SPECTINOMYCIN

First draft prepared by
Dr. Richard L. Ellis
Food Safety and Inspection Service
U.S. Department of Agriculture
Washington, DC, USA

Dr. Robert C. Livingston
Center for Veterinary Medicine
U.S. Food and Drug Administration
Rockville, MD, USA

ADDENDUM
to the spectinomycin residue monograph prepared by the 42nd meeting of the Committee and published in FAO Food and Nutrition Paper 41/6, Rome 1994,

INTRODUCTION

Spectinomycin is an aminocyclitol antibiotic. In veterinary medicine it is used therapeutically for bacterial respiratory and enteric infections. Its broad spectrum bactericidal activity is based on its ability to inhibit protein synthesis in the 30S ribosomal sub-unit of the cell. It is administered to cattle, pigs, sheep and poultry as injectable solutions, orally as aqueous solutions or in feed.

Spectinomycin was reviewed previously by the 42nd Committee in 1994 at which time a full ADI was established (0-40μg/kg) based on a microbiological endpoint. Temporary MRLs were recommended for cattle, pigs and chickens in kidney, liver, muscle, fat and cattle milk as parent drug. The MRLs were designated as temporary because many of the residue depletion data were either from interim progress reports or from pilot studies. At that time the Committee was aware that additional metabolism studies were being conducted to confirm that the microbiologically active portion of the residues in edible tissues was predominantly spectinomycin. The Committee recommended that results of these studies be available for review by 1996.

Sponsors have submitted results of new studies to the Committee for its consideration including:
a) studies in species considered by the 42nd meeting of the Committee;
b) studies to support adding sheep to the list of species;
c) a proposal for an MRL in chicken eggs; and
d) studies to propose adjusted MRL's in muscle and fat.

These new studies provide data on pharmacokinetics in cattle, pigs and sheep; residue data from cattle, pigs, chicken and sheep; and information on assay methodology validation including a comparison between microbiological inhibition and chemical methods.

These new studies provide data on:
a) rapid absorption following IM and SC dosing as well as high bioavailability from these dosing regimens;
b) kidney tissues containing the highest residues of parent drug for long withdrawal periods, while in liver, dihydrospectinomycin is the primary residue that persists for about as long a time as parent drug in kidney;
c) identification of eight metabolites showing that spectinomycin is the major residue in kidney and dihydrospectinomycin is the major metabolite in liver; and
d) concentrations of metabolites in edible tissues indicating muscle and fat have very low concentrations except for skin and underlying fat in chickens.

The new studies also provide additional residue data. They provide information that verifies that:
a) kidney is the target tissue and parent spectinomycin is the marker residue;
b) almost all the microbiological activity in kidney and other edible tissues except liver is accounted for by parent drug; in liver dihydrospectinomycin accounts for most of the microbiological activity; and
c) quantities of microbiologically active residues in other tissues are very low.

The data are most complete for cattle, however, where comparable data are available in other species, it indicates profiles similar to that found in cattle. One sponsor acknowledges that they have not conducted extensive, contemporary species-specific studies to generate data for all species for this old compound. The sponsor suggests the

existing database is satisfactory. Using available data on kidney as the target tissue as an example, they acknowledge that they do not have direct comparisons of the microbiological activity of parent drug in kidney from other species including sheep and pigs. However, data from a radiolabel spectinomycin oral dosing study in pigs indicates very similar absorption characteristics and dose excretion patterns as in cattle, as well as distribution of residues in the tissues. Residue analysis from the pig study indicates that the highest concentrations of spectinomycin residues are in the kidney at all time periods and the highest concentrations of residues in the liver are dihydrospectinomycin. Details are described below.

Pharmacokinetic Data

Cattle

A new pharmacokinetic study of spectinomycin in calves following a single IM, IV and SC injection at a dose of 10 mg spectinomycin/kg BW has been reported using six male Friesian calves about seven weeks old (Caputo, et al., 1995). Each animal was injected once by each route with a washout period of seven days between each treatment. Results are summarized in Table 1. Spectinomycin is rapidly absorbed with IM and SC administration and has a relatively short elimination half-life in calves. It is also completely bioavailable by the IM and SC routes.

Table 1. Pharmacokinetic data using a single dose of 10 mg ^{3}H-spectinomycin/kg BW in calves. by IV, IM and SC administration, respectively

Pharmacokinetic parameter	Intravenous	Intramuscular	Subcutaneous
C_{max}(μg/mL)		27	19.9
t_{max}(h)		0.61	1.06
$AUC_{0-\infty}$ (μg·h/ml)	65.1	76.7	77.3
$T_{1/2}$ (terminal) (h)	1.76	1.52	1.83
T (h)	2.26	2.69	3.04
F (%) (bioavailability)		118	120

Using a full dose disposition study with 16 animals and the highest recommended dose (15 mg ^{3}H-spectinomycin free base equivalents/kg BW) once daily during five consecutive days, data for urine, blood, tissues and feces are summarized in Table 2 (Hornish, et al., 1996a, 1996b). There was a small sex difference accounted for by the method of collection. Greater than 90% of the excreted dose was eliminated within 24 hours after the last dose. The terminal elimination phase of total plasma pharmacokinetic residue had a half-life of about 8 days. The tritiated water accounted for less than 4% of the dose. There appeared to be appreciable retention of drug residues in tissue, primarily in liver and kidney, while muscle and fat retained lesser amounts of total residue (most of which in the latter withdrawal times was accounted for as tritiated water) as shown in Table 3 (Hornish, et al., 1996b). Results are the mean residue concentration in mg/kg (n = 4 animals per group). These data are corrected for tritiated water.

Table 2. Summary of dose accountability of ^{3}H-spectinomycin in cattle receiving five daily subcutaneous doses (% recovered)

Withdrawal Time (days)	Urine	Faeces	Tissues	Total
1	69.35	7.67	3.30	80.31
5	84.48	5.32	1.42	91.23
10	77.07	6.26	0.89	84.21
15	77.17	8.35	0.71	86.22

The spectinomycin related HPLC residue profiles of the urine metabolites were determined in the day 1-5 (on-treatment) urine samples. Eight metabolites were identified by HPLC/APCI (atmospheric pressure chemical ionization) mass spectrometry. Parent drug accounted for approximately 62-64% of the on-treatment urinary residues with all other metabolites being less than 9%. The only major residue identified in kidney was spectinomycin, and in liver the major metabolite was dihydrospectinomycin. For all 16 animals in the study the actual ranges of spectinomycin in kidney were 6.6-15.3% total residues, whereas the maximum amount in liver tissues was less than 4.2% of all residues. The concentration of residues in muscle and fat were too low to allow for meaningful metabolite profiling and identification.

Since the ADI is based on a microbiological endpoint, identification of the metabolites, other than their microbiological activity equivalents is less important.

Table 3. Summary of total residue concentrations (mg/kg) in cattle tissue using a single dose of ^{3}H-spectinomycin subcutaneously daily for five days

Withdrawal Time (days)	Liver	Kidney	Muscle	Fat
1	32.4	59.6	1.03	1.27
5	18.8	14.2	0.36	1.06
10	7.54	4.50	0.36	0.83
15	4.54	2.66	0.29	0.77

Pigs

One new study was reported comparing the plasma pharmacokinetics and bioavailability of spectinomycin sulfate and hydrochloride salts in pigs after a single IM injection of 15 mg spectinomycin free base equivalents/kg BW (Cameron, *et al.*, 1997a). The study used 12 pigs and used a two-week washout period between treatments. Blood samples were collected at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post treatment. The results are summarized in Table 4 and should be compared to data in Table 1. Available data indicate comparable pharmacokinetic results with cattle.

Table 4. Pharmacokinetic data in pigs after IM injection with 15 mg spectinomycin free base equivalents/kg BW

Pharmacokinetic Parameter	Hydrochloride	Sulfate
$AUC_{0-\infty}$ (μg·h/ml)	88.7	107.6
C_{max} (μg/ml)	43.1	47.7
T_{max} (h)	0.40	0.45

Sheep

The pharmacokinetic data in sheep was generated using a similar design as was used for cattle (Craigmill, *et al.*, 1995a). Ten sheep were treated with a single IV and single and multiple IM injection using a dose of 15 mg/kg (5 mg lincomycin + 10 mg spectinomycin/kg BW. The two injections were separated by a three-week washout period. Blood samples were collected before each injection and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours. Results are summarized in Table 5 and data represent mean values (n = 5 animals per group).

The spectinomycin concentration versus time data following IV dosing were fit to a one compartment open model as were the data following the IM administration (with first order kinetics). Spectinomycin was completely bioavailable after IM dosing. Again the data are comparable to the cattle data in Table 1. After multiple dosing of 15 mg spectinomycin for three consecutive days, there were no significant differences in C_{max} values, AUC and accumulation ratios from dose 1 to dose 3. There was no accumulation following multiple dosing based on the accumulation ratios calculated from C_{max} and C_{min}.

Table 5. Pharmacokinetic parameters of spectinomycin in sheep after a single IV dose and a single IM dose with Linco-Spectin®

Pharmacokinetic Parameter	IV Administration	IM Administration
C_{max} (μg/mL)		23.1
t_{max}	-	0.78
$AUC_{0-\infty}$ (μg-h/mL)	71.2	72.7
$t_{1/2}$ (h)	1.34	1.62
MRT (h)	2.1	2.6
F (%) (bioavailability)	-	104

Residue Data

Cattle

Residue depletion was studied using radiolabeled and unlabeled spectinomycin. The radiolabeled study was the same study used to generate some of the pharmacokinetic data in cattle (Hornish, *et al.*, 1996a, 1996b). For this reason, details will not be repeated other than to indicate that 16 animals were used in the study. Total residues and spectinomycin parent drug were determined with parent drug residues reported using an HPLC method. Results are summarized in Table 6. Results are mean values (n = 4) and concentrations are in mg/kg.

Table 6. Total (parent) spectinomycin residues in bovine kidney and liver after five consecutive daily 15 mg/kg BW subcutaneous doses

Withdrawal Time(days)	Kidney	Liver	Muscle	Fat
1	59.6 (9.12)	32.4 (1.36)	1.03	1.27
5	14.2 (1.74)	18.8 (0.58)	0.36	1.06
10	4.50 (0.42)	7.54 (0.20)	0.36	0.83
15	2.66 (0.20)	4.54 (0.14)	0.29	0.77

The linear regression curves for mean residue concentrations of spectinomycin in kidney and liver are shown below:

- Kidney: $y = -0.5774x + 7.3451$ $r = -0.8310$ y = concentration (mg/kg), x = days
- Liver: $y = -0.0841x + 1.2215$ $r = -0.9096$

Two residue depletion studies were reported using unlabeled spectinomycin. The first residue depletion study was conducted with 24 beef cattle treated subcutaneously once daily for five consecutive days with 15 mg/kg BW unlabeled spectinomycin per day with six animals in each group (Hornish, *et al.*, 1996d). Residues are summarized in Table 7 and reported as mean concentrations in mg/kg. The dose regimen is the sponsor's highest recommended treatment dose in cattle. Residues were determined by the sponsor's proposed HPLC method. Fat did not contain detectable concentrations of parent drug (LOQ = 0.10 mg/kg) at any post treatment time. Liver residues declined to below the LOQ by day 15.

Table 7. Residues of parent spectinomycin in bovine tissue after 5 consecutive daily 15 mg/kg BW subcutaneous doses

Withdrawal Time (days)	Kidney	Liver	Muscle	Inj. Site	Fat
5	3.97	0.28	0.23	0.38	<0.10
10	0.95	0.08	0.15	0.14	<0.10
15	0.27	<0.04	0.13	0.20	Not analyzed
20	0.16	<0.04	0.13	0.20	Not analyzed

The regression equations for the mean residue concentrations of spectinomycin are as follows:

- Kidney: $y = -0.2818x + 5.025$ $r = -0.8604$
- Muscle: $y = -0.0064x + 0.24$ $r = -0.8677$

Because the ADI is based on a microbiological endpoint, a study was reported that evaluated the relationship of a sponsor's microbial inhibition assay for residues in liver and kidney to parent drug (by HPLC) and to total residues (Hornish, *et al.*, 1996c). The tissues used were those from a previous pharmacokinetic study. The microbial inhibition assay was measured by a cylinder plate assay that was not very sensitive to either parent drug or its metabolites. The microbiological inhibition assay was modified from a method used to assay feeds and used *E. coli* #UC527. As used, the assay LOQ was about 4 mg/kg. The LOQ limited the method to analysis of day 1 kidney and day 1 and day 5 liver samples only. The chemical assay was an HPLC method recommended by the sponsor for analysis of spectinomycin residues. For kidney, the ratio of microbiological to parent drug was 0.986 and for liver, the corresponding ratio was 5.61 (day 1) and 3.99 (day 5). This result supports the HPLC method for residue analysis in kidney tissue. Since the only major residue found in liver is dihydrospectinomycin, this metabolite is responsible for almost all the activity

found in liver, even though it is less than 10% of the activity of the parent drug (Salmon, *et al.*, 1994). Results support liver residues as spectinomycin equivalents.

As the above study used almost two year old tissue, a repeat study was conducted with fresh incurred residues from 20 treated and 2 control animals (Hornish, *et al.*, 1997a). Results from residues analyzed from five time frames (1, 2, 3, 5, 10 days) gave a ratio of microbial inhibition versus the HPLC assay of 0.89 to 0.97 for kidney, again supporting the HPLC assay for residue analysis. For liver tissues, only one value was determined because residues beyond one day were below the LOQ of the microbiological inhibition assay. The ratio for day 1 residues in liver was 3.62, consistent with the previous study. Results are summarized in Table 8. The ratios reported in Table 8 are an average of the individual ratios (Hornish, *et al.*, 1997a, 1997b).

Table 8. Comparison of residues determined by HPLC and microbiological assays in cattle tissues following five daily doses at 15 mg/kg SC

Withdrawal Times (days)	Kidney			Liver		
	HPLC (mg/kg)	Micro CP (mg/kg)	Ratio MIC/HPLC	HPLC (mg/kg)	Micro CP (mg/kg)	Ratio MIC/HPLC
1	17.90	17.1	0.95	1.18	3.7	3.62
2	9.42	8.7	0.91	0.67	<0 2	n/a
3	6.75	6.1	0.89	0.54	<0 2	n/a
5	4.34	4.2[a]	0.97	0.55	<0 2	n/a
10	1.09	< LOQ	n/a	0.16	<0 2	n/a

a. One sample result was below the LOQ of 4 mg/kg. One-half the LOQ was used to calculate the mean value for the four samples. See Hornish, 1997a, page 38.

Residues in muscle tissue were determined using the sponsors HPLC method only because residues were below the sensitivity of the microbiological inhibition assay. For the five withdrawal periods noted above, the mean residue concentrations in muscle tissue in mg/kg were 0.42, 0.38, 0.34, 0.26 and 0.12, respectively.

The second residue depletion study with unlabeled spectinomycin was carried out using 20 calves with an average weight of 123 ± 7.1kg. The calves (n = 4 per group) were administered Spectam® G.A. as an injectable solution containing 10% spectinomycin free base (as the dihydrochloride salt) at 30 mg/kg BW spectinomycin daily for five days. Animals were sacrificed at 1, 3, 7, 10 and 14 days post treatment. The results are summarized in Table 9 (Guyonnet, 1995a). Residues in fat were below the limit of quantitation (0.25 mg/kg) at all withdrawal times. For liver and kidney the LOQ was 0. 5 mg/kg and for muscle the LOQ was 0.15 mg/kg.

Table 9. Spectinomycin residues (mg/kg) in calf tissues after five daily doses at 30 mg/kg BW

Withdrawal Time (days)	Kidney	Liver	Muscle	Muscle Inj. Site
1	105.94	6.41	1.15	19.17
3	43.05	4.65	0.67	16.76
7	9.55	1.55	0.36	4.15
10	4.18	1.37	0.25	4.33
14	2.75	0.90	0.20	1.31

Milk

One new study on milk was reported (Guyonnet, 1995b). Eight Holstein cows weighing 638 ± 61 kg, ages 2-8 years with milk production of 30-35 liters (n = 4) and 17-22 liters (n = 4) were treated with Spectam® G.A. at 30 mg/kg BW per day as three 10 mg/kg doses daily for five days. Doses were intramuscular in the neck. The LOQ for milk was

reported as 0.10 mg/L. Residues at 48 hours and at later milkings were below the LOQ. Residues are summarized in Table 10 (n = 8) in mg/L. Residues at 48 hours are for four cows.

Table 10. Residues in milk (mg/L) for dairy cows dosed IM at 30 mg/kg BW for five days

Milking Time (h)	Mean	Minimum/Maximum
12	1.59	0.89-2.11
24	0.45	0.21-0.90
48	0.14	0.13-0.16

Pigs

Three residue studies were reported by the sponsors - one with radiolabeled and two with unlabeled spectinomycin. The radiolabeled study used 20 animals (31.2 ± 7.2 kg) with 88 mg/kg medicated feed (containing 44 mg/kg lincomycin and 44 mg/kg spectinomycin) for seven consecutive days (Jaglan, *et al.*, 1994). This feeding regimen provided an average consumption of 2.73 mg/kg BW per day. Average consumption of the medicated feed was 12.9 ± 1.7 kg/day. Excretion was primarily in the faeces (72.3%) and urine (7.2%). Total residues of ^{3}H-spectinomycin free base in tissues in mg/kg (n = 4 animals per group) are summarized in Table 11. Residue concentrations are corrected for tritiated water. Total residues of spectinomycin in the medicated feed treatment are low, consistent with the poor bioavailability (ca. 10%) by this route of administration.

Table 11. Total residues (mg/kg) of ^{3}H-spectinomycin in pigs after 7 days continuous medicated feed treatment

Withdrawal Time (days)	Kidney	Liver	Muscle	Fat
0 (8 h)	0.64	0.21	0	0.16
1	0.46	0.14	0	0.14
3	0.24	0.10	0	0.17
7	0.06	0.06	0	0.17
10	0.02	0.02	0	0.14

A study using 12 pigs administered unlabeled spectinomycin compared residues in kidney and the injection site after a single IM dose of spectinomycin as the sulfate and hydrochloride salt, each at 15 mg/kg BW (Cameron, et al., 1997b). Mean concentrations of spectinomycin residues by the sponsors HPLC method (LOQ = 0.1 mg/kg) were determined at 1, 2 and 5 days post treatment (n = 4 animals per group). These results are summarized in Table 12.

Table 12. Spectinomycin residues (mg/kg) in pigs following a single dose 15 mg/kg BW IM injection[a]

Withdrawal	Spectinomycin hydrochloride		Spectinomycin sulfate	
Time (days)	Kidney	Injection Site	Kidney	Injection Site
1	9.6	4.8	10.7	3.5
2	6.4	3.4	7.3	1.9
5	1.9	0.8	2.3	0.7

a. Residues at the injection site deplete to <0.3 mg/kg by day 5.

The second study was in 20 piglets (4.82 ± 1.13kg, 16 days old) using an oral solution containing 5% spectinomycin free base (as the dihydrochloride salt) with a pump delivery dose of 1 ml containing 50 mg spectinomycin (Guyonnet, 1996). This equates to mean doses of 29.1 mg/kg BW every 12 hours at the beginning of the trial and 25.0 mg/kg BW

every 12 hours at the end of the five day dosing study. Mean residues (mg/kg) are summarized in Table 13 using the sponsor's HPLC method (LOQ = 0.5 mg/kg in kidney and liver; 0.25 mg/kg for fat/skin; and 0.3 mg/kg for muscle).

Table 13. Spectinomycin residues (mg/kg) in piglets dosed orally for five days at 25-29 mg/kg BW

Withdrawal time, days	Kidney	Liver	Muscle	Skin/Fat
1	18.15	2.15	0.64	0.69
3	7.64	1.03	<0.3	0.39
7	4.41	0.84	<0.3	<0.25
10	1.90	<0.5	<0.3	<0.25
14	<0.5	<0.5	<0.3	<0.25

Chickens

Two residue depletion studies were reported. In one study using 7-8 week old broiler chickens (84 chickens, BW = 1412-2057 g) were dosed with Linco-Spectin® soluble powder (100 mg spectinomycin and 50 mg lincomycin per kg BW) in the drinking water (Cameron, *et al.*, 1996, Nappier, et al., undated). Groups of 12 birds were sacrificed at 0, 6, and 12 hours, 1, 2, 4 and 8 days post treatment. Each data point is a two bird composite (n = 6 sampling units per sampling time). Results are reported in mg/kg as mean values. The authors noted that there was an interfering peak in analyzing liver samples for spectinomycin using the proposed HPLC method (LOQ = 0.1 mg/kg). Residues in liver are reported using a modified version of the original method (Nappier, et al., undated). Results are summarized in Table 14.

Table 14 Residues of spectinomycin (mg/kg) in broiler chickens after oral administration of 100 mg/kg BW spectinomycin and 50 mg/kg BW lincomycin for seven days

Withdrawal time	Kidney	Liver	Muscle	Skin plus underlying fat
0 hour	2.0	0.43	0.5	2.9
6 hour	4.2	0.38	0.3	1.7
12 hour	1.0	0.27	0.3	1.3
1 day	0.6	0.22	0.1	0.7
2 days	0.7	0.12	0.1	0.5
4 days	<0.1	<0.1	<0.1	0.2
8 days	<0.1	<0.1	<0.1	0.3

The second residue study used 36 broiler chickens (859-1255 g, 44 days old) treated five days with 50 mg/kg BW of spectinomycin (Guyonnet, 1997). The drug was administered as an oral powder containing 50% spectinomycin, mixed into the drinking water. The birds were sacrificed at days 1, 4, 7, 11 and 14 post treatment. No spectinomycin residues could be quantified in any sample at any of the withdrawal times based on the sponsor's LOQ of 0.5 mg/kg in liver and kidney; 0.3 mg/kg in muscle and 0.25 mg/kg in fat.

These two data sets show relatively lower amounts of residues in edible tissues compared with residues from subcutaneous treatments, consistent with oral dosing in other food animal species.

Eggs

No new data were reported for eggs, however, a study was referenced in the 42nd meeting of the Committee (Keppens and DeSutter, 1992). In that study, birds received four different treatments for seven days. Three treatments were 1:1 mixtures of spectinomycin-lincomycin at 440, 330 and 220 mg/kg in feed. The fourth treatment was 0.5 g/L (0.333 g/L spectinomycin and 0.167 g/L lincomycin) in water. No residues of spectinomycin were detected in eggs from any treatment group either the last two days of treatment or three consecutive days following withdrawal of drug. The sensitivity (limit of determination) of the microbiological method was 2 mg/L.

Sheep

Two new studies were reported in sheep. One new study (Craigmill, *et al.*, 1995b) using twenty crossbred sheep (57-87.5 kg) that were injected IM once daily for three consecutive days at the recommended dose level of 15 mg/kg BW (10 mg/kg spectinomycin and 5 mg/kg lincomycin). Groups of five sheep were sacrificed at 8 hours, 7, 14 and 21 days post treatment. Results (mg/kg) are summarized as mean values in Table 15. The detection limit of the HPLC method was 0.04 mg/kg.

Table 15. Tissue residues of spectinomycin (mg/kg) in sheep following multiple IM injections at 15 mg/kg BW (10 mg/kg BWspectinomycin and 5 mg/kg BW lincomycin)

Withdrawal Time	Kidney	Liver	Muscle	Fat	Injection Site
8 hours	11.99	0.63	0.29	0.19	4.56
7 days	0.51	0.10	<0.04	<0.04	0.08
14 days	0.10	0.08	<0.04	<0.04	<0.04
21 days	<0.04	<0.04	<0.04	<0.04	<0.04

The linear regression curve for mean residues of spectinomycin in kidney is shown below:

$$\text{Kidney: } y = -0.5166x + 8.5542 \quad r = -0.7746$$

The second study employed 24 sheep (43.9 ± 3.4 kg, ages 6-7 month) treated with 30 mg/kg spectinomycin BW two times per day by IM injection for five days (Guyonnet, 1995c). Withdrawal times were 1, 3, 7, 10, 14 and 18 days. Mean residue concentrations (n = 4 animals per group) are summarized in Table 16. For this study, the LOQs are 0.5 mg/kg in liver and kidney; 0.15 mg/kg for muscle; and 0.25 mg/kg for fat.

Table 16. Spectinomycin residues (mg/kg) in sheep following IM injections of 30 mg/kg BW twice daily for 5 days

Withdrawal Time (days)	Kidney	Liver	Muscle	Muscle (Inj. Site)	Fat
1	99.96	4.78	0.43	16.30	0.41
3	47.42	3.18	0.25	4.09	<0.25
7	10.31	1.24	<0.15	2.25	<0.25
10	3.89	0.90	<0.15	0.86	<0.25
14	1.75	0.83	<0.15	0.46	<0.25
18	0.78	<0.5	<0.15	0.17	<0.25

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

One sponsor has developed and reported on the performance assessment and validation of their quantitative method for determining residues of spectinomycin in food animal tissue (Jaglan and Haagsma, 1993, Haagsma, 1995, Weist and Hornish, 1995a). The method involves isolation involving solvent extraction and solid phase extraction clean-up using a citrate buffer solution followed by HPLC analysis. The HPLC procedure employs a one-column gradient elution procedure with post column oxidation using hypochlorite solution and derivatization with o-phthalaldehyde to form fluorescent derivatives for detection of spectinomycin. The method was evaluated for potential interferences with seven other antibiotics: ceftiofur, erythromycin, lincomycin, neomycin, penicillin G, sulfadimethoxine and tylosin. No interferences were noted.

Results of the method validation (concentration limits of 0.10-10.0 mg/kg) demonstrated that there was acceptable day-to-day variability, acceptable recoveries in all tissues, and the method has a limit of quantification (LOQ) of 0.10 mg/kg. Specifically, in kidney the recoveries were 81-87% with a Coefficient of Variation (CV) of 6.2%; in liver, recoveries were 85-90%; in muscle and fat, recoveries were 89-93%. For kidney, the target tissue, the LOQ was validated at 0.1 mg/kg with a recovery of 87.2 ± 17.0% (CV=19.5%). Day-to-day variability in kidney was from 2.2-

9.8% at 2.5-10.0 mg/kg. The quantitative performance was successfully compared in bovine kidney samples obtained from fortified control tissue and from biologically incurred residue tissue. Two laboratories were involved in the method performance studies. The Haagsma-based HPLC method (Weist and Hornish, 1995a) was compared with the original method developed by Haagsma (Jaglan and Haagsma, 1993, Haagsma, 1995) and found to be less repeatable over different days, but both methods give analytical results that were not statistically different. The sponsors concluded that with 95% confidence, the two methods provided the same average analytical results for spectinomycin in bovine kidney within 5.6% ± 4.6% (Hornish, *et al.*, 1996e). A set of 12 samples can be processed in 6-8 hours.

The sponsor for the method described above noted the HPLC method is applicable to and has been validated for all tissues, however, the potential exists in some liver samples for interference from dihydrospectinomycin, the major metabolite, and an endogenous liver component. To address this potential interference, a minor modification in the chromatography is suggested (Hornish and Weist, 1997b). The modification for liver samples employs a reverse phase separation rather than an ion-exchange separation to completely resolve parent spectinomycin from the interferences for accurate quantification. The sample preparation and the HPLC post-column detection system remained the same for all tissues.

The second sponsor also has reported an HPLC method as well for spectinomycin (Guyonnet, 1995a, p38, *et seq.*). The procedure involves homogenization, followed by liquid-liquid extraction, elution using a C_{18}-solid phase extraction cartridge, derivatization with 2,4-diphenylhydrazine, and quantification by HPLC on a C_{18}-column using ultraviolet detection at 405 nm (except in chicken tissue where 465 nm was used). Performance was determined by an intralaboratory one analyst procedure. Although no ruggedness testing was indicated, interference was checked against lincomycin, gentamicin and trospectinomycin. For kidney and liver, performance testing was evaluated using fortified tissues at 0.5-20 mg/kg; in muscle, 0.15-5.0 mg/kg; in fat, 0.25-10 mg/kg and in milk, 0.1-10 mg/L. Recoveries in muscle, kidney and liver were 72-80%; in milk, 85-92%; and in fat, 80-90%. The limit of quantification in kidney and liver was 0.5 mg/kg; in fat, 0.25 mg/kg; in milk, 0.1 mg/L; in cattle and sheep muscle, 0.15 mg/kg; in chicken muscle, 0.25 mg/kg; and in pig muscle, 0.3 mg/kg. The LOQ's were determined using a CV of 15%. Though the method was indicated as being suitable as a regulatory method for routine analysis, there was no indication of the number of samples that could be analyzed per day.

A confirmatory method was also reported by one sponsor (Wiest and Hornish, 1995b). It is a two-dimensional method that employs HPLC and atmospheric pressure chemical ionization (APCI) collision induced dissociation (CID) mass spectrometry (MS/MS). Three criteria were met for confirmation: the signal-to-noise ratio for four reaction product ions at m/z 98, 116, 158 and 189 (derived from the m/z 333 for the protonated ion of spectinomycin) were greater than 3-to-1; an HPLC retention time of spectinomycin in the tissue sample within ± 2 minutes relative to an external spectinomycin standard; and a relative abundance of four reaction products ions in the sample within ±10% relative to the external standard. The method was shown to confirm spectinomycin residues to a lower limit concentration of 0.05 mg/kg. Routine confirmation for bovine kidney is 0.1-10 mg/kg, and for other tissues, concentrations of 0.05-1 mg/kg.

APPRAISAL

Spectinomycin was reviewed previously by the 42nd meeting of the Committee at which time a full ADI was established (0-40 μg/kg) based on a microbiological endpoint. Temporary MRL's were recommended for cattle, pig and chickens in kidney, liver, muscle, fat, and cattle milk as parent drug. The MRL's were designated as temporary because many of the residue depletion data were either from interim progress reports or from pilot studies.

The two sponsors have submitted new studies that provide data on pharmacokinetics in cattle, pigs and sheep; residue depletion data for cattle, pigs, chickens, sheep, and milk in cattle; studies to support adding MRL's in sheep; and studies to propose new MRL's in muscle and fat. Information was provided on two residue methods for quantification, including validation data on one of the quantitative methods, and a confirmatory method. A study comparing the microbiological inhibition and chemical assay methods also was provided.

Pharmacokinetic Studies

Pharmacokinetic studies in cattle and sheep using radiolabeled spectinomycin at 10 mg/kg BW as an injectable solution show nearly identical pharmacokinetic parameters, including rapid uptake of the drug and complete bioavailability. There was little difference between intramuscular or subcutaneous treatments. In a full disposition study in cattle given 15 mg/kg BW radiolabeled spectinomycin subcutaneously daily for five days, more than 90 percent of the excreted radioactivity was eliminated within 24 hours after the last dose. The terminal elimination phase of plasma residues had a half-life of about 8 days. Residues from urine, faeces and tissues accounted for 80-91% of the dose with urine accounting for 69-84% of the total, faeces 5-8% and tissues 1-3%. The residues in tissue for 1-15 days post treatment

are highest in kidney and liver, with muscle and fat having much lower amounts. Approximately 85-90% of the residues are found in liver and kidney.

Cattle Eight spectinomycin metabolites were identified by HPLC/mass spectrometry in the urine from a radiolabeled calf study using 16 animals. Parent drug accounted for 62-64% of the on-treatment urinary residues with all other metabolites being less than 9% each. The major residue identified in kidney was spectinomycin, and in liver the major metabolite was dihydrospectinomycin. The actual amounts of spectinomycin in kidney were 6.6-15.3% of the total residues, whereas the maximum amount in liver tissues was less than 4.2% of all residues. The concentration of residues in muscle and fat were too low to allow meaningful metabolite profiling and identification. Since the ADI is based on a microbiological endpoint, identification of the metabolites other than their microbiological activity equivalents is less important. These studies support the studies reviewed at the 42nd meeting of the Committee, identifying the kidney as the target tissue and spectinomycin as the marker residue.

Pigs Pharmacokinetic studies in pigs given 15 mg/kg BW spectinomycin free base equivalents as either the hydrochloride or sulfate salt by intramuscular injection gave comparable but somewhat higher area-under-the-curve values and shorter times to maximum concentration in plasma than in cattle indicating a more rapid absorption in pigs.

A radiolabeled study in pigs using 88 mg/kg medicated feed (1:1 ratio of lincomycin and spectinomycin) for seven days was reported. This feeding regimen provided an average daily consumption of 2.73 mg/kg BW. Excretion of radioactivity was mainly in the faeces (72.3%) and urine (7.2%). Total residues in kidney at day 1 were 0.46 mg/kg and residues declined to 0.02 mg/kg on day 10. In liver tissue, the total residues at day 1 were 0.21 mg/kg and declined to 0.02 mg/kg at day 10. No residues were detectable in muscle tissue after correcting for tritiated water. Values in fat remained constant between days 1-10 at about 0.15 mg/kg. Total residues were low, consistent with the poor bioavailability by this route of administration.

Sheep The pharmacokinetic data in sheep were generated using a design that was used for cattle. Pharmacokinetic parameters were nearly identical by either subcutaneous or intramuscular injection using a dose of 15 mg/kg BW. Spectinomycin was bioavailable and after single doses for three consecutive days there was no significant difference in the pharmacokinetic parameters measured and also no indication of accumulation of residues from day 1 to day 3.

Residue Depletion Studies

Cattle Three residue depletion studies in cattle were reported - one using radiolabeled drug and two with unlabeled spectinomycin. In the radiolabeled (s.c.) study using 15 mg/kg BW, residues were determined at days 1, 5, 10 and 15 post treatment. Total residues in kidney depleted from 59.6 mg/kg on day 1 to 2.66 mg/kg on day 15. Spectinomycin residues in kidney by the HPLC method [limit of quantification (LOQ) was 0.1 mg/kg] were 9.12 mg/kg and 0.20 mg/kg on day 1 and 15, respectively. For liver, total residues on day 1 were 32.8 mg/kg and declined to 4.54 mg/kg on day 15. Spectinomycin residues were 1.36 mg/kg on day 1 and 0.14 mg/kg on day 15. Only total residues in muscle and fat could be determined. In muscle, total residues on day 1 were 1.03 mg/kg and on day 15 they were 0.29 mg/kg. The corresponding total residues in fat were 1.27 mg/kg on day 1 and 0.77 mg/kg on day 15. The unlabeled spectinomycin study using 24 animals treated with five consecutive daily subcutaneous doses of 15 mg/kg BW gave similar amounts of spectinomycin residues in all four tissues. The second study using 30 mg/kg BW unlabeled spectinomycin per day in calves by injection for five days gave higher residues. In kidney, spectinomycin residues on day 1 were 106 mg/kg, declining to 2.75 mg/kg on day 14. In liver, residues on day 1 and day 14 were 6.41 mg/kg and 0.90 mg/kg, respectively. For muscle, residues on day 1 and day 14 were 1.15 mg/kg and 0.20 mg/kg, respectively; residues in fat were below the limit of quantification.

Because the ADI is based on a microbiological endpoint, one sponsor reported two studies that evaluated the relationship of the microbiological inhibition assay with their HPLC method in liver and kidney. The microbiological analysis used a cylinder plate assay that was not very sensitive to spectinomycin (LOQ was 4 mg/kg), while the HPLC assay had a reported limit of quantitation of 0.1 mg/kg. For kidney the ratio of the microbiological assay value to the HPLC assay was approximately 0.98; for liver the ratio was approximately 3.6. This result supports the HPLC method for residue analysis in kidney tissue. Since the only microbiologically active residue in liver is dihydrospectinomycin, this metabolite is responsible for almost all the activity found in liver, even though it is less than 10% of the microbiological activity of parent drug.

One new study in lactating cows using three 10 mg/kg BW spectinomycin injections per day for five days confirmed study results from the 42nd meeting of the Committee. Residues depleted to below the limit of quantification (0.10 mg/L) at 48 hour post treatment milkings.

Two residue studies were reported in pigs using unlabeled drug. In one study using 12 pigs residues were determined following a single intramuscular injection of 15 mg/kg BW. of spectinomycin hydrochloride and spectinomycin sulfate. Residues using the hydrochloride salt on day 1 in kidney were 9.6 mg/kg and declined to 1.9 mg/kg on day 5; at the injection site residues declined from 4.8 mg/kg on day 1 to 0.8 mg/kg on day 5. With the sulfate salt, corresponding residues in kidney on day 1 were 10.7 mg/kg and declined to 2.3 mg/kg on day 5; injection site residues on day 1 and day 5 were 3.5 mg/kg and 0.7 mg/kg. The second study used an oral solution of spectinomycin that ranged from 29-25 mg/kg BW twice daily for five days. Residues in kidney on day 1 were 18.15 mg/kg and declined to less than 0.5 mg/kg on day 14. In liver the residues on day 1 were 2.15 mg/kg and declined to less than 0.5 mg/kg on day 10. Residues in muscle tissue were 0.64 mg/kg on day 1 and less than 0.3 mg/kg on day 3. For fat, residues were 0.69 mg/kg on day 1 and less than 0.25 mg/kg on day 7.

One residue depletion study was reported using 7-8 week old broiler chickens using an oral solution of 100 mg spectinomycin and 50 mg lincomycin per kg BW in the drinking water for seven days. Residues in kidney declined from 2.0 mg/kg at time of withdrawal (0 h) to less than 0.1 mg/kg on day 4. Corresponding residues in liver declined from 0.43 mg/kg at 0 h to less than 0.1 mg/kg on day 4. Residues in muscle were almost identical to residues in liver at all time points. Residues in skin and adhering fat were 2.9 mg/kg at the time of withdrawal, declining to 0.3 mg/kg on day 8. In the second residue study, broiler chickens were treated five days with 50 mg/kg BW of spectinomycin. No spectinomycin residues could be quantified in any sample using the sponsor's method (limit of quantification was 0.5 mg/kg in kidney and liver, 0.3 mg/kg in muscle and 0.25 mg/kg in fat) on day 1 withdrawal. These residue amounts are consistent with other food animal species with relatively low residues from oral administration of spectinomycin.

No new studies were reported for eggs, however, a study was reviewed by the 42nd meeting of the Committee. In that study, laying birds were treated with four different doses of 1:1 mixture of spectinomycin and lincomycin at 440, 330 and 220 mg/kg in feed and a drinking water treatment using a 2:1 mixture of spectinomycin and lincomycin at 0.5 g/L. No residues were detected in eggs in any group during the last two days of treatment and three consecutive days following withdrawal. Residues were assayed using a method with a limit of quantification of 2 mg/kg.

In sheep, two new studies were reported. In the first study, animals were treated for three days by intramuscular injection at 15 mg/kg BW per day using a 2:1 mixture of spectinomycin and lincomycin. Residues in kidney declined from 12.0 mg/kg at 8 hours withdrawal to 0.1 mg/kg on day 14. In liver, residues declined from 0.63 mg/kg at 8 hours to 0.08 mg/kg on day 14. Residues in muscle were 0.29 mg/kg at 8 hours withdrawal and less than 0.04 mg/kg on day 7 withdrawal. Residues in fat were 0.19 mg/kg at 8 hours withdrawal and less than 0.04 mg/kg on 7 days withdrawal. The second study treated the animals with 30 mg/kg BW of spectinomycin two times per day by intramuscular injection for five days. Residues in kidney were 100 mg/kg on day 1 and declined to 0.78 mg/kg on day 18. Corresponding residues in liver were 4.78 mg/kg on day 1 and less than 0.5 mg/kg on day 18. For muscle, residues declined from 0.43 mg/kg on day 1 to less than 0.15 mg/kg on day 7. In fat residues were 0.41 mg/kg on day 1 and less than 0.25 mg/kg on day 3.

Analytical Methods

One sponsor reported results of the method performance trials for two quantitative and one confirmatory method. The preferred quantitative method from one sponsor involves solvent extraction and solid phase extraction followed by an HPLC procedure employing a gradient elution for separation with post column oxidation and derivatization to allow fluorescence detection. The method did not show any potential interference with seven other antibiotics examined. The method was performance tested in two laboratories using fortified and incurred residue samples over a concentration range of 0.1-10 mg/kg in kidney, 0.1-5 mg/kg in liver, and 0.1-1.0 mg/kg in muscle and fat. Performance values for recovery, day-to-day variability and limit of quantification were evaluated. Recoveries in all tissues were greater than 80%. The Coefficient of Variation for all tissues and species evaluated were less than 20%, even at the limit of quantification of 0.1 mg/kg. A set of 12 samples can be analyzed in 6-8 hours. The second method developed by this sponsor was not as reliable as the above method; however, with 95% confidence the two methods provided the same average analytical results for spectinomycin in bovine kidney within 5.6%. This sponsor's confirmatory method was based on atmospheric pressure chemical ionization (APCI) collision induced dissociation (CID) mass spectrometry. Criteria for confirmation included satisfactory ratios for four product ions, HPLC retention time and relative abundance of the reaction product ions compared to an external standard of spectinomycin. These methods are satisfactory for residue analysis.

The second sponsor also reported an HPLC method for quantification of spectinomycin residues using a similar extraction and isolation procedure, derivatization and quantification by HPLC using ultraviolet detection. Performance in all tissues and species noted above was determined in one laboratory. Although no study was reported on optimizing performance of the method, no interference was noted when evaluated with three other antimicrobial drugs. Recoveries in all species and tissues as well as Coefficients of Variation were satisfactory for routine residue analysis, however, the limits of quantification were higher than with the HPLC method noted above. There was no indication of the number of

samples that could be analyzed in one day. The method may be suitable for routine analysis depending on the analytical equipment available.

Maximum Residue Limits

The new pharmacokinetic and residue depletion studies in cattle, pigs, sheep and chickens verify data from the 42nd meeting of the Committee, indicating that kidney is the target tissue and spectinomycin is the marker residue. However, considering the practical limitations of collecting kidney tissue from chickens for residue analysis, skin/adhering fat may be the more appropriate target tissue in chickens.

In reaching its decision on MRLs, the Committee took into account the following information:

- Based on the ADI of 0-40 μg/kg BW for spectinomycin that was established at the 42nd meeting of the Committee, the allowable daily intake of spectinomycin is 2400 μg for a 60-kg person.
- The ADI is based on a microbiological and point.
- The only microbiologically active residues are parent drug and dihydrospectinomycin.
- Parent drug is the only microbiologically active residue in muscle, kidney and fat.
- Dihydrospectinomycin is the major microbiologically active residue in liver.
- The ratio of microbiological activity in liver compared to HPLC-determined spectinomycin is approximately four to one.
- Dihydrospectinomycin microbiological activity is approximately 10% of parent drug.

The Committee recommended the following MRL's:

For cattle, sheep, pigs and chickens: muscle, 500 μg/kg; liver, 2000 μg/kg; kidney, 5000 μg/kg; and fat, 2000 μg/kg. The recommended MRL for cattle milk is 200 μg/L and for eggs, 2000 μg/kg. Residues are expressed as parent drug.

Using these values, the theoretical maximum daily intake of spectinomycin residues is 1800 μg.

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