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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLORFENICOL
(Extension to chicken)

SUMMARY REPORT (3)

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 bovine by intramuscular route of administration and in fish by administration via drinking water.

   A microbiological ADI of 3 µg/kg bw, i.e. 180 µg per person and a toxicological ADI of 10 µg/kg bw, i.e. 600 µg per person had previously been established by the Committee for Veterinary Medicinal Products.

   Currently, florfenicol is included in Annex I and 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
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol-amine	Bovine	200 µg/kg 3000 µg/kg 300 µg/kg	Muscle Liver Kidney	

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	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

   An application has now been submitted for an extension of the MRLs to poultry, however data were provided on broiler chicken only. The recommended dose is 30 mg/kg bw/day for 3 days via drinking water.

2. After a single intravenous administration of 54.4 mg of florfenicol/kg bw to broiler chicken, the concentrations of florfenicol in serum declined from 58 500 µg/l at 5 minutes after the injection to 69 µg/l at 8 hours post dose. Ten hours after the intravenous administration, no florfenicol could be detected in serum. The half-time of elimination is 37 minutes. The volume of distribution at steady state was 1387 ml/kg indicating wide-spread distribution of florfenicol into tissues.

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After the first oral gavage administration of florfenicol of 42 mg/kg bw/day for 3 days to broiler chicken, the highest concentration of florfenicol in serum (12 200 µg/l) was measured after 30 minutes and then declined to reach 810 µg/l, 8 hours later. Florfenicol could not be detected in the serum, 60 hours after the last administration.

During a continuous treatment of broiler chicken with florfenicol in drinking water at a daily dose of 26 mg/kg bw for 18 hours for 3 days, the estimated mean serum concentration of florfenicol was 711 µg/l. No florfenicol was detected in the serum, 72 hours after the terminal dose.

After repeated oral administration of ^{14}C-florfenicol, given twice daily 12 hours apart at 20 mg/kg bw/dose for three days, 93.7% and 98.2% of total radioactivity administered were excreted within 1 day and 7 days after the last dose. In excreta, at 7 days, the parent compound represented the major fraction of the radioactivity (42%), florfenicol amine (25%), florfenicol oxamic acid (5%) and florfenicol alcohol (10%) and the remaining part of radioactivity being represented by a small percentage of three unknown compounds. No monochloroflorfenicol was detected in excreta.

3. In a radiometric study, chicken received ^{14}C-florfenicol by oral gavage twice daily 12 hours apart at 20 mg/kg bw/dose for three days (i.e. 40 mg/kg bw/day for three days).

   Twenty-four hours after the end of oral administration, the levels of radioactivity in edible tissues were 146 µg equivalents florfenicol/kg in muscle, 475 µg/kg in skin + fat, 11 148 µg/kg in liver and 3125 µg/kg in kidney. Then, they declined to reach approximately 50 µg/kg in muscle and in skin + fat 5 days after the end of treatment, whereas in liver and kidney significant amounts of residues were still found: 2403 and 844 µg equivalents florfenicol/kg respectively.

   After acid digestion followed by extraction with ethylacetate (pH higher than 10), the major fraction of the radioactivity could be extracted, the percentages of extraction being in the magnitude of 70% for muscle, 60 to 76.3% for skin + fat, 65 to 80% for liver and about 80% for kidney.

4. Florfenicol amine was the major metabolite measured in edible tissues of chicken, florfenicol, the only microbiologically active compounds, being only detected in skin + fat in low concentrations (4%) and in kidney (1%). The other metabolites already identified for the other species. florfenicol oxamic acid, florfenicol alcohol and monochloroflorfenicol and one unknown compound (unknown metabolite 2) in significant amounts, representing 3.45%, 11.30% and 13.05% of total radioactivity at day 1 in skin + fat, liver and muscle, respectively. This unknown metabolite detected in chicken is also present in the rat as unknown metabolite U5. After acid hydrolysis the metabolite is also converted to florfenicol amine. These results indicate that the current determinative assay for chicken liver would quantitatively include the unknown metabolite which seems to be structurally close to the other metabolites of florfenicol. Another metabolite (unknown 1) was found only in liver at very low levels (less than or equal to 1.1%).

5. From the radiometric study, where chickens received, by gavage, 40 mg/kg bw of ^{14}C-florfenicol per day for 3 days, the ratios of the marker residue/total residue were determined by comparing the values of florfenicol-amine after assaying the matrixes with the analytical method proposed for monitoring to the total radioactivity levels and were at 1 day after the end of the treatment: 50%, 41%, 52% and 50% in muscle, skin + fat, liver, and kidney, respectively.

6. In a non-radiometric study, broiler chickens received florfenicol according to the recommended regimen via drinking water at concentrations equivalent to 17 to 30 mg/kg bw/day for 3 days. At 0.5 day after the end of the treatment the concentrations of florfenicol amine were lower than the limit of quantification for muscle (less than 50 µg/kg) and for skin + fat (less than 109 µg/kg) whereas in liver and kidney, significant amounts of florfenicol-amine were measured 2862 and 1161 µg/kg respectively. Florfenicol amine concentrations declined to reach 2038 and 679 µg/kg in liver and kidney, 1 day after the end of the treatment and to 1215 and 484 µg/kg in liver and kidney, 3 days after the end of the treatment. Seven days after the end of the treatment, the concentrations of florfenicol amine were below the limit of quantification for liver (less than 461 µg/kg) and could be still measured in kidney (136 µg/kg).

7. An HPLC analytical method proposed to monitor residues of florfenicol in edible tissues of chickens was fully validated according to the requirements of Volume VI of The Rules Governing Medicinal Products in the European Community. The limits of quantification are 100, 200, 150 and 1500 µg/kg for muscle, skin + fat, kidney and liver respectively, the limits of detection being 5, 39, 250 and 5 µg/kg for muscle, skin + fat, liver and kidney, respectively. A confirmative method, based on HPLC-MS/MS has also been developed.

8. For the establishment of MRLs for cattle and fish, the MRLs were based on the microbiological ADI (180 µg per person), the only microbiologically active compound being florfenicol.

   In the metabolism study carried out in chicken after single oral administration, the microbiologically active compound could not be detected in muscle and liver, and represented 4% and 1% in skin + fat and kidney, respectively. Therefore, 12 hours after the end of the administration via drinking water, the microbiological active compounds will represent only 0.66% of the microbiological ADI. Thus, it was also considered relevant to compare the amount of residues likely to be ingested to the toxicological ADI.

Conclusions and recommendation

Having considered that:

- the toxicological ADI is 10 µg/kg bw, i.e. 600 µg per person and the microbiological ADI is 3 µg/kg bw, i.e. 180 µg per person,
- the tissue distribution is based on results obtained at 1 day after the end of the treatment at the therapeutic regimen,
- the ratio of the marker residue florfenicol-amine towards total residues is known for all edible tissues: 50% for muscle, 41% for skin + fat, 52% for liver and 50% for kidney,
- as the analytical method is based on the conversion of florfenicol and its metabolites to florfenicol-amine, it was therefore appropriate to establish MRLs on the basis of florfenicol-amine,
- a validated analytical method, based on HPLC with UV detection, for the routine determination of florfenicol-amine in edible tissues of chicken was provided,
- since residue levels in skin + fat and in muscle were very low, an MRL was established at the limit of quantification of the analytical method for these tissues;

the Committee for Veterinary Medicinal Products recommends the inclusion of florfenicol for chicken 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 tissues	Other provisions
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol-amine	Chicken	100 µg/kg 200 µg/kg 2500 µg/kg 750 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

Based on these MRLs values, the daily intake will represent 99.95 % of the toxicological ADI but less than 1% of the microbiological ADI.