



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SPIRAMYCIN (Extension to pigs)

SUMMARY REPORT (3)

1. Spiramycin is a macrolide antibiotic used for the treatment and control of a number of bacterial and mycoplasmal infections in cattle and chickens.

A microbiological ADI of 50 µg/kg bw (i.e. 3000 µg/person) had been previously established by the Committee for Veterinary Medicinal Products.

Currently, spiramycin is included 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
Spiramycin	Sum of spiramycin and neospiramycin	Bovine	200 µg/kg 300 µg/kg 300 µg/kg 300 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	
		Chicken	200 µg/kg 300 µg/kg 400 µg/kg	Muscle Skin + fat Liver	

However, for pigs it had not been possible to include spiramycin in Annex I at the date of expiry of the provisional MRLs, as only a microbiological method was available and as there was no information on the ratio of spiramycin and neospiramycin to total microbiologically active residues.

An application has now been submitted for an extension of the MRLs to pigs. Spiramycin will be administered via the feed at dose levels ranging from 15 to 25 mg/kg bw per day for 7 days.

2. In the previous application, 5 depletion studies carried out in groups of 3 or 4 pigs per slaughtering time point, had been provided.

In 3 depletion studies, spiramycin was administered alone or in combination with other antimicrobials (sulfadimidine or oxytetracycline) in feed at a dose level of 16 mg/kg bw/day for 7 days. The concentrations of spiramycin and its active metabolites in tissues were determined by the agar diffusion method with *Micrococcus luteus* as test organism. At 0.5 day withdrawal, the following concentrations of microbiologically active compounds expressed as spiramycin equivalents were measured: 120 µg equivalents/kg in muscle, less than 100 µg equivalents/kg in fat, 6300 µg equivalents/kg in liver and 8900 µg equivalents/kg in kidney. They then declined to 1400 µg equivalents/kg in liver, 1300 µg equivalents/kg in kidney, and less than 100 µg equivalents/kg in muscle and fat by 3 days after the end of the treatment.

At 7 days post treatment, the concentrations of microbiologically active compounds ranged from 500 to 650 µg equivalents/kg in liver and from 150 to 250 µg equivalents/kg in kidney. For longer withdrawal periods, the levels were below the limits of quantification of 300 µg equivalents/kg and 150 µg equivalents/kg for liver and kidney, respectively.

In a 4th depletion study, 7 days after administration of spiramycin via the feed at a level of 25 mg/kg bw/day for 7 days, the concentrations of microbiologically active compounds declined from 1430 and 560 µg equivalents/kg in liver and kidney, respectively, and to 870 and 190 µg equivalents/kg in liver and kidney, 10 days post treatment. No microbiologically active compounds could be detected in muscle and fat (concentrations below 100 µg equivalents/kg).

In another study, 2 hours after the end of spiramycin treatment via the feed at a dose level of 20 mg/kg bw/day for 7 days, spiramycin (approximately 400 µg/kg) and neospiramycin (approximately 150 µg/kg) could be measured in liver; 60 µg/kg of spiramycin were quantified in muscle. At later sampling time, 3 and 10 days post dosing, neither spiramycin nor neospiramycin could be detected in liver or in muscle. The concentrations were quantified using a HPLC method equipped with an Automatic Advanced Sample Process. In liver, the limits of quantification were 200 and 100 µg/kg for spiramycin and neospiramycin, respectively and 25 µg/kg in muscle, for both compounds. No information was available for the other edible tissues.

3. In a metabolism study submitted previously, groups of 4 pigs received spiramycin in feed at a dose level of 22 mg/kg bw/day for 7 days. Liver samples were collected and spiramycin and its metabolites were assayed by an HPLC method which could measure spiramycin- and neospiramycin-cysteine conjugates or non conjugated compounds. The microbiologically active residues were determined by a microbiological assay, using *Micrococcus luteus*.

Two hours after the end of the treatment, 5225 µg/kg of microbiologically active residues were measured in liver. The concentrations of spiramycin 1 and spiramycin 3 expressed as the sum of conjugates and non conjugates were 2800 µg/kg and 1950 µg/kg, respectively. Neospiramycin was found as traces. Neospiramycin 3 could not be detected whereas its conjugates form was eluted with spiramycin 1. The sum of metabolites assayed by HPLC and spiramycin 1 represented about 96.17% and 54% of the microbiologically active residues, respectively.

After 3 days the levels determined by the microbiological assay (1275 µg equivalents/kg) were higher than the concentrations of spiramycin 1 measured by HPLC (approximately 400 µg/kg).

In a further study, after repeated administration of spiramycin at the recommended dose of 20 mg/kg bw/day of spiramycin base in feed (as embonate salt) for 7 days, liver concentrations of spiramycin and its 3 metabolites were quantified immediately after the end of the treatment (4 pigs). The mean concentrations of spiramycin 1, spiramycin 3, neospiramycin 1 and neospiramycin 3 were 6527.5, 4753, 1130 and 968 µg/kg, respectively. Spiramycin 1 was the major compound and represented 48.79% of the total residues measured by HPLC.

From these 2 studies, it was concluded that spiramycin 1 represents about 50% of the microbiologically active compounds.

4. In a further study, 4 piglets were treated by spiramycin at the recommended daily dose of 20 mg/kg bw of spiramycin base in feed (as embonate salt) for 7 days. The concentrations of spiramycin in muscle and kidney were assayed by a microbiological method and a validated HPLC method. The microbiological analytical method was based on agar diffusion using *Micrococcus luteus* as test organism, the limit of quantification being 100 µg/kg. The HPLC method allowed the quantification of spiramycin 1 and its 3 metabolites (spiramycin 3, neospiramycin 1, neospiramycin 3). The limits of detection and quantification were 150 and 250 µg/kg, respectively.

Immediately after the end of the treatment, the concentrations of microbiologically active residues measured were 450 µg and 19 925 µg equivalents/kg in muscle and kidney, respectively.

Using the HPLC method, only spiramycin 1 could be measured in muscle (about 272.5 µg/kg), whereas spiramycin 1, spiramycin 3, neospiramycin 1 and neospiramycin 3 could be quantified at levels of 6473, 5323, 680 and 1368 µg/kg, respectively, in kidney.

5. In a further study, 4 piglets received daily doses of 200 mg/kg bw of spiramycin base in feed (as embonate salt) for 7 days. Immediately after the end of the treatment, 28 950 and 3 433 500 µg equivalents/kg were measured by a microbiological method in muscle and in kidney, respectively.

Using the HPLC method, spiramycin 1 (18 925 µg/kg), spiramycin 3 (3523 µg/kg), neospiramycin 1 (2433 µg/kg) and neospiramycin 3 (1723 µg/kg) could be measured in muscle.

In kidney, significant amounts of these four compounds could be quantified: 1 009 115 µg/kg of spiramycin 1, 220 703 µg/kg of spiramycin 3, 267 623 µg/kg of neospiramycin 1 and 80 963 µg/kg of neospiramycin 3.

From this set of data, spiramycin 1 was considered as the marker residue and the percentage of the total antimicrobial activity represented by spiramycin 1 in pig muscle and kidney was estimated at 65% and 30%, respectively.

6. At the 47th meeting held in June 1996, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established definitive MRLs for pig tissues. This assessment was based on data obtained from microbiological methods for analysing residues in pigs. The microbiological method was considered as the reference and the routine method for residue quantification in pig tissues. JECFA considered it appropriate to harmonize MRLs for spiramycin and neospiramycin residues of different food producing animals and made the following recommendation: cattle, pigs, chicken: muscle, 200 µg/kg; fat, 300 µg/kg; liver, 400 µg/kg; kidney, 300 µg/kg (and 800 µg/kg for chicken).

7. Having considered:

- the improvement of the analytical method in pig edible tissues,
- the further information submitted on the nature of the metabolites in porcine edible tissues, particularly for muscle and kidney, which had not been available to JECFA at the time of their assessment of spiramycin,
- that the additional information shows that neospiramycin represents only a small fraction of the microbiologically active residues in porcine tissues;

the Committee for Veterinary Medicinal Products can not follow the JECFA approach with regard to pigs and retains spiramycin 1 as the marker residue, which is different from that retained by JECFA (spiramycin plus neospiramycin).

8. A validated routine analytical method for spiramycin 1 based on an HPLC method (UV detection) using an internal standard (triacetylated spiramycin) is available. Residues of spiramycin 1 were converted into cysteine derivative by adding cysteine before the extraction by methanol. The limits of quantification, established according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community, are 250 µg/kg for muscle and kidney and 1000 µg/kg for liver, the limits of detection ranging from 70 to 173 µg/kg according to the matrix.

Conclusions and recommendation

Having considered that:

- the microbiological ADI is 50 µg/kg bw (i.e 3000 µg/person),
- the tissue distribution is based on results obtained 3 days after the end of the treatment at the therapeutic regimen,
- spiramycin 1 is the appropriate marker residue in porcine tissues,
- the percentage of the marker residue spiramycin 1 towards total microbiologically active residues is known for all edible tissues except fat and equals 65% for muscle, 50% for liver and 30% for kidney,
- a validated analytical method, based on HPLC with UV detection, for the routine determination of spiramycin 1 in edible tissues of pigs was provided,
- since no microbiologically active residues could be detected in skin + fat, no MRL was allocated to this target tissue,
- since residue levels in muscle were very low, an MRL was established at the limit of quantification of the analytical method;

the Committee for Veterinary Medicinal Products recommends the inclusion of spiramycin 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
Spiramycin	Spiramycin 1	Porcine	250 µg/kg 2000 µg/kg 1000 µg/kg	Muscle Liver Kidney	

Based on these MRLs values, the daily intake will represent around 23% of the microbiological ADI.