FLUMEQUINE

José Luis Rojas, Lanaseve, Costa Rica

Stefan Soback, Beit Dagan, Israel

ADDENDUM

To the monographs prepared by the 48th and 54th meetings of the Committee published in the FAO Food and Nutrition Paper 41/10, Rome 1998 and 41/13, Rome 2000, respectively.

IDENTITY

Chemical name:	9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1 H,5H-quinolizine-2-carboxylic acid
Synonyms:	R-802, Apurone
Molecular formula:	$C_{14}H_{12}NFO_3$
Molecular weight:	261.26
Pure active ingredients:	Flumequine
Appearance:	White microcrystalline powder
Melting point:	253-255°
Solubility:	Soluble in aqueous alkaline solutions and alcohol, insoluble in water

INTRODUCTION

Residues of flumequine were evaluated by the Committee at the 42nd (WHO 1998), 48th (WHO 1998) and in the 54th meeting (WHO 2001). The Committee established an ADI of 0-30 µg/kg of body weight based on a toxicological end-point (hepatotoxiciy in male CD-1 mice in the 13-week study) and recommended MRLs for flumequine of 500 µg/kg for muscle and liver, 3000 µg/kg for kidney and 1000 µg/kg for fat in cattle, pigs, sheep and chickens, expressed as parent drug. The Committee also recommended an MRL of 500 µg/kg for trout muscle with skin in their natural proportions.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Flumequine is a quinolone with antimicrobial activity against Gram negative organism and is used for the treatment of enteric infections in domestic species. It has also a limited use in the treatment of urinary tract infection in man.

Disposition and residues of flumequine in black tiger shrimp

Data was submitted to the Committee for its consideration including: the disposition and residue pattern of flumequine in black tiger shrimp (Penaeus monodon) and for the establishment of MRL in giant prawn or black tiger shrimp. In response to a question from the Committee the sponsor indicated that there is no recommended dose of flumequine for giant prawns.

The disposition and residue data were generated with shrimps (Penaeus monodon) with an average weight of 20-30 gm which were maintained in 5 x 10 m concrete tanks in the open with shading to bring the water temperature to 28-32 grades centigrade at a pH of 8.0. Flumequine was administered to the shrimps at 12 mg/kg shrimp bodyweight by intramuscular injection, forced oral dosing using feeding needle, or mixed to the pelleted feed and given ad libitum for 5 consecutive days.

After drug administration, nine shrimp samples were randomly, collected at 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336 and 360 hours post-dosing. In the groups given feed treatment, shrimps were sampled at daily interval from the tanks, before medicated feed was administred in the morning for a 15 day period.

Samples of the whole edible part of muscle tissue from each shrimp were packed in plastic bag and kept frozen at -20 grades centigrade until assay.

Injection, single oral administration

The absorption and excretion of flumequine in black tiger shrimp following a single intramuscular and oral administration are shown in figure 1. The maximum peak concentration obtained in shrimp muscle, 2616.45 µg/kg at 2 hours following injection and decreased below LOQ at 216 hours post dosing and the maximum peak concentration obtained in shrimp muscle, 365.8 at twelve hours after oral administration and decreased below LOQ after 144 hours post dosing. The mean drug concentrations versus time are presented in table 1.

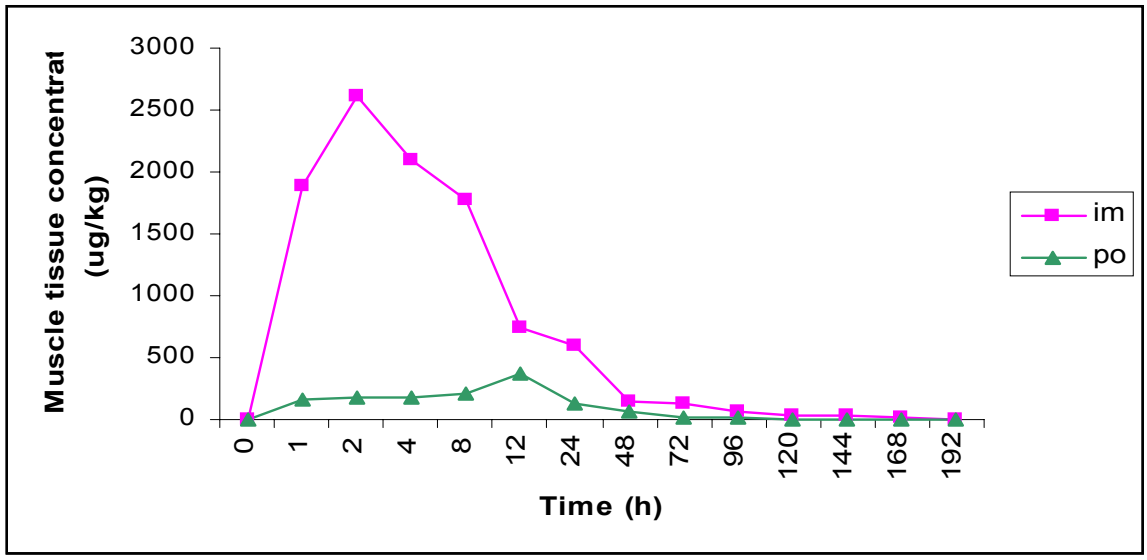
Table 1. Flumequine concentration in shrimp muscle after intramuscular and oral administration of a single dose of 12 mg/kg shrimp.

Flumequine concentration (µg/kg) by HPLC		
Time (hours) post dosing	**Intramuscular [a]Mean (SD)**	**Oral dosing [a]Mean (SD)**
1	1885.80 (754.45)	162.22 (20.40)
2	2616.45 (811.09)	178.16 (95.17)
4	2101.87 (394.20)	183.01 (26.58)
8	1777.95 (1084.3)	210.76 (15.44)
12	744.48 (70.82)	365.80 (136.44)
24	600.76 (404.74)	124.56 (24.72)
48	149.91 (42.10)	65.41 (45.30)
72	121.84 (109.90)	15.75 (1.30)
96	64.64 (24.15)	11.62 (11.06)

Flumequine concentration (µg/kg) by HPLC		
Time (hours) post dosing	**Intramuscular [a]Mean (SD)**	**Oral dosing [a]Mean (SD)**
120	38.42 (19.15)	7.67 (6.18)
144	27.36 (11.90)	<5[b]
168	12.22 (3.29)	<5
192	6.66 (3.64)	<5
216	<5[b]	<5
240	<5	0
264	<5	0
288	0	0
312	0	0

a. pooled muscle tissue from 9 shrimp
b. limit of quantification 5 µg/kg

Figure 1. Mean muscle concentrations versus time profiles of flumequine in black tiger shrimp following single intramuscular and oral administration of 12 mg/kg



Medicated feed

Flumequine solution was mixed with pelleted feed to represent an oral dose equivalent to 12 mg/kg. The feed was ad libitum to the shrimp in the experiment for 5 consecutive days. Residue concentrations were determined daily for 15 days or until they fell under detection limit of 5 µg/kg. The results are presented in table 2. The maximum peak concentration was observed at day 3 of the treatment and decrease below the LOQ at 96 hours post-treatment. After intramuscular administration, the estimation half live (T1/2) was 33.4 hours, the relative bioavailability (F) after forced oral administration was 21.6% (table 3)

Table 2. Flumequine concentration in shrimp muscle following medicated feed application for 5 consecutive days at the dose of 12 mg / kg b.w

Time	Flumequine concentration (µg/kg) Mean (SD)
Day 1 of treatment	0
Day 2 of treatment	43.8 (15.2)
Day 3 of treatment	45.5 (14.1)
Day 4 of treatment	45.0 (9.17)
*Day 5 of treatment	29.8 (7.63)
24 hours	28.5 (14.5)
48 hours	22.7 (6.46)
72 hours	9.29 (5.73)
96 hours	<5
120 hours	<5
144 hours	0
168 hours	0

* Last day of medicated feed treatment

Table 3. Pharmacokinetic parameter for flumequine in shrimp

Parameters	Intramuscular	Oral dosing	Feed treatment
Dose (mg/kg shrimp or feed)	12	12	12
Water pH	8	8	8
$T_{1/2\beta}$ (h)	33.45	60.21	
MRT (h)	28.17	35.07	
AUC (µg.h^{-1}/kg)	56.55	12.23	
F_x %		21.63	
C_{max} (µg/kg)		365.81	45.52
T_{max} (h)		12.0	Day 3

$T_{1/2\beta}$: elimination half life; MRT: mean residence time; AUC: area under curve; F: availability of administered dose; Cmax: maximum tissue concentration; Tmax: time of peak tissue concentration

According the guidelines of Stamm (1989) concerning antibiotics used in aquaculture the plasma concentration of the drug should exceed its minimum inhibitory concentration (MIC) value by a factor of 3-4 times in the fish. The MIC of Aeromonas salmonicida for flumequine is below 0.063 mg/ml (Tsoumas et al., 1989). A single intramuscular injection of 12 mg/kg of flumequine maintained the flumequine above 250 µg/kg corresponding to 4 times the MIC values for 24 hours in shrimp. The maintained levels in excess of MIC value of 62.5 µg/kg are found for 48 hours in muscle, which the peak muscle concentration of 365 µg/kg at 12 hours after oral dosing. In comparison, medicated with flumequine in feed (12 mg/kg) for 5 consecutive days, the levels of drugs are low, only 30-45 µg/kg found in muscle which is below the MIC values at all sampling time. Therefore no drug efficacy using feed treatment at 12 mg/kg can be expected.

METHOD OF ANALYSIS

Samples of muscle tissues were homogenized following a modified procedure reported by Samuelsen (1990). The concentration of flumequine in muscle tissues were determined by high-performance liquid chromatography using a fluorescence detector set at excitation wavelength of 327 nm and emission wavelength of 369 nm (following Samuelsen and Ervik, 2001).

The method used ethyl acetate extraction. After evaporation the analyte was reconstituted in mobile phase. The mobile phase consisted of oxalic acid,acetonitrileand methanol. The separation was achieved by use of a reversed phase column in isocratic mode and the fluorescence detector was adjusted at 327 nm excitation and 369 nm emission.

Calibration curves were established in muscle tissues fortifying the samples to represent a range of 0.5-30 µg/kg and 100-200 µg/kg. The LOQ was 5 µg/kg and the curves were linear over the tested range. The linear correlation coefficients were 0.99968 and 0.99998, respectively. The recovery of flumequine was from 99.8% (2000 µg/kg) to 104.4% (5 µg/kg). The determination of flumequine metabolites was not carried out.

APPRAISAL

Considering the data available at the present meeting the Committee concluded that:

- The study showed that flumequine in an aqueous solution is relatively poorly absorbed by shrimp.
- Based on MRL of 500 µg/kg for muscle tissue in fish, and the results from the study using medicated feed at 12 mg/kg, flumequine concentration in tiger shrimps were below the tolerance all samples analyzed.
- The tissue residue depletion studies utilized methodology based on HPLC for separation and fluorescence detection. The LOQ, linearity and recoveries appeared acceptable.

Also the Committee concluded that they would justify establishing a MRL of 500 µg/kg in muscle in Black tiger shrimp provided the following information would be made available:

1. Detailed information on a regulatory method including method performance characteristics and method validation
2. Information on the approved dose for treatment of Black tiger shrimp and results of residue data from studies using the recommended dose.

In view of the recommendation to withdraw the ADI for flumequine, the Committee agreed to withdraw the MRLs for all species which had been established at previous meetings.

REFERENCES

Alderman, DJM. (1992). Chemotherapy in aquaculture today: from theory to Reality. OIE, Paris.

Elema, M.O., et. al., (1995). Bioavailability of flumequine after oral administration to Atlantic to Salmon. Aquaculture, 136,209.

Gibaldi, M., Perrier, D. (1982). Pharmacokinetics. 2nd edition.,Marcel Dekker, NY

Guyonnet, j. (1998). HPLC and microbiological determination of flumequine residues in edible tissues after single oral administration of flumequine to rainbow trout. Sanofi dossier to the 54 JECFA Meeting

Hansen, M.K., Horsberg, T.E. (2000). Single dose pharmacokinetics of flumequine in cod and goldsinny wrasse. J. Vet. Pharm. Therap., 23, 163.

Inglis, V. (2000). Antibacterial chemoteraphy in aquaculture. Proceedings of the meeting on the use of chemicals in aquaculture in Asia, Philippines.

Martinsen, B., Horsberg, T.E. (1995) Comparative single dose pharmacokinetics of 4-quinolones, oxxolinic acid, flumequine, sarafloxacin and enrofloxacin in Atlantic salmon held in seawater at 10°C. Antimicrob. Ag. Chemo., 39, 1059.

Rasmussen, K.E., et al. (1989). Solid phase extraction and HPLC determination of flumequine and oxolinic acid in salmon plasma. J. Chromato. Appl., 496,355.

Rogstad, A., et al. (1993). Pharmacokinetics and bioavailability flumequine and oxolinic acid after various routes of administration to Atlantic salmon in seawater. Aquaculture., 110, 207.

Samuelsen, O.B. (1990). Simple and rapid method for determination of flumequine and oxolinic acid in salmon plasma by HPLC and fluorescence detection. J. Chromato., 530, 452.

Samuelsen, O.B. Ervik, A. (1997). Single dose pharmacokinetics study of flumequine after intravenous, intraperitoneal, and oral administration to Atlantic halibul held in seawater at 9 degrees. Aquaculture, 158, 215.

Samuelsen, O.B. Ervik, A. (2001). Absorption, tissue distribution and excretion of flumequine and oxolinic acid in corking wrass following a single intraperitoneal injection or bath treatment. J. Vet. Pharma. The., 24, 111.

Stamm, J. M (1989). In vitro resistance by fish pathogens to aquaculture antibacterials, including the quinolones difloxacin and sarafloxacin (A-56620) J. Aqua. Ani. Health, 1, 135.

Tsoumas, A., et al. (1989). Aeromonas salmonicida: development of resistance to 4-quinolone antibacterials. J. Fish Disease, 12, 493.

World Health Organization. (1995). Evaluation of certain veterinary drug residues in food. WHO Technical Series No. 851. Geneva

World Health Organization. (1998). Evaluation of certain veterinary drug residues in food. WHO Technical Series No. 879. Geneva

World Health Organization. (2001). Evaluation of certain veterinary drug residues in food. WHO Technical Series No 900. Geneva