FLUMEQUINE

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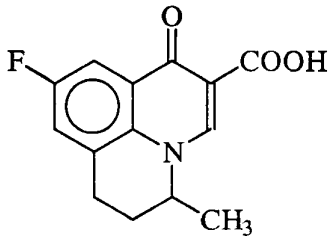
ADDENDUM
to the flumequine residue monograph
prepared by the 48th meeting of the Committee and published in
FAO Food and Nutrition Paper 41/10, Rome 1998.

INDENTITY

Chemical names: 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-*1H,5H*-benzo[ij]-quinolizine-2-carboxylic acid.

Synonyms: R-802, Apurone.

Structural formula:



Molecular formula: $C_{14}H_{12}NO_3$

Molecular weight: 261.26

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: flumequine.

Appearance: white microcrystalline powder.

Melting point: 253-255°C.

Solubility: soluble in aqueous alkaline solutions and alcohol, insoluble in water.

Optical rotation: produced and used as a racemic mixture.

INTRODUCTION

The Committee has previously considered the antimicrobial agent flumequine at its forty-eighth meeting. At that meeting the Committee recommended MRLs for flumequine in cattle of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug. In the absence of data on the contribution of parent drug to the total residues in sheep, chickens and pigs, the Committee recommended temporary MRLs in these species of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug. The Committee also recommended a temporary MRL of 500µg/kg for trout muscle (including normal proportions of skin) expressed as parent drug.

In reaching its decision on MRLs for flumequine, the forty-eighth meeting of the Committee took the following factors into consideration:

- An ADI of 0-30 μg /kg of body weight was established, based on a toxicological end-point. This corresponds to a maximum theoretical daily intake of 1800 μg for a 60-kg person.
- The parent drug was identified as the marker residue.
- Muscle and kidney were considered to be the appropriate target tissues. For practical reasons, however, liver is recommended as the target tissue for chickens in place of kidney.
- On the basis of data from studies in calves, non-extractable residues accounted for 20% of the total residues.
- In calf muscle, kidney and fat, the parent drug accounted for approximately 80% of the extractable residues.
- 7-Hydroxyflumequine and the unknown metabolite, M1, together account for 80% of the total radioactivity in calf liver after 168 hours.
- On the basis of the contribution of parent drug in extractable residues in calves, the parent drug accounts for 50% of the total residues in muscle, kidney and fat, and 25% of the total residues in liver.
- No data were provided for milk or eggs and no MRLs are recommended.
- No data were provided on the amount of parent drug as a percentage of the total residues in sheep, chickens, pigs or trout.

From the MRLs for cattle, the maximum theoretical intake of flumequine residues would be 1100 μg per day, which is compatible with a maximum of 1800 μg for a 60-kg person based upon the ADI of 0-30 μg/kg body weight.

The Committee requested that studies be conducted with radiolabeled flumequine in pigs, sheep, chickens and trout to estimate the proportion of the total residues accounted for by the parent drug. The results of these studies were required for evaluation in 2000.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of Use

Flumequine is a first generation drug of the fluoroquinolone group of antibiotics. It is used primarily for the treatment of enteric infections in domestic species. Flumequine also has a limited use in man for the treatment of urinary tract infections.

METABOLISM

Previous studies of the metabolism of flumequine in calf liver

Metabolites were isolated from calf liver at different times after the last drug administration by extraction with both ethyl acetate and methanol. The efficiency of recovery of radioactivity was not significantly affected by enzymatic deconjugation for early time points (6 and 24h) but an increasing fraction of radioactivity was recovered in the ethyl acetate layer after deconjugation with *Helix promatia* as time increased. About 20% of the isolated metabolite radioactivity could be ascribed to conjugated metabolites. Acceptable recoveries of the residual radioactivity remaining in tissue after solvent extraction could be achieved using pronase digestion prior to repetition of the extraction process.

Radio profiling of peaks obtained from HPLC identified 13 other metabolites (M1-M13) in addition to flumequine and 7-hydroxyflumequine. Only flumequine and 7-hydroxyflumequine and metabolite M1 contributed significantly to total extractable radioactivity and M1 was the major metabolite identified at times of 24 h and later. Table 1 shows results of metabolite distribution after metabolite deconjugation with *Helix promatia*. The concentration of flumequine decreased rapidly and was not observable after 24 h. 7-Hydoxyflumequine decreased somewhat more slowly during the first 72 h (FAO, 1998).

Table 1. **Time dependence of the 3 major metabolites of flumequine in calf liver following hydrolysis with *Helix promatia***

Time(h) post dosing	Mean total radioactivity (mg equiv/kg)	Mean (%) total radioactivity measured	Analytes (% of total)		
			M1	(1)	(2)
6	7.73	65.5	9-15	42-58	9-13
24	5.41	51.0	25-41	10-34	2-12
72	4.19	48.2	26-43	ND	6-9
120	3.98	41.4	52-57	ND	ND
168	3.00	48.5	35-63	ND	0-22

Note: (1) = flumequine, (2) = 7-hydroxyflumequine. ND = not detected.

New metabolism studies with [^{14}C]-flumequine

Cattle

Three male and three female beef cattle, seven months old and weighing 125-135 kg, were given ^{14}C-flumequine for five consecutive days at a dose of 12 mg/kg body weight by subcutaneous injection into the neck. The first 3 of 4 doses were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 18 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 2, showing that at the time of sacrifice about 83% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 21% of the radioactivity in both muscle and kidney while in fat and at the injection site, flumequine was recovered almost entirely as parent drug (Guyonnet, 1999; Lynch and McLean, 1998)

Table 2. **Ratio of flumequine to total radioactivity in cattle tissues after the s.c. administration of ^{14}C-labelled flumequine for 5 consecutive days**

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (µg/kg)	SD (µg/kg)	Mean (µg/kg)	SD (µg/kg)	
Muscle	321	89	374	92	0.79 (0.04)
Liver	1200	280	7110	1380	0.17 (0.06)
Kidney	4200	981	5320	1270	0.79 (0.05)
Fat*	1080	666	1180	954	1.00 (0.14)
Injection site	455000	456000	464000	434000	1.04 (0.07)

Note: * fat samples were a composite of renal, omental and subcutaneous fat.

Pigs

Three male and three female pigs, weighing 45-49 kg were given ^{14}C-flumequine intramuscularly into the neck twice daily for five consecutive days. The first dose was 15 mg/kg body weight and the subsequent nine doses were 7.5 mg/kg body weight. Injections 1-9 were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 16 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 3, showing that at the time of sacrifice about 93% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 56% of the radioactivity in kidney, 45% in skin with fat and about 25% in both muscle and fat. (Guyonnet, 1998a; Lynch and Speirs, 1998a)

Table 3. **Ratio of flumequine to total radioactivity in pig tissues after i.m. administration of ^{14}C-labelled flumequine twice-daily for 5 consecutive days**

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	199	45	262	42	0.75 (0.05)
Liver	468	78	7140	964	0.07 (0.02)
Kidney	2360	861	5270	796	0.44 (0.10)
Fat*	364	113	483	141	0.76(0.09)
Skin with fat	246	41	446	59	0.55 (0.05)
Injection site	104000	57600	125000	68000	0.83 (0.20)

Note: * fat samples were a composite of total fat isolated.

Sheep

Three male and three female sheep, weighing 38-50 kg, were dosed twice daily with ^{14}C-flumequine intramuscularly into the neck for five consecutive days. The first dose was 12 mg/kg body weight and the subsequent nine doses were 6 mg/kg body weight. Injections 1-9 were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 16 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 4, showing that at the time of sacrifice about 94% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 65% of the radioactivity in kidney, 51% in muscle and 44% in fat. At the left and right injection sites, lower observed levels of metabolites were accompanied by high amounts of flumequine (Guyonnet, 1998b; Lynch and Speirs, 1998b).

Table 4. **Ratio of flumequine to total radioactivity in sheep tissues after i.v. administration of ^{14}C-labelled flumequine twice-daily for 5 consecutive days**

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	49	15	100	21	0.49(0.08)
Liver	139	30	2520	787	0.06(0.01)
Kidney	710	148	2230	682	0.35 (0.05)
Fat*	120	77	160	131	0.56 (0.05)
Injection site L	17500	14300	17800	13300	0.86 (0.24)
Injection site R	13300	12000	12500	10000	0.96 (0.21)

Note: * fat samples were a composite of total fat isolated. R = right, L = left.

Chickens

Six broiler chickens, three males and three females, 5 to 5 weeks old and weighing 2.16-2.64 kg were dosed with ^{14}C-labelled flumequine for five consecutive days. The drug was administered orally as a solution by gavage into the crop at a dose of 18 mg/kg body weight. All animals were killed and exsanguinated 12 h after the final injection. The entire kidney, liver, breast and thigh muscle, omental fat pad and skin with fat were retained for HPLC analysis, determination of total radioactivity and microbiological activity.

The results of HPLC and radiochemical analysis are summarised in Table 5, showing that at the time of sacrifice about 30% of the radioactivity in liver is flumequine either as metabolites or bound residues. Metabolites or bound residues account for about 24% of the radioactivity in kidney, 6% in muscle, 23% in skin with fat. There was no measurable metabolism of flumequine in omental fat (Guyonnet, 1998c; Lynch and Speirs, 1998c).

Table 5. Ratio of flumequine to total radioactivity in chicken tissues after the oral administration of ^{14}C-labelled flumequine to broiler chickens daily for 5 consecutive days

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (µg/kg)	SD (µg/kg)	Mean (µg/kg)	SD (µg/kg)	
Muscle	509	154	553	183	0.94 (0.10)
Liver	1080	397	1550	557	0.70 (0.04)
Kidney	1560	488	2060	624	0.76 (0.06)
Skin with Fat	275	95	361	127	0.77 (0.06)
Omental fat	129	38	123	44	1.09 (0.20)

Note: * fat samples were a composite of total fat isolated.

Trout

Two groups of 20 rainbow trout, with an average weight of 90.1 ± 8.1 g and 100.2 ± 10.3 g, respectively, were maintained in separate tanks with water temperatures of 7°C and 16°C, respectively. A single dose of ^{14}C-labelled flumequine was administered by gavage via a syringe, as a 2% formulation in lactose, enclosed in a gelatine capsule at a dose level of 12 mg/kg body weight. The method of administration was investigated prior to the study to confirm that the gelatine capsule ruptured in the stomach of the fish within 3 h of administration. Doses for individual fish were calculated based on weight at slaughter. Five trout from the group maintained at 16 °C were slaughtered at 18 h and five at 36 h after treatment and five trout from the group maintained at 7 °C were slaughtered at 36 h and five at 96 h.

The results of HPLC and radiochemical analysis are summarised in Table 6, showing that at the time of slaughter there is no evidence of metabolism of flumequine at any time point at either temperature (Guyonnet, 1998d, Caley, 1998).

Table 6. Ratio of flumequine to total radioactivity in trout muscle after the oral administration of a single dose of ^{14}C-labelled flumequine to trout maintained at different water temperatures

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (µg/kg)	SD (µg/kg)	Mean (µg/kg)	SD (µg/kg)	
Muscle with skin, 16°C , 18 h, n = 8	3320	2210	3160	2030	1.04 (0.06)
Muscle with skin, 16°C , 36 h, n = 8	3160	1300	3110	1140	1.00 (0.07)
Muscle with skin, 7°C , 36 h, n = 8	3680	1750	3670	1490	0.98(0.11)
Muscle with skin, 7°C , 96 h, n = 8	1700	555	1760	585	0.97 (0.05)

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE

A routine high performance liquid chromatographic (HPLC) analytical method for flumequine and its metabolite 7-hydroxyflumequine using fluorescence detection similar to the method presented to the 48th Committee was used to obtain data presented in the residue studies. Flumequine and 7-hydroxyflumequine were extracted from 2 gram sample with ethyl acetate without any prior deconjugation. Ibafloxacine was added to the sample as an internal standard to monotor retention time after clean up by liquid-liquid extraction. An extra step was performed for all samples with a high fat content where the extract was partioned between acetonitrile and hexane. Detection and quantification of flumequine was achieved by HPLC, employing gradient elution, using various mixtures of acetonitrile and 2.7 x 10^{-3} M aqueous oxalic acid (pH 2.5), on a C_{18} column using fluorescence detection with an excitation wavelength of 252 nm and an emision wavelength of 356 nm. Quantification was achieved by comparison of results with a calibration curve constructed from information obtained by the analysis of samples fortified with flumequine. The linearity, accuracy, repeatability, quantification and lower limits of detection of the method have been assessed in a single laboratory. No interlaboratory studies have been performed to test reproducibility. All data originate from one laboratory and one analyst. Within laboratory (day-to-day) precision data were all obtained from fortified blank tissues. No incurred tissues were included in the method validation. No ruggedness tests have been applied.

The specificity of the method was checked against some other quinolone antibiotics. Other quinolones tested for and showing no interference are oxolinic acid, nalidixic acid, marbofloxacin, danofloxacin, enrofloxacin and ciprofloxacin. Interference from "endogenous" compounds has been tested for each species/matrix combination with at least 20 "representative" blank samples.

The sponsor does not refer in the dossier as such to a routine regulatory analytical method for the determination of flumequine in edible tissues of pig, sheep, chicken, cattle and trout. However, in the dossier the HPLC-fluorescence-method(s) for the determination of flumequine are described that have been used to support residue depletion studies and are considered by the sponsor as potential regulatory methods (Guyonnet et al, 1996a and 1996b, CEVA, 2000). All these methods have the ability to determine the flumequine metabolite 7-hydroxyflumequine, however, no validation data for this application were supplied. The determination of 7-hydroxyflumequine was not carried out in studies discussed above.

All method descriptions are in conformity with ISO 78/2 Standard for drafting Standard Operating Protocols. The performance characteristics of the method, as they apply to pig and shep tissues, are detailed in Table 7 below (Guyonnet, 1998b, CEVA, 2000). The sponsor also provided similar data for calf and chicken tissues.

Table 7. The performance characteristics of routine high performance liquid chromatographic method for flumequine as determined for pig tissues

Validation parameter	Tissue							
	Kidney		Liver [b]		Muscle [a]		Skin/fat	fat[a]
	Pig[a]	Sheep[c]	Pig[b]	Sheep[c]	Pig[a]	Sheep[c]	Pig[a]	Sheep[c]
Range (µg/kg)	50-2500	5-100	50-2500	5-100	50-2500	5-100	50-2500	5-100
Precision (%)[e]	0.5-6.0	3.79-6.24	0.3-6.1	5.45-9.42	0.5-5.9	4.78-9.53	0.2-12.2	2.65-7.76
Recovery (%)	85.8-94.8	87.5[d]	76.1-93.7	78.3[d]	83.2-89.4	89.7[d]	78.9-91.4	83.8[d]
Accuracy (%)	-1.8/+5.5	-	-3.7/+0.2	-	-0.4/+6.6	-	-0.3/+8.7	-
LOQ (µg/kg)	50	5	50	5	50	5	50	5
LOD (µg/kg)	15	1	10	2	24	2	21	0.5

a = Samples homogenized with anhydrous Na_2SO_4, subsequent extraction with ethyl acetate.
b = Samples homogenized overnight with 1 molar HCl , subsequent extraction with ethyl acetate.
c= Samples homogenized in water with ultrasound, subsequent extraction with ethyl acetate.
d = "overall value"
e = day to day precision
- = no data supplied

APPRAISAL

The sponsor has provided the information on new residue studies that allow the estimation of the proportion of the total residues that accounted for parent drug at one time point for each species. The information provided was conducted to GLP and fully satisfies the provision of new data requested by the 48th Meeting.

The mean ratios of flumequine to total radioactivity in various tissues, derived from Tables 2-5, are summarised in Table 8. The new data provided show that:

- ratios of flumequine to total radioactivity in cattle kidney, muscle and fat (0.79 - 1.0) were somewhat above the average value of 0.5 estimated from residue depletion studies by the 48th Committee.
- The ratio of flumequine to total radioactivity in cattle liver (0.17) was somewhat below the average value of 0.25 estimated from residue depletion studies by the 48th Committee.
- ratios of flumequine to total radioactivity in sheep kidney, muscle and fat (0.35 - 0.56) were of the same order, or somewhat less than the value of 0.5 estimated from residue depletion studies by the 48th Committee used to recommend temporary MRLs.

- ratios of flumequine to total radioactivity in pig kidney, muscle and fat (0.44 - 0.75) were of the same order, or somwhat less than the value of 0.5 estimated from residue depletion studies by the 48th Committee and used to set temporary MRLs.

- ratios of flumequine to total radioactivity in pig and sheep fat (0..07 and 0.06, respectively) were about 4 times less than the value of 0.55 estimated from residue depletion studies by the 48th Committee and used to set temporary MRLs.

- there appears to be less metabolism of flumequine in chickens than in mammalian species. New data show ratios of flumequine to total radioactivity in chichen tissues (0.70 - 0.94), greater than the value of 0.5 estimated from residue depletion studies by the 48th Committee.

- there appears to be no measurable metabolism of flumequine in trout and correction factors (a ratio) does not need to be applied to residue data in considering appropriate MRL values

Table 8. Mean ratios of flumequine to total radioactivity in various tissues, derived from Tables 2 – 5.

Species	Tissue			
	Muscle	Liver	Kidney	Fat
Cattle	0.79	0.17	0.79	1.00
Pig	0.75	0.07	0.44	0.55*
Sheep	0.49	0.06	0.35	0.56
Chickens	0.94	0.70	0.76	0.77*
Trout	1.00**, 0.97***			

* = skin with fat ** = muscle with skin, 36 h, 16°C *** = muscle with skin, 96 h, 7°C

The sponsors method is suitable for regulatory purposes. Some methods, previously to other quinolone antibiotics (FAO, 1995), have also been applied to flumequine. Especially for *multi* quinolone residue analysis in food of animal origin a variety of useful HPLC methods has been published (Charriere et al, 1993; Ellerbroek, 1993; Haagsma et al, 1993; Heijden et al, 1993; Moretti et al, 1996; Samuelsen, 1990; Yorke, 1998) as well as planar chromatography (Juhel-Gaugain, 1996). For more efficient automated analyses aquous on-line dialysis and quinolone residue enrichment methods have been developed (Andresen & Rasmussen, 1990; Cohen et al, 1999; Eng et al, 1998; Leeuwen & Gend, 1989; Maxwell & Cohen, 1998). Also fast multi quinolone separation techniques are reported based on capillary zone electrophoresis (Perez-Ruiz et al, 1999; Sun & Chen, 1997). For final confirmation of test results in regulatory multi residue control an increasing number of methods based on hyphenated gas or liquid chromatography-mass spectrometry is available (Alvarez et al, 1997; Delepine et al, 1998; Doerge & Bajic, 1995; Plakas et al, 1999; Rose et al, 1998; Volmer et al, 1997; Munns et al, 1995; Pfenning et al, 1996).

If the Committee uses the same procedure to allocate MRLs to tissues of pigs, sheep, chickens and trout as it did to cattle, a problem arises with the data for the mean ratios of flumequine to total radioactivity in pig and sheep liver. Data provided suggests that since flumequine only accounted for about 6 - 7% of total residues, a correction factor (ratio) of 16 might be appropriate in TMDI calculations. Other data for pigs and sheep would suggest that the correction factor of 0.5 used for kidney, muscle and fat by the 48th Committee would be an appropriate figure to retain (with the possible exception of sheep kidney).

Reviewing the analytical data presented in Tables 3 and 4 for the determination of flumequine in pig and sheep liver, respectively, would suggest that it would not be possible to reliably quantify flumequine residues in sheep for more that two days after withdrawal of drug. This is because after 16 h, mean flumequine residues shown in Table 4 were only 2.5 times higher than the LOQ of the analytical method. Using similar logic, it should not be possible to reliably quantify flumequine residues in pigs for more than three days after withdrawal of drug.

MAXIMUM RESIDUE LIMITS

On the basis of the above considerations, the Committee recommended MRLs for edible tissues of cattle, pigs, sheep and chickens as follows: muscle and liver, 500 µg/kg; kidney, 3000 µg/kg and fat, 1000 µg/kg. The Committee also recommends 500 µg/kg for trout muscle, including skin in natural proportions. The MRL for flumequine in cattle liver and all temporary MRLs recommended by the Committee at its forty-eighth meeting are withdrawn.

Applying the values of food intake established by JECFA to the MRLs recommended above would result in a theoretical maximum daily intake of 1655 µg (see Table 9). This TMDI is based on residues in sheep, the species for which data indicates the most extensive metabolism of flumequine. This value is compatible with a maximum daily intake of 0-1800 µg

Table 9. Theoretical maximum daily intake (TMDI) of flumenquine residues

Food item	MRL (µg/kg)	Food Basket (g)	µg	MR/TR[1]	TMDI (µg)
Muscle	500	300	150	0.5	300
Liver	500	100	50	0.06	835
Kidney	3000	50	150	0.35	430
Fat	1000	50	50	0.55	90
Total					1655

Note: MR = marker residue; TR = total residue

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