kg bw/day for 18 month (group 1), 800 mg/kg bw during week 1 to 6 and week 13 to 18 (group 2), 800 mg/kg bw during the weeks 1 to 6 (group 3). In all treated groups, an increase in the incidence of both benign and malignant hepatic tumours was reported.

11. There is evidence of compound-related tumorigenic effects in the liver of mice. In order to explain the mechanism of liver tumour induction, the dosage of a preneoplastic marker  $\gamma$ GT and of a detoxification enzymes, GSH S-transferase, were performed on liver samples collected in the 90-toxicity study carried out in mice. No variations of  $\gamma$ GT were noted whatever the dosage used. However, an increase of the GSH S-transferase activity in females dosed at 400 and 800 mg/kg bw and in males dosed at 800 mg/kg bw showed that flumequine induced detoxification phenomena, showing cells hepatotoxicity. However, this phenomena was not correlated with the number of tumours incidence.

As the tumorigenicity is considered to be a consequence of hepatotoxicity, it was concluded that the NOEL of 25 mg/kg bw/day covered both end-points.

12. Flumequine is used in humans at a dose level of 1200 mg per day divided into three intakes. In a pharmacovigilance study (40, 722, 119 tablets delivered for a therapeutic treatment) carried out from 1984 to 1993, the following side-effects were reported : allergy ( $2.43 \times 10^{-6}$ ), digestive ( $3.43 \times 10^{-6}$ ), neurological ( $1.88 \times 10^{-6}$ ) and neuro-sensorial ( $1.18 \times 10^{-6}$ ) effects.

13. Having considered that :

- flumequine is not genotoxic in the four recommended tests,
- flumequine can not act through interference with mammalian topoisomerases,
- flumequine is not carcinogenic in the rats,
- flumequine increases the incidence of hepatocellular tumours occurring spontaneously in CD-1 mice,
- the mechanism by which flumequine increases hepatocellular tumours might be due to hepatotoxicity;

it was possible to retain a toxicological ADI based on the absence of hepatotoxicity in 3-month mice study. Based on the NOEL of 25 mg/kg bw/day and retaining a safety factor of 100 and using an additional safety factor of 10 to account for the fact that the explanation of tumorigen mechanism could be not completely resolved, a toxicological ADI of 0.025 mg/kg bw or 1.5 mg for a person weighing 60 kg bw was set.

14. Data on the activity of flumequine and one of its metabolites, 7-hydroxy-flumequine, towards bacterial strains of human origin were provided. A microbiological ADI of 8.25 µg/kg bw, i.e. 495 µg per person per day, was calculated applying the CVMP formula :

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} (\mu\text{g/ml}) \times \text{daily faecal bolus (150 ml)}}{\frac{\text{fraction of an oral dose available for microorganisms}}{\text{weight of human (60 kg)}}} (\mu\text{g/kg bw})$$

- CF1 : MIC<sub>50</sub> of the most sensitive predominant microorganism (0.33 µg/ml *E.coli*). In this case, no correction is warranted and consequently this factor is equal to 1.
  - CF2 : The geometric mean MIC<sub>50</sub> should be multiplied by a correction factor (CF2) in order to correct for differences in growth conditions between the *in vitro* and in the *in vivo* situation and to take into account the influence of bacterial density on MICs. As the size of the inoculum (10<sup>7</sup> or 10<sup>9</sup> bacteria/ml) did not significantly modify the MIC value, 1 was retained for CF2 factor.
  - Fraction of an oral dose available for microorganisms : 0.1 determined from human data.
15. After repeated administration of <sup>14</sup>C-flumequine to ruminant calves (54-82 kg bw) at the therapeutic regimen (a dose level of 12 mg/kg bw/day for the first dose and then at a dose level of 6 mg/kg bw/day for doses 2-10) the plasma concentration levels appeared to attain a steady state of 1.3 to 2.6 mg equivalent flumequine/l after intramuscular administration and of 0.8 to 1.8 mg equivalent flumequine/l after oral administration. After the last dose, the concentration of the total radioactivity decreased slowly to 0.2 mg equivalent flumequine/l, 7 days post last dose.
16. Whatever the administration route used, about 90% of radioactivity administered was excreted within 168 hours : about 55% via urine, 35% via faeces. The major portion (98%) of the radioactivity excreted is recovered within 24 hours.
17. The two major metabolites identified either in urine or faeces are the parent compound and its hydroxylated metabolite. Both in urine and faeces, the flumequine represented about 80% of the radioactivity measured, its hydroxylated metabolite 10 to 20% after intramuscular and oral routes respectively. Another unknown compound (12%) was only seen in urine of animals treated by intramuscular route.
18. *In vitro* metabolism studies were performed on the liver microsomes of rat, mouse, calf, pig, chicken, trout and sheep. Flumequine was slightly metabolised by the enzymes of phase I : the main metabolite was 7-hydroxy-flumequine which represented less than 6% of total radioactivity. Furthermore, flumequine was glucuronidated, the glucuronide represented less than 12.5% of total radioactivity in all species.
19. After methanol extraction, at twenty-four hours withdrawal period, the extraction efficiencies of the radioactivity were close to 90% for kidney, fat and 43% for liver. The ratios of flumequine towards the total radioactivity were evaluated in edible tissues of calves : 57% for fat, 8% for liver, 50-68% for kidney. The corresponding ratio for 7-hydroxy-flumequine were : 40% for fat, 86% for liver and 33-29% for kidney.
- For muscle, flumequine represented 98% of the sample radioactivity (extraction efficiency, 99%), 6 h after the end of the treatment.
20. However, another extraction procedure (ethyl acetate and methanol extractions) tested on calf liver samples gave different figures for these ratios : for example, on 24 hour liver samples, flumequine represented 10 to 34% of the radioactivity extracted, 7-hydroxy-flumequine 2.12%.
- Another metabolite M1 representing about 50% was detected but not identified. Several minor metabolites were also quantified. The choice of the extraction solvent during the analytical method leads to a different estimation of these compounds.

21. In calf liver samples, the flumequine and 7-hydroxy-flumequine levels measured by the HPLC method retained for the depletion studies (ethyl acetate extraction and then recuperation in acetonitrile-oxalic acid) represented hardly 10% of the total radioactivity : 0.665 mg/kg flumequine, less than 0.018 mg/kg 7-hydroxy-flumequine vs. 7.4 mg equivalent flumequine. As the concentrations of flumequine were higher than those of 7-hydroxyflumequine, flumequine was selected as marker residue.

There is no information about the real percentage measured in other edible tissues.

22. An attempt was made to assess the microbiological activity of liver collected at 6 hours after administration. However due to technical difficulties, this activity could only be measured on 4 samples. In 3 of the four samples assayed, the microbiological activity was slightly higher than the flumequine concentrations determined by HPLC.
23. In a radiometric depletion study, at 24 hours after the end of the treatment, at the therapeutic regimen, the levels of radioactivity in edible tissues of calves were of the same magnitude whatever the administration route : 0.2 mg equivalent flumequine/kg in muscle, 0.9-0.4 mg equivalent flumequine/kg in fat, 5.8 mg equivalent flumequine/kg in liver, 2.5 mg equivalent flumequine/kg in kidney. At 168 hours after the end of the treatment, <sup>14</sup>C-flumequine could not be detected in muscle nor at the injection site. The concentrations were 0.1 mg equivalent flumequine/kg in fat and in kidney and ranged from 2.6 to 3.6 mg equivalent flumequine/kg for liver.
24. Non radiometric depletion studies were conducted in all the other target species : cattle, pigs, broilers, trout and sheep.
25. In non ruminant calves, forty-eight hours after the end of the oral therapeutic treatment by flumequine (12 mg/kg bw/day for dose 1 and 6 mg/kg bw for doses 2 to 10 administered 12 hours apart), the following levels of flumequine were measured in edible tissues : 0.12 mg/kg in muscle, 0.31 mg/kg in fat, 0.32 mg/kg in liver and 0.50 mg/kg in kidney. No 7-hydroxy-flumequine could be detected in muscle, fat and liver and only traces (less than 0.050 mg/kg) in kidney.

In ruminant calves, forty-eight hours after the end of the intramuscular therapeutic treatment by flumequine (12 mg/kg bw/day for dose one and 6 mg/kg bw for doses 2 to 10 administered 12 hours apart), the following levels of flumequine were measured in edible tissues : 0.05 mg/kg in muscle, 0.07 mg/kg in fat, 0.07 mg/kg in liver and 0.34 mg/kg in kidney. No 7-hydroxy-flumequine could be detected in muscle, fat and liver and only traces (0.053 mg/kg) in kidney.

26. In pigs, forty-eight hours after the end of the intramuscular therapeutic treatment by flumequine (15 mg/kg bw/day for dose 1 and 7.5 mg/kg bw for doses 2 to 10 administered 12 hours apart), the following levels of flumequine were measured in edible tissues: 0.08 mg/kg in muscle, 0.07 mg/kg in skin/fat, 0.164 mg/kg in liver and 0.37 mg/kg in kidney. Significant amounts of 7-hydroxy-flumequine, 0.19 mg/kg, were measured in liver and kidney whereas only traces were detected in muscle (less than 0.024 mg/kg), and in fat (less than 0.050 mg/kg).

Seventy-two hours after the end of the treatment, only traces of flumequine could be detected in muscle and skin/fat (less than 0.050 mg/kg). Flumequine could be measured in liver (0.075 mg/kg) and in kidney (0.155 mg/kg), the concentrations of 7-hydroxyflumequine being in the same magnitude.

27. In broilers, after treatment via drinking water at the therapeutic regimen (12 mg/kg bw/day of flumequine for 5 days), residues in tissues declined rapidly. At 6 hours after cessation of the treatment, the concentrations of flumequine were significant : 1.5 mg/kg in muscle, 0.72 mg/kg in skin/fat, 2.45 mg/kg in liver, 2.50 mg/kg in kidney. The following levels of 7-hydroxy-flumequine were reported : 0.17 mg/kg for muscle, 0.10 mg/kg for skin/fat, 1.10 mg/kg for liver and 1.90 mg/kg for kidney.

After a forty-eight hour withdrawal period, the concentrations of flumequine and hydroxy-flumequine were of the same magnitude than those assayed in pigs.

Seventy-two hours after the end of the treatment, only traces of flumequine and 7-hydroxyflumequine could be detected in all tissue samples.

28. Rainbow trout were treated orally with the equivalent of 12 mg/kg bw/day of flumequine divided into 2 administrations for 5 days.

In the group kept at 7.4°C, the concentrations of flumequine in muscle plus skin were 5.60 mg/kg, 0.90 mg/kg, 0.08 mg/kg at 1, 4, 7 days after the end of the treatment respectively.

In the group kept at 16.4 °C the concentrations of flumequine in muscle plus skin decreased more rapidly : 1.67, 0.08, at 1 and 4 days after the end of the treatment and could not be detected (less than 0.018 mg/kg) for the further sampling times.

7-hydroxy-flumequine could not be detected in those samples collected in trout kept either at 7.4°C or 16.4°C.

29. In sheep, forty-eight hours after the end of the intramuscular therapeutic treatment (12 mg/kg bw/day for dose 1 and 6 mg/kg bw for doses 2 to 10 administered 12 hours apart), the mean flumequine concentrations for all tissues were lower than 0.075 mg/kg except for kidney (0.110 mg/kg). Traces of 7-hydroxyflumequine could only be detected in muscle, fat, liver (less than 0.010 mg/kg) and kidney (less than 0.100 mg/kg).

After a seventy-eight hour withdrawal period, the concentrations of flumequine were 0.009 mg/kg for muscle, 0.052 mg/kg for fat, 0.014 mg/kg for liver and 0.039 mg/kg for kidney.

30. Analytical methods, using HPLC with fluorescence detection, have been validated according the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community to measure residues of flumequine and 7-hydroxy-flumequine. For the method proposed for cattle, trout and broiler tissues, the limit of quantification for both substances ranged from 0.025 mg/kg to 0.050 mg/kg except for liver and kidney broiler (0.10 mg/kg). However, for sheep edible tissues, the limits of quantification for flumequine and 7-hydroxy-flumequine were 0.005 and 0.010 mg/kg for liver, muscle and fat and 0.005 and 0.1 mg/kg for kidney, respectively.

## Conclusions and recommendation

Having considered that :

- the microbiological ADI is 8.25 µg/kg bw per day, i.e. 495 µg mg per person of 60 kg;
- 85 % of <sup>14</sup>C flumequine is eliminated within 24 hours,
- flumequine is the marker residue,
- the comparison between total residues with regard to the flumequine determined by the HPLC method can be only made for liver,
- in the worst case scenario, flumequine might represent 10-20 % of the microbiological compounds in liver,
- this ratio was assumed for all tissues in all species,
- validated analytical methods are available for monitoring residues in edible tissues;

the Committee recommends the inclusion of flumequine 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 tissue	Other provisions
Flumequine	Flumequine	Bovine, ovine, porcine, chicken	50 µg/kg 50 µg/kg 100 µg/kg 300 µg/kg	Muscle Fat or fat + skin Liver Kidney	Provisional MRL expire on 01.01.2000
		Salmonidae	150 µg/kg	Muscle and skin	

Based on these MRLs values, the daily intake will represent about 42.9 % to 85.86 % of the microbiological ADI.

## LIST OF QUESTIONS

To enable a definitive evaluation of flumequine residues, the following studies and other information should be available by July 1999 :

1. The experimental report on the induction of arthropathy in dogs.
2. The final report on the pharmacovigilance investigation conducted in humans from 1984 to 1993.
3. As the concentrations of liver samples evaluated by the reference HPLC method are not in accordance with the concentrations which could be extrapolated from the radiometric studies carried out in calves, and as this HPLC method measured hardly 10 % of the total radioactivity, the applicant should establish for all edible tissues and for all the target species the ratio of flumequine and 7-hydroxy-flumequine measured with regard to the total radioactivity.
4. The applicant should identify the structure of the metabolite M1 which represents about 50 % of the extractable radioactivity, the solvents used being ethyl acetate (x2) and then methanol (x2).
5. Information about the microbiological activity of the metabolite M1 should be given.
6. The applicant should provide information about the stability of flumequine and its metabolites and about their microbiological activity according the duration of the storage of samples.
7. The applicant should validate a limit of quantification of 0.025 mg/kg for pig muscle and for the fat in all target species.