SPIRAMYCIN

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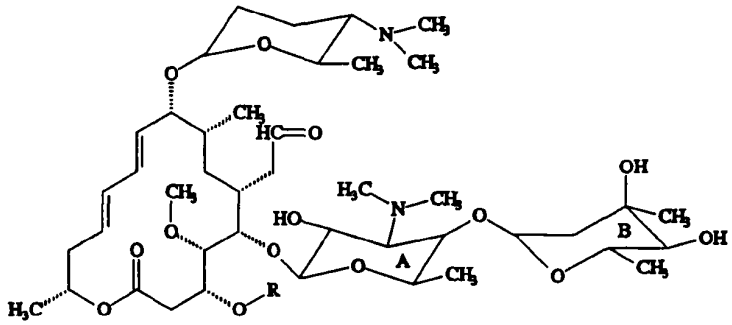
IDENTITY

Chemical name: Spiramycin

Synonyms: CAS 8025-81-8; Foromacidin; Sequamycin; Selectomycin; Rovamycin; Provamycin

Structural formula:



Molecular formula:

Spiramycin I	$(C_{43}H_{73}N_2O_{13})$-O-R;	R = H
Spiramycin II	$(C_{43}H_{73}N_2O_{13})$-O-R;	R = $COCH_3$
Spiramycin III	$(C_{43}H_{73}N_2O_{13})$-O-R;	R = $COCH_2CH_3$

Formula weight:

Spiramycin I	843.07
Spiramycin II	885.11
Spiramycin III	899.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: White or faintly yellowish powder

Melting point: 128-137°C

Solubility: Barely soluble in water, freely soluble in acetone, ethanol and methanol, sparingly soluble in ether

Optical rotation: [a]S(20,D) -80° (c = 1 in methanol)

UV_{max}: 231 nm (ethanol)

pH: 8.5-10.5 (0.5% solution)

SUMMARY OF THE 38TH JECFA

METABOLISM

The data available to the Committee indicated that spiramycin could be metabolised into neospiramycin in tissue and that neospiramycin has similar antibacterial activity to the parent drug. There was no structure identification of the neospiramycin metabolite.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabel Residue Depletion Studies

No radiolabel residue depletion studies were submitted by the sponsor for the 38th meeting of the Committee.

Residue Depletion Studies (with Unlabeled Drug)

Cattle

The major study in cattle consisted of an 18 animal study, in which the animals received two intramuscular injections of 100,000 IU/kg body weight (31 mg/kg BW) Suanovil at 48 hour intervals. Groups of three animals were used for each withdrawal period. Male animals were used for the 14 and 21 day withdrawal period; female animals were used for the 28, 35, 42, and 49 day withdrawal periods. Tissues were collected from injection sites, muscle, liver, kidney and fat as noted in Table 1 (Sanders and Delepine, 1990a). Injection site residues were 20.91 and 35.12 μg/g at day 14, 10.23 and 10.30 μg/g at day 21. Residues declined rapidly to 0.47-0.60 μg/g at day 28 and 0.13-0.16 μg/g at day 42. Residues were below the limit of quantification of the liquid chromatographic method by day 49. Neospiramycin residues were reported as spiramycin equivalents using a correction factor of 0.88, the ratio of neospiramycin and spiramycin titers. Concentrations of residues are expressed in μg/g using the WHO standard titer of 3200 IU/mg.

Table 1. Concentrations of Residues in Spiramycin Equivalents (μg/g) in Cattle

Days Post Treatment	Muscle	Liver	Kidney	Fat
14	0.09	0.48	0.47	NS
21	<0.06	0.30	0.17	NS
28	<0.03	0.14	0.05	ND
35	<0.03	<0.12	<0.03	0.05
42	ND	<0.12	<0.03	<0.03
49	ND	<0.06	ND	ND

ND: not detected (<0.015 mg/kg); NS: not sampled.

Although not shown in the Table, residues of neospiramycin were approximately the same concentration as those of spiramycin.

Using 6 cows, residues of spiramycin in milk were determined following intramuscular administration of 30,000 IU/kg body weight (9.3 mg/kg). Milk samples were collected for 25 milkings. Residues were determined using the microbiological agar diffusion method with *Micrococcus luteus* (Moulin and Sanders, 1989a). Results are shown in Table 2.

Table 2. Spiramycin Residues (μg/ml) in Milk

Milking	Concentration (mean ± SD)
1	16.54 ± 7.50
2	10.00 ± 3.18
3	5.27 ± 1.62
4	2.74 ± 0.90
5	1.50 ± 0.40
6	1.06 ± 0.25
7	0.74 ± 0.57
8	0.57 ± 0.09
9	0.43 ± 0.08
10	0.40 ± 0.05
11	0.32 ± 0.06
12	0.30 ± 0.06
13	0.24 ± 0.10
14	0.19 ± 0.03
15	0.16 ± 0.03
16	0.14 ± 0.03
17	0.09 ± -
18-25	ND

ND: Not detected (<0.06 μg/ml)

Residues of spiramycin were measured in 42 female Friesian/Holstein calves (aged 8 - 15 days) divided into three groups. Group 1 (4 calves) served as controls. Group 2 (22 calves) received 25 mg/kg BW spiramycin per day for seven days in their feed. Group 3 (16 calves) received 25 mg/kg BW spiramycin per day plus 40 mg/kg BW oxytetracycline per day in their feed for seven days. Residues were determined by the microbiological method (Pascal et al., 1990a). Results are shown in Table 3. There was no indication that the admixture with oxyetracycline effected residue concentrations of spiramycin and its metabolites. In addition, oxytetracycline was not found to interfere significantly with the assay of spiramycin residues in tissue.

Table 3. Residues in Calves (μg/g) Receiving Spiramycin Alone or in Combination with Oxytetracycline

Days Post Treatment	Muscle	Liver	Kidney	Fat
3	0.2	9.17	13.7	0.15
7	<0.1	3.03	5.3	<0.1
14	<0.1	1.1	1.7	<0.1
24	<0.1	0.2	<0.1	ND
35	ND	<0.1	ND	ND

ND: Not detected

Pigs

Four studies were reported in pigs, one using intramuscular dosing, two using oral administration and one using subcutaneous injection and oral administration. In the intramuscular dosing study, 15 pigs 10-12 weeks old, received 25 mg spiramycin per kg body weight per day for three days. Three animals were used in each withdrawal period. Tissue samples were collected from liver, kidney, muscle, fat, skin, heart and brain. Tissue samples were analyzed using a microbiological assay with *Micrococcus luteus* (May and Baker, 1967). The results are summarized in Table 4.

Table 4. Spiramycin Residues (μg/g) in Pigs Receiving 25 mg/kg BW per day Intramuscularly for Three Days

Days Post Treatment	Liver	Kidney	Muscle	Fat	Skin	Heart	Brain
1	9.01	21.59	0.29	0.03	0.11	0.32	0.04
3	2.26	4.75	0.20	0.09	0.20	0.20	<0.025
5	0.68	1.24	0.05	0.05	0.07	0.10	<0.025
7	0.49	0.48	<0.025	0.04	0.04	0.07	<0.025
14	<0.025	0.04	<0.025	<0.025	<0.025	<0.025	<0.025

The two residue studies using oral dosing with spiramycin embonate were conducted in 25-30 kg male and female White X Landrace pigs (Pascal et al., 1990b). In each study, three animals were used for each withdrawal period. The dose in each study is indicated in the tables below. Analysis of spiramycin and its active metabolites in tissues was conducted using the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. In animals receiving spiramycin (16 mg/kg BW/day) combined with sulfadimidine or oxytetracycline, there was no change in the elimination kinetics as compared with animals receiving spiramycin alone at the same dose. Sulfadimidine and oxytetracycline did not interfere significantly with the assay of spiramycin in the tissues. Results are listed in Tables 5 and 6.

Table 5. Tissue Residues (μg/g) in Pigs Receiving 16 mg/kg BW per day Spiramycin Embonate for Seven Days

Days Post Treatment	Liver	Kidney	Muscle	Fat
0.5	6.26 ± 1.26	8.90 ± 1.75	0.12 ± 0.01	<0.10
3	1.44 ± 0.24	1.30 ± 0.56	<0.10	<0.10
7	0.58 ± 0.19	0.23 ± 0.07	<0.10	<0.10
10	<0.30	<0.15	ND	<0.10
15	<0.30	<0.15	ND	ND
20	<0.30	<0.15	ND	ND

ND: Not determined

Table 6. Tissue Residues (μg/g) in Pigs Receiving 25 mg/kg BW per day Spiramycin Embonate for Seven Days

Days Post Treatment	Liver	Kidney	Muscle	Fat
7	1.45 ± 0.40	0.56 ± 0.11	<0.10	<0.10
10	0.89 ± 0.35	0.19 ± 0.05	ND	<0.10
20	<0.30	<0.15	ND	ND

ND: Not determined

Excretion and tissue concentrations of spiramycin were measured in pigs following oral administration (4 animals) or subcutaneous injection (10 animals) (Ferrot and Videau, 1971). Subcutaneous injection gave significantly greater bioavailability than the oral administration at an equivalent dose. However, the residue concentrations in organ tissues and bile resulting from oral administration was considerably higher than reported in other studies, whereas concentrations in muscle by both routes was much lower than in the other tissues and depleted rapidly by comparison to the other studies.

Poultry

One study was reported by Banazet and collaborators (1960). Thirteen broiler chickens approximately 1.2 kg each, were treated with 300 ppm spiramycin in the feed for 10 days. The average daily intake was calculated to be 43 mg per bird. Samples of liver and muscle were analyzed using a microbiological assay using *Micrococcus luteus* ATCC 9341. The results are noted in Table 7.

Table 7. Tissue Residues (μg/g) in Broilers Receiving 300 ppm Spiramycin in Feed for 10 Days

Days Post Treatment	Liver	Muscle
0	3.78	0.19
1	1.67	0.08
3	0.89	0.04
5	0.21	<0.02
8	<0.02	<0.02

Note: The assay had a sensitivity of 0.02 μg/g

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE

Microbiological and high performance liquid chromatography methods have been reported for the determination of spiramycin residues in plasma, serum, tissues and milk.

The microbiological agar gel diffusion method uses *Micrococcus luteus* ATCC 9341 as the test organism. Tissue samples are extracted and purified prior to adding to the test organism. For serum, samples are diluted with phosphate buffer as needed. Agar plates are prepared using Difco #9 medium and seeded with the test organism. Standards are prepared in blank serum. Cylinders are placed on the seeded plates and 200 μl of material (sample or standard) is added. The method has a sensitivity of approximately 0.1 IU/ml (about 0.03 μg/g) (Huet et al., 1988).

Milk samples are prepared in much the same way as serum samples. Milk samples may be analyzed directly or diluted with phosphate buffer as necessary. Plates are prepared as noted above. The test samples (250 μl) are placed in the test cylinders and incubated. Zones of microbial inhibition are measured and the concentration of spiramycin determined by extrapolation with known samples. The quantification limit in milk is 0.2 IU/ml (about 0.06 μg/g) (Moulin and Sanders, 1989b).

High performance liquid chromatography (HPLC) has been used to measure spiramycin residues in plasma (Mourot and Sanders, 1988). Test samples and standards added to blank plasma are processed through C2 cartridges using 4% acetonitrile. A 5u reverse phase RP 8 column with a RP 8 precolumn is used for separation of analytes. Samples are eluted with 0.5% sulfuric acid and acetonitrile (79:21, v/v). Detection is by UV, using 231 nm. The method is linear from 0.2 - 104 IU/ml (0.06 - 32 ug/ml) with average recoveries of about 85%. The limit of quantification is 0.2 IU/ml (0.06 μg/ml).

Tissues are also analyzed for spiramycin and neospiramycin residues by reverse phase HPLC. Tissue samples and standards prepared in blank tissue are extracted using chloroform, and the extracts processed through solid phase extraction cartridges using chloroform. A 5u reverse phase PR 8 column is used as noted above for separation. Samples are eluted with 0.5% sulfuric acid in acetonitrile (80:20 v/v) and detection of residues is monitored with a UV detector using 231 nm. The method is linear for concentrations between 0.2 - 10 IU/g (0.06-3 μg/g) with recovery of spiramycin from extracted standards of 76, 85, and 97% for muscle, liver and kidney, respectively. Recovery for neospiramycin is 83, 72, and 80% respectively, for the three tissues. The quantification limit was 0.1 IU/g (0.03 μg/g) in muscle and kidney and 0.2 IU/g (0.06 μg/g) in liver. The limit of detection was estimated to be 0.05 IU/g (0.015 μg/g) (Sanders and Delepine, 1990b).

APPRAISAL

In the assessment on MRL's for spiramycin, the Committee took into consideration the temporary ADI and recommended temporary MRL's for liver, kidney, and muscle tissue in pigs and cattle, and in milk. No MRL was assigned for fat because sufficient data were not available. The Committee requested results on two studies for evaluation in 1994:

1. Radiometric studies on the concentrations of spiramycin and its metabolites as proportions of the total residues in edible tissues of cattle, pigs and poultry.

2. Studies on the pharmacokinetics of spiramycin residues in the fat of cattle and pigs and the edible tissues of poultry.

1994 DATA SUPPLEMENT

RESIDUE STUDIES OF SPIRAMYCIN AND ITS METABOLITES IN EDIBLE TISSUES

Instead of radiometric studies, the sponsor presented extensive studies using microbiological and HPLC methods deemed appropriate to assess residues of spiramycin and its metabolites as proportions of the total residues in edible tissues of the target species. The HPLC method, as noted above, is specific for parent drug and its primary metabolite, neospiramycin. The bioassay indicates the presence of microbiologically active metabolites. The hypothesis of the studies presented is that all spiramycin metabolites possess a certain amount of microbiological activity and that the microbiological method is representative of the total residues (metabolites) in biological fluids and tissues. The original spiramycin submission estimates the microbiological activity of neospiramycin at 88% of spiramycin titre.

Sanders and collaborators (1991) studied residues in plasma from cattle administered Spiramycin intravenously using HPLC and microbiological assays on the same plasma samples. The area under the curve (AUC) was calculated from the microbial inhibition assay and results compared to the HPLC data. The ratio of AUC versus HPLC was estimated at about 1.2:1, indicating the presence of microbiologically active metabolites in plasma. Data presented in the study indicates that approximately 70% of the activity is due to spiramycin at post treatment times up to 96 hours.

Similar studies were reported in cattle tissues. Animals received two intramuscular doses of 100,000 IU/kg (approximately 32 mg/kg) at 48 hour intervals. Microbiological residues were determined using *Micrococcus luteus* (*Sarcina luteus*) ATCC 9341 (Huet et al., 1988). Spiramycin and neospiramycin residues were measured by reverse phase HPLC (Sanders and Delepine, 1990a; Sanders et al., 1990). Neospiramycin was unambiguously identified in cattle muscle by co-elution with an analytical standard with structure confirmed by mass spectrometry. A comparison of the concentrations in the same samples is presented in Table 8. Ratios of the two sets of results are also tabulated. Each data point represents the mean of three tissue samples.

Table 8. Residues of Spiramycin and Neospiramycin (μg/kg) in Cattle Tissue Determined by HPLC and Total Active Metabolites by *Micrococcus luteus* ATCC 9341

Days Post Treatment	Assay Method	Liver	Kidney	Muscle	Fat
14	HPLC	480	470	90	NS
	ATCC 9341	718	742	66	NS
	Ratio (%)	67	63	100	-
21	HPLC	300	170	<60	NS
	ATCC 9341	220	172	53	NS
	Ratio (%)	100	99	100	-
28	HPLC	140	<50	<30	<30
	ATCC 9341	151	85	<25	85
	Ratio (%)	93	59	100	35
35	HPLC	<120	<30	<30	50
	ATCC 9341	118	48	27	69
	Ratio (%)	100	62	100	72

NS: Not sampled.

Values for the HPLC assay represent the sum of spiramycin and neomycin residues.
Ratios expressed as %: (spiramycin + neospiramycin/total active metabolites) x 100

Comparing residue concentrations by the two methods indicates that spiramycin and neospiramycin represent the majority of the total active residues in cattle tissue. In muscle tissue, spiramycin and neospiramycin are the only detectable residues in muscle tissue. Similarly, ratios in liver indicate that these two analytes represent 67%, 100%, 93%, and 100% of total active residues at days 14, 21, 28 and 35, respectively. In kidney, the results are somewhat more variable, with ratios of spiramycin and neospiramycin ranging from 60% to 100% of total active metabolites. Data in fat is limited to results from 28 and 35 days only, with ratios of 35% and 72%, respectively. Results from cattle plasma and tissues are in general agreement with the pharmacokinetic properties of spiramycin in cattle (i.e., large body distribution and a long elimination half-life) (Sanders et al., 1992). The presence of minor quantities of unknown active metabolites in the liver and kidney may be accounted for by a further metabolic transformation of neospiramycin to polar metabolites (noted later in pig and poultry studies) by formation of adducts of neospiramycin with L-cysteine. The proposed metabolism of spiramycin in cattle will be summarized following the discussion of the poultry metabolism studies, since data strongly suggest a similar metabolic scenario.

Poultry

Studies on spiramycin metabolism in chickens were carried out using two medicated diets of 10 g (the therapeutic dose for growth promotion) and 100 g of spiramycin per ton of food for about 60 days (Jolles and Terlain, 1968). Muscle, liver, and kidney tissues were sampled at the end of the treatment period. The withdrawal time was not reported by the authors. Residues were analyzed by thin layer chromatography (TLC) following solvent extraction, with detection by bioautography. Detection limits for this assay was about 0.01 and 0.02 μg/g for spiramycin and neospiramycin, respectively. Results indicate that these two analytes were the major microbiologically active residues in all three tissues. The polar metabolites were tentatively identified as conjugates or bound forms of neospiramycin. Jolles and Terlain indicate that these metabolites appear to be neither glucuro- nor sulfoconjugates. Neospiramycin could be partially regenerated from the conjugates on treatment of the residues at 50 to 60°C with organic solvents or storage at room temperature for longer periods of time. Results are summarized in Table 9.

Table 9. Residues of Spiramycin (μg/g) in Chicken Tissues

Tissue	Spiramycin	Neospiramycin	Polar Derivatives
Muscle	0.010 - 0.015	<0.010	ND
Liver	1.0 - 1.3	0.8 - 1.0	<2.3
Kidney	0.12 - 0.15	0.10 - 0.12	<2.7

ND: Not detected
Data represents the highest dose group (100 g per ton)

Jolles and Terlain (1968) estimated concentrations of residues by comparison with fortified blank tissue with known quantities of each analyte, however, this could not be done with the polar metabolites. The authors concluded that the inhibition area attributed to the polar substances on *Bacillus subtilis* bioautographs was smaller than the sum of the inhibition zones of the other two analytes. The polar derivatives, based on this observation and the data in Table 9 indicate that the polar metabolites do not contain more than 50% of the total residues.

A major residue study in poultry is described by Bosc and collaborators (1993) and Mignot and collaborators (1993). Bosc and collaborators conducted the animal dosing studies by addition of Suanovil 50 in the drinking water at 0.8 g/l for three days. Mignot and collaborators determined spiramycin and neospiramycin residues in plasma, muscle, liver, kidney, and skin with fat using an HPLC method. Residues were measured at 5, 10, 15 and 20 days after the last treatment. Six birds were used in each sampling period. Mignot and collaborators (1993) measured residues in liver in five samples at day 5 but were only able to detect residues in two samples at day 10. In kidney, residues were measured in only two samples at day 5; in the four remaining samples spiramycin was detected but residues were below the limit of quantification. Concentrations of spiramycin and neospiramycin were most often below the limit of quantification in muscle and skin with fat at day 5 after the last treatment. Residues were highest in liver but were below the limit of quantification by day 15; for kidney, by day 10 after the last treatment. Results are summarized in Table 10.

Table 10. Residues of Spiramycin and Neospiramycin in Poultry Tissues (ng/g)

Days Post Treatment	Muscle		Liver		Kidney		Skin with fat	
	SP	NSP	SP	NSP	SP	NSP	SP	NSP
5	55 (n=2)	+	699 (n=5)	579	280 (n=2)	+	99 (n=3)	+
10	+	+	423 (n=2)	491	+	+	+	+
15	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+

SP is spiramycin; NSP is neospiramycin.
Limit of quantification for spiramycin and neospiramycin was 50, 100, 200, and 75 ng/g, respectively for muscle, liver, kidney and skin with fat. A "+" indicates results below the limit of quantification.

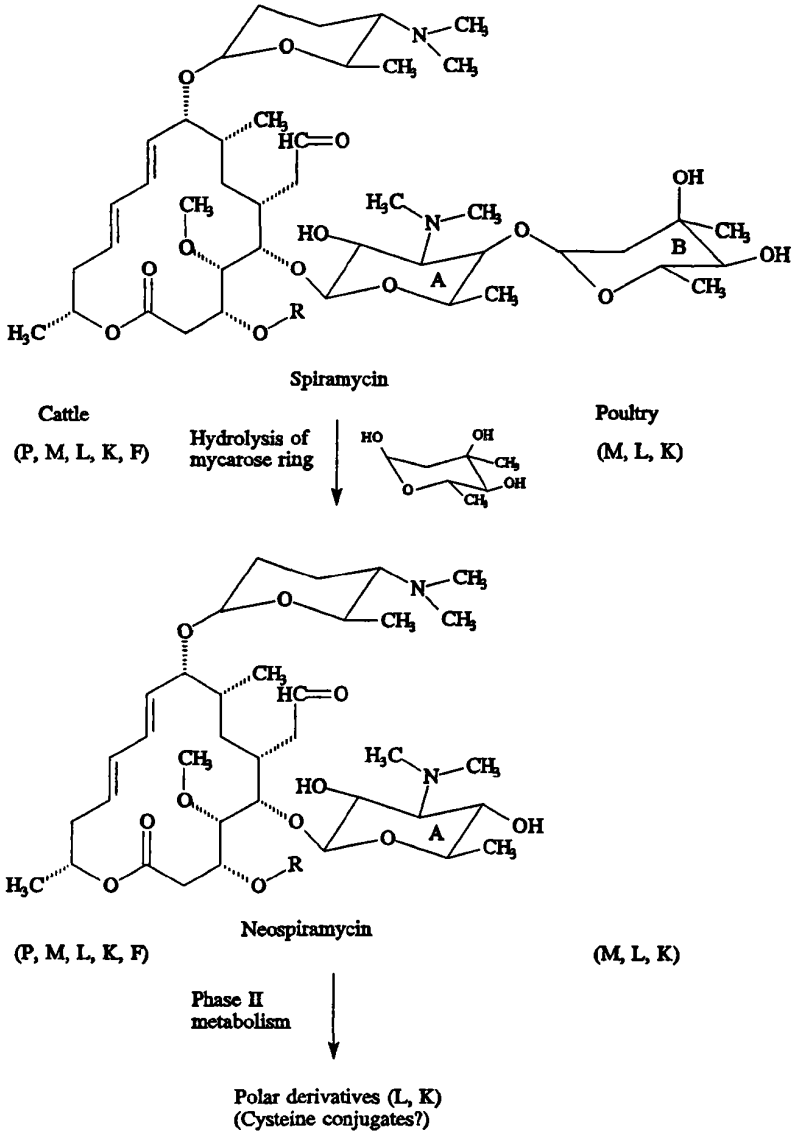
Based on the collective data of these studies, spiramycin metabolism occurs by hydrolysis to neospiramycin by loss of the mycarose moiety. Neospiramycin residues are present at lower concentrations than parent drug and polar residues were noted by the authors in liver, kidney and fat. Total residues represent the microbiologically active residues. The authors noted that the proportion of spiramycin and neospiramycin versus total microbiologically active residues in skin and fat is a conservative estimate as an additional safety margin. These results are summarized in Table 11.

Table 11. Residues of Spiramycin and Neospiramycin (μg/g) Versus Total Residue in Edible Tissues of Chicken

Tissue	Ratio (%) (Spiramycin + Neospiramycin/Total Residues) x 100
Muscle	100
Liver	50
Kidney	50
Fat	50

Based on these metabolism studies, the proposed metabolic pathway for cattle and poultry is noted in Figure 1.

Figure 1. Proposed Metabolic Pathway for Spiramycin in Cattle and Poultry



P = plasma, M = muscle, L = liver, K = kidney, F = fat

Pigs

Mourier (1993) conducted a metabolism study in pigs using spiramycin embonate to evaluate and identify the polar metabolites in pig liver. The initial work was an in vitro study to identify the principal biotransformations. Results indicated that L-cysteine (present in relatively large quantities in pig liver), reacts with the macrocycle aldehyde, resulting in the formation of a thiazolidine carboxylic acid. Additional studies showed that only spiramycin derivatives whose aldehyde function is modified do not undergo this transformation. In a subsequent study with pigs treated orally with 50 mg/kg BW per day for seven days, and using an HPLC assay method (detection limit of 0.2 μg/g) six spiramycin metabolites were detected. (See page 1 of this monograph to identify the structure of spiramycin I and III.)

Spiramycin I	0.2 μg/g
Spiramycin III	0.2 μg/g
Spiramycin I with cysteine	6.4 μg/g
Spiramycin III with cysteine	4.1 μg/g
Neospiramycin I with cysteine	1.2 μg/g
Neospiramycin III with cysteine	1.0 μg/g
Total Spiramycin residues	**13.1 μg/g**

This study provides strong support for the proposed metabolism noted in Figure 1. It suggests, though there is not metabolism data in other pig tissues, that the metabolism shown in Figure 1 is representative of metabolism in pig muscle and kidney.

A definitive study of spiramycin residues in pig tissues was reported by Cuypers and collaborators (1994). Twelve castrated piglets weighing 15-18 kg received spiramycin embonate feed dosed at 450 ppm. Daily ingested doses were measured at 21-23 mg/kg live weight of spiramycin base. Groups of four animals were analyzed at each withdrawal time. Spiramycin residues were analyzed by HPLC and the microbiological assay using *Micrococcus luteus* ATCC 9341. The HPLC method had a limit of quantification of 0.1 μg/g, and analyte extraction recovery of 89%. The tissue concentrations represent spiramycin and its metabolites in pig liver receiving 22 mg/kg spiramycin embonate per day for seven days. At all three withdrawal times, the highest residue concentrations were the cysteine derivative of spiramycin I and III. Residues of neospiramycin and its cysteine derivatives were very low. Results are summarized in Table 12. These data support the hypothesis that spiramycins are metabolized to neospiramycins in pig liver to only a slight extent. Spiramycin and neospiramycin combine readily with L-cysteine to yield thiazolidine derivatives.

Table 12. Residues of Spiramycin and Metabolites (μg/g) in Pig Liver Measured by the HPLC and Microbiological Methods

Days Post Treatment	Residues by HPLC	Microbiologically Active Residues
0	5.0	5.2
3	0.9	1.3
10	0.1	<0.2

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Both microbiological and HPLC methods are available for residues of spiramycin in cattle tissues. The microbiological method is based on the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. An organic extraction and purification process is employed prior to application of the microbiological assay (Pascal, et al., 1990). The HPLC method for the determination of spiramycin and neospiramycin has been referenced previously (Sanders and Delepine, 1990b). A microbiological method is available for residue analysis of milk samples (Moulin and Sanders, 1989b) and employs the same agar diffusion method noted above. The limit of quantification is 62 μg/l of total residues.

For pig tissues, a microbiological method is available for residue studies virtually identical to those referenced above (Pascal, et al., 1990). An HPLC method is available for liver tissue only.

For poultry, an HPLC method for residue analysis in edible tissues has been reported (Mignot, et al., 1993). It is suitable for both spiramycin and neospiramycin.

Tables 13 and 14 summarize the limits of quantification for cattle, pigs and chickens tissues, of analytes of interest.

Table 13. Limits of Quantification for Residues of Spiramycin (μg/kg)

Species	Method	Muscle	Liver	Kidney	Fat	Milk
Cattle	ATCC 9341 HPLC	100 30	100 62.5	100 30	100 47	62
Pig	ATCC 9341	100	300	150	100	
Chicken	HPLC	50	100	200	75	

Table 14. Limits of Quantification for Residues of Neospiramycin (μg/kg)

Species	Method	Muscle	Liver	Kidney	Fat	Milk
Cattle	ATCC 9341 HPLC	100 25	100 50	100 15	100 30	62
Pig	ATCC 9341	100	300	150	100	
Chicken	HPLC	50	100	200	75	

The *Micrococcus luteus* ATCC 9341 residue assay measures total microbiological activity. The estimated microbiological activity of neospiramycin is 88% of spiramycin activity.

Although the *Micrococcus luteus* ATCC 9341 methodology has ample data to verify its use for measuring residues of spiramycin biologically active residues, it is not an unambiguous method for use in a regulatory control program. The USDA's Food Safety and Inspection Service Microbiology Laboratory Guidebook uses this same test organism, as well as other test organisms, to specifically identify several classes of antibiotics, including penicillin, streptomycin, erythromycin and neomycin . To specifically identify the analyte of interest requires development of a fingerprint profile of each class of antibiotics with a set of several test organisms.

APPRAISAL

Residues of spiramycin in cattle are highest in liver tissue following intramuscular injection at all withdrawal times up to 49 days, with quantifiable residues up to 28 days. With oral treatment, the results indicate that residues in kidney are somewhat higher than in liver tissue for at least 14 days and comparable at longer withdrawal times. In pigs following intramuscular treatment residues are approximately two fold higher in the kidney through the first seven days post treatment, but are below the limits of quantitation by day 14.

With pigs receiving medicated feed, residues are similar in the liver and kidney at three days withdrawal but are higher in the liver by day seven. Residues tend to deplete more rapidly in pigs than in cattle and that may be due to the relatively high amounts of L-cysteine in pigs resulting in more extensive thiazolidine carboxylic acid derivative formation. The data suggest that liver is the most reasonable target tissue for residue analysis. The parent drug and the neospiramycin or the microbiologically active residues are suitable as the marker residue.

In poultry, the residue data at withdrawal times of 5, 10, 15 and 20 days were studied by Bosc and Mignot and collaborators (1993). Concentrations of residues measured by an HPLC method were often below the limit of quantification in muscle and skin with fat even at day 5 after the last treatment. Residues were higher in liver tissue than in kidney, while residues appear to be low (ng/g) at all withdrawal times. On this basis, liver is indicated as the target tissue for poultry and spiramycin would be the appropriate marker residue. A microbiological method is available (Benazet, et al., 1960) using *Micrococcus luteus* ATCC 9341 as the test organism.

With the exception of muscle tissue, total microbiological activity is greater than residues detected as spiramycin or neospiramycin by HPLC, indicating other metabolites exhibiting biological activity are likely to be present. In cattle, for example, HPLC measured residues are 59-100% of the residues determined by the microbiological assay in liver and kidney tissue. In chickens, residues by HPLC in liver, kidney and fat are conservatively estimated at about 50% of microbiologically active residues. In pigs, the residues by HPLC in liver are 71-96% of microbiologically active residues for spiramycin I and III, respectively, depending on the withdrawal time.

Maximum Residue Limits

Based on the ADI of 0-50 μg per kg body weight established by the Committee using microbiological data, the permitted daily intake of spiramycin and its antimicrobially active residues would be 3000 μg for a 60-kg person.

In reaching its decision on MRLs, the Committee also took into account the available residue and metabolism data on spiramycin and neospiramycin, the percentage of the total antimicrobial activity representing these residues, and the limits of quantification for the HPLC and microbial methods.

The Committee calculated the minimum residue levels that could be considered as a basis for MRL calculations using twice the limits of quantification for spiramycin and neospiramycin residues in cattle, pigs and chickens (Table 15).

Table 15. Minimum Residue Levels that could be considered as a basis for MRL calculations for Spiramycin (μg/kg) in cattle, pigs and chickens[a]

Species	Analytical method	MRL levels				
		Muscle	Liver	Kidney	Fat	Milk
Cattle	Agar diffusion *M. luteus* ATCC 9341	200	200	200	200	124
	HPLC	60[b] 50[c]	124[b] 100[c]	60[b] 30[c]	94[b] 60[c]	26[b] 12[c]
Pigs	Agar diffusion *M. luteus* ATCC 9341	200	600	300	200	NA
Chickens	HPLC	100[b] 100[c]	200[b] 200[c]	400[b] 400[c]	150[b] 150[c]	NA NA

NA: not applicable; [a] based on the two-fold quantification limits of the analytical methods, for the agar diffusion method, values refer to the sum of residues of spiramycin and neospiramycin; [b] values refer to spiramycin residues; [c] values refer to neospiramycin residues

These values were not adjusted to account for the percentage of antimicrobial activity represented by the sum of spiramycin and neospiramycin residues. In cattle these two residues accounted for about 100% of the total antimicrobial activity in muscle, 85% in liver, 70% in kidney and 50% in fat. For pig tissues, the residues were determined by a validated microbial method. A validated chemical method for the determination of spiramycin and neospiramycin was not available, hence it was not possible to estimate the percentage of the total antimicrobial activity due to spiramycin and neospiramycin residues. In chickens, these two residues accounted for about 100% of total antimicrobial activity in muscle, and 50% in liver, kidney and fat.

The recommended MRLs for spiramycin in cattle, pigs and chickens are shown in Table 16. For cattle and chickens the MRLs are expressed as the sum of spiramycin and neospiramycin. For pigs, the MRLs are expressed as spiramycin equivalents and are temporary for liver, kidney and fat based on antimicrobial

activity, pending the availability of a validated chemical method and the estimation of the percentage of antimicrobially active residues represented by spiramycin and neospiramycin in these tissues.

Table 16. Recommended MRLs for Spiramycin (μg/kg) in cattle, pigs and chickens[a]

Species	MRL				
	Muscle	Liver	Kidney	Fat	Milk[b]
Cattle	100	300	200	300	100
Pigs	200[c]	600[c,d]	300[c,d]	200[c,d]	NA
Chickens	200	400	800	300	NA

[a] Expressed as the sum of spiramycin and neospiramycin, if not otherwise stated;
[b] Expressed as μg/l; [c] Expressed as spiramycin equivalents; [d] Temporary MRL;
NA: not applicable

If these values are used for MRLs, the theoretical maximum daily intake of residues of spiramycin and neospiramycin from cattle and pigs would be less than that from chickens. The theoretical maximum daily intake of antimicrobially active residues from chickens is 250 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, and 50 g of each of kidney and fat. For bovine milk, theoretical maximum daily intake of spiramycin and neospiramycin residues is 150 μg, based on a daily consumption of 1.5 l.

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