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

EMEA/MRL/251/97-FINAL
July 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLORFENICOL
(Extension to fish)

SUMMARY REPORT (2)

1. Florfenicol is a wide spectrum, synthetic antibacterial. It is structurally related to D(-)threo chloramphenicol, but differs from it in two fundamental aspects: firstly, presence of a p-methyl sulfonyl group instead of the p-nitro group, secondly, presence of a fluorine atom instead of the hydroxyl group in the terminal primary alcohol function of chloramphenicol. It is used in cattle by the intramuscular route of administration. MRLs were previously established in the European Union and published in Commission Regulation (EC) No. 2703/94 of 7 November 94 as follows:

Pharmacalogically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	Bovine	200 µg/kg 3000 µg/kg 300 µg/kg	Muscle Liver Kidney	

These MRLs were elaborated by reference to the microbiological ADI of 0.003 mg/kg bw, based on the MIC-value for the most sensitive microorganism of the human gut flora (0.36 µg/ml for *Fusobacterium* spp).

2. Florfenicol is proposed for the treatment and the control of sensitive infections in Atlantic Salmon at a level dose of 10 mg/kg bw/day for 10 days.

3. In two poorly described tolerance studies, it was shown that florfenicol administered via feed for 10 days was well tolerated at 1, 5 and 10 times the therapeutic dosage.

4. After a single oral administration of 10 mg florfenicol/kg bw to fish held at 10°C, the highest plasma concentration of florfenicol, 4 mg/l, occurred at 10.3 hours post dose. The oral bioavailability of florfenicol was estimated to be 96.5%.

   During the repeated administration of medicated feed at a level of 10 mg/kg bw/day for 10 days to fish held in sea water at 3-5°C, the plasma concentrations of florfenicol were much higher than those of florfenicol-amine (19.09 mg/l vs 3.37 mg/l). After the end of the treatment, the concentrations declined rapidly to attain 2.75 mg florfenicol/l and 0.60 mg florfenicol-amine/l, 24 hours post dose. Florfenicol and florfenicol-amine could be detected 4 days and 18 days after the end of the treatment respectively (limit of detection = 0.075 mg/l).

5. Two radiometric depletion studies using ^{14}C-florfenicol were conducted in Atlantic Salmon held at 10°C after a single administration of 10 mg/kg bw.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

In the first study carried out in 194 g fish, the highest level of radioactivity in tissues was measured at 12 hours post dose: 6130 µg equivalent florfenicol/kg for muscle and 5480 µg equivalent florfenicol/kg for skin. Seven days post dose, the radioactivity measured in the skin (400 µg equivalent florfenicol/kg) was much higher than that measured in muscle (50 µg equivalent florfenicol/kg).

In the second study carried out in 400-1000 g fish, the highest levels of radioactivity were measured at 12 hours post dose : 8600 µg equivalent florfenicol/kg for muscle and skin. Five days post dose, the levels measured in the skin were of the same magnitude as those in the muscle (300-500 µg equivalent florfenicol/kg).

The combination of florfenicol residues could be readily quantified for muscle/skin samples in natural proportion by combining the available data using a ratio of 10:90 corresponding to the percentage of skin and muscle in natural proportions. This ratio was determined from data obtained from the 106 fish used in the radiolabelled studies.

6. In muscle, at a 24-hour withdrawal period after the end of 10-day treatment at the therapeutic dosage (10 mg/kg bw per day), about 90% of the radioactivity could be extracted. However, depending on the temperature of the water, the ratios of the different metabolites to total radioactivity were slightly different. For fish maintained at 10°C, florfenicol represented 33%, florfenicol-amine 50 %, florfenicol-alcohol 5 % and florfenicol-oxamic acid 0.70% whereas for salmon maintained at 5°C, florfenicol represented 45%, florfenicol-amine 37%, florfenicol-alcohol 3% and florfenicol-oxamic acid 1%.

   In skin about 80-90% of the radioactivity could be extracted at 3 hours after cessation of the treatment. Whatever the temperature, florfenicol represented 65-70% of the radioactivity extracted, florfenicol-amine 11-14%, florfenicol-alcohol 2.50% and florfenicol-oxamic acid 0.4-1.2%

   Considering that the skin represents 10% of the muscle mass in fish, the following mean values to express the relative percentage of the different metabolites were calculated from results obtained at 7 days for the skin and 15 days for muscle and were of the magnitude of 4.0% for florfenicol, 50.70% for florfenicol-amine, 18.50% florfenicol-alcohol, 23.50% florfenicol-oxamic acid and 3.20% for monochloroflorfenicol.

7. In two radiometric depletion studies carried out in fish given 10 mg/kg bw/day/10 days via the feed and held either in sea water at 10°C or 5°C, no differences in the measurements of total radioactivity were observed. One day after the end of the treatment, the levels of radioactivity in skin and muscle were of the same magnitude (4500-5850 µg equivalent florfenicol/kg) and 3 days later close to 1150 µg equivalent florfenicol/kg for muscle and 1650-1770 µg equivalent florfenicol/kg for skin.

   In the radiometric studies, the analytical method used is based on the conversion of all the metabolites of florfenicol to one compound, florfenicol-amine. The ratio of conversion of metabolites into florfenicol-amine was estimated to be close to 100%. The limit of quantification was 300 µg/kg. The concentrations of florfenicol-amine were determined in skin and muscle.

   When fish were held at 10°C, one day after the end of the treatment the skin and muscle concentrations of florfenicol-amine were 9770 µg/kg and 6710 µg/kg respectively. They then declined to reach to 930 µg/kg in skin and 1310 µg/kg in muscle, 7 days post-dose. Large individual variations were observed. Only groups of 6 animals per time-point were used. At 15 days after the end of treatment, florfenicol-amine could only be measured in the muscle of some animals whereas significant levels of florfenicol-amine (670 µg/kg) were still measured in skin.

When fish were held at 5°C, one day after the end of the treatment the concentrations of florfenicol-amine measured were high: 17200 µg/kg in skin and 14800 µg/kg in muscle. Seven days after the end of the treatment, the concentrations of florfenicol-amine had declined to 1370 µg/kg in skin and to 440 µg/kg in the muscle. Large individual variations were observed. Only groups of 6 animals per time-point were used. At 15 days after the end of the treatment, florfenicol-amine could only be measured in the muscle of some animals whereas significant levels of florfenicol-amine (1190 µg/kg) were still measured in skin.

8. In two other non radiometric depletion studies conducted at the therapeutic dosage (10 mg/kg bw/day/10 days), the concentrations of florfenicol and florfenicol-amine were determined in fish tissues by an HPLC method which separated these two compounds.

In the first study, in fish held at 10°C, 24 hours after the end of the treatment florfenicol concentrations were 1800 µg/kg in muscle and 690 µg/kg in skin, whereas florfenicol-amine concentrations were 7270 µg/kg in muscle and 6350 µg/kg in skin. Florfenicol-amine could be detected in skin until 49 days post medication (22 µg/kg).

In the second study, in fish kept at 5°C, 24 hours after the end of the treatment 4300 µg of florfenicol/kg and 2810 µg florfenicol-amine/kg were measured in muscle, whereas 970 µg of florfenicol/kg and 1600 µg of florfenicol-amine/kg were quantified in skin. The first time points when florfenicol and florfenicol-amine could not be detected, were 11 and 21 days for muscle and 11 and 56 days for skin respectively.

9. As the performances of the analytical methods used for assaying the residues of florfenicol in the radiometric and the non radiometric studies were different, no comparison between the amounts of residue measured in muscle and skin in the four depletion studies can be made. Therefore, only the results of the two radiometric studies should be taken into account to describe the depletion of florfenicol residues in fish tissues. However, as the distribution of the concentrations according to the time is not normal, as the variance between groups is not homogeneous and in the absence of log-linearity, it will not be possible to apply the current guideline on the statistical determination of a withdrawal period.

10. A validated HPLC analytical method was proposed to monitor residues of florfenicol in fish skin and fish muscle. The limit of quantification is 300 µg/kg. However, no analytical method as been validated for muscle and skin in natural proportions.

Conclusions and recommendation

Having considered:

- the microbiological ADI of 180 µg per person per day,
- the percentage of the microbiologically active residue at 15 days after the end of treatment (4% of florfenicol),
- the results of the radiometric studies conducted at 5°C and 10°C on groups of 6 animals (instead of 10 as recommended) at 15 days after the last administration,
- florfenicol-amine as the residue maker due to the analytical method leading to a total conversion of florfenicol and its metabolites to florfenicol amine,
- the molecular weights of the microbiological active residue florfenicol (358.21) and of the marker residue florfenicol-amine (247.28),
- that a validated method, based for residues monitoring residues monitoring purposes is available for muscle or skin of fish, but not for muscle and skin in natural proportions,

the Committee recommends the inclusion of florfenicol in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacalogically active substance(s)	Marker residue	Target Species	MRLs	Target tissue	Other provisions
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	Fish	1000 µg/kg	Muscle and skin in natural proportions	Provisional MRLs expire on 1.7.2001

Based on this MRL value, the daily intake will represent about 10% of the microbiological ADI. This margin balances the fact that the depletion studies were not conducted according to the requirements of Volume VI of the Rules Governing medicinal Products in the European Community.

LIST OF QUESTIONS

1. The applicant should provide a validated analytical method according to the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community for the monitoring of residues in fish muscle and skin in natural proportions.

   The analytical method for fish muscle and skin in natural proportions should be presented according to an internationally recognised format (e.g. ISO 78/2).