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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUMEQUINE

SUMMARY REPORT (2)

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, however the establishment of MRLs was only requested for non-lactating cattle, pigs, sheep, chicken 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.

   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 data were provided to support an entry of flumequine in Annex I of Council Regulation (EEC) No 2377/90.

2. After repeated administration of ^{14}C-flumequine to 20 ruminant calves (body weight of 54 to 82 kg) at the therapeutic regimen of 12 mg/kg bw/day for the initial dose, followed by 6 mg/kg bw/day for doses 2 to 10) the plasma concentrations appeared to attain a steady state of 1300 to 2600 µg equivalents flumequine/l after intramuscular administration and of 800 to 1800 µg equivalents flumequine/l after oral administration. After the last dose, the concentration of the total radioactivity decreased slowly to 200 µg equivalents flumequine/l, 7 days post last dose.

   In cattle, whatever the administration route used, about 90% of the administered radioactivity was excreted within 168 hours, about 55% via urine and 35% via faeces. The major portion (98%) of the radioactivity excreted is recovered within 24 hours.

   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.

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In vitro metabolism studies were performed using liver microsomes of rat, mouse, calf, pig, chicken, trout and sheep. The metabolism of flumequine was limited, the main metabolite was 7-hydroxy-flumequine which represented less than 6% of total radioactivity. Furthermore, flumequine was glucuronidated, the glucuronide representing less than 12.5% of total radioactivity in all species.

In humans, after administration of a single oral dose (832 mg ^{14}C-flumequine), 84% of the dose administered was recovered within 5 days, 75% of the radioactivity being excreted via urine, and about 10% via faeces.

3. Acute oral and parenteral toxicity studies in various animal species including mice, rats, rabbits and dogs show that flumequine has low toxicity. LD_{50} values exceed 1 g/kg bw. Subacute toxicity tests involving repeated administration over periods of 2 to 3 weeks confirm this low toxicity.

4. Several 90-day repeated dose toxicity studies were carried out on rats, young dogs and mice.

   In rats after oral administration of 200, 400 or 800 mg flumequine/kg bw/day effects mainly on the relative weight of the live, were recorded 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 effect, after oral administration up to 100 mg/kg bw/day.

   In order to test the effects of flumequine on articular cartilage, groups of 10 dogs aged 3 months received flumequine orally, as pellets, at doses of 0, 15, 30, 60 and 150 mg/kg bw/day for 3 and 13 weeks. In each group, 4 out of 10 animals were sacrificed after 3 weeks and the 6 remaining dogs after 13 weeks of treatment. In the 150 mg/kg bw/day dose group, after 3 weeks of treatment, 2 females and 2 males showed synovial focal hyperplasia and articular lesions (cavitation in the cartilage was reported for 2 of them). After 13 weeks of treatment, 1 female showed macroscopical articular lesion (erosion in shoulder), 2 females showed histological articular lesions (slight erosion of the cartilage) and 1 male, synovial focal hyperplasia. In the 60 mg/kg bw/day dose group, after 3 weeks of treatment, 1 male had microscopical erosion of the cartilage. In the 30 mg/kg bw/day dose group, no compound related macroscopical and microscopical findings were reported at any time of sacrifice. In the 15 mg/kg bw/day dose group, after 13 weeks of treatment, one female showed apparent macroscopical erosion of the hips which was not associated with histological findings. Having considered that the gross lesions reported in one animal of the lowest dose group are without toxicological significance, the NOEL for the induction of arthropathy in young dogs was 30 mg/kg bw/day.

   In a 90-day repeated dose toxicity in CD-1 mice, flumequine was administered orally at doses of 0, 25, 50, 100, 400 and 800 mg/kg bw/day for males and doses of 0, 100, 400 and 800 mg/kg bw/day for females. In the two high doses groups histopathological examination of the livers revealed in both males and females, periacinar single cell necrosis, inflammation, periacinar pigment laden macrophages, increased ploidy of hepatocytes, hepatocytic intranuclear inclusions and increased periacinar hepatocytic fatty vacuolation. However, a periacinar hepatocytic hypertrophy was only observed in male, 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 of the 50 mg/kg bw dose group and these lesions were dose related. In addition an inhibition of the activity of NADPH-cytochrome P450 for females of the two highest dose group and of UDP-glucuronosyltranferase for males at 50 mg/kg bw was also reported. A NOEL of 25 mg/kg bw/day was retained for the hepatotoxicity in mice.

5. A 1-year oral toxicity test in dogs (0, 50, 100 and 200 mg/kg bw/day) showed that convulsions may be caused at dose-related frequency. No effect was observed at a dose of 50 mg/kg bw/day.

6. In teratology studies flumequine was administered orally to 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 bw/day it does have an effect on bone formation (incomplete ossification). The NOELs for the most sensitive species, rats and mice, was 100 mg/kg bw/day.

7. The mutagenic potential of flumequine was explored in *in vitro* tests such as the *Salmonella* microsomal assay (Ames test) with *Salmonella typhimurium* strains TA1515, TA1537, TA1538, TA98 and TA100, mammalian point mutation assay (HRPT locus) in mouse L5178Y lymphoma cells, a mammalian gene mutation assay in Chinese hamster ovary cells (CHO) and an *in vivo* mammalian chromosome aberration test in rat bone marrow (1000 mg/kg bw orally). Negative results obtained in all test systems enabled the conclusion that flumequine was not genotoxic.

8. Two carcinogenicity and 1 long-term toxicity studies were carried out in rats and mice.

   In a 2-year carcinogenicity study in rats dosed orally with flumequine at 200, 400 or 800 mg/kg bw/day, no carcinogenic effects were observed.

   In an 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 versus 9% in the control group for males and 13% in the high dose females versus 0% for the control and the low dose groups. Dose related changes in the hepatocytes, which paralleled the liver tumor incidence, occurred in the low dose males and in the high dose males and females.

   A further dietary study with flumequine was carried out in male mice receiving 0 (control), 800 mg/kg bw/day for 18 month (group 1), 800 mg/kg bw/day during week 1 to 6 and week 13 to 18 (group 2), 800 mg/kg bw/day 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.

   There is evidence of compound-related tumourigenic effects in the liver of mice. The concentrations of γ-glutamyl transpeptidase and of a detoxification enzyme, glutathione-S-transferase, were measured in liver samples collected in the 90-toxicity study carried out in mice. No variations of γ-glutamyl transpeptidase were noted at any dose. However, an increase of the glutathione-S-transferase activity in females treated with 400 and 800 mg/kg bw and in males treated with 800 mg/kg bw showed that flumequine induced detoxification phenomena, with cells hepatotoxicity. However, this phenomenon was not correlated with the number of tumours incidence.

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

9. Flumequine is used in humans at a dose level of 1200 mg/day divided into three doses. In a pharmacovigilance study (40 722 119 tablets delivered for a therapeutic treatment) carried out from 1984 to 1993, the following incidence of side-effects were reported: allergy (3.43×10^{-6}), digestive (2.33×10^{-6}), neurological (1.88×10^{-6}) and neuro-sensorial (1.18×10^{-6}) effects.

10. At the 48th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) held in February 1997 a toxicological ADI of 0-30 µg/kg bw was established, based on the NOEL of 25 mg/kg bw/day for hepatotoxicity in the 3-month study in mice and a safety factor of 1000. This factor was chosen to reflect the short duration of the study and the lack of histo-chemical characterisation of the foci of altered hepatocytes. The ADI was rounded to one significant value.

11. Having considered that :

    - flumequine is not genotoxic in the test systems which addressed all relevant end points,
    - flumequine is not carcinogenic in the rats,
    - flumequine increases the incidence of hepatocellular tumours occurring spontaneously in CD-1 mice, by a mechanism which may be related to its hepatotoxicity;

    the Committee for Veterinary Medicinal Products retained a toxicological ADI based on the absence of hepatotoxicity in a 3-month mice study. Based on the NOEL of 25 mg/kg bw/day and using a safety factor of 1000, a toxicological ADI of 0.025 mg/kg bw, i.e. 1.5 mg/person was set.

12. The MIC of bacteria isolated from healthy human faeces were determined under aerobic and/or anaerobic conditions. Ten strains of each species were tested : *Escherichia coli, Bifidobacterium spp, Bacteroides fragilis, Eubacterium spp, Clostridium spp, Streptococcus spp, Fusobacterium spp, Lactobacillus spp, Proteus spp* and *Peptostreptoccus spp.* The MIC_{50} ranged from 0.33 µg/ml to values higher than 32 µg/ml, the most sensitive predominant micro-organism being *Escherichia .coli.* The activity of 7-hydroxy-flumequine on human gut microflora was negligible when compared with flumequine.

13. At the 48th meeting held in February 1997, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a microbiological ADI of 37 µg/kg bw based on MIC_{50} of the most sensitive relevant genera from the gastrointestinal tract, *Fusobacterium spp* (1.0 µg/ml), applying 1 as safety factor and 0.1 as fraction of an oral dose available for micro-organism.

14. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP.

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

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\frac{0.33 \times 1}{1} \times 150}{0.1 \times 60} = 8.25 \text{ µg/kg bw i.e.} = 495 \text{ µg/person}$$

and the following assumptions were made:

- MIC_{50} of the most sensitive micro-organism, *Escherichia coli,* was retained: 0.33 µg/ml
- CF1 = 1, because the MIC_{50} of the most sensitive micro-organism was retained, and therefore no correction is warranted;
- CF2 = 1, as the size of the inoculum (10^7 or 10^9 bacteria/ml) did not significantly modify the MIC value (the geometric mean MIC_{50} should be multiplied by a correction factor (CF2) in order to correct for differences in growth conditions between the *in vitro* and the *in vivo* situation and to take into account the influence of bacterial density on MICs);
- Fraction of an oral dose available for micro-organisms: 0.1, determined from human data.
- 150 g was the weight of the daily faecal bolus;

The difference reported for the microbiological ADI established by the Committee for Veterinary Medicinal Products and the JECFA is due to a different MIC_{50} and to the value given to the daily fecal bolus (150 ml versus 220 ml).

15. Following administration of ^{14}C-flumequine to 20 ruminant calves (initial dose of 12 mg/kg bw/day, followed by 6 mg/kg bw/day for doses 2 to 10), the ratio of flumequine to total residues was evaluated. After methanol extraction, at 24 hours after intramuscular injection, 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 57% for fat, 8% for liver, 50 to 68% for kidney. The corresponding ratio for 7-hydroxy-flumequine were 40% for fat, 86% for liver and 33 to 29% for kidney.

Another extraction procedure (ethyl acetate and methanol extractions) tested on calf liver samples gave different figures in 24 hour liver samples, flumequine represented 10 to 34% of the radioactivity and 7-hydroxy-flumequine amounted to 2.12%. Another metabolite 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.

16. In a further radiometric study in beef cattle, 6 animals received 12 mg/kg bw/day of ^{14}C-flumequine for 5 days by subcutaneous route. Eighteen hours after the last injection, the animals were sacrificed. Total radioactivity, microbiologically active residues and parent compound were simultaneously analysed. The concentrations of parent compound were determined by the HPLC method proposed for monitoring purposes, based on ethyl acetate extraction and then recuperation in acetonitrile-oxalic acid. The limits of quantification of this method were 50 µg/kg for kidney, liver and fat, and 25 µg/kg for muscle. The concentrations of microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification being 100 µg/kg for kidney, liver and muscle, and 250 µg/kg for fat.

    The mean levels of radioactivity were 374 µg equivalents flumequine/kg in muscle, 1175 µg equivalents flumequine/kg in fat, 7110 µg equivalents flumequine/kg in liver and 5316 µg equivalents flumequine/kg in kidney. At the injection site, 464 018 equivalents flumequine/kg were measured.

    The mean concentrations of flumequine were 321 µg/kg in muscle, 1081 µg/kg in fat, 1191 µg/kg in liver, 4196 µg/kg in kidney and 49 5089 µg/kg at the injection site. The ratios of flumequine towards total radioactivity were 0.85 in muscle, 1 in fat, 0.17 in liver and 0.79 in kidney.

    The mean concentrations of microbiologically active residues were 414 µg microbiologically active residues/kg in muscle, 1110 µg/kg in fat, 1794 µg/kg in liver and 4547 µg/kg in kidney. The ratios of flumequine towards total microbiologically active residues were 0.77 in muscle, 1 in fat, 0.69 in liver and 0.94 in kidney.

    Flumequine is the main residue with antimicrobial activity in all edible tissues and was retained as the marker residue.

17. In 2 radiometric depletion studies using the therapeutic regimen, the levels of radioactivity in edible tissues of calves were in the same magnitude whatever the administration route (oral or intramuscular route) after 24 hours: 200 µg equivalents flumequine/kg in muscle, 650 µg equivalents flumequine/kg in fat, 5800 µg equivalents flumequine/kg in liver, 2500 µg equivalents flumequine/kg in kidney. At 168 hours after the end of the treatment, ^{14}C-flumequine could not be detected in muscle or at the injection site. The concentrations were 100 µg equivalents flumequine/kg in fat and in kidney and ranged from 2600 to 3600 µg equivalents/kg in liver.

    In a non-radiometric depletion study, non-ruminant calves received flumequine in therapeutic doses (12 mg/kg bw/day for dose 1 and 6 mg/kg bw for doses 2 to 10, administered 12 hours apart) by oral route. Twenty-four hours after the end of the treatment, the mean concentrations of flumequine were 281, 594, 1117 and 1815 µg/kg in muscle, fat, liver and kidney respectively, then they declined to 120, 310, 320 and 590 µg/kg at 48 hours post dose. Seventy-two hours after treatment, the mean concentrations of flumequine were 91, 118, 181 and 237 µg/kg in muscle, fat, liver and kidney, respectively. No 7-hydroxy-flumequine could be detected in muscle, fat and liver and only small amounts in kidney (171 and less than 50 µg/kg at 24 hours and 48 hours, respectively).

    In a second non-radiometric depletion study, ruminant calves received flumequine by intramuscular route at the therapeutic regimen (12 mg/kg bw/day for dose 1 and 6 mg/kg bw for doses 2 to 10, administered 12 hours apart). Twenty-four hours after the end of the treatment, the concentrations of flumequine were 353, 1920, 241 and 1058 µg/kg in muscle, fat, liver and kidney respectively, then they declined to 52, 70, 74 and 394 µg/kg, 48 hours post dose. At 72 hours post dosing, the mean concentrations of flumequine were 30, less than 50, 82 and 298 µg/kg in muscle, fat, liver and kidney respectively. At 96 hours post dose, flumequine could only be quantified in muscle and kidney (47 and 90 µg/kg respectively) while in fat and liver the concentrations of flumequine were below the limit of quantification (50 µg/kg). The residues at the injection site declined from 58 926 µg/kg at 24 hours post dosing to 190 and 101 µg/kg at 48 and 96 hours. No 7-hydroxy-flumequine could be detected in muscle, fat and liver and only small amounts were measured in kidney (108, 53 and less than 50 µg/kg at 24 hours, 48 hours and 72 hours, respectively).

18. In sheep given ^{14}C-flumequine by intramuscular route twice daily for 5 days (12 mg/kg for dose 1 and 6 mg/kg bw for doses 2 to 10), the total radioactivity, the microbiologically active residues and the parent compound were simultaneously assayed. The concentrations of parent compound were determined by the HPLC method proposed for monitoring purposes, the limit of quantification being 5 µg/kg for all edible tissues. The concentrations of microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification being 100 µg/kg for kidney, liver and muscle, and 250 µg/kg for fat. The 6 animals treated were sacrificed 16 hours after the last injection.

    Flumequine is the main residue with antimicrobial activity in all edible tissues and was retained as the marker residue.

19. In a non-radiometric study, groups of 4 sheep received flumequine by intramuscular route according to the recommended regimen (12 mg/kg bw/day for dose 1 and 6 mg/kg bw for doses 2 to 10, administered 12 hours apart). Eighteen hours after the end of the treatment, the concentrations of flumequine were 180, 87, 487 and 1355 µg/kg in muscle, fat, liver and kidney, respectively. Then they declined to 30, 74, 55 and 246 µg/kg 48 hours post dose and to 9, 52, 14 and 39 µg/kg at 78 hours post dosing. The residues at the injection site declined rapidly from 2519 µg/kg at 18 hours post dosing to 38 and 8 µg/kg at 48 and 78 hours post dosing.

20. In pigs given ^{14}C-flumequine by intramuscular route twice daily for 5 days (15 mg/kg bw/day for dose 1 and 7.5 mg/kg bw for doses 2 to 10), the total radioactivity, the microbiologically active residues and the parent compound were simultaneously assayed. The concentrations of parent compound were determined by the HPLC method proposed for monitoring purposes, the limit of quantification being 50 µg/kg for all edible tissues. The concentrations of microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification being 100 µg/kg for kidney, liver and muscle, and 250 µg/kg for skin + fat. The 6 animals treated were sacrificed 16 hours after the last injection.

    Sixteen hours after the last administration, the mean levels of radioactivity were 262 µg equivalents flumequine/kg in muscle, 446 µg/kg in skin + fat, 7135 µg/kg in liver, 5265 µg/kg in kidney and 125 252 µg/kg at the injection site.

    The mean concentrations of flumequine measured at the same time were 199 µg/kg in muscle, 246 µg/kg in skin + fat, 468 µg/kg in liver, 2360 µg/kg in kidney and 10 3527 µg/kg at the injection site. The ratios flumequine towards total radioactivity were 0.75 in muscle, 0.55 in skin + fat, 0.06 in liver and 0.44 in kidney.

    The concentrations of residues with antimicrobial activity were 378, 288, 803, 3024 and 131 422 µg /kg in muscle, skin + fat, liver, kidney and at the injection site. The ratios of flumequine towards total microbiologically active residues were 0.53 in muscle, 1 in skin + fat, 0.58 in liver and 0.78 in kidney.

    Flumequine is the main residue with antimicrobial activity in all edible tissues, therefore, it was retained as marker residue.

21. In pigs given flumequine by intramuscular route at the recommended regimen (15 mg/kg bw/day for dose 1 and 7.5 mg/kg bw for doses 2 to 10 administered 12 hours apart), the mean concentrations of flumequine in muscle, skin + fat, liver and kidney declined from 194, 190, 448 and 1879 µg/kg 12 hours after dosing, to 65, 56, 125, and 460 µg/kg, respectively, 24 hours post dosing. Seventy-two hours after the end of the treatment, only traces of flumequine could be detected in muscle and skin + fat (less than 50 µg/kg) whereas it could be measured in liver (75 µg/kg) and in kidney (155 µg/kg).

22. In broiler chickens given ^{14}C-flumequine by oral gavage at a daily dose of 18 mg/kg bw for 5 days, the total radioactivity, the microbiologically active residues and the parent compound were simultaneously assayed in edible tissues. The concentrations of parent compound were determined by the HPLC method proposed for monitoring purposes, the limit of quantification being 25 µg/kg for muscle, for skin + fat and for liver, and was 100 µg/kg for kidney. The concentrations of microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification being 100 µg/kg for kidney, liver and muscle, and 250 µg/kg for skin + fat. The 6 animals treated were sacrificed 12 hours after the last injection.

    Twelve hours after the last administration, the mean levels of radioactivity were 553 µg equivalents flumequine/kg in muscle, 361 µg equivalents flumequine/kg in skin+fat, 1553 µg equivalents flumequine/kg in liver and 2062 µg equivalents flumequine/kg in kidney.

    The concentrations of flumequine measured at the same time by HPLC method were 509 µg/kg in muscle, 275 µg/kg in skin+fat, 1082 µg/kg in liver and 1557 µg/kg in kidney. The ratios of flumequine towards total radioactivity were: 0.93 in muscle, 0.77 in skin+fat, 0.69 in liver and 0.75 in kidney.

    The microbiologically active residues in muscle, skin+fat, kidney and liver were 629, 528, 1519 and 2013 µg/kg respectively. The ratios of flumequine towards total microbiologically active residues were 0.81 in muscle, 0.5 in skin+fat, 0.72 in liver and 0.79 in kidney.

    Flumequine supports the main microbiological activity in all edible tissues, therefore it was retained as marker residue.

23. In broilers chickens, 6 and 12 hours after the end of a treatment via drinking water at the therapeutic regimen (12 mg/kg bw/day of flumequine for 5 days), the concentrations of flumequine were in the same magnitude: 1500 µg/kg in muscle, 700 µg/kg in skin + fat, 2250 µg/kg in liver and 2400 µg/kg in kidney. Then, they declined to 158, 90, 178 and 215 µg/kg in muscle, skin + fat, liver and kidney at 36 hours post dosing and to 69, 79, 185 and 161 µg/kg, respectively at 48 hours. At later sampling all the concentrations were below the limits of quantification (50 µg/kg for skin + fat and 100 µg/kg for liver and kidney) except for muscle (37 µg/kg).

    At 6 hours post dosing, the following levels of 7-hydroxy-flumequine were reported: 170 µg/kg for muscle, 100 µg/kg for skin + fat, 1100 µg/kg for liver and 1900 µg/kg for kidney. Then they declined to be lower than 10 µg/kg and 50 µg/kg in muscle and skin + fat and to 185 and 165 µg/kg in liver and kidney at 48 hours post dosing.

24. Groups of 10 rainbow trout, maintained at 2 different water temperatures, 7°C and 16°C, received once 12 mg/kg bw of ^{14}C-flumequine by gavage. The concentrations of parent compound were determined by the HPLC method proposed for monitoring purposes, the limit of quantification being 50 µg/kg for muscle and skin in natural proportions. The concentrations of microbiologically active residues were measured by a microbiological method based on agar diffusion using *Morganella morganii* as test organism, the limits of quantification being 100 µg/kg.

    In the group kept at 16°C, the total radioactivity measured in muscle plus skin in natural proportions was 3160 and 3110 µg equivalents flumequine/kg at 18 and 36 hours after treatment, respectively. The concentrations in flumequine were in the same magnitude and the ratio of flumequine towards total radioactivity at both time points was 1.

    The concentrations of microbiologically active residues were 4046 and 4415 µg equivalents flumequine/kg after 18 and 36 hours, and the ratios of flumequine towards total microbiologically active residues were 0.83 and 0.75.

    In the group kept at 7°C the values of total radioactivity flumequine in muscle plus skin in natural proportions were 3670 and 1756 µg equivalents flumequine/kg at 36 and 96 hours after treatment, respectively. The concentrations of the parent compound were in the same magnitude and the ratio of flumequine towards total radioactivity was 0.97.

The concentrations of residues with antimicrobial activity were 4495 and 2541 µg equivalents flumequine/kg after 36 and 96 hours, and the ratios flumequine towards total microbiologically active residues were 0.79 and 0.67.

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

    In the group kept at 7°C, the concentrations of flumequine in muscle plus skin in natural proportions were 5600 µg/kg , 900 µg/kg, 80 µg/kg at 1, 4,7 days after the end of the treatment, respectively.

    In the group kept at 16°C the concentrations of flumequine in muscle plus skin in natural proportions decreased more rapidly: 1670 and 80 µg/kg at 1 and 4 days after the end of the treatment and could not be detected (less than 18 µg/kg) for the further sampling times. 7-Hydroxy-flumequine could not be detected in those samples collected in trout kept either at 7°C or 16°C.

26. At the 48th meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) held in February 1997 recommended definitive MRLs for flumequine of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat in cattle expressed as parent compound. In absence of data on the contribution of parent compound to the total residues in sheep, chickens and pigs, the JECFA recommended temporary MRLs of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat. A temporary MRL of 500 µg/kg was set for trout muscle plus skin.

27. The Committee for Veterinary Medicinal Products could not retain the same MRL values as JECFA considered that the toxicological end point was the most appropriate to assess the safety profile of this compound. From the JECFA MRL values, the maximum theoretical intake was estimated at 1100 µg per person and per day, a value compatible with the toxicological ADI of 1800 µg/person. However, those MRLs were not compatible with the microbiological ADI of 495 µ/person retained by the Committee for Veterinary Medicinal Products.

28. Analytical methods, using HPLC with fluorescence detection, have been validated according the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community to measure residues of flumequine and 7-hydroxy-flumequine. The limits of quantification for flumequine were 50 µg/kg for all edible tissues of pigs and for trout, 50 µg/kg for bovine kidney, liver and fat and 25 µg/kg for bovine muscle. For all edible chicken tissues, it was 25 µg/kg except for kidney (100 µg/kg). For sheep edible tissues, the limit of quantification for flumequine was 5 µg/kg.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 8.25 µg/kg bw (i.e. 495 µg/person);
- 85% of flumequine related residues are eliminated within 24 hours,
- flumequine is the marker residue and the respective ratios towards total residues with microbiological activity are:
  - 0.77, 1, 0.69 and 0.94 in muscle, fat, liver and kidney of bovine,
  - 1, 1, 0.85 and 1 in muscle, fat, liver and kidney of ovine,
  - 0.53, 1, 0.58 and 0.78 in muscle, skin + fat, liver and kidney of porcine,
  - 0.81, 0.50, 0.72, 0.79 in muscle, skin + fat, liver and kidney of broiler chickens,
  - 0.67 of the microbiologically active residues in muscle plus skin in natural proportions in salmonidae,
- in cattle, sheep and pigs, the relative tissue distribution at 16 to 24 hours was 1:1.5:2:8 for muscle, fat or skin + fat, liver and kidney respectively while for chicken, having considering the individual variations, the tissue distribution could not be exactly estimated,
- validated analytical methods are available for monitoring residues in edible tissues;

the Committee recommends the inclusion of flumequine 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 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	

Based on these MRL values, the daily intake will represent approximately 64% of the microbiological ADI.