



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SPIRAMYCIN (2)

SUMMARY REPORT

Spiramycin had been scientifically assessed again in November 1994 by the members of JECFA.

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

Spiramycin adipate and embonate were of low acute toxicity in the laboratory animals after oral or subcutaneous administrations (LD₅₀ (adipate) per os - 3120 mg/kg bw for mouse, rat > 3000 mg/kg bw).

In a short-term dietary study in which rats were given the equivalent of up to 3900 mg/kg bw for 13 weeks, the only major effects noted were a reduction in neutrophil counts in some mid- and high-dose animals, and dilatation of the caecum : the latter was attributed to antibiotic effects on the rodent gut flora. The NOEL was equivalent to 140 mg/kg bw/day. In another dietary study in the rat, animals were given up to the equivalent of 720 mg/kg bw/day for one year. The only notable effects were reductions in the bodyweights of females receiving the higher doses, and increases in relative liver, kidney, and adrenal weights at high dose levels in animals of both sexes. Hepatic glycogen depletion occurred at all dose levels but not in controls. However, the significance of this was unknown.

Oral doses of 200 and 500 mg/kg bw/day of spiramycin given to dogs for 28 days produced no adverse effects. However, in a second study, when mongrel dogs were given 500 mg/kg bw/day for up to 56 days, reductions in spermatogenesis and testicular atrophy occurred. Kidney damage was also seen. A NOEL could not be established, as only a single dose level was used. When beagles were given orally spiramycin at up to the equivalent of 150 mg/kg bw/day for two years, testicular damage was not seen although degenerative changes occurred in other organs. The NOEL in this study was 75 mg/kg bw/day. No adequate reproduction studies were available to the Committee.

In teratogenicity studies in mice, oral doses of spiramycin of up to 400 mg/kg bw given over days 5-15 of gestation had no effects on the outcome of pregnancy. Intravenous doses of up to 84 mg/kg bw/day given on days 6-15 of gestation to rats and days 6-19 to rabbits had no effect on development, but oral doses of 200 and 400 mg/kg bw/day in rabbit produced caecal enlargement in mothers and significance for human hazard assessment, because this species is known to be particularly susceptible to the effects of antibiotics on the gut micro flora. The embryotoxicity was probably related to maternal toxicity as neither was evident at 100 mg/kg bw/day.

The genotoxic potential of spiramycin was investigated in a range of studies. Negative results were obtained with spiramycin adipate and embonate in a forward-mutation test in mammalian cells *in vitro*, in an *in vitro* cytogenic assay, and in the mouse micronucleus test.

Adverse reactions in humans following spiramycin treatments are uncommon but, when encountered, the most frequently reported are mild gastro-intestinal disturbances.

In order to study the effects of spiramycin on the human gut flora *in vivo* (on gnotobiotic mice implanted with human flora) and *in vitro* experiments were carried out. MIC values for spiramycin were conducted using bacterial species isolated from healthy human volunteers. Dominant flora tested, consisted of strictly anaerobic bacteria (10⁷ bacteria/ml) while the subdominant flora included facultative aerobic and anaerobic bacteria (10⁷ bacteria/ml). In a total of 110 strains tested all the MIC values were higher than or equal to 1 µg/ml.

Based on the *in vitro* studies results and applying a safety factor of 1 instead of the previous value of 10, an ADI of 50 µg/kg bw was calculated according the following formula:

$$\text{ADI } (\mu\text{g/kg bw}) = \frac{\text{Concentration without effect on human gut flora (1 } \mu\text{g/ml)} \times \text{Daily faecal bolus (150 g)}}{\text{Fraction of dose bioavailable (0.05)} \times \text{Safety factor (1)} \times \text{Human bodyweight (60 kg)}}$$

Spiramycin was metabolised to neospiramycin by hydrolysis of the mycarose sidechain fragment, followed by conjugation of the aldehyde with L-cysteine to yield thiazolidone carboxylic acid derivatives, tentatively identified as the major polar metabolites.

Neospiramycin, the primary metabolites has an estimated antimicrobial activity of 88% compared to spiramycin.

In cattle studies, spiramycin and neospiramycin residues in tissues determined by HPLC were evaluated to total antimicrobial residues. At day 28 post-treatment, the sum of these 2 compounds represents respectively 100%, 87%, 59-63% of antimicrobial activity in muscle, liver and kidney. In fat, the parent drug and its primary metabolite represented 35-72% (mean = 54%) of total antimicrobial activity at days 28-35.

After intramuscular administration of 30000 IU per kg bw to 6 dairy cows, the concentration of spiramycin was below 1 mg/l after 8 milkings (4 days) and at 17 milkings (8.5 days) was 0.09 mg/l.

In chickens, the ratios of spiramycin and neospiramycin, determined by bioautography in muscle, liver, kidney, and skin with fat tissues were approximately 100%, 50%, 50%, and 50%, respectively, when compared with total antimicrobial activity. Polar derivatives may account for up to 50% of total antimicrobial activity. After administration of 0.8 g/l spiramycin via drinking water for three days, neospiramycin and spiramycin residues were lower than the limit of quantification in all tissues except for liver (400 µg/kg for each compound) at ten days after the end of the administration.

In pigs, there is no information about the ratio spiramycin plus neospiramycin towards total active microbiological residues, in pigs treated with 50 mg/kg bw/day for seven days, six quantifiable residues were identified and their structure confirmed by mass spectrometry. Residues of parent drug accounted for 0.4 mg/kg, while residues of spiramycin adducts with L-cysteine represented 10.5 mg/kg, and neospiramycin adducts with L-cysteine accounted for an additional 2.2 mg/kg of residues. However, the transformation into cysteine compound is reversible and depends on chromatographic conditions.

For cattle and chicken HPLC are available. For cattle, the limits of quantification are 0.050 µg/kg for both compounds whichever the edible tissue retained and 50 µg/l for milk. For chicken, the limits of quantification vary according to the tissues : 50 µg/kg for muscle, 75 µg/kg for fat, 100 µg/kg for liver and 200 µg/kg for kidney;

An antimicrobial method using *Micrococcus luteus* ATCC 9341 as the test organism was the only method available for residue analysis of pig tissues. The limits of quantification were 200, 600, 300 and 200 µg/kg, respectively for muscle, liver, kidney and fat.

The Working Group considered the MRLs for spiramycin as proposed by the 43th meeting of JECFA, but also took into account the actual tissue distribution. It recognised that the actual residues in chicken kidney are far below the MRLs proposed by JECFA. Therefore, the JECFA MRLs for the chicken kidney tissue were not adopted.

The following MRLs for the sum of spiramycin and neospiramycin are proposed :

Cattle

Muscle	200 µg/kg
Liver	300 µg/kg
Kidney	300 µg/kg
Fat	300 µg/kg
Milk	200 µg/l

Chicken

Muscle	200 µg/kg
Liver	400 µg/kg
Fat+skin	300 µg/kg

Due to the fact that for pig tissues, only a antimicrobial method is available and that there is no information on the ratio of spiramycin and neospiramycin towards total antimicrobial residues, the following provisional MRLs, covered by all active antimicrobial residues and expressed in spiramycin equivalent, are proposed for pigs.

Muscle	300 µg/kg
Liver	600 µg/kg
Kidney	300 µg/kg
Fat	200 µg/kg

As this compound is antibiotic, it should be keep in mind that the activity of spiramycin depends on the nature of the reference compound : 3200 IU/mg for the WHO standard.

The following information is required before a time period terminating on 1 July in 1997 :

- A validated analytical method for spiramycin and neospiramycin in pig tissues, described according a standard layout (ISO 78/2 or a similar international standard);
- Residue data to estimate the percent of total antimicrobial activity represented by spiramycin and neospiramycin for pig liver, kidney, fat and muscle.