Apramycin

First draft prepared by
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Identity

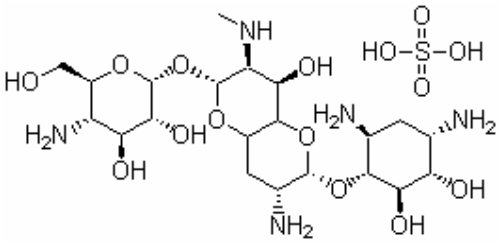
International Non-proprietary names (INN): Apramycin

Synonyms: Laboratory Code EL-820 (EL-857, free base). Compound 47657.
Apralan® is the registered trademark for Elanco® products containing apramycin

IUPAC Names: D-Streptamine, 4-0- [(8R)-2-amino-8-0-(4-amino-4-deoxy-a-D-glucopyranosyl)-2, 3, 7-trideoxy-7-(methylamino)-D-glycero-a-D-allo-octodialdo-1,5: 8, 4-dipyranos-1-yl] -2-deoxy-sulphuric acid salt

Chemical Abstracts Service Number: 41194-16-5

Structural formula of main components:



Molecular formula: $C_{21}H_{41}N_5O_{11}$. 5/2 H_2SO_4

Molecular weight of the salt: 784.8

Molecular weight of the base: 539.6

Other information on identity and properties

Pure active ingredient

Apramycin is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. It is extracted from the fermentation medium as apramycin sulphate with a purity of at least 85%. A microbiological assay is used to determine activity as equivalents of apramycin base. Apramycin is synthesized stereospecifically by *Streptomyces tenebrarius*. It exists as a single enantiomer for which absolute configurations have been determined.

Degree of impurity of Apramycin (produced by Elanco Animal Health)

Total impurities described below are not to exceed 15% (specifications for the release of the product).

Qualitative and quantitative composition of impurities

3 O-Hydroxyapramycin ($C_{21}H_{41}N_5O_{12}$) has a biological spectrum which is very similar to apramycin; microbiological activity is one-half to one-quarter that of apramycin.

Lividimine ($C_{21}H_{41}N_5O_{12}$) is structurally related to apramycin, containing 2-deoxystreptamine and part of the bicyclic portion of the apramycin molecule. Its contribution to the biological activity of apramycin is negligible.

2-Deoxystreptamine ($C_6H_{14}N_2O_3$) is a structural unit of most common aminocyclitol antibiotics, and is biologically inactive.

Compounds A and B are defined on the basis of their thin-layer chromatography. These compounds have not been identified.

Caerulomycin ($C_{12}H_{11}N_3O_3$) is determined as a dipyridyl, as the dipyridyl moiety is a more suitable measurement for the caerulomycin content since a part may be complexed or bound.

Solubility: >300 g/L in water.

Residues in food and their evaluation

Conditions of use

The Committee evaluated apramycin to establish an ADI and recommend MRLs in cattle, pig and chicken tissues at the request of the 19th session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The request for MRLs in rabbits was not addressed as there was no approved use of apramycin in rabbits.

Dosage

Indications, recommended doses and duration of treatment for apramycin are presented in Table 2.3, below, in the tissue residue depletion studies section. An injectable form for use in cattle and an oral dose form for use in neonatal lambs, calves and pigs are no longer marketed. Apramycin is not used in human medicine.

Registered uses

Apramycin is registered for use in more than twenty countries in cattle, pigs and chickens. Individual country withdrawal periods for the three species exhibit substantial differences. In cattle, withdrawal periods vary between zero and 42 days. For pigs, the withdrawal periods are 10 to 42 days, and for chickens the withdrawal periods are zero to 14 days.

Pharmacokinetics and metabolism

Pharmacokinetics in food-producing animals

Cattle

Calves weighing 38 to 52 kg were administered 20 or 40 mg apramycin activity/kg bw in milk replacer once per day for 5 days (3 male and 3 female calves per dose level) (Van Duyn and Handy, 1978). Following the first, third and fifth doses, blood samples were analysed for apramycin by a microbiological assay up to 24 h post-treatment for the first and third treatments and until 48 h following the fifth treatment. Serum concentrations were broadly similar for each treatment level in both dosages. With the 20 mg/kg bw dose, serum levels peaked at 4 to 6 h after treatment, with mean values from 0.38 to 0.88 µg/ml. Values at 24 h after treatment ranged from undetectable to 0.13 µg/ml. After the fifth and last dose, apramycin serum levels were undetectable 24 h post treatment. After the 40 mg/kg bw dose, serum levels of apramycin were higher than those for the 20 mg/kg treatment. Serum levels peaked at approximately 6 h after treatment, with mean values of 2.49 and 2.40 µg/ml for the first and fifth daily doses, respectively. Mean values 24 h after treatment ranged from 0.15-0.31 µg/ml and duration of activity following the fifth and last dose was between 24 and 36 h.

Male and female Israeli-Friesian calves, 3 to 5 weeks of age, were fed an antibiotic-free milk replacer once a day (Ziv *et al.*, 1985). Apramycin was administered orally as a drench at 20, 30 and 40 mg/kg bw to 10, 9 and 10 calves, respectively. Blood samples were taken 0.5, 1, 2, 3, 4, 6, 8 and 12 h after treatment. Six calves were placed in metabolism cages that facilitated total collection of urine and were administered 20 mg/kg bw apramycin in milk replacer. Maximum serum levels occurred 1 hour after administration. The oral dose of 20 mg/kg bw resulted in mean serum drug levels lower than 0.25 µg/ml and the drug was not detected 12 h post-treatment. After oral dosing of 30 and 40 mg/kg bw, mean peak serum drug concentrations were 1.42 and 1.84 µg/ml, respectively.

Apramycin is very poorly absorbed by the oral route in calves. Even where the area under the serum curves was not reported, it is evident from inspection of the curves that availability of apramycin by the oral route was low. Concentration of apramycin in urine was determined by a microbiological assay. Urine concentrations were very low, i.e. 2.5 µg/ml, but the drug was still detected at 128 h post-treatment. Only 11% of the dose was recovered in urine.

Pigs

A series of studies was conducted in which pigs were administered single doses of apramycin sulphate in aqueous solution by oral gavage and blood levels measured during the next 24 h (Van Duyn and Kline, undated). The pigs ranged in weight from approximately 1.3 to 21 kg and doses ranged from 2.27 to 100 mg apramycin activity per kg bw. Blood levels, determined by a microbiological assay, were low and highly variable. Generally, where measurable levels were found, they peaked between 30 minutes and 4 h after dosing, and declined to below the sensitivity of the assay within 12 to 24 h. In one of the studies, blood levels were compared in 2-day, 4- and 8-week old piglets after dosing with the same (mg/kg bw) apramycin doses. Absorption of apramycin appeared to be most efficient in neonatal pigs, and to decline with age.

Another study (Shang *et al.,* 2004) reported pharmacokinetics of apramycin after single oral or intravenous (i.v.) dosing of pigs with 20 mg apramycin/kg bw. Blood levels fitted a one-compartment open model and a two-compartment open model after oral and i.v. dosing, respectively. For oral treatment, the half-life of elimination was 7.36 h and area under the curve was 4.14, while for i.v. treatment, the half-life of elimination was 3.17 h and area under the curve was 130.62. Oral availability was 3.2%.

One female and two castrated male pigs, weighing approximately 10 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days (Zornes, Herberg and Donoho, 1979), and 82% to 92% of the dose was recovered in excreta, mostly in faeces. Less than 10% of the dose was found in urine.

Two female and four castrated male pigs, weighing approximately 8 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days (Herberg *et al.,* 1979). Groups of 1 female and 2 castrated males were slaughtered 4 h or 7 days after the last dose. Balance-excretion data were

collected for the female in the 7-day withdrawal group; 86.9% of the dose was recovered in excreta, of which 81.6% in faeces and 5.3% in urine.

Chickens

Five chickens were administered a single dose of 75 mg/kg apramycin orally into the crop and another five were administered the same dose intramuscularly (i.m.) (Afifi and Ramadan, 1997). Two weeks later, all of the chickens were administered 75 mg/kg i.v. to study drug availability and protein binding. Apramycin was rapidly absorbed when it was administered orally or i.m.; mean times to reach maximum serum concentration after the dose were 0.2 and 0.76 h, respectively. Average half lives for elimination were 1.22 and 2.31 h for the same respective treatments. The maximum concentration reached after oral treatment was only 0.79 µg/ml, compared with 11.6 µg/ml for i.m. treatment, and the respective areas under the curve were 0.81 µg/h/ml and 23.18 µg/h/ml. Bio-availability of apramycin was approximately 2% by the oral route and 58% by the i.m. route.

No excreta data were presented for chickens.

Pharmacokinetics in other species

Rats

Adult rats were given single oral or s.c. doses of 4 mg ^{14}C-apramycin (Donoho *et al.*, 1976). Following oral treatment, 99.5% of the dose was recovered in faeces and 0.5% in urine, while 93% was recovered in urine and 7% in faeces after s.c. dosing.

Dogs

Male and female Beagle dogs with initial weights of 7.2 to 14.6 kg were administered apramycin at 0, 25, 50 or 100 mg/kg bw/day for 6 months (Howard *et al.,* 1977). Dogs were dosed orally with apramycin in gelatin capsules. Blood samples, collected from 2 males and 2 females per treatment group on days 1, 64, 119 and 182 of the study, were analysed for apramycin using a microbiological assay. Mean peak serum levels occurred 2 h after dosing and were 2.2, 5.5 and 11.0 µg/ml for the 25, 50 and 100 mg/kg bw treatments, respectively. All samples collected 24 h after dosing contained <0.5 µg/ml. There was no evidence of accumulation or altered plasma concentrations after prolonged treatment. Urine voided over a 24-hour period was collected from 2 males and 2 females per treatment group on test days 1, 64, 119 and 182 and analysed for apramycin using a microbiological assay. The average proportion of the daily dose that was recovered in urine was 4.0% (standard deviation = 2.6%) with a range of 0.3 to 10.5%.

Summary of pharmacokinetic studies

In calves, pigs, chickens, rats and dogs, apramycin is rapidly yet poorly absorbed by the oral route and quickly eliminated. Mean levels in serum are found a few hours after treatment (up to 6 h) and undetectable at times between 24 and 36 h, depending on the study. Reported availability is poor (3.2% in pigs; < 2% in chickens). More than 80% is primarily recovered in excreta in pigs and rats. The main excreta is in faeces for the mentioned species (more than 82% in pigs and 99.5% in rats), with very low concentrations in urine (11% for cattle; less than 10% for pigs, rats and dogs). Binding of apramycin to serum proteins was 26%, similar to the value found for cows, sheep and goats by Ziv *et al.* (1995).

Metabolism in food-producing animals

Cattle

A calf of approximately 40 kg body weight was given 20 mg/kg ^{14}C-apramycin by i.m. injection on two successive days (Donoho *et al.,* 1976). Total radiolabel dose of apramycin for the calf was 18 µCi. The calf was slaughtered 5 days after the last dose, with the bulk of the radioactivity collected by the end of day 2. Kidney contained the most radioactivity (40.9 mg apramycin equivalent/kg), followed by liver (4.7 mg/kg) and muscle (0.3 mg/kg); fat contained no radioactivity above background. Residues determined by the microbiological assay were compared with the radiolabel results, and 71% of the radioactivity in kidney and 62% of radioactivity in liver was un-metabolized apramycin as determined by the microbiological assay. In blood, levels of radioactivity reached a peak at approximately 30

minutes after injection (maximum concentration was approximately 35 mg/kg-equivalents) and declined to near baseline levels by 24 h. Of the total radioactive dose administered, 84% was recovered in excreta by the end of 5 days following the last dose. More than 93% of the excreted radioactivity was found in the urine within 24 h, and microbiological assay indicated that 90–91% the radioactivity was accounted for as apramycin.

Pigs

A 1979 two-part radiolabel study was conducted by Herberg *et al*. (1979) and Zornes, Herberg and Donoho (1979). In the first part, six piglets weighing approximately 8 kg each were given 25 mg ^{14}C-apramycin/kg by oral gavage daily for 5 days (Herberg *et al.,* 1979). One female and two castrated males were slaughtered at 4 h withdrawal and a similar group was terminated at 7 days withdrawal. Edible tissues were assayed for total radioactivity and selected samples were assayed for apramycin. At 4 h withdrawal, all of the radioactivity in kidney samples was characterized as apramycin After 7 days withdrawal, 80% of the radioactivity in one kidney sample was apramycin, while the other samples contained too little residue for analysis. At 4 h withdrawal, liver samples of the two male piglets contained approximately 85% of unchanged apramycin, while the liver from the female contained at least 50% of apramycin. None of the other tissues contained sufficient radioactivity for apramycin characterization.

In the second part of the study, one female and two castrated male piglets, weighing approximately 10 kg, were given 25 mg ^{14}C-apramycin/kg bw by oral gavage daily for 5 days; they were slaughtered 14 days after the last dose (Zornes, Herberg and Donoho, 1979). Approximately 66% of the kidney residue was unchanged apramycin. In the one liver sample that contained enough residue for characterization, approximately 36% of the total residue was apramycin. In both the faeces and the urine collected, radioactivity was predominantly accounted for by unchanged apramycin (75%)

Chickens

Broiler chickens that were approximately 4 weeks old were given ^{14}C-apramycin (500 mg/L) in the drinking water for five days (Zornes, Herberg and Thomson, 1985). Three chickens were slaughtered 6 h, 7, 10 and 14 days after withdrawal from treatment. Samples with sufficient total activity (all kidney samples and the 6-hour liver samples) were analysed for parent apramycin by bio-autography. Apramycin accounted for 80% or more of total activity in all of the samples.

Summary of metabolism studies

In cattle, pigs and chicken, levels of radioactivity are highest in kidney, followed by liver, with very low quantities in muscle and fat. Little biotransformation occurs; the drug remains in tissues mostly as unchanged apramycin. In general, tissues contained insufficient residues for characterization.

In calf studies, 71% of the radioactivity in kidney and 62% in liver were unchanged apramycin. In pigs, apramycin in kidney ranged from 66–80% of the radioactivity and 85% in the liver samples tested, with exception of two animals where levels of 50% and 36% were found. In chickens, in kidney and liver samples analysed, unchanged apramycin accounted for 80% or more of total activity. It is excreted mainly as unmetabolized apramycin in cattle (>90%) and pigs (75%).

Tissue residue depletion studies

Radiolabelled residue depletion studies

Pigs

In radiolabel depletion studies, sampling in the first part was at 4 h and 7 days with six piglets weighing about 8 kg (Herberg *et al.,* 1979), and in the second part, sampling was only done at 14 days, with 6 piglets weighing about 10 kg (Zornes, Herberg and Donoho, 1979). Analysis of fortified ^{14}C apramycin for all control tissues fortified at 0.5, 0.1 and 0.05 mg/kg gave recoveries of all tissues of 98, 107 and 135%, respectively. Balance excretion data at 7 days was 86.9% with 81.6% in faeces and 5.3% in urine. At 14 days the total excreted radioactivity was 82–92% with less than 10% in urine.

Approximately 66% of the radioactivity in kidney was apramycin. None of the other samples contained sufficient residue for characterization. Results are presented in Table 2.1.

Table 2.1. Residues in pig tissues following administration of ^{14}C-apramycin in water (25 mg/kg bw) for 5 days

Withdrawal time	^{14}C Residue (mg apramycin-equivalents/kg)			
	Muscle	Liver	Kidney	Fat
4 hours[(1)]	0.06	0.20	1.39	0.13
	0.22	4.01	70.99	0.30
	0.11	1.94	9.74	0.13
7 days[(1)]	0.04	0.10	0.11	0.09
	0.06	0.19	0.52	0.12
	0.04	0.08	0.17	0.11
14 days[(2)]	0.02	0.08	0.17	0.06
	0.02	0.04	0.05	0.10
	0.05	0.14	0.29	0.15

SOURCES: (1) Herberg *et al.*, 1979. (2) Zornes, Herberg and Donoho, 1979.

Chickens

Zornes, Herberg and Thomson (1985) gave twelve 4-week old Hubbard × White Mountain broiler chickens 500 mg/L ^{14}C-apramycin in the drinking water for five days (maximum recommended dose). Three birds (two male and one female in each group) were terminated at each sampling time of 0 (6 h), 7, 10 and 14 days (body weights of the chickens were not provided). Total radioactivity was determined by direct solubilization followed by liquid scintillation counting. All residue samples were adjusted to 100% counting efficiency. Detection and recovery of radioactivity was checked by fortifying control tissues digested in the same manner as the treated chickens with a nominal radio-activity equivalent to 0.1 mg/kg bw from a solution with a mean value of 109.35 dpm/ml. At zero day withdrawal, average total residues were 3.23, 0.42, 0.20 and 0.07 mg apramycin equivalent/kg in kidney, liver, skin and muscle, respectively. After 14 days, average total residue had declined to 0.47, 0.08, 0.03 and 0.02 mg/kg in the same respective tissues. Apramycin accounted for more than 80% of the total residue in the samples that contained sufficient residue for assay (all kidney samples and the 6 h liver samples). Results are summarized in Table 2.2.

Table 2.2. Residues in chicken tissues following administration of ^{14}C-apramycin in drinking water (500 mg/L) for 5 days

Withdrawal time	^{14}C-Apramycin (mg apramycin equivalents/kg)			
	Muscle	Liver	Kidney	Fat+Skin
0 (6 hours)	0.07	0.24	2.35	0.22
	0.08	0.51	4.47	0.26
	0.07	0.50	2.87	0.13
7 days	0.02	0.20	1.93	0.05
	0.03	0.21	1.60	0.06
	0.02	0.05	0.88	0.06
10 days	0.02	0.14	1.32	0.05
	0.02	0.18	0.70	0.04
	0.02	0.07	0.70	0.04
14 days	0.01	0.11	0.37	0.03
	0.02	0.02	0.50	0.04
	0.02	0.11	0.55	0.03

Residue depletion studies with unlabelled drug

To assist in the evaluation of the residue depletion studies it is relevant to consider the approved treatments and use of apramycin in pigs, cattle and poultry (Elanco, 2011). The relevant information is provided in Table 2.3 (revised apramycin sponsor submission, 24 October 2011). In some pig studies, the maximum recommended doses in the original dossier did not appear to have been used. The sponsor provided updated information on maximum dose rates in pigs based on regulatory approvals (e.g. Australia and New Zealand) where the highest label dose rate is 25 mg/kg bw/day for neonatal piglets and 12.5 mg/kg bw/day for weanlings. For feed medication in pigs, the maximum dose rate of 8 mg/kg bw/day, using a maximum feed inclusion of 200 mg/kg, implies a voluntary feed intake of 4% of body weight. The sponsor noted that this is a very good estimate for pigs weighing from about 50 kg to slaughter weight. Younger pigs will regularly consume more than 6% of body weight, and as a consequence, higher mg/kg bw/day doses may be found in studies that used younger animals. For example, in the study of Kido *et al*. (1983) noted below, the average weight of the pigs during the period of medication was approximately 26 kg. The pigs consumed approximately 6% of body weight daily, giving average daily doses of 12 and 36 mg/kg bw/day for feed inclusion levels of 200 and 600 mg/kg. As a result, reported studies using neonate or weanling pigs represent considerably higher doses on a mg/kg bw/day basis than would normally be encountered in pigs of market weight.

Table 2.3. Indications and posologies for apramycin

Species	Indication	Formulation	Dose (mg/kg bw/d; inclusion rate in water or feed)	Duration (days)
Calves*	Colibacillosis, Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water or milk replacer	20–40	5
		Premix; incorporated in feed	20–40	5
Pigs	Colibacillosis Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water	7.5–12.5 (1)	7
		Premix; incorporated in feed	4–8 (80–200 mg/kg feed)	≤ 28
Poultry(2)	*E. coli* septicaemia, Colibacillosis, Salmonellosis and other bacterial infections	Soluble powder; incorporated in drinking water	20–80 (250–500 mg/L)	5–7
		Premix; incorporated in feed	(2–5mg/kg feed)	5
Rabbits	Bacterial enteritis, including colibacillosis	Soluble powder; incorporated in drinking water	10–15 (50–100 mg/L)	5–8
		Premix; incorporated in feed	5–10 (50–100 mg/kg feed)	≤ 21

NOTES: (1) Correction to maximum dose for market-ready pigs. (2) Not for use in animals producing milk or eggs for human consumption.

Additional comment regarding posology of apramycin.

- The highest label dose rate for pigs is 25 mg/kg/day in Australia and New Zealand for neonatal piglets. This dose reverts to 12.5 mg/kg/day for weanlings.
- Most labels require dosing to an inclusion rate in feed or water. Dose rates in terms of mg apramycin per kg per day are typically calculated from "consumption" data, which is often skewed by spillage and waste, particularly in the case of pigs.
- Older animals eat and drink less per body weight than younger animals. Thus, typical market-weight animals will tend toward the lower end of the dose rate range.

It is also relevant to consider the effects of the reported limit of detection (LOD) and limit of quantitation (LOD). The analytical method LOD and LOQ as reported in the sponsor dossier provide preliminary guidance to interpret the results in the residue depletion studies for each animal species and tissue (See Table 2.4). The LOD values were determined in accord with EC directive 93/256EEC, while the LOD values were based on lowest concentration of fortified control tissues used in the

method validation and may not reflect true LOQs. Studies conducted in the early 1970s may be reported as analytical range values based on bio-autography and/or paper chromatography. Details regarding the analytical methods are presented in the methods section of this report.

Table 2.4. Limits of detection (µg/kg) and limits of quantitation (µg/kg) of apramycin for different species×matrix combinations (analytical method based on HPLC separation and fluorimetric detection)

Species	Tissue	LOD (µg/kg)	LOQ (µg/kg)	Ratio LOQ/LOD
Cattle	Muscle	268	500	1.9
Cattle	Liver	396	5000	12.6
Cattle	Kidney	229	5000	21.8
Cattle	Fat	129	500	3.9
Pig[(1), (2)]	Muscle	280/314	500/500	1.8/1.6
Pig	Liver	250/253	5000/500	20.0/2.0
Pig	Kidney	220/212	5000/2500	22.7/11.8
Pig	Fat	20/23	500/500	25.0/21.7
Pig	Skin+Fat	50/60	500/500	10.0/8.3
Poultry	Muscle	319	500	1.6
Poultry	Liver	470	500	1.1
Poultry	Kidney	133	500	3.8
Poultry	Skin+Fat	32	500	15.6
Rabbit[(3), (4)]	Muscle	54/500	500/500	9.2/1.0
Rabbit	Liver	57/100	500/500	8.8/5.0
Rabbit	Kidney	24/600	2500/2500	105/4.2
Rabbit	Fat	38/200	500/500	13.3/2.5

NOTES: (1) Values reported in validation study by Parker, 1995b. (2) Values reported in validation study by Parker, 1995c. (3) Values reported in method validation study by Heal, 2007. (4) Values reported in method validation study by Villa and Brightwell, 1998.

Cattle

Two early studies used a semi-quantitative microbiological method to determine tissue residues after oral administration of apramycin to Holstein bull calves for five days. The calf body weights were 36–52 kg. In each study, three or four calves were terminated at intervals of approximately 1 h, 7, 14, 21 and 28 days after the last dose.

Van Duyn and Handy (1977) treated 16 Holstein dairy calves (body weights 36.3–46.7, mean = 42.1 kg) with bolus doses of 39 mg apramycin/kg bw/day by gavage (maximum recommended dose). Residues in kidney were 50–100 mg/kg at zero day withdrawal, depleting to 1–4 mg/kg after 28 days. Residues in liver were 2–8 mg/kg at zero day withdrawal, depleting to 0.4 mg/kg or less by 21 days. The maximum residue in any muscle sample was 0.5–1.0 mg/kg at zero withdrawal and no residues were detectable by 28 days. Results are summarized in Table 2.5. ND means no residue detected at test sensitivity of <0.1 mg/kg and IS means insufficient sample

Table 2.5. Residues in calf tissues after administration of bolus doses of apramycin (39 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 (ca. 1 h)	<0.5	2.0 to 4.0	50.0 to 100.0	IS
	<0.5	4.0 to 8.0	50.0 to 100.0	
	0.5 to 1.0	4.0 to 8.0	50.0 to 100.0	
7 days	<0.1	<0.2	2.5 to 5.0	IS
	0.1 to 0.2	4.0 to 8.0	40.0 to 80.0	
	0.1 to 0.2	4.0 to 8.0	20.0 to 40.0	
14 days	0.1 to 0.2	1 to 2	8.0 to 16.0	IS
	<0.1	2.0 to 4.0	5.0 to 10.0	
	<0.1	2.0 to 4.0	8.0 to 16.0	
21 days	0.1 to 0.2	0.2 to 0.4	10.0 to 20.0	IS
	ND	<0.2	5.0 to 10.0	
	ND	<0.2	5.0 to 10.0	
28 days	ND	<0.2	4.0	IS
	ND	Lost sample	1.0 to 2.0	
	ND	<0.2	4.0	
	ND	<0.2	4.0	

NOTES: ND means no residue detected at test sensitivity of <0.1 mg/kg. and IS means insufficient sample

Table 2.6. Residues in calf tissues after administration of apramycin in milk replacer once daily (40 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 (1 hour)	0.1–0.2	2.0–4.0	40.0–80.0	1.0–2.0
	0.1–0.2	2.0–4.0	40.0–80.0	
	0.1–0.2	2.0–4.0	40.0–80.0	
7 days	ND	0.4–0.8	10.0–20.0	0.1–0.2
	<0.05	2.0–4.0	5.0–10.0	
	ND	2.0–4.0	5.0–10.0	
14 days	ND	0.2–0.4	4.0–8.0	IS
	<0.05	1.0	4.0–8.0	
	ND	2.0–4.0	4.0–8.0	
21 days	ND	1.0	1.6–3.2	<0.05
	ND	1.0–2.0	1.6–3.2	
	ND	1.0–2.0	1.6–3.2	
28 days	ND	1.6	2.0–4.0	0.1–0.2
	ND	0.8–1.6	2.0–4.0	
	ND	0.8–1.6	2.0–4.0	

NOTES: ND = no residue detected at the method sensitivity of <0.05– <0.10 mg/kg; IS = insufficient sample.

Handy and Van Duyn (1978) treated 25 × 2–7-day-old calves with apramycin dissolved in reconstituted milk replacer, which was bottle-fed once per day at a dose rate of 40 mg/kg bw (maximum recommended dose). Weights of the calves were 37–52 kg (mean = 42.9 kg) at the beginning of the study. Three animals were sampled at each withdrawal time of 0, 7, 14, 21 and 28 days. Analysis was done using bioautography. Residues in kidney were 40–80 mg/kg at zero withdrawal, depleting to 4 mg/kg or less after 28 days. Residues in liver at zero withdrawal were 2–4 mg/kg, depleting to 1.6 mg/kg or less after 28 days. In muscle, the highest level was 0.2 mg/kg at zero withdrawal and residues were not detected after 21 days. Because of insufficient fat in each animal, a composite fat sample was analysed at each withdrawal time. At zero withdrawal, the residue in fat was 1–2 mg/kg and declined approximately 10-fold by 28 days. The very young age of the animals may not be representative of calves in general because of their immature metabolic status. Results are shown in Table 2.6.

Table 2.7. Residues in male calves following oral dosing with apramycin (40 mg/kg- body weight/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 (4 h)	ND	2.80	118.7	0.900
	ND	0.90	161.8	
	ND	2.00	153.6	
	ND	0.60	75.3	
7 days	ND	ND	12.4	0.100
	ND	ND	15.5	
	ND	1.20	21.7	
	ND	ND	2.80	
14 days	ND	0.40	3.50	ND
	ND	0.40	17.3	
	ND	ND	2.90	
	ND	0.50	3.00	
21 days	0.80	0.60	9.40	ND
	ND	0.60	2.00	
	ND	0.40	3.60	
	ND	0.70	4.40	
28 days	ND	ND	3.90	ND
	ND	ND	1.50	
	ND	ND	1.50	
	ND	ND	0.90	
35 days	ND	0.40	9.20	ND
	ND	ND	2.70	
	ND	ND	0.40	
	ND	ND	1.40	
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.27	0.40	0.23	0.13

NOTES: ND = not detected.

A GLP-compliant residue depletion study in calf tissues was conducted by Parker (1995a). In the two-part study, one group of 20 calves was given 20 mg/kg bw by i.m. injection (Group A) and one group of 24 male Friesian crossbred calves were given oral doses of 40 mg/kg bw apramycin daily (maximum recommended dose for oral treatment) for five consecutive days (Group B). Because oral treatment is the only approved treatment for use, results are only provided for group B (with animals weighing 48–68 kg, mean = 55.5 kg). Groups of four calves were sacrificed at withdrawal intervals of zero (4 h), 7, 14, 21, 28 and 35 days. Apramycin residues in edible tissues were determined using a validated HPLC method (Parker, 1995d). Laboratory analysis provided estimates of residue concentrations that were between the LOQ and the LOD. Residues in liver were always less than the LOQ (5 mg/kg); estimated concentrations were less than 1 mg/kg by 14 days and residues were detected in only one sample collected at 28 and 35 days withdrawal. Because of limited fat, samples at each withdrawal time were composited for analysis. Apramycin was not detected in fat at or beyond the 14 day sample (LOD = 0.13 mg/kg). Residues were not detected in muscle (LOD = 0.27 mg/kg) except one sample at 21 days (<1 mg/kg). Apramycin residues in kidneys were less than 20 mg/kg at 14 days and the majority of the residues were below the limit of quantitation (LOQ = 5.0 mg/kg) by 21 days. Results below the LOQ are estimated values. Results are shown in Table 2.7.

Table 2.8. Residues in calf tissues following oral dosing with apramycin (40 mg/kg bw/day) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Fat**
7 days	0.35	1.47	4.40	0.33
	0.29	1.71	6.52	0.46
	ND	1.81	6.47	0.14
	0.60	1.48	7.16	0.14
14 days	0.39	1.51	1.57	0.39
	0.52	1.74	1.89	0.84
	ND	1.58	2.21	0.16
	0.43	1.42	3.97	0.62
21 days	ND	2.20	2.97	0.13
	0.27	2.02	2.35	0.23
	0.29	1.77	1.95	0.14
	0.40	1.20	3.37	0.17
28 days	0.35	1.35	1.86	ND
	0.46	1.02	1.79	ND
	0.47	1.48	2.48	ND
	0.58	1.34	2.42	ND
35 days	ND	1.51	2.02	ND
	ND	1.27	2.20	ND
	ND	1.80	3.22	ND
	ND	1.43	1.92	ND
42 days	ND	1.31	1.41	ND
	ND	1.50	1.82	ND
	ND	1.42	2.64	ND
	ND	1.68	3.25	ND
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.27	0.40	0.23	0.13

NOTES: ND = not detected.

An additional GLP-compliant residue study was carried out in young calves (Parker, 1999b), with 24 male and female Friesian crossbred calves given oral doses of 40 mg/kg bw apramycin daily for five days (maximum recommended dose) and groups of four calves were sacrificed at withdrawal intervals of 7, 14, 21, 28, 35 and 42 days. Animal weights were 38–69 kg (mean = 49.0 kg). Apramycin residues were determined using a validated HPLC method (Parker, 1995d). Apramycin was detected at all withdrawal times in liver and kidney, although residues in liver were all below the LOQ. In kidney, three of the four samples collected at 7 days withdrawal contained apramycin above the LOQ, but no residues above the LOQ were found at 14 days or later withdrawal times. A limited number of muscle and fat samples contained residues above the LOQ at 7 or 14 days or both. Residues were undetectable in fat from 28 days and in muscle from 35 days. Results reported below the validated LOQ are estimated values. Results are shown in Table 2.8.

Residue studies in calves provide some useful data for residue depletion analysis as doses were at the maximum recommended amounts; however, as in the pig studies described below, the time frames are generally too long to provide useful information for residue depletion analysis.

Pigs

In a very old study, 27 pigs were treated with apramycin in drinking water at the recommended dose with 12 serving as controls (and three sacrificed in each sampling timeframe at 4, 7, 14, 28 and 42 days). Pigs were given unlabelled apramycin at 1 g activity per US gallon (approximately 264 mg activity/L) as the sole source of drinking water for 7 days (VPR-164-766, 1972). Body weights of the pigs were not provided, thus dose per kg bw was not reported. Residues in tissues were analysed with a semi-quantitative microbiological assay. No apramycin residues were detected in muscle, skin or fat at any withdrawal interval. The study report shows that the zones of inhibition on the bio-autography plates for these tissues were similar to controls, but the sensitivity of the assay was not defined. There was approximately 0.1 mg/kg in liver at zero withdrawal and <0.1 mg/kg at subsequent time points. Kidney contained ca. 1.3–1.7 mg/kg at zero withdrawal, declining to <0.1 mg/kg by 28 days. Results are tabulated in Table 2.9.

In a similar study, Van Duyn and Johnson (undated) administered apramycin in drinking water at 1 g activity per US gallon (264 mg/l) to 50 piglets for 7 days, with 18 pigs serving as controls and two groups of 16 provided apramycin in the drinking water. The mean initial weight of the piglets was approximately 13 kg. Based on the weight of the piglets and average water consumption, the average daily dose was calculated to be 11.4 mg/lb bw/day, or approximately 25 mg/kg bw/day (200% of maximum recommended dose of 12.5 mg/kg bw/day). Following the seven days of treatment, the medicated pigs were combined into one group of 32 pigs. At days 14, 28, 35 and 42 post-treatment, three randomly selected medicated pigs and two control pigs were sacrificed and tissues collected for residue analysis. No residue above approximately 0.1 mg/kg was found in liver or muscle at any withdrawal interval. One skin+fat sample contained a residue estimated at 0.1–0.2 mg/kg at zero withdrawal (immediately off-treatment), but the other two zero-day samples, and all subsequent withdrawal samples, contained no measurable residue. Kidney contained approximately 1.3 to 5 mg/kg at zero withdrawal, 0.1 to 1 mg/kg at 14 days and <0.1 mg/kg at subsequent withdrawal intervals. Results are in Table 2.10.

Table 2.9. Residues in pig tissues following treatment with apramycin in drinking water (264 mg/l) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin/Fat
0 days	ND	0.10	1.29	ND
	ND	0.08	1.43	ND
	ND	0.12	1.74	ND
4 days	ND	0.03	1.01	ND
	ND	0.02	0.53	ND
	ND	0.02	0.59	ND
7 days	ND	ND[a]	0.44	ND
	ND	0.03	0.31	ND
	ND	0.06	0.44	ND
14 days	ND	0.01	0.17	ND
	ND	0.04	0.28	ND
	ND	ND	0.16	ND
28 days	ND	ND	ND	ND
	ND	ND	Negligible	ND
	ND	ND	0.05	ND
42 days	ND	ND	ND	ND
	ND	ND	0.03	ND

NOTES: ND = residue not detected (limit of detection not defined)

Table 2.10. Residues in pig tissues following treatment with apramycin in drinking water (264 mg/L) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 days	ND	ND[a]	2.5–5.0	0.1–0.2
	ND	ND	2.5–5.0	ND
	ND	ND	1.25–2.5	ND
14 days	ND	ND	0.5–1.0	ND
	ND	ND	0.5–1.0	ND
	ND	ND	0.1–0.2	ND
28 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
35 days	NA	ND	ND	NA
	NA	ND	ND	NA
	NA	ND	ND	NA
42 days	NA	NA	ND	NA
	NA	NA	ND	NA
	NA	NA	ND	NA

NOTES: ND = residue not detected (limit of detection 0.1 mg/kg); NA = not analysed.

An additional study that used a microbiological assay was conducted in Japan (Kido *et al.,* 1983). Thirty-six castrated male crossbred pigs were medicated with two levels of apramycin in drinking water for 7 days. Actual doses, based on average water consumption and body weights, were approximately 10 and 29 mg/kg bw/day (80% and 230% of the maximum recommended dose). For each treatment level, 3 pigs were slaughtered at 0 (2 h), 7, 14, 21, 28 and 35 days after withdrawal from medication. The sensitivity of the assay was 0.06 mg/kg for all tissues. No residues above the assay sensitivity were detected in muscle or fat from any pig regardless of medication level or withdrawal time. Only one of the liver samples at the 29 mg/kg bw/day treatment level (2 h withdrawal time) contained a residue above the assay sensitivity. Mean kidney residues at 2 h withdrawal time were 0.62 and 1.57 mg/kg for the 10 and 29 mg/kg bw/day dose levels, respectively, while mean levels at 7 days withdrawal were 0.10 and 0.25 mg/kg for the same respective doses. All kidney samples from later withdrawal intervals were below the sensitivity of the assay. Results are provided in Table 2.11.

Table 2.11. Residues in pig tissues following treatment with apramycin in drinking water (10 or 29 mg/kg bw/day) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
	Dosed at 10 mg/kg bw/day			
0 (2 h)	ND	ND[a]	0.52	ND
	ND	ND	1.06	ND
	ND	ND	0.27	ND
7 days	ND	ND	0.09	ND
	ND	ND	0.12	ND
	ND	ND	0.08	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	Dosed at 29 mg/kg bw/day			
0 (2 h)	ND	ND	1.22	ND
	ND	0.28	1.52	ND
	ND	ND	1.96	ND
7 days	ND	ND	0.20	ND
	ND	ND	0.20	ND
	ND	ND	0.36	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

A GLP-compliant study was conducted in 1995 using 24 (12 barrow and 12 gilts) Duroc × Landrace piglets medicated with a single daily dose of apramycin in water at 20 mg/kg bw (160% maximum recommended dose, see sponsor comments above on piglets up to 26 kg) by stomach tube for 7 days (Parker, 1995b). Body weights at the beginning of the study were 15–20 kg. Samples of muscle, liver, kidney and skin with fat were collected from four animals at each of the withdrawal periods (1, 4, 7, 14, 21 and 28 days) and residues were determined using a validated HPLC method (Parker, 1995c and addendum). No residues were detected at any withdrawal interval for muscle, liver or fat samples. The validated LOQ for apramycin in kidney was 5 mg/kg, but the authors of the report provided estimates of concentrations that were between the LOD and LOQ. Residues in kidney declined to below the limit of quantitation by 7 days withdrawal. Results are summarized in Table 2.12.

Table 2.12. Residues in pig tissues following administration of apramycin in water (20 mg/kg bw) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin+fat
1 day	ND	ND	5.80	ND
	ND	ND	6.90	ND
	ND	ND	15.30	ND
	ND	ND	6.30	ND
4 days	ND	ND	4.50	ND
	ND	ND	3.60	ND
	ND	ND	5.80	ND
	ND	ND	6.40	ND
7 days	ND	ND	2.60	ND
	ND	ND	2.90	ND
	ND	ND	3.10	ND
	ND	ND	2.50	ND
14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	0.70 (1)	ND
	ND	ND	ND	ND
21 and 28 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	5.00	5.00	0.50
Tissue LOD	0.28	0.25	0.20	0.05

NOTES: ND = not detected. (1) Estimated value.

The results of these studies show that oral treatment of pigs with apramycin results in very low concentrations of residues in edible tissues. The older studies that used microbiological assays are in general agreement with newer studies that used HPLC methods of analysis. Zero-day withdrawal kidney residues were lower after natural intake of drinking water containing apramycin, compared with single daily bolus doses given by gavage.

In an early study, 27 pigs were fed a ration medicated with 100 g apramycin activity per U.S. ton (110 mg/kg) for 56 days (Experiment SW-396, 1971) (55% of maximum recommended treatment in feed). Two males and one female were terminated at 0, 4, 7, 14, 28, 37 and 42 days after withdrawal of the medicated ration; six pigs served as controls. Body weights of the pigs were not reported. Analysis of tissues using a semi-quantitative microbiological assay found no detectable residue of apramycin in muscle, fat or skin at any withdrawal time. Residues in kidney were estimated to be approximately 2–3.4 mg/kg at zero withdrawal, depleting to negligible or undetectable levels by 28 days. Residues in liver were always less than 0.1 mg/kg; they were detected in all samples through 7 days withdrawal, in none of the pigs at 14 days, in one pig at 28 days and in no animals at 37 or 42 days. Results are provided in Table 2.13.

Table 2.13. Residues in pig tissues following treatment with apramycin in feed (110 mg activity/kg) for 56 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 day	ND	0.07	3.40	ND
	ND	0.09	3.40	ND
	ND	0.08	2.00	ND
4 days	ND	0.04	0.50	ND
	ND	0.04	0.85	ND
	ND	0.05	0.38	ND
7 days	ND	0.03	0.19	ND
	ND	0.03	0.30	ND
	ND	0.04	0.27	ND
14 days	ND	ND	0.09	ND
	ND	ND	0.03	ND
	ND	ND	0.25	ND
28 days	ND	ND	ND	ND
	ND	Negligible	ND	ND
	ND	ND	ND	ND
37 days	ND	ND	ND	ND
	ND	ND	Negligible	ND
	ND	ND	ND	ND
42 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

NOTES: (1) Limit of detection in liver not defined.

In another study (Handy and Van Duyn, 1979), pigs of mixed breed and sex, ranging in weight from 8 to 22 kg, were fed a ration medicated with 110 mg/kg apramycin activity for 28 days (55% of maximum recommended treatment in feed). Six randomly selected pigs were slaughtered at 0 (approximately 1 h) 7, and 14 days after withdrawal of the medicated ration and three randomly selected pigs were terminated at 21, 28 and 35 days after withdrawal. For the 0, 14 and 28 day intervals, tissue samples from two pigs were composited before analysis. Apramycin residues were determined using a semi-quantitative assay involving thin layer chromatography and bio-autographic detection with *Bacillus subtilis*. No residues were found above the test sensitivity of 0.1 mg/kg in fat+skin or muscle tissues at any withdrawal time. Kidneys contained 0.5 to 1.0 mg apramycin/kg at zero withdrawal, depleting to 0.2 mg/kg at 7 and 14 days; no residue was detected in kidney after 21, 28 or 35 days withdrawal. Liver contained <0.1 mg/kg at zero withdrawal; except for a trace of residue in one of the 3 samples analysed after 7 day withdrawal, no other liver sample contained a detectable apramycin residue. The report states that, based on the average feed consumption during 28 days and the final weight of the pigs, the average dose of apramycin was 2.83 mg/kg bw/day (23% of the maximum recommended dose/kg bw/day). However, if the calculation is based on the average weight of the pigs during medication, the mean dose is approximately 4.65 mg/kg bw/day. Results are presented in Table 2.14.

Table 2.14. Residues in pig tissues following treatment with apramycin in feed (110 mg activity/kg feed) for 28 days

Withdrawal time	Apramycin (mg/kg) LOD = 0.1 mg/kg			
	Muscle	Liver	Kidney	Fat
0 (1 hour)	ND	<0.1	0.5–1.0	ND
	ND	<0.1	0.5–1.0	ND
	ND	<0.1	0.5–1.0	ND
7 days	ND	ND	0.1	ND
	ND	<0.05	0.1–0.2	ND
	ND	ND	0.1–0.2	ND
14 days	ND	ND	0.1–0.2	ND
	ND	ND	0.1 -0.2	ND
	ND	ND	0.1 -0.2	ND
21, 28 and 35 days	NA	ND	ND	NA
	NA	ND	ND	NA
	NA	ND	ND	NA

NOTES: ND = not detected; NA = not analysed.

The study of Kido *et al.* (1983), discussed earlier, also included treatment groups given apramycin in feed. Thirty-six castrated male crossbred pigs were fed a ration medicated with 200 or 600 mg/kg apramycin *ad libitum* (100% or 300% of the maximum recommended treatment in feed). Actual doses, based on average feed consumption and average body weights, were 12 and 36 mg/kg bw/day (150 and 450% of the maximum recommended dose). Three pigs were slaughtered at 0 (2 h), 7, 14, 21, 28 and 35 days after withdrawal from treatment. The sensitivity of the assay was 0.06 mg/kg for all tissues. No residue was detected in muscle or fat from any pig regardless of dose level or withdrawal time. In kidney, residues at 2 h withdrawal were 0.38–0.54 mg/kg for the 12 mg/kg bw/day dose and from 0.97–1.63 mg/kg for the 36 mg/kg bw/day dose. No residues were detected in kidney at 7 days or later withdrawal intervals. No residues were detected in liver from pigs that received the 12 mg/kg bw/day dose at any withdrawal time. Liver samples from two of the pigs that received 36 mg/kg bw/day and terminated at 2 h withdrawal contained 0.18 or 0.07 mg/kg apramycin. Liver from the third pig terminated at 2 h and from all pigs at later withdrawal intervals contained no residue above the sensitivity of the assay. Results are provided in Table 2.15.

A GLP marker residue study was conducted in pigs using apramycin premix at the highest recommended dose (Parker, 1999a). Sixteen Large White Cross pigs, 12 weeks old (plus one control pig) with body weights 19–31 kg (mean = 21.9 kg) at the initiation of the study were dosed at a nominal rate of 200 mg apramycin/kg of feed for 28 days (body weights of 28–52 kg [mean = 34.7 kg] at the end of the study). Estimated daily doses based on feed consumption ranged from 13.1 to 16.7 mg/kg bw (105–134% of the maximum recommended dose/kg bw/day). Samples of muscle, liver, kidney and skin with fat were collected from four animals at withdrawal periods of 3, 6, 9 and 12 days. Residues were determined using the validated HPLC method that reported the LOQ in liver tissue at 0.5 mg/kg, lower than in other studies. In this study, in contrast to other pig studies, the highest residues were found in liver tissue. The results are summarized in Table 2.16.

Table 2.15. Residues in pig tissues after treatment with apramycin in feed (12 or 36 mg/kg bw/day) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
		12 mg/kg bw/day dosage		
0 (2 h)	ND	ND	0.43	ND
	ND	ND	0.54	ND
	ND	ND	0.38	ND
7 and 14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
		36 mg/kg bw/day dosage		
0 (2 h)	ND	0.18	0.97	ND
	ND	ND	1.11	ND
	ND	0.07	0.63	ND
7 and 14 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND

NOTES: ND = not detected.

Table 2.16. Residues of apramycin in pig tissues following administration of apramycin in feed (200 mg/kg feed) for 28 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Skin/Fat
3 days	ND	1.2 8	0.39	ND
	ND	1.31	0.83	ND
	ND	1.25	ND	ND
	ND	1.40	ND	ND
6 days	ND	1.55	ND	ND
	ND	1.41	ND	0.16
	ND	1.62	ND	0.17
	ND	1.43	1.31	0.14
9 days	ND	1.37	0.41	ND
	ND	1.20	1.39	0.16
	ND	1.22	0.79	0.14
	ND	1.09	ND	0.13
12 days	ND	1.14	ND	0.19
	ND	0.99	ND	0.18
	ND	1.02	ND	0.33
	ND	1.22	0.53	0.19
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.31	0.25	0.21	0.06

NOTES: ND = not detected

The apramycin residue studies of pigs following treatment in feed are in general agreement with the pattern of distribution observed in studies with apramycin through drinking water or by gavage dosing, with residues monitored through ^{14}C-labelling, HPLC or microbiological assay. Residues are usually highest in kidney, followed by liver (see, however, Table 2.16) with residues depleting rapidly after withdrawal of treatment. Residues are generally not detectable or very low in muscle and

skin+fat. Each of the studies, however, is deficient in the multiple day selection of withdrawal times, compromising assessment of residue depletion studies.

Chickens

Thirty-six Hubbard crossbred broiler chickens (12 males and 12 females in trial; 12 birds as controls), approximately 30 days old, were administered apramycin in drinking water (559 mg/L, 110% maximum recommended treatment, in drinking water) for five days (Handy and Thomson, 1985). Three males and three females were terminated at 0 (5 h), 7, 10, 14, 21 and 28 days after withdrawal of medication, and residues in edible tissues were determined using a microbiological assay. Based on use of medicated water and the total pen bird weights on the day before treatment was initiated, the average dose was estimated to be 102 mg/kg bw/day (127% of maximum recommended dose). Weights of the individual groups of chickens were 1.36 kg at day 0; 1.75 kg at day 7; 2.02 kg at day 10; 2.12 kg at day 14; 2.71 kg at day 21; and 2.93 kg at day 28. Five hours after withdrawal, residues of apramycin in kidney were 2.7–6.9 mg/kg; these declined to ≤1 mg/kg after 7 days, and to ≤0.48 mg/kg after 28 days. Residues in liver were 0.26–0.54 mg/kg after 5 h withdrawal, ≤ 0.21 mg/kg after 7 days and <0.05 mg/kg by 21 and 28 days. Residues in skin were 0.06–0.2 kg/kg after 5 h and no residues were detected at later withdrawal intervals. In this study the authors used a method labelled as AM-AA-CA-R100-AA-775. The reported LOQ was 0.05 mg/kg in all tissues with the exception of 7 and 10 day withdrawal skin samples with a LOQ of 0.1 kg/kg. These LOQs are notably lower than other apramycin methods and studies. One fat sample at 5 h withdrawal contained 0.15 mg/kg; the remaining samples, and all samples from subsequent samplings, contained no detectable residue. Residues in muscle were <LOQ at all withdrawal intervals. An earlier study using a less developed microbiological assay gave similar results (Handy, 1985). Results are presented in Table 2.17.

Because of limited sample sizes, tissues from chickens were sometimes pooled before analysis. Skin and fat were analysed separately and the value shown in the table is the higher of the two values. Below LOQ means <0.05 mg/kg, except 7-day skin tissue samples where the LOQ = 0.01 mg/kg. No residue detected (ND) means no response below the LOQ (0.01 mg/kg).

In a GLP-compliant study, apramycin was administered in drinking water at 500 mg/L to 48 four-week-old Ross broiler chickens for 5 days (Parker, 1998a). Body weights were tabulated for each of the four sampling points (day 3 birds, 0.98 kg; day 6 birds, 0.95 kg; day 9 birds, 1.03 kg; and day 12 birds, 0.92 kg). Delivery of medicated water was also calculated: day 1, 118.7 mg/kg bw; day 2, 94.4 kg/kg bw; day 3, 112.0 mg/kg bw; day 4, 132.1 mg/kg bw; day 5, 134.3 mg/kg bw). The average dose was estimated to be 118 mg/kg bw/day (about 150% of the maximum recommended dose). Tissues from ten birds were analysed for apramycin using a validated HPLC method (Parker, 1998b). The reported LOQ was 5 mg/kg for all tissues (see Table 2.4). Allowing for the differences in withdrawal times and the precision of the assays, the results of the chicken radiometric residue studies and the microbiological method studies are in general agreement with the Parker (1998a) study using the HPLC assay. Results are reported in Table 2.18.

Table 2.17. Residues in chicken tissues following administration of apramycin in drinking water (559 mg/L) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Skin/Fat**
0 (5 h)	BLQ	0.26	3.30	0.20
	ND	0.35	6.90	0.11
	ND	0.33	—	0.08
	BLQ	0.54	8.40	0.06
	ND	0.29	2.70	0.09
	ND	0.35	—	0.10
7 days	ND	0.21	1.00	ND
	ND	0.14	0.56	ND
	ND	0.08	—	ND
	ND	0.09	0.79	ND
	ND	0.06	0.97	ND
	ND	0.11	—	ND
10 days	ND	0.15	0.81	ND
	ND	0.08	0.59	ND
	ND	0.06	—	ND
	ND	BLQ	1.05	ND
	ND	0.11	0.53	ND
	ND	0.12	—	ND
14 days	NA	0.08	0.45	NA
	NA	0.12	0.58	NA
	NA	0.16	—	NA
	NA	BLQ	0.82	NA
	NA	0.05	0.61	NA
	NA	0.23	—	NA
21 days	NA	BLQ	0.49	NA
	NA	BLQ	0.88	NA
	NA	BLQ	—	NA
	NA	BLQ	0.52	NA
	NA	BLQ	0.37	NA
	NA	BLQ	—	NA
28 days	NA	BLQ	0.14	NA
	NA	ND	0.31	NA
	NA	BLQ	—	NA
	NA	BLQ	0.31	NA
	NA	BLQ	0.48	NA
	NA	ND	—	NA

NOTES: NA = not analysed; BLQ = below limit of quantitation; ND = not detected.

Table 2.18. Residue in chicken tissues following administration of apramycin in drinking water (500 mg/L) for 5 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	**Liver**	**Kidney**	**Skin+fat**
3 days	ND	ND	BLQ	BLQ
	ND	ND	BLQ	0.62
	ND	ND	0.85	BLQ
	ND	ND	0.58	BLQ
	ND	ND	1.48	BLQ
	ND	ND	0.62	BLQ
	ND	ND	0.90	BLQ
	ND	BLQ	1.10	0.55
	ND	ND	BLQ	BLQ
	ND	ND	0.66	ND
6 days	ND	ND	1.03	ND
	ND	ND	BLQ	ND
	ND	ND	1.40	ND
	ND	ND	0.77	BLQ
	ND	ND	0.62	ND
	ND	ND	0.54	BLQ
	ND	ND	BLQ	BLQ
	ND	ND	BLQ	BLQ
	ND	ND	BLQ	ND
	ND	ND	BLQ	BLQ
9 days	ND	ND	BLQ	ND
	ND	ND	0.56	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	0.60	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
12 days	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	ND	BLQ
	ND	ND	BLQ	ND
	ND	ND	0.58	ND
	ND	ND	BLQ	ND

Notes: ND = not detected; BLQ = below limit of quantitation.

Rabbits

In a GLP-compliant study, apramycin was administered in the drinking water at 100 mg/L (maximum recommended dose) for 7 days to 30 New Zealand White rabbits. Weight ranges at the beginning of the study for males was 2.2–2.5 kg and for females, 2.2–2.6 kg. The estimated mean concentration of medicated water was determined to be 108.6 mg/L/day for the 7-day study. Liver, kidney, muscle and fat were analysed for apramycin using a validated HPLC method with a LOQ of 0.50 mg/kg (Villa and Brightwell, 1998). One liver sample at day zero withdrawal had a value of 0.6 mg/kg; all other samples at day zero were <LOD. All day 3 and subsequent withdrawal time samples were <LOD. Results are in Table 2.19

Table 2.19. Residues in rabbit tissues following administration of apramycin in drinking water (100 mg/L) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0	ND	0.600	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
3, 7, 14 and 21 days	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.50	0.10	0.60	0.20

NOTES: ND = not detected; BLQ = below limit of quantitation.

In a second GLP compliant study, 36 New Zealand White rabbits were medicated with apramycin at nominal rates of 100 mg/kg bw or 300 mg/kg bw in the feed (100% or 300% of the maximum recommended treatment dose) for 21 days (Heal, 2008). Calculated medicated doses were 85.6 mg/kg and 258 mg/kg in feed. Body weights of the rabbits at the beginning for the study were 0.9–1.5 kg (mean 1.36 kg). The achieved apramycin dose was 6.6 ± 1.0 mg/kg bw/day for the low-dose group and 20.1 ± 2.3 mg/kg bw/day for the high-dose group. At the end of the 21-day study, mean body weight of the low-dose group was 2.07 kg and the high-dose group was 2.16 kg. Feed intakes were calculated for each group at day 21 of treatment: for the low-dose group the estimated daily feed intake was 138.1 g/day and the high-dose group was 139.7 g/day. The overall mean medicated daily intake of apramycin in the low-dose group was 11.82 mg/kg bw/day and for the high-dose group the mean was 37.0 mg/kg bw/day. Muscle, liver, kidney and fat were sampled from six animals at each of the withdrawal periods (0, 24 and 48 h). In both treatment groups, all samples were below the LOD for muscle (49.5 µg/kg), liver (51.7 µg/kg) and fat (34.3 µg/kg). In the high-dose group, all but three kidney samples contained residues between the LOD (21.7 µg/kg) and the LOQ (2.28 mg/kg), while the remaining three kidney samples residues were non-detectable. All kidney samples in the low-dose group had non-detectable residues. Residues were determined using a validated HPLC fluorescence method (Heal, 2007). Because of the limited number of positive residue findings, only those from the high treatment group are reported in Table 2.20.

These studies indicate that apramycin is very poorly absorbed by rabbits, either by medication in drinking water or in medicated feed.

Table 2.20. Residues in rabbit tissues following administration of apramycin in feed (300 mg/kg feed) for 7 days

Withdrawal time	Apramycin (mg/kg)			
	Muscle	Liver	Kidney	Fat
0 hours	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
24 hours	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
48 hours	ND	ND	BLQ	ND
	ND	ND	ND	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	BLQ	ND
	ND	ND	ND	ND
Tissue LOQ	0.50	0.50	2.50	0.50
Tissue LOD	0.05	0.06	0.02	0.04

NOTES: ND = not detected; BLQ = below limit of quantitation.

Methods of analysis for residues in tissues

During the last decade, several laboratories have worked on the development of analytical methods for the analysis of aminoglycosides (e.g. Cheng *et al.,* 2010; Ishii *et al.,* 2008; Bogialli *et al.,* 2005; van Holthoona *et al.,* 2009; Stead, 2000). These methods cover a wide range of matrices (mainly for which MRLs have been established). Generally, most protocols use an extraction solvent containing trichloroacetic acid and sample clean-up is performed with solid phase extraction (SPE) on weak cation exchange cartridges such as CBA or CBX. Chromatographic separation is commonly performed using reversed-phase ion-pair principles. Although some groups describe the use of ultraviolet (UV) detection and fluorescence detection (FD), the lack of a suitable chromophore requires a derivatization step to detect aminoglycosides. Chemiluminescence has been described as a rapid and robust detection technique for aminoglycosides without the need for derivatization. Mass spectrometric detection has the same benefits as chemiluminescence but with higher selectivity and sensitivity especially in the selective reaction mode (SRM) of a triple quadrupole LC–MS system. Most methods use tobramycin as an internal standard, because deuterated or chemical analogues of the aminoglycosides are not available. Some research groups have synthesized internal standards (such as dimethylspectinomycin, methyldihydrostreptomycin and octamethylkanamycin A) that provide incomparable performance for quantification.

Methods used in absorption and bio-availability studies

Regarding methods submitted by the sponsor, blood levels (in relevant species) were investigated by a microbiological assay. This assay used Mueller-Hinton (Difco) media adjusted to pH 8.0 and *Bacillus subtilis* ATCC 6633 as test organism, against apramycin residues in tissue. The paper disc procedure was used. Plates were kept overnight at ambient temperature and were then incubated at 37°C until

distinct growth of the assay organism was apparent. Semi-logarithmic plots of known apramycin concentrations versus diameters of inhibition zones were linear with typical correlation coefficients (r) >0.93. The intra-day and inter-day variability were <7.5% and <2.5% through the concentration ranges studied. Few additional technical details are given in the experimental section of the studies. The semi-quantitative microbiological assay is adapted to produce qualitative data, i.e. 'presence or absence'. No quantitative measurement is available by this approach [see Van Duyn and Johnson (undated) "…the assay for aprymicin … does not provide for the establishment of a finite residue level for each sample… The assay is more applicable to verifying the absence of apramcyin than the exact measurement of residue level…"]. The threshold of detection was established at 0.1 mg/kg (standard recovery samples fortified at 1.0 mg/kg could always be distinguished from negative control tissue samples), but details (e.g. number of replicates and concentrations studied) are lacking. The semi-quantitation is done by measuring the spot intensity onto the plate. The nature of the bio-autographic tissue residue procedure is more appropriately suited to the detection of antimicrobial residues rather than the estimation of amount present. The semi-quantitative residue activity estimations are frequently reported as activity ranges. It is difficult to know if the apramycin residues were found to be highly variable between animals (the dose response was difficult to assess) due to individual animal variability or to the analytical method itself. The specificity of the detection is not described; false negative and false positive rates are unknown.

Methods used in residue depletion studies

The data used in the residue depletion (distribution) studies were provided mainly by a LC-fluorescence detection method. Tissue is treated with ammonium hydroxide/methanol solution to release apramycin. The methanolic solution is evaporated to dryness, re-suspended in aqueous buffer, and ion-pair extracted into ethyl acetate/di-(2-ethylhexyl)phosphate (DEHP). Apramycin is back extracted into 0.75M aqueous hydrochloric acid and subsequently neutralized with sodium hydroxide. The neutralized aqueous solution is then washed with toluene and aliquots transferred to vials for analysis. Apramycin is determined by HPLC with fluorescence detection after pre-column derivatization with o-phthaldehyde. The reaction acts on the apramycin primary amines to produce a fluorescent imine derivative (see Figure 2.1).

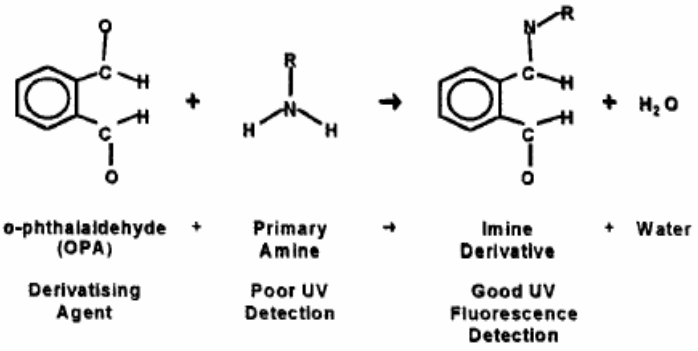


Figure 2.1. The reaction with the apramycin primary amine to produce a fluorescent imine derivative

All compounds bearing an amino group will be modified by the derivatization step, and will produce a detectable signal by the fluorescence detector. Any residual molecule from the clean-up procedure containing a similar primary amine will also undergo the derivatization step. No internal standard is used in the method.

The specificity is passable to medium, as attested by the chromatograms shown in Figure 2.2 (copied from the sponsor submission) for different blank and fortified kidney extracts (0.5 mg/kg and 1.0 mg/kg of apramycin).

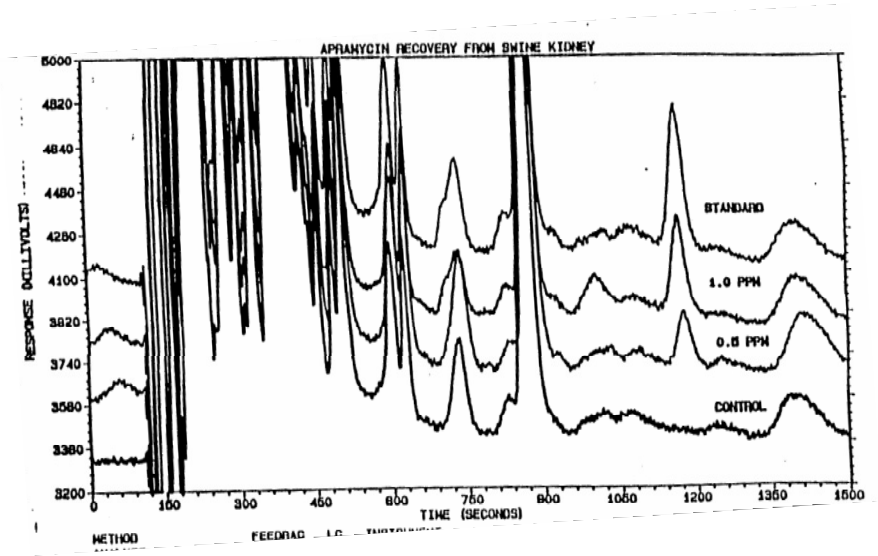


Figure 2.2. Chromatograms corresponding to pig kidney extracts (blank, 0.5 mg/kg and 1.0 mg/kg fortified samples, standard)

Limits of detection have been calculated as described in European Community Decision 93/256 (replaced in 2002 by the 2002/657/EC decision), i.e. on the calculation of the noise observed in blank tissues plus 3 standard deviations of the noise. Twenty chromatograms of negative control tissue were used to determine the LODs. They are generally good (see Table 2.4), especially for fat or skin+fat samples. LODs in liver and muscle are less satisfactory; sometimes the offset (20 mU in the region of elution of apramycin) is high for liver extracts (a complex matrix and the cleanliness of the extract being worse).

The LOQ for the sponsor-based methods used the lowest concentration for which the method has been validated to a stated level of confidence (first point of calibration). For this reason, LOQs are sometimes very high and are probably far from the true analytical LOQs. Reasonably, and without additional information provided by the studies, the Committee considered it possible to deduce (or estimate) LOQs from LODs by applying a basis of blank tissue mean residue finding plus ten standard deviations, resulting in a factor of approximately LOQ/LOD = 3. The Committee also critically reviewed the quality of the analytical tracings of the residue validation studies in its evaluation of method performance. Accordingly, the Committee re-calculated the LODs and LOQs in the different tissues for the different species for the studies provided by the sponsor. The analytical signal information was taken on representative chromatograms available in the submission. The method of calculation was performed according to the principle shown in Figures 2.3 and 2.4.

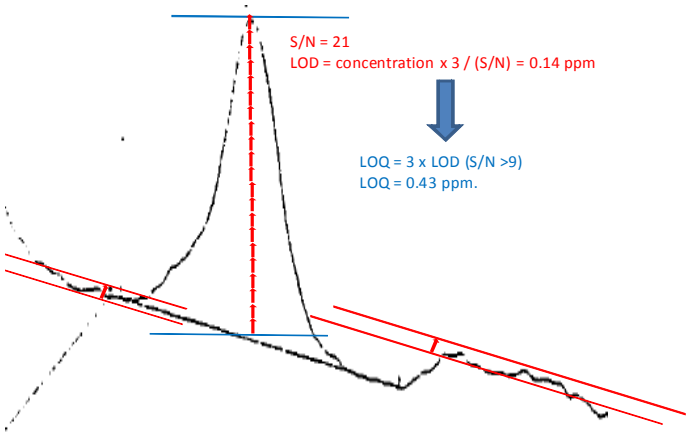


Figure 2.3. Method applied to re-assess LODs and LOQs. Illustration given on a chromatogram corresponding to a blank porcine skin+fat fortified with apramycin at 1 mg/kg (Parker, 1995c).

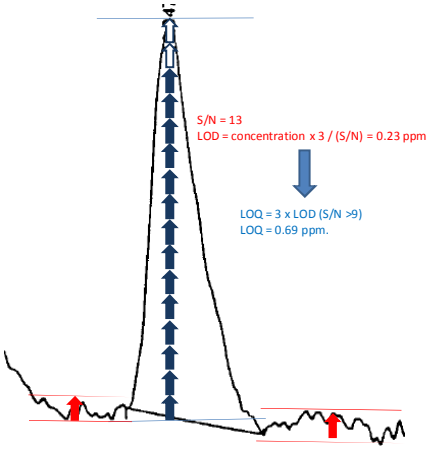


Figure 2.4. Method applied to re-assess LODs and LOQs. Illustration given on a chromatogram corresponding to a blank bovine fat fortified with apramycin at 1 mg/kg.

The noise has been measured in a retention time region as close as possible to the target signal of apramycin (i.e. retention time ± 5 peak widths at 50% height of the peak). The determination of the noise amplitude was determined to be as representative as possible, avoiding any over- or under-estimation of the corresponding value. The LOD has been calculated at a signal-to-noise ratio of 3. The LOQ has been deduced by applying a factor of 3 to the LOD. A summary of the values are given in Table 2.21 below. Assessment of the signal has been given in two separate columns; the offset level gives an indication of the probable complexity of the extract whereas the signal quality refers to the interpretability of the chromatographic peak (resolution, co-elution, etc). Finally, depending on the quality of the chromatograms (the consequence of both the PDF version readability and method specificity), a confidence level has been attributed to the interpretation; it reflects the level of certainty we may have on the LOD/LOQ recalculation. LOD/LOQ values are given in mg/kg.

Table 2.21. Summary of the committee-calculated values of LODs and LOQs in representative blank tissues fortified in different species' chromatograms from the sponsor submission.

SPECIES	TISSUES	LOQ SPONSOR	LOQ REVISITED	LOD SPONSOR	LOD REVISITED	OFFSET LEVEL	SIGNAL QUALITY	CONFIDENCE IN ASSESSMENT	STUDY REFERENCES
Cattle	Liver	5000	5000	396	1700	LOW	GOOD	GOOD	Parker 1995d
Cattle	Kidney	5000	2600	229	900	LOW	GOOD	GOOD	Parker 1995d
Cattle	Muscle	500	750	268	250	VERY HIGH	WEAK	GOOD	Parker 1995d
Cattle	Fat	500	370	129	230	VERY HIGH	GOOD	GOOD	Parker 1995d
Pig	Liver	5000***/500***	2500***/900***	253***/250***	830***/300***	MEDIUM***/LOW***	GOOD***/GOOD***	GOOD***/GOOD***	Parker 1995c/***Parker 1995c Addendum***
Pig	Kidney	2500	1900	212	625	MEDIUM	MEDIUM	MEDIUM	Parker 1995c
Pig	Muscle	500	1800	314	600	HIGH	BAD-COELUTION	LOW	Parker 1995c
Pig	Skin/fat	500	450	60	150	HIGH	MEDIUM	MEDIUM	Parker 1995c
Pig	Fat	500	390	23	130	MEDIUM	GOOD	GOOD	Parker 1995c
Poultry	Liver	500	750	133	250	MEDIUM	BAD-COELUTION	MEDIUM	Parker 1998b
Poultry	Kidney	500	1300	470	430	MEDIUM	BAD-COELUTION	MEDIUM	Parker 1998b
Poultry	Muscle	500	1140	319	380	MEDIUM	GOOD	MEDIUM	Parker 1998b
Poultry	Skin/fat	500	480	32	160	LOW	GOOD	GOOD	Parker 1998b
Rabbit	Liver	500***/500***	1500***/4500***	57***/100***	500***/1500***	LOW***/MEDIUM***	MEDIUM***/WEAK***	GOOD***/GOOD***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Kidney	2500***/2500***	3750***/9000***	24***/600***	1250***/3000***	LOW***/LOW***	MEDIUM***/GOOD***	LOW***/GOOD***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Muscle	500***/500***	*900* ***/9000***	54***/500***	*300* ***/3000***	LOW***/LOW***	MEDIUM***/GOOD***	MEDIUM***/WEAK***	Heal(2008)/***Villa and Brightwell (1998)***
Rabbit	Fat	500***/500***	120***/1800***	38***/200***	40***/600***	LOW***/LOW***	EXCELLENT***/GOOD***	GOOD***/WEAK***	Heal(2008)/***Villa and Brightwell (1998)***

Reproducibility studies were not routinely carried out in the validation exercise. Repeatability (recovery) has been assessed through intra- and inter-batch variations. The recovery has been calculated at different concentration levels and in different batches of samples [64% in liver (Repeatability standard deviation (rsd) = 10%), 70% in kidney (rsd = 7%), 68% in muscle (rsd = 11%), 77% in fat (rsd = 9%) and 74% in skin+fat (rsd = 10%)]. The repeatability is generally acceptable.

Most recent methods are based on extraction by a solvent containing trichloroacetic acid; this method unexpectedly used ammonium hydroxide and methanol to release target residues from tissue. Instead of using an SPE ion exchange strategy, the method used a complex liquid-liquid extraction with an ion-pair agent. In conclusion, the analytical methods combine a non-specific purification followed by a weak specific detection (i.e. all co-extracted 'amino interfering compounds'), derivatized generating a fluorescent signal resulting in a more complex chromatogram, and generating results that are more difficult to interpret. The only identification criterion is the chromatographic retention time. Internal standards are not used for identification and quantification of apramycin.

The quality of the data generated by the LC-fluorescence detector method is of medium quality, but may be used for the risk assessment exercise. The Committee is more reserved regarding use of the data generated from the microbiological assay.

A limited set of quality criteria (Table 2.22) were applied to each sample batch (retention time, repeatability of the standard signal, linearity of the calibration curve, recovery).

Table 2.22. Acceptance criteria used to validate batches of samples

Acceptance criterion	Action if unacceptable
1. Retention time of analyte peak within 20 seconds of nearest reference standard	Sample negative.
2. Percentage coefficient of variation of standards through run >10	Re-run HPLC. If still unacceptable, run batch again with fresh standards.
3. Regression of matrix curve <0.950	Re-extract batch.
4. Percentage recovery within the acceptable range for that particular species+tissue type. (see validation reports)	Re-extract batch.

Appraisal

Apramycin is an old drug with a long history of use. It has not been reviewed previously by the Committee. Apramycin is a broad spectrum aminocyclitol antibiotic produced stereospecifically by a strain of *Streptomyces tenebrarius*. It is extracted from the fermentation medium as apramycin sulphate at a purity of at least 85% and total impurities are not to exceed 15%. Six impurities have been identified and one impurity, 3 O-hydroxyapramycin ($C_{21}H_{41}N_5O_{12}$), has a biological spectrum that is very similar to apramycin, with microbiological activity of one-half to one-quarter compared with apramycin. A microbiological assay was used to determine activity as equivalents of apramycin base.

Apramycin is used in veterinary medicine, effective against both Gram-positive and Gram-negative bacteria, some strains of mycoplasma and most field strains of *E. coli* and *Salmonella* spp.. It is bactericidal at minimum inhibitory concentrations. The drug exerts its antibacterial effect by inhibiting protein synthesis at the level of peptidyl translocation. It is mostly used for treating gastrointestinal infections. Apramycin is available in soluble powder and feed premix formulations.

In calves, apramycin is intended to be administered at a dose of 20 to 40 mg/kg bw/day in drinking water, milk replacer or feed for 5 days. In pigs, it is intended to be administered at a dose of 7.5 to 12.5 mg/kg bw/day in drinking water or as premix incorporated in feed at a dose of 4 to 8 mg/kg bw/day (80–200mg/kg feed) for less than 28 days. In poultry, it is intended to be administered at a dose of 20 to 80 mg/kg bw/day (250 to 500 mg/L) in drinking water for 5–7 days or as premix incorporated in feed at a dose of 2 to 5 mg/kg feed for 5 days. In rabbits, it is intended to be administered at a dose of 10 to 15 mg/kg bw/day (50 to 100 mg/L) in drinking water for 5 to 8 days or as premix incorporated in feed at a dose of 5 to 10 mg/kg bw/day (50–100 mg/kg feed) for less than 21 days. It is not to be used in animals producing eggs or milk for human consumption.

Apramycin is a weak organic base which is highly polar with low solubility in lipids and a poor ability to penetrate membranes. Metabolism data are consistent across species. Levels in blood after oral dosing were much lower than after parenteral treatment, indicating low oral availability in pigs, calves and chickens. In calves, pigs, chicken, rabbits, rats and dogs, apramycin is rapidly and poorly absorbed by the oral route and quickly eliminated. Main levels in serum are found a few hours after treatment (until 6 h) and are undetectable between 24 and 36 h. Pharmacokinetic studies indicate oral availability of approximately 3% in pigs and 2% in chickens. Oral doses are extensively excreted in faeces (more than 82% in pigs and 99.5% in rats) while parenteral doses are mostly excreted in urine in a low percentage of the given dose (11% for cattle, less than 10% for pigs, rats and dogs). Binding of apramycin to serum proteins was 26%.

Chromatographic analysis of the studies with radiolabelled apramycin did not identify any major metabolites in blood, excreta or tissues. Most of the radioactivity in blood, urine, faeces and tissues was unmetabolized apramycin. The distribution of residues in edible tissues was similar for oral and parenteral routes of administration, but levels were much lower with oral treatment. Kidney contained the highest concentration of residue, followed by liver. Muscle and fat (or skin+fat) contained little or no apramycin residue. Kidney was generally the tissue from which apramycin depleted most slowly. In cattle, pigs and chicken, levels of radioactivity are highest in kidney, followed by liver and very minor quantities in muscle and fat. Little biotransformation occurs, the drug remains in tissues mostly as unchanged apramycin. In general, tissues contained insufficient residues for characterization.

Sixteen residue depletion studies (both GLP-compliant and studies prior to GLP regulations) were provided by the sponsor: four in young calves, eight in young pigs, two in chickens and two in rabbits. However, as there were no regulatory approvals noted by the sponsor for rabbits, these are not included in the appraisal (i.e. 14 studies are considered). A summary of residue study findings at or above the reported LOQ is tabulated in Table 2.23, irrespective of the time points (see individual studies for details).

Table 2.23. Summary of residue findings above reported limits of quantitation in four9teen residue depletion studies

Animal	Study report	Muscle	Liver	Kidney	Fat or Skin+fat
Calves	Van Duyn and Handy, 1977	Values reported as ranges	Values reported as ranges	Values reported as ranges	0
	Handy and Van Duyn, 1978	Values reported as ranges	Values reported as ranges	Values reported as ranges	Values reported as ranges
	Parker, 1995a	1	0	10	1
	Parker 1995b	3	0	3	2
Pigs	VPR-164-766, 1972	0	0	0	0
	Van Duyn and Johnson, undated	0	0	0	0
	Kido *et al.,* 1983 (drinking water)	0	0	6 at 2.3× recom-mended dose	0
	Parker 1995b	0	0	6 at 1.6× recom-mended dose	0
	SW-396, 1971	0	0	0	0
	Handy and Van Duyn, 1979	0	0	0	0
	Kido *et al.,* 1983 (feed)	0	0	0	0
	Parker, 1999a	0	16	0	0
Chicken	Handy and Thompson, 1985[1]	0	22	24	6
	Parker, 1998a	0	0	0	0

NOTES: (1) Reported LOQs were 0.05 mg/kg for all tissues except 7 and 10 day skin+fat

In these fourteen studies, with the exception of one study, the majority of the positive residue values were in kidney tissues. Liver tissue contains the second highest concentrations of residues in tissue, and in one pig study liver contained the highest amounts of apramycin. Residues in muscle and fat or fat+skin were universally very low. These conclusions are consistent with the radiolabel studies. The studies indicate that apramycin is the suitable marker residue and kidney as the appropriate target tissue.

Of primary concern with the analytical methods is the disparity in how the limit of detection and limit of quantitation were determined. Limits of detection were based on the calculation of the noise observed in blank tissues plus 3 standard deviations of the noise. Twenty chromatograms of negative control tissue were used to determine the LODs. They are generally good expected values, especially for fat or skin+fat samples. LODs in liver and muscle are less good; sometimes the offset (20 mU in the region of elution of apramycin) is high for liver extracts (a complex matrix) and the cleanliness of the extract is poor. The LOQ approach employed by the sponsor studies used the lowest concentration to which the method has been validated to a stated level of confidence, and consequently may not represent true limits of quantitation. This can be readily seen in Table 2.4. The Committee therefore re-calculated method performance LOD and LOQ values and re-assessed the positive residue findings in the studies. In reviewing the residue findings for estimating recommendations on MRLs, the Committee concluded that it was limited to applying the previously described statistical procedures from the 66th and 70th meetings of the Committee. Only two tissue residue data sets were available that provided sufficient positive residue findings greater than the reported LOQ. They were calf kidney and chicken kidney. The results are presented in the Figures 2.5 and 2.6, respectively.

The consequence of the concerns relating to the analytical methods and the limited set of residue determinations for the fourteen studies in calves, pigs and chickens present a complex determination of recommended MRLs for apramycin.

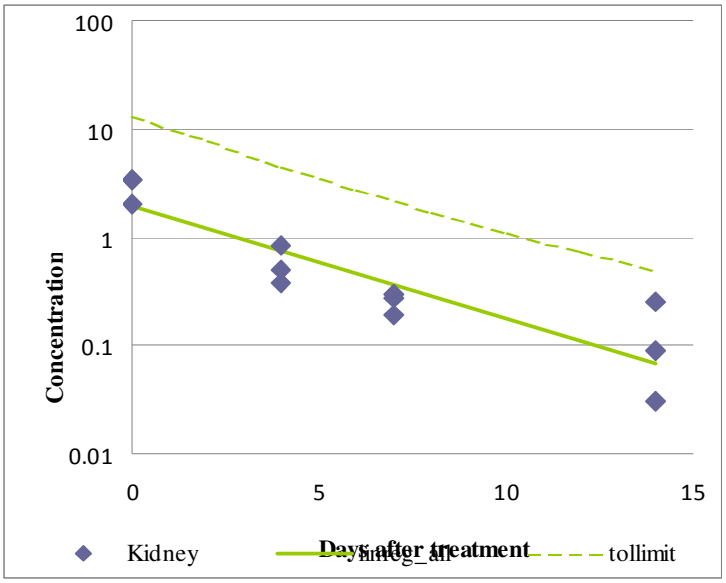


Figure 2.5. Tolerance limit considerations for calf kidney residues of apramycin

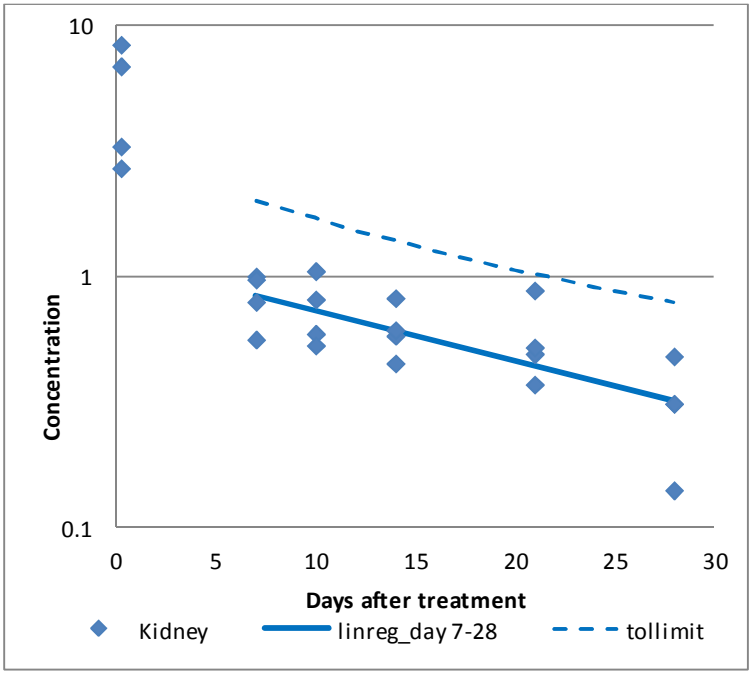


Figure 2.6. Tolerance limit considerations for chicken kidney residues of apramycin

Maximum residue limits

In recommending MRLs for apramycin, the Committee considered the following factors:

- A microbiological based acceptable daily intake (ADI) was established at 0–30 µg/kg bw, equivalent to an upper bound of 1800 µg per day for a 60 kg person.
- Apramycin is produced as a fermentation product and acceptable purity is ≥85%.
- Apramycin is poorly absorbed orally in calves, pigs and chickens.
- LOQs revised by the Committee were used to identify the values in the residue depletion studies which could be used for the assessment.
- Considering the revised LOQs, in four calf studies, 3 muscle, 24 kidney and 5 fat values were greater than the LOQ; in eight pig studies, 16 liver and 15 kidney samples were greater than the LOQ with almost all at doses of 1.6–2.3 times the recommended dose; in chicken, 24 kidney samples were above the LOQ.
- There are only sufficient residues above the LOQs in calf kidney and chicken kidney to estimate tolerance limits based on statistical approaches. In all other species and tissues, the low number of reported values above the respective LOQs made unachievable the assessment of tolerance limits based on statistical approaches.
- Residues are consistently highest in kidney tissues in the residue depletion studies, with the exception of one study. Kidney is the appropriate target tissue.
- Apramycin remains mostly unchanged and is therefore the appropriate marker residue.

The Committee recommended temporary MRLs at 5 mg/kg only in cattle and chicken kidney, measured as apramycin based on statistical approaches. If the MRLs were calculated according to the LOQs provided by the sponsor or the LOQs re-calculated by the Committee, the maximum estimated daily intake of apramycin residues in the worst case scenario would be around 1400 µg/day and would not exceed the upper bound of the ADI.

The sponsor is requested to provide improved analytical methods with better performance with lower LOQs, and residue depletion studies with appropriate sampling points close to the zero withdrawal periods for all tissues and species. The validated analytical method(s) and residue depletion studies are requested by the end of 2014.

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