# Single-dose pharmacokinetics of flumequine in the eel (*Anguilla anguilla*) after intravascular, oral and bath administration

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Knowledge of the pharmacokinetic properties of drugs to combat bacterial infections in the European eel (*Anguilla anguilla*) is limited. One antimicrobial agent likely to be effective is flumequine. The aim of this study was to investigate the pharmacokinetic properties of flumequine in European eels in fresh water. Flumequine was administered to eels (*Anguilla anguilla*) intravenously (i.v.) and orally (p.o.) at a dose of 10 mg/kg body weight, and as a bath treatment at a dose of 10 mg/L water for 2 h. The study was performed in fresh water with a temperature of $23 \pm 0.3$ °C, pH 7.15. Identical experimental designs were used. Two additional bath treatments were also performed, one in which the pH in the water was lowered by approximately 1 unit to 6.07 (dose: 10 mg/L) and one at a dose of 40 mg/L for 2 h in a full-scale treatment. Following i.v. administration, the volume of distribution at steady state was 3.4 L/kg. Total body clearance was 0.012 L/h per kg and the elimination half-life ($t_{1/2\lambda z}$) was calculated to be 314 h. Mean residence time was 283 h. Following oral administration, the $t_{1/2\lambda z}$ was 208 h. Maximal plasma concentration ($C_{max}$) was 9.3 mg/L, at 7 h after administration ($C_{max}$). The oral bioavailability ($F$) was calculated to be 85%. Following bath administration in 10 mg/L for 2 h, maximal plasma concentration was 2.1 mg/L, observed immediately after the end of the bath. The 'bioavailability' in eel following a 2-h bath treatment was 19.8%. Reducing the pH in the bath to 6.07 produced a maximal plasma concentration of 5.5 mg/L, observed immediately after the end of the bath. The 'bioavailability' was increased to 41% by the lowering of the pH. A similar effect was observed in a full-scale treatment (1 kg eels/L water). The $CO_2$ produced by the eel lowered the pH and increased 'bioavailability' to 35%.

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## INTRODUCTION

The production of eels by aquaculture in Europe in 1998 was approximately 9000 tons (Federation of European Aquaculture Producers, 1999), the main producer countries being Italy, Denmark and the Netherlands. The production systems in Denmark and the Netherlands use wild-caught elvers put into intensive farm systems with recirculation of the water. In Norway, several attempts to farm have been made using wild-caught yellow eel (approximately 150 g), but in intensive farm systems, this fish is very prone to parasitic and bacterial infections. Infections with *Vibrio vulnificus*, atypical *Aeromonas salmonicida* and *Edwardsiella tarda* have produced both serious outbreaks with high mortality and more chronic disease with moderately increased mortality.

No commercial vaccines are available against these diseases. To combat these infections, antimicrobials have been used, mainly as bath treatments. To be able to apply the correct agent and the optimal dosage regime for successful treatment, and to minimize environmental hazards, knowledge of the pharma-

 

cokinetic and pharmacodynamic properties of the drug(s) in the actual species is vital. Knowledge of the pharmacokinetic properties of these agents in eels, following different routes of administration, is sparse. One antimicrobial agent likely to be effective in eel is flumequine. Flumequine is a broad-spectrum synthetic antimicrobial agent belonging to the 4-quinolones and has the properties of a weak acid.

The aim of this study was to investigate the pharmacokinetic properties of flumequine in the eel.

## MATERIALS AND METHODS

### *Formulation of test substance*

There are no commercial formulations of flumequine available in Norway for intravenous (i.v.) or bath administration. For this study, flumequine was obtained from Sigma Chemical Co., St. Louis, MO, USA. The solution for i.v. administration was prepared by dissolving 1 g flumequine in 10 mL 1.0 M NaOH, with subsequent regulation of the pH to 10.3 with 6 M HCl. Further lowering of the pH resulted in precipitation of flumequine. The final volume was adjusted with 0.9% saline to a concentration of 10 g/L. Flumequine for oral administration was mixed into a 4:3 emulsion of crushed pelleted fishfeed:cod liver oil at a concentration of 10 g/L (1 mL = 1.006 g, SD = 0.012 g) after first being dissolved in 1.0 M NaOH. Flumequine for bath administration was prepared by dissolving 5 g flumequine in 100 mL 0.1 M NaOH. The appropriate amount of the stock solution was added to the fish tank. The concentrations in the fish tanks were confirmed with a high-performance liquid chromatography (HPLC) assay.

### *Test facilities and test fish*

The study was conducted at Farsund Aqua as, Farsund, Norway. The eels had been caught 3 months earlier outside the coast of southern Norway, and were held in fiberglass tanks of 10000 L supplied with recirculated (90%) and oxygenated water at a temperature of $23 \pm 0.3$ °C and pH of 7.1. The eels weighed $183 \pm 26$ g (mean $\pm$ SD).

One hundred and fifty fish were randomly allocated into five groups of 30, one group for each route/dose of administration.

### *Intravenous administration*

The fish to receive flumequine by the i.v. route were allocated into groups of six, which were administered flumequine i.v. at a dose of 10 mg/kg individually into the caudal vein. The i.v. injection was performed with the fish positioned on a damp cloth, after approximately 5 min. sedation with benzocaine (10 mg/L water). Each fish was weighed individually. The flumequine solution was injected slowly using a 1-mL disposable syringe and a $0.5 \times 25$-mm needle (Terumo, Leuven, Belgium). The position of the needle was confirmed by aspiration of blood before, during and after the injection. Fish in which the needle dislocated during the injection were discarded and replaced. Each group of six fish was blood-sampled at two different time points after administration.

### *Oral administration*

The fish for oral administration were also allocated into groups of 6, and each fish was given flumequine orally at a dose of 10 mg/kg through a stomach tube (Martinsen *et al.*, 1993). The fish were lightly sedated with benzocaine (10 mg/L water) and weighed before administration. After administration the fish were observed for regurgitation. Each group of 6 fish was blood-sampled at two different time points after administration.

### *Bath administrations*

The experimental bath administrations were carried out in 1000-L fiberglass tanks with 100 L of static aerated water. Flumequine from the stock solution was diluted with 1 M NaOH and added to the water to a final concentration of 10 mg/L. In one bath administration, pH in the water was lowered from 7.15 to 6.07 by adding HCl before starting the bath treatment. The fish were kept in the flumequine bath solution for 2 h and then transferred to flow-through water tanks. Each fish was blood-sampled at two different timepoints after the bath.

In the full-scale commercial bath treatment, flumequine was added to produce a final concentration of 40 mg/L, and 400 kg eel was treated in a tank containing 400 L water for 2 h. After the administration, 30 eels were randomly allocated to the study.

### *Sampling*

Blood samples from six fish were collected at 1, 3, 7, 12, 18, 24, 48, 96, 168, 288 and 480 h after administration. In the groups given bath treatment, the first blood sample was collected immediately after the treatment. The fish were sedated with benzocaine (10 mg/L water) before blood sampling by caudal venipuncture using a $0.5 \times 25$-mm needle and a 1-mL syringe. The blood was sampled caudal to the injection site in the i.v.-administered group. Each sample consisted of 100 μL blood. The samples were centrifuged and the plasma was kept frozen at $-80$ °C until analyzed.

No mortalities were recorded in the experimental fish during the study.

### *Analytic procedures*

The plasma samples were cleaned by solid-phase extraction on a column of the Bond Elute type, size 1 mL, with C2 sorbent material, according to a previously published method (Rasmussen *et al.*, 1989). The concentrations of flumequine in plasma were determined by means of HPLC using a fluorescence detector operated at an excitation wavelength of 325 nm and an emission wavelength of 360 nm. Oxolinic acid was added before cleanup/extraction and used as an internal standard.

The HPLC system used consisted of a Perkin Elmer LC 250 pump connected to a Waters wisp 710 B autoinjector and a Perkin Elmer LC 240 fluorescence detector. A 150 × 4.6-mm 5 µm PLRP-S analytic column with a 5.0 × 3.0-mm 36-µm PLRP-S pre-column was used. The integrator was the Analytic workstation, Omega-2, V2.60, Perkin Elmer, Norwalk, CT, USA. The system was operated at room temperature with mobile phase containing 0.001 M $H_3PO_4$:tetrahydrofuran:acetonitrile (13:3:4). The flow was 0.7 mL/min.

The lower limit of quantitation of the method was 10 µg/L, and it was linear over a tested range of 100–4000 µg/L. The linear correlation coefficient was 0.9999. The linearity of the calibration curve was also tested on a residual plot, revealing no bias. The recovery of flumequine was from 99% (4000 µg/L) to 105% (400 µg/L). Recovery of 105% is probably due to some evaporation of organic solvent during elution with vacuum.

Because of an interfering peak in the chromatogram, the following results had to be omitted: 480 h after administration in the pH 6.07 and commercial bath treatment group, four of the results from i.v.-administered group at 480 h, and one of the results from 288 h in the bath-administered group. Due to technical problems, one result from the commercial bath treatment group at 18 h and two at 96 h was lost.

### *Pharmacokinetic analysis*

Pharmacokinetic modeling was performed using the computer program WIN-NONLIN, version 1.1 (Statistical Consultants Inc., Lexington, KY, USA), in a least-square non-linear regression analysis. Standard pharmacokinetic parameters were calculated according to a non-compartment model. In the i.v. group, the intercept with the y-axis was calculated by back-extrapolation of the curve, using the first two data points. The terminal elimination-rate constant, $\lambda_z$, was estimated according to the algorithm of Dunne (1985). The concentration versus time curve was extrapolated to infinity using the $\lambda_z$.

The bioavailabilities were calculated comparing the areas under the concentration time curves $(AUC)_{\text{i.v.}}$ $(0-\infty)$ and $AUC_{\text{p.o.}}$ $(0-\infty)$ or $AUC_{\text{bath}}$ $(0-\infty)$. In the bath-administered groups, the concentration in the water was used when adjusting for different dose in the calculation of 'bioavailability'.

## RESULTS

After i.v. administration, the estimated elimination half-life ($t_{1/2\lambda z}$) was 314 h. The observed volume of distribution at steady state ($V_{ss}$) was 3.4 L/kg, and the plasma clearance ($Cl_t$) was 0.012 L/h per kg.

Oral administration gave a maximal plasma concentration ($C_{max}$) of 9.3 mg/L at 7 h after administration ($t_{max}$). $t_{1/2\lambda z}$ after oral administration was calculated to 208 h. Bioavailability ($F$) was 85%.

After bath administration, a $C_{max}$ of 2.1 mg/L was observed at 0 h after treatment ($t_{max}$) and 'bioavailability' was 19.8%. Lowering of pH from 7.15 to 6.07 gave a $C_{max}$ of 5.5 mg/L at 7 h after treatment and a 'bioavailability' of 41%. The large-scale commercial bath treatment with a dose of 40 mg/L water gave a $C_{max}$ of 24 mg/L at 3 h after treatment and a 'bioavailability' of 35%. The pharmacokinetic parameters are listed in Table 1, and the plasma concentration versus time of flumequine administered i.v., orally and by bath are shown in Fig. 1. The plasma concentrations versus time of flumequine after different bath treatments are shown in Fig. 2.

## DISCUSSION

In pharmacokinetic studies in humans and domestic animals, each individual is normally blood-sampled throughout the

**Table 1.** Pharmacokinetic parameters in eels held in fresh water at 23 °C. The parameters were calculated by a non-compartment model

| Pharmacokinetic parameter | Intravenous administration | Oral administration | Bath administration | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| Dose (mg/kg or mg/L) | 10 | 10 | 10 | 10 | 40 |
| Water pH: start | | | 7.15 | 6.07 | 7.15 |
| Water pH: end | | | 7.15 | 6.05 | 6.0 |
| $AUC_{0-\infty}$ (mg/h per L) | 831 | 707 | 165 | 338 | 1158 |
| $t_{1/2\lambda z}$ (h) | 314 | 208 | 126 | | |
| *MRT* (h) | 283 | 171 | 181 | | |
| $V_{ss}$ (L/kg) | 3.4 | | | | |
| *Cl* (L/h per kg) | 0.012 | | | | |
| $C_{max}$ (mg/L) | 11.2 | 9.3 | 2.1 | 5.5 | 24 |
| $t_{max}$ (h) | 0 | 7 | 0 | 7 | 2 |
| $F$ (%) | | 85 | 19.8* | 41* | 35* |

* Bioavailability of bath treatments calculated using dose = concentration of flumequine in the water.

$AUC_{0-\infty}$, area under plasma concentration time curve extrapolated to infinity; $t_{1/2\lambda z}$, elimination half life; *MRT*, mean residence time; $V_{ss}$, volume of distribution at steady state; *Cl*, total body clearance; $C_{max}$, maximum plasma concentration; $t_{max}$, time of peak plasma concentration; *F*, availability of administered dose in plasma.

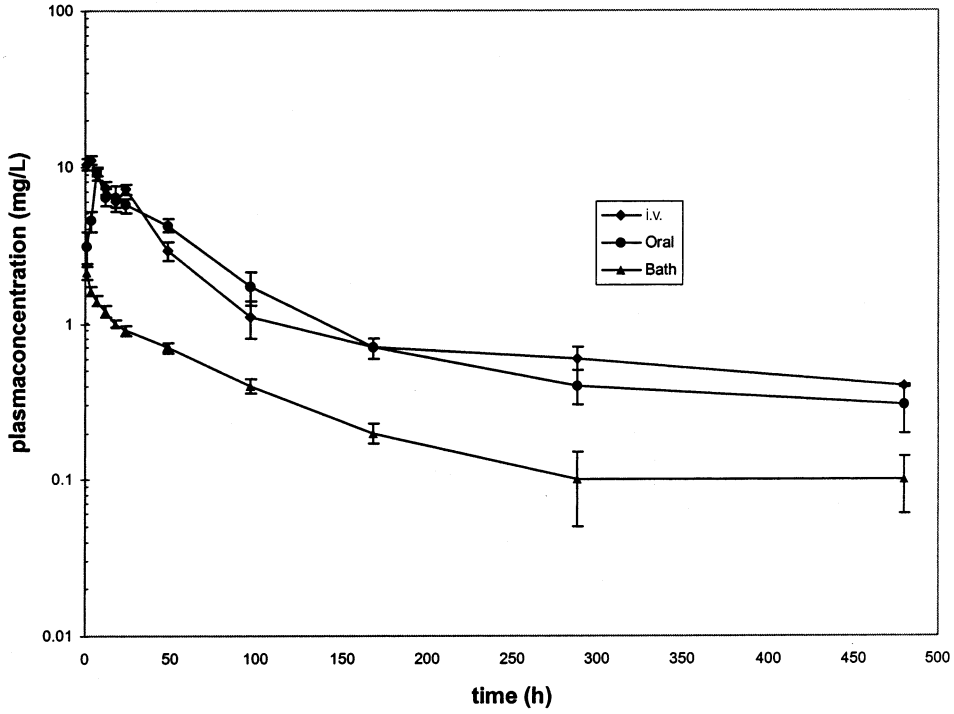


**Fig. 1.** Mean ( ± SEM) plasma concentration profiles of flumequine in eels after a single 10-mg/kg dose administered intravenously (i.v.), a 10-mg/kg dose by the oral route, and 10 mg/L for 2 h administered as a bath (pH 7.15).

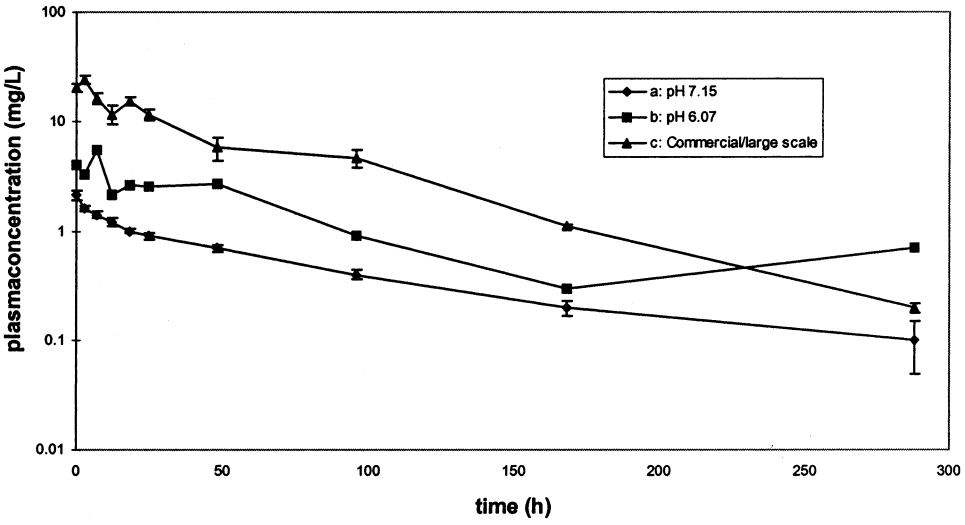


**Fig. 2.** Mean ( ± SEM) plasma concentration profiles of flumequine in eels after 2 h of bath treatment at different water pH levels and fish density: a) pH 7.15 in 10 mg/L, 0.06 kg eels/L water; b) pH 6.07 in 10 mg/L, 0.06 kg eels/L water; and c) pH 7.15 in a 40-mg/L commercial treatment, 1 kg eels/L water.

whole study period. This experimental protocol is very difficult to use in studies with fish. In the current study, only small experimental eels were available (average weight: 183 g), and the fish had to be anesthetized during administration and blood sampling. Frequent blood sampling of each fish was considered impossible. This is why we chose a design in which each fish was blood-sampled only twice. The dose of 10 mg/kg for i.v. administration was chosen to make our results as comparable as possible with previous studies in eel (Boon *et al.*, 1991: 9 mg/kg intramuscularly; and Van der Heijden *et al.*, 1994: 18 mg/kg i.v.), and other fish species (Hiney *et al.*, 1995: Atlantic salmon; Sohlberg *et al.*, 1994: rainbow trout; O'Grady & Smith, 1992: Atlantic salmon; O'Grady *et al.*, 1988: Atlantic salmon and brown trout).

The same dose (10 mg/kg) was chosen for the oral administration to make the comparison between the different ways of administration as good as possible.

The bath administrations were carried out with a dosage of 10 mg/L flumequine in the water. The dosage was chosen to make the comparison between the different methods of administration as valid as possible, but with the amount of flumequine related to the amount of water instead of body weight.

A non-compartment model was used in the pharmacokinetic calculations. Other ways of modeling the data were also tested. Based on the minimal Akaike's information criterion estimation (Yamaoka *et al.*, 1978), the data from the i.v.- and oral-administered groups could also be interpreted using a two-compartmental model. In this model, the $t_{1/2\beta}$ values were estimated to

be 534 h (i.v.) and 389 h (oral). The $V_{ss}$ was calculated to be 5.3 L/kg (i.v.). Total body clearance was calculated to be 0.010 L/h per kg (i.v.).

There are few pharmacokinetic studies on the administration of flumequine in fish in fresh water. Great inter-species variation in flumequine absorption and disposition in freshwater fish has been reported (Van der Heijden *et al.*, 1994). The pharmacokinetic properties of flumequine have been more intensively studied in some fish species in saltwater. It seems clear that in addition to inter-species variation, quinolones show reduced absorption and increased elimination rates in seawater as compared to the same species in fresh water (Ishida, 1992: oxolinic acid in rainbow trout; Elston *et al.*, 1994: difloxacin in Atlantic salmon). It is thus necessary to be cautious when comparing pharmacokinetic parameters from different studies on flumequine in different fish species. Sohlberg *et al.* (1994) administered 5 mg/kg flumequine intra-arterially in cannulated rainbow trout (*Oncorhynchus mykiss*) in fresh water. At 13 °C, they reported a *Cl* of 0.0018 L/h per kg and a $V_{ss}$ of 3.2 L/kg. Using a three-compartmental model, $t_{1/2\beta}$ was 10.3 h and $t_{1/2\gamma}$ was 137 h. The rainbow trout were cannulated, which tends to slow the elimination process (Sohlberg *et al.*, 1994) In our study we found $t_{1/2\lambda z}$ to be 314 h, which is considerably longer, even considering that the temperature and the metabolic rate of the rainbow trout in that study was lower than in ours.

The high $V_{ss}$ of 3.4 L/kg indicates that flumequine is widely distributed in eel, as it is in rainbow trout. $Cl_t$ was estimated to be 0.012 L/h per kg.

The reason for the slow elimination in eels can probably be explained partly by the lack of biotransformation. Van der Heijden *et al.* (1994) found only traces of a 7-OH flumequine metabolite and no glucuronide metabolites after administering flumequine orally to eels. Boon *et al.* (1991) found the protein binding of flumequine in eel during the elimination phase to be 41%, and it is therefore unlikely to contribute to the slow elimination. However, the very small gill surface of the eel (Byczkowska-Smyk, 1958) and other possible physiological inter-species differences in excretory organs, tissue composition and vascularization could contribute, as these are factors known to cause differences in drug elimination (Ingebrigtsen, 1991).

The $C_{max}$ after oral administration was observed after 7 h at 9.3 mg/L. This is higher than the $C_{max}$ of 4.1 mg/L observed between 2 and 16 h after administration of 18 mg/kg reported by Van der Heijden *et al.* (1994). The lower $C_{max}$ in this study might be caused by the different formulation of the flumequine solution. Van der Heijden *et al.* (1994) administered flumequine in an aqueous solution. In our study, we dissolved flumequine in cod liver oil and ground, pelleted fish feed (3:4). The bioavailability in our study was calculated to be 85%, which is higher than in other studies. A similar influence of formulation on oral bioavailability of sarafloxacin in Atlantic salmon has been reported (Martinsen *et al.*, 1993), in which a formulation in an edible oil showed the highest bioavailability.

The results of the analysis after bath administration showed that flumequine was relatively poorly absorbed from water, with a 'bioavailability' of 19.8%. No formulas for calculating bioavailability of bath treatments have been described. The 'bioavailability' of the bath treatments in our study was calculated by dividing *AUC* bath × dose i.v. by the *AUC* i.v. × flumequine concentration water. The 'bioavailabilities' reported from our bath treatments are therefore only valid for comparison within this context. A $C_{max}$ of 2.1 mg/L immediately after bath treatment (0 h) is also lower than reported by Hiney *et al.* (1995) for Atlantic salmon smolts. These authors reported a $C_{max}$ of 22.5–40.8 mg/L after 18 min in a bath containing 25 mg/kg flumequine, pH 6.5, at 7 °C. O'Grady & Smith (1992) reported in brown trout (*Salmo trutta*) a $C_{max}$ of 35 mg/L after a 75-min bath containing 50 mg/L flumequine, pH 7, at 11 °C. The apparently lower and slower uptake of flumequine in eels could be caused by physiological and anatomical differences, such as the very small gill surface of the eel and the fact that the eel has the ability to absorb up to 60% of its total oxygen uptake through its skin (Krogh, 1904) and thus reduce water flow through the gills. The gills are probably the most important site for drug absorption from water, and flumequine is not likely to be absorbed rapidly through intact skin.

O'Grady & Smith (1992) reported a progressive decline in the uptake of flumequine with increasing water pH. Flumequine is a weak acid, and the proportion of the undissociated form decreases with increasing pH. By lowering pH from 7.15 to 6.07, we achieved a two-fold increase in absorbed flumequine, increasing the 'bioavailability' of bath treatment to 41% with a $C_{max}$ of 5.5 mg/L. The $t_{max}$ of bath treatments is expected to be 0 h. The $t_{max}$ in our study of 7 h is probably due to inter-individual variation. O'Grady & Smith (1992) and O'Grady *et al.* (1988) reported serum levels of flumequine that were consistently higher in Atlantic salmon and brown trout treated in field trials and commercial fish farm treatments compared to what was obtained in laboratory experiments. We included in our study a group of eels that was given a standard 'commercial' treatment of flumequine of 40 mg/L for 2 h in stagnant oxygenated water. The fish density is often very different in a commercial treatment compared to a laboratory experiment due to practical considerations. In our commercial treatment, 400 kg eel was treated in 400 L water (1 kg bodyweight/L water), whereas the fish density in our laboratory studies was 0.06 kg bodyweight/L water. A $C_{max}$ of 24 mg/L was obtained in the commercial bath treatment, which is much higher than expected from the increase in dose alone. Measurements of water pH showed a lowering during the commercial treatment from 7.15 (start) to 6.0 (end). This is probably caused by acidification of the water due to $CO_2$ from respiration and the often low buffering capabilities of fresh water. This is probably the main reason for the increased absorption.

No studies of minimum inhibitory concentration (*MIC*) of flumequine for susceptible strains of pathogenic bacteria for eels have been conducted. *MIC*s of susceptible strains of bacteria pathogenic to Atlantic salmon (*Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri*) have been reported to range from 0.005 to 0.5 mg/L, for most strains $< 0.1$ mg/L (Barnes *et al.*, 1990; Martinsen *et al.*, 1992). Atypical *Aeromonas salmonicida*

and different vibrio strains are some of the bacteria producing disease problems in eel culture. The *MIC*s for these bacteria are likely to be within the same range as the ones producing disease in Atlantic salmon.

Blaser *et al.* (1987) reported bacterial regrowth of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus in vitro* unless the peak concentration:*MIC* ratio exceeded 8:1 for the fluoroquinolone enoxacin. Using this guideline in the evaluation of the plasma concentrations of flumequine in our study, eels exceed this peak concentration:*MIC* ratio for most susceptible strains after oral and bath treatment administration of 10 mg/kg flumequine. In the eel, an adequate plasma concentration is maintained for 48 h (bath) and 168 h (oral). By lowering pH in the bath treatment water or increasing the dose, it is possible to maintain adequate plasma concentration after a single bath treatment for an even longer period. The slow elimination of flumequine in the eel in fresh water does, however, raise problems. Withdrawal times have to be long, as pointed out earlier by Boon *et al.* (1991) and Van der Heijden *et al.* (1993). There is also an increased risk of developing resistant bacteria due to the prolonged period of sub *MIC* levels.

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