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 pigs)

SUMMARY REPORT (4)

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 tables:

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 proportion	The provisional MRLs expire on the 1.1.2001

   In addition the Committee recently recommended the inclusion of florfenicol in Annex I of Council Regulation (EEC) No 2377/90 as follows:

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

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An application has now been submitted for an extension of the MRLs to pigs. Florfenicol will be administered in drinking water for 5 consecutive days at a target dose of 20 mg/kg bw or by intramuscular route at 15 mg/kg bw with a second dose 48 hours later.

2. After a single intravenous administration of 15 mg of florfenicol/kg bw, the concentrations of florfenicol in serum declined from 18000 µg/l, 10 minutes after the injection to 1110 to 2040 µg/l at 8 hours. Twenty-four hours post dose, the concentrations of florfenicol were below the limit of quantification (80 µg/l) in 9 out of 11 treated animals. The half-life of elimination is 2.2 hours. The volume of distribution at steady state was in the magnitude of 950 ml/kg indicating wide-spread distribution of florfenicol into tissues.

   After repeated oral administrations of 15 mg/kg bw/day for 5 days, the concentrations of florfenicol in plasma depleted from 4270 µg/l, 1 hour after the last gavage administration, to 2240 µg/l 6 hours later, and to 210 µg/l, 24 hours after the last administration.

   During a continuous treatment by florfenicol via drinking water at daily dose of approximately 16 mg/kg bw for 5 days, the serum concentrations of florfenicol ranged from 1500 to 2500 µg/l.

   In a pharmacokinetic study where pigs received two intramuscular administrations of florfenicol at the dose of 15 mg/kg bw, 48 hours apart, the concentrations of florfenicol in serum declined from 9750 µg/l, 1 hour after the first injection, to 2040 µg/l, 8 hours later, and to 910 µg/l, 24 hours post dosing. After the second intramuscular administration, the concentrations were in the same range. Thirty-six and 48 hours after the second administration, florfenicol could be still measured in serum: 290 and 90 µg/l, respectively.

3. After repeated oral doses (gavage) of ^{14}C-florfenicol, given once a day at 20 mg/kg bw for five days, 80 to 91.45% of the administered dose were recovered in excreta within 3 to 19 days, depending on the experimental animals, the major fraction being excreted via urine (76%) and a minor portion (24%) in faeces.

   Within 12 days after two intramuscular administrations of 20 mg/kg bw of ^{14}C-florfenicol given at a 48-hour interval, 67.81% of the administered dose was excreted: 81% of the recovered radioactivity in urine, 19% in faeces.

   Whatever the route of administration for florfenicol, the parent compound represented the major fraction of the radioactivity (45 to 60%) in urine, followed by florfenicol amine (11.2 to 17%), florfenicol oxamic acid (less than 10%), florfenicol alcohol (1.10%), monochloroflorfenicol (1.90%) and the remaining part of radioactivity being represented by a small percentage of three unknown compounds. In faeces, the same metabolites were found.

4. In a first radiometric study, groups of 3 animals received ^{14}C-florfenicol in gelatine capsules at doses of 20 mg/kg bw/day for 5 days. They were sacrificed 3, 6, 9, 12, 15 and 19 days after the last administration. Three days after the end of the treatment significant levels of radioactivity were measured in edible tissues: 396 µg equivalents of florfenicol amine/kg in muscle, 325 µg equivalents/kg in skin + fat, 10 594 µg equivalents/kg in liver and 3401 µg equivalents/kg in kidney. They then declined to 256 and 165 µg equivalents/kg in muscle and skin + fat, and to 2948 and 1148 µg equivalents/kg in liver and kidney at 12 days. Fifteen days post dose, 193, 122, 2124, and 658 µg equivalents of florfenicol amine/kg were still measured in muscle, skin + fat, liver and kidney, respectively.

   In this study, the ratio florfenicol amine to total residues was only determined for muscle and liver: 44%, 45%, 59% and 60% at 3, 6, 9 and 12 days for muscle, and 63%, 59%, 62%, 58% at 3, 6, 9 and 12 days for liver.

   Florfenicol, the only microbiologically active compound, represents only a small fraction of the total radioactivity: 4.3, 7.0, 0.2 and 1.1% of the total radioactivity in muscle, skin + fat, liver and kidney 3 days after the end of the treatment and 1.2, 8.8, 0.1, and 0.5% after 12 days.

5. In a second radiometric study, groups of 3 pigs received by intramuscular route 2 doses of 20 mg/kg bw of ^{14}C-florfenicol given 48 hours apart. Animals were slaughtered 3, 6, 9 and 12 days after the end of the treatment. Three days after the end of the treatment, significant levels of radioactivity were measured: 366 µg equivalents of florfenicol amine/kg in muscle, 269 µg equivalents/kg in skin + fat, 9612 µg equivalents/kg in liver and 3788 µg equivalents/kg in kidney. They then declined to 138 µg equivalents/kg in muscle and skin + fat, 4085 µg equivalents/kg in liver and 1235 µg equivalents/kg in kidney, 9 days post dosing. Twelve days after the end of the treatment, 97, 131, 2222, 738 µg equivalents of florfenicol amine/kg were still measured in muscle, skin + fat, liver and kidney, respectively.

   Significant high levels of radioactivity were measured at the injection sites (3 samples per injection site) ranging from 449 to 24 627 µg equivalents of florfenicol amine/kg 3 days post dosing, from 117 to 1249 µg equivalents/kg 9 days post dosing, and from 83 to 1097 µg equivalents/kg at 12 days post dosing.

   The ratios florfenicol amine towards total residues were determined for all edible tissues: more than 100% for muscle (data only available at 3 days, as the concentrations were too low to allow the determination of ratios at later sampling), 99%, 70% and 53% at 3, 6, 9 days for skin + fat, 94%, 91%, and 72% at 3, 6 and 9 days for liver, and 68%, 59% and 63% at 3, 6 and 9 days for kidney. At 12 days, the ratios were 43% for skin + fat, 75% for liver and 55% for kidney.

   Florfenicol, the only microbiologically active compound, represents only a small fraction of the total radioactivity: 10.3, 23.3, 0.54% of the total radioactivity in muscle, skin + fat, and liver 6 days after the end of the treatment, and 2.99 and 18.1% in muscle and skin + fat 9 days post dosing. At the injection sites, florfenicol was the major compound identified. In methanol extracts, the percentages of the parent compound to total radioactivity were within the following ranges: 67 to 95% at day 3, 40 to 100% at day 6, 0 to 31% at day 9, and 56% at day 12.

6. Two non-radiometric studies were conducted in pigs.

   In the first, groups of 6 pigs received florfenicol via drinking water at daily doses of 12.99 mg/kg bw for 5 days and were sacrificed 1, 3, 6, 9, 12, 15, or 21 days after the end of the treatment. Six animals (3 animals per sex) were used per time point. One day after the end of the treatment the concentrations of florfenicol amine in edible tissues were 527 µg/kg in muscle, 884 µg/kg in skin + fat, 9860 µg/kg in liver and 3270 µg/kg in kidney. After 9 days they declined to 218, 2410 and 385 µg florfenicol amine/kg in skin + fat, liver and kidney, respectively. In muscle at this time point, and at later sampling times, the concentrations of florfenicol were too low to be quantified (less than 150 µg/kg, the limit of quantification). Twelve days post dosing, 237 µg florfenicol amine/kg were measured in skin + fat, 2410 µg florfenicol amine/kg in liver and 385 µg florfenicol amine/kg in kidney. Twenty-one days after the administration significant amounts of florfenicol amine could be still measured in fat (122 µg/kg) and in kidney (123 µg/kg). In liver the concentrations were below the limit of quantification (less than 1000 µg/kg)

   In the second study, pigs received 2 intramuscular administrations of 15 mg/kg bw of florfenicol, 48 hours apart. Groups of 4 animals were sacrificed at 3, 6, 9, 12 and 15 days after the second injection. At day 1 after the end of the treatment, 5040 and 1210 µg/kg of florfenicol amine were measured in liver and kidney. Then the levels declined to reach 1810 and 474 µg of florfenicol amine/kg, in liver and kidney at 9 days post treatment. In muscle, the concentrations were below the limit of quantification (150 µg/kg) in all samples, whereas at the injection site residues declined rapidly to reach the limit of quantification 9 days after dosing. In skin + fat, the residues of florfenicol did not significantly decrease during the experimental study and ranged from 192 to 369 µg of florfenicol amine/kg. Twelve days post dosing, 369 µg florfenicol amine/kg were measured in fat, 1270 µg/kg in liver and 332 µg/kg in kidney. Fifteen days after the last injection, florfenicol amine could still be measured in kidney (145 µg/kg) and in fat (233 µg/kg), while for liver the concentrations were below the limit of quantification of the HPLC method (1000 µg/kg).

7. The HPLC analytical method based on UV detection proposed to monitor residues of florfenicol in edible tissues of pigs 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 50 µg/kg for kidney and skin + fat, 150 µg/kg for muscle and 1000 µg/kg for liver, the limits of detection being 7, 15, 64 and 16 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 pigs after oral and intramuscular administration, the only microbiologically active compound, florfenicol, represented only a small fraction of the radioactivity extractable by methanol after 12 days: 1.2% of the extractable radioactivity in muscle, 11% in skin + fat, 0.1% in liver, 0.5% in kidney and 56% in the injection site. For this reason florfenicol can not be considered as the marker residue in the edible tissues, especially for liver and kidney. At the sampling time point retained to establish the MRLs for pigs, the microbiologically active compounds will represent a low fraction of the microbiological ADI (4.89% without considering the nature of the residues at the injection site, or 50.56% when the residues at the injection site are considered). 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 of residues at 12 days is suitable for the establishment of MRLs,
- the analytical method is based on conversion of florfenicol and its metabolites to florfenicol amine; it was therefore appropriate to consider florfenicol-amine as marker residue,
- at 12 days after the end of the treatment, the percentage of florfenicol-amine towards total residues is known for all edible tissues: 60% for muscle, 43% for skin + fat, 58% for liver and 55% for kidney,
- a validated analytical method, based on HPLC with UV detection, for the routine determination of florfenicol-amine in edible tissues of pigs was provided;

the Committee for Veterinary Medicinal Products recommends the inclusion of florfenicol for pigs 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	Porcine	300 µg/kg 500 µg/kg 2000 µg/kg 500 µg/kg	Muscle Skin + fat Liver Kidney	

Based on these MRLs values, the daily intake will represent 99.74% of the toxicological ADI but less than 5% of the microbiological ADI.