The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

APRAMYCIN

SUMMARY REPORT (1)

1. Apramycin is an aminoglycoside antibiotic which is used in the treatment of colibacillosis and salmonellosis in calves, bacterial enteritis in pigs, colibacillosis in lambs and *E. coli* septicaemia in poultry. It is also administered to rabbits. Apramycin is not authorised for use in laying birds nor for use in cattle or sheep producing milk for human consumption. The available products include an oral doser intended for oral administration to neonatal pigs at a dose of 10 to 20 mg apramycin sulphate per kg bw per day for 3 to 5 days; and neonatal lambs at a dose of 10 mg/kg bw per day for 3 to 5 days, a premix for incorporation into pig feed at a rate of 100 mg/kg feed (equivalent to 3 to 7 mg/kg bw) for 28 days; a soluble powder for administration via the drinking water or milk replacer for calves at a rate of 20 to 40 mg/kg bw per day for 5 days to pigs at a rate of 7.5 to 12.5 mg/kg bw per day for 7 days; poultry at a rate of 250 to 500 mg active ingredient/litre (equivalent to 25-50 mg/kg bw) for 7 days and an injectable formulation for intramuscular administration to calves at a rate of 20 mg/kg bw per days for 5 days. In rabbits, apramycin is administered in the drinking water at a concentration of 50 to 100 mg/litre (equivalent to 10 to 15 mg/kg bw) for 5-8 days or in the feed at a concentration of 50 to 100 mg/kg feed (equivalent to 5 to 10 mg/kg bw) for up to 3 weeks.

2. Apramycin had no secondary pharmacological effects in a series of *in vitro* and *in vivo* studies designed to investigate possible effects on the cardiovascular, respiratory, renal and neuromuscular systems and isolated muscle preparations.

3. Following oral administration of 4 mg ^{14}C-apramycin to rats, 99.5% of the administered dose was recovered from the faeces and around 0.5% from the urine. When the same dose was administered subcutaneously, 93% was recovered from the faeces and around 7% from the urine. Small amounts of radioactivity remained in the kidney. The residues in the kidney and the urine consisted almost entirely of unmetabolised apramycin. In dogs, serum concentrations were monitored in the 6-month and 1-year repeated dose studies in which doses of 25 to 100 mg/kg bw were employed. Peak serum concentrations were directly proportional to the dose administered and were achieved 1 to 2 hours after dosing and were below the limit of detection 24 hours later. Urinary excretion corresponded to 0.3 to 10.5% of the administered dose.

4. Apramycin was of low acute toxicity when administered orally. Acute oral LD_{50} values were greater than 1250 mg/kg bw in Hartley guinea pigs, greater than 1600 mg/kg bw in NZW rabbits, greater than 1000 mg/kg bw in Beagle dogs, greater than 8000 mg/kg bw in Wistar rats and greater than 10000 mg/kg bw in ICR mice. There were no deaths at any of the dose levels used in the rat, mouse or dog studies. Apramycin was more toxic when administered parenterally. Acute intravenous LD_{50} values ranged from 162 to 1640 mg/kg bw and depended on the purity of the material tested.

5. Three repeated dose toxicity studies were carried out in rats using oral administration and duration of 3 to 6 months. The adverse effects were generally limited to mild anaemia and diarrhoea which may have resulted from the effects of the substance on the gut flora. Evidence of nephrotoxicity was found only in one study, in 3 out of 15 males fed the top dose level of 1% apramycin in the feed. The overall NOEL from these studies was 20.9 and 26.1 mg/kg bw for males and females, respectively.

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6. Three repeated dose toxicity studies were carried out in dogs using oral administration and durations of 3 months to 1 year. The adverse effects were mild reductions in haematology values and some changes in organ weights which did not correlate with any pathological findings. 25 mg/kg bw was a NOEL in a 6-month study and 50 mg/kg bw was a NOEL in a 1-year study.

7. In the classical repeated dose toxicity studies, there was little evidence for either ototoxicity or nephrotoxicity. This was probably due to the poor absorption of the substance following oral administration. A special study was carried out in 2 male and 2 female cats using daily subcutaneous administration of a 20% aqueous solution of apramycin for up to 30 days. The righting reflex remained normal in all the cats and the duration of post-rotatory nystagmus was unaffected in 3/4 cats. At termination, histopathological examination of the kidneys revealed severe nephrosis. It can be concluded that apramycin has greater potential for inducing nephrotoxicity when administered parenterally.

8. Target species tolerance studies were carried out in all the target species. The tolerance studies included special tests for ototoxicity, in swine no such effects were observed. The most frequent adverse effects were irritation at the injection site and diarrhoea following the administration of large doses.

9. No adverse effects on reproductive performance were observed in a multigeneration study, in which diets containing up to 1.0% apramycin (equivalent to 785 mg/kg bw per day) were fed to Fischer 344 rats, throughout 4 successive generations.

10. In a teratogenicity study, there was no evidence of maternal toxicity, foetotoxicity or teratogenicity following oral doses of up to 1000 mg/kg bw apramycin to pregnant Wistar rats.

11. Oral doses of 0, 2, 8 and 32 mg/kg bw were used in a rabbit teratology study. There were dose-related reductions in maternal food consumption and bodyweight gain in all treated groups. There was dose-related increase in the numbers of dams aborting. The incidence of resorptions was significantly increased at the top dose. Foetotoxicity was apparent at all dose levels with a dose-related reduction in foetal weight. One case of cleft palate and one case of omphalocele were found at 32 mg/kg bw; the significance of these findings was unclear because only 5 dams with 24 foetuses in this group survived to the scheduled kill. There was a dose-related increase in the incidence of supernumerary ribs though only the incidence in the 32 mg/kg bw group exceeded that of the "historical" controls. No NOELs were established for maternal toxicity or foetotoxicity. In this study the effects on the foetus were probably secondary to the severe maternal toxicity. Rabbits are highly susceptible to the effects of antibiotics on their gut flora. For this reason, the rabbit is not a suitable species for teratogenicity studies with antibiotics.

12. Apramycin was not mutagenic in 3 *in vitro* assays. Negative results were obtained in an *in vitro* bacterial assay for gene mutation, an *in vitro* assay for gene mutation in mammalian cells (mouse lymphoma assay) and an *in vitro* UDS assay in primary rat hepatocytes.

13. There was no evidence of carcinogenicity in a study in which B6C3F1 mice were fed diets containing up to 4.5% apramycin for up to 2 years. Pathological changes involving the non-brush border cortical tubular epithelium were observed in mice given 0.5% apramycin and above. A NOEL was not established due to the reduced bodyweight gain at 0.15%, the lowest dose level tested. There was no evidence of carcinogenicity in a study in which Fischer 344 rats were fed diets containing up to 5.0% apramycin for 2 years. The NOEL was 0.25%, equivalent to 124 mg/kg bw in males and 154 mg/kg bw per day in females.

14. Apramycin was not a sensitiser in 2 non-standard tests for delayed hypersensitivity in the guinea pig.

15. Apramycin is not used in human medicine. There were no data concerning possible effects in humans.

16. A NOEL of 25 mg/kg bw per day was established in a 6-month repeated dose toxicity study in Beagle dogs, based on mild anaemia and slight changes in organ weights. A similar NOEL was established in a 90-day repeated dose toxicity study in Wistar rats. A toxicological ADI of 0-250 µg/kg bw per day was calculated by applying a safety factor of 100 to the NOEL of 25 mg/kg.

17. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI (µg/kg bw)} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} \text{ (µg/ml)} \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

In vitro MIC data were provided for a wide range of bacteria of both human and veterinary origin. Many of the strains were insensitive to apramycin. It was decided to base the microbiological ADI on the MIC_{50} value for *Escherichia coli* because:

- an adequate number of human isolates had been studied;
- data supplied for veterinary strains indicated that the veterinary strains were also sensitive to apramycin and gave MIC values in the same range as the human isolates;

although some strains of human gut bacteria gave lower MIC values, e.g. *Bacteriodes ureolyticus*, only this one strain was sensitive; all the other strains of *Bacteriodes* were insensitive with MIC_{50} values greater than 64 µg/ml.

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\frac{8 \times 2}{1} \times 150}{1 \times 60} = 40 \text{ µg/kg bw i.e. } = 2400 \text{ µg/person}$$

The following assumptions were made:

- 8 µg/ml was the *in vitro* MIC_{50} for the most relevant, sensitive, gut organism (*Escherichia coli*) tested under aerobic conditions.
- A correction factor CF2 = 2 was used to convert from aerobic to anaerobic conditions; it was not considered necessary to correct for changes in pH or bacterial density. The MIC_{50} value used was the one obtained at the higher (10^8 CFU/ml) of the 2 bacterial densities tested.
- CF1 = 1 because the MIC_{50} for the most sensitive predominant and relevant organism was used.
- 1 = proportion of dose available to the microorganisms at the distal part of the gastrointestinal tract.
- 150 g = weight of daily faecal bolus
- 60 kg = human bodyweight

18. Pharmacokinetics and metabolism studies were carried out in which 2 pigs and a calf were given intramuscular injections of ^{14}C-labelled apramycin. Peak radioactivity concentrations were found in blood 0.5 hours after treatment and declined to around the limit of detection 24 hours later. Almost all of the radiolabelled material in blood and more than 90% of the material in excreta was microbiologically-active and appeared to be unmetabolised apramycin. The pigs were slaughtered 6 days after treatment and the calf was slaughtered 5 days after treatment and the total residues in tissues were determined. Residues of apramycin in liver and kidney were also determined using a microbiological assay. The majority of the residues in these tissues of both species appeared to consist of unmetabolised apramycin. Another study in pigs and calves using oral administration of ^{14}C-labelled apramycin gave similar results. The excreta consisted almost entirely of unmetabolised apramycin and greater than 75% of the radio-labelled material in liver and kidney consisted of unmetabolised apramycin. It can be concluded that apramycin is an appropriate marker residue for bovine and porcine tissues.

19. A residues depletion study was carried out in which pigs were given apramycin in the drinking water at a dose equivalent to 25 mg/kg bw per day for 7 days. The residues in tissues were determined using a microbiological assay with a limit of detection of 100 µg/kg. Detectable residues were found only in kidney and in fat samples taken within one day of treatment. Within one day of the end of treatment, mean residues in kidney were 2500 µg/kg and declined to 200 µg/kg, 14 days after cessation of treatment. In a modern GLP study, pigs were given daily oral doses of 20 mg/kg bw per day (the maximum indicated dose) for 7 consecutive days. The pigs were killed (4/time) at 1, 4, 7, 14, 21 and 28 days after the last treatment and residues in tissues were determined using HPLC. Detectable residues were found only in kidney. Residues of apramycin depleted from 5800 to 15300 µg/kg, one day after treatment, to 2500 to 3100 µg/kg, 7 days after treatment and were below the limit of detection (210 µg/kg), 14 days after treatment.

20. A residue depletion study was carried out in which 2 to 7 day old calves were given daily intramuscular injections of 20 mg/kg bw apramycin for 5 days. Residues in tissues were determined using a microbiological assay. The residues declined from mean values of 60,000 µg/kg in kidney, 5000 µg/kg in liver, 1500 µg/kg in fat, 1000 µg/kg in muscle and 1000 µg/kg at the injection site, 7 days after the end of treatment, to 8000 µg/kg in kidney, 6000 µg/kg in liver, 300 µg/kg in fat, 200 µg/kg in muscle and less than 200 µg/kg at the injection site, 28 days after treatment. In a modern GLP study, using the same dosage regime in 3-week old calves, the residues in tissues were determined using HPLC. The residues declined from 296,600 to 435,300 µg/kg in kidney, 8700 to 14,700 µg/kg in liver, 6200 µg/kg (mean value) in fat, 1900 to 3400 µg/kg in muscle and 23,600 to 65,100 µg/kg at the injection site, 4 hours after the last treatment, to 1200 to 14500 µg/kg in kidney, 3500 to 4200 µg/kg in liver, 400 µg/kg (mean value) in fat, below the limit of detection (268 µg/kg) in muscle and less than 268 to 4600 µg/kg at the injection site, 28 days after treatment. The pattern of residues depletion monitored using HLPC paralleled that obtained using the microbiological method.

21. A residue depletion study was carried out in which 2 to 7 day old calves were given daily oral doses of 40 mg/kg bw per day of apramycin, for 5 consecutive days, in the milk replacer. Residues in tissues were determined using a microbiological assay. The residues in tissues declined from 5000 to 20,000 µg/kg in kidney, 400 to 4000 µg/kg in liver, 100 to 200 µg/kg in fat and less than 50 µg/kg in muscle, 7 days after treatment, to 1600 to 3200 µg/kg in kidney, 1000-2000 µg/kg in liver and less than 50 µg/kg in fat and muscle, 21 days after treatment. In a modern GLP study, 3-week old calves were given daily oral doses of 40 mg/kg bw per day for 5 days and the residues in tissues were determined using HPLC. Residues in kidney declined from 2800 to 21,700 µg/kg, 7 days after treatment, to 200 to 9400 µg/kg, 21 days after treatment. Residues in bulked fat samples declined from 900 µg/kg 4 hours after treatment, to 100 µg/kg, 7 days after treatment and were below the limit of detection on subsequent occasions. Residues in muscle samples were below the limit of detection except for one sample (out of 4) taken, 21 days after treatment; a residue of 800 µg/kg was found in this sample and may have resulted from contamination. Residues in liver were very variable between individual animals and ranged from less than the limit of detection to 1200 µg/kg 7 days after treatment to 400 to 700 µg/kg 21 days after treatment. Again the pattern of residue depletion monitored using HPLC paralleled that obtained using the microbiological method.

22. A residue depletion study was carried out in which lambs were given daily oral doses of 10 mg/kg bw apramycin on 3 consecutive days. The lambs were killed (3 per time-point) within one day of the end of treatment, 21, 28 and 35 days after the end of treatment and residues in tissues were determined using a microbiological assay. Residues in liver and muscle were less than 500 µg/kg at all time points. Residues in fat were below the limit of detection (less than 500 µg/kg) at all time points except in the samples taken 21 days after treatment when concentrations in the range of less than 500 to 960 µg/kg was found. Residues in kidney were less than 500 to 2860 µg/kg immediately after the end of treatment and declined from 1200 to 1730 µg/kg 21 days after treatment to less than 500 µg/kg, 35 days after treatment.

23. A total residue depletion study was carried out in which broilers were given drinking water containing 500 mg per litre of ^{14}C-apramycin for 5 days. Mean total residues in kidney declined from 3230 µg/kg within one day of the end of treatment, to 1470 µg/kg 7 days later, and 470 µg/kg 14 days after the end of treatment. Over the same time period, mean total residues in liver declined from 420 µg/kg to 150 µg/kg to 80 µg/kg and mean total residues in skin from 200 µg/kg to 60 µg/kg to 30 µg/kg. Mean total residues in muscle declined from 70 µg/kg within one day of the end of treatment to 20 µg/kg 7 days after treatment. The liver and kidney samples were also analysed using a microbiological assay. From the results it was concluded that more than 80% of the residues in liver and kidney consisted of unmetabolised apramycin. It can be concluded that apramycin is an appropriate marker residue for chicken tissues.

24. Broilers were given drinking water containing 559 mg/l apramycin sulphate for 5 days. 6 birds per time point were killed within one day of the end of treatment and 7, 10 and 14 days after cessation of treatment. The residues in tissues were measured using a microbiological assay. Residues in all samples of muscle were less than 50 µg/kg. Residues in skin were detectable only in samples taken on the day of the last treatment (range 60 to 200 µg/kg). Mean residues in fat were 150 µg/kg on the day of the last treatment and were less than 50 µg/kg on subsequent occasions. Residues in liver were variable between individual birds and were 260 to 540 µg/kg on the day of treatment, 60 to 210 µg/kg 7 days later, and 50 to 230 µg/kg, 14 days after treatment.

25. No residue depletion data were provided for rabbits and there was no information to justify a choice of marker residue for this species.

26. Kidney was shown to be the main target tissue for pigs, calves, lambs and chickens. Residues were much higher following parenteral administration when compared with oral administration. Residues were much higher in calf tissues when compared with pig tissues; this was thought to be attributable to the higher doses used in calves and the enhanced absorption in the very young calves which were studied.

27. The proposed routine analytical method was based on HPLC with fluorescence detection. The method had satisfactory specificity. Validation data were provided for all edible bovine and porcine tissues and also for ovine kidney, chicken kidney and rabbit kidney. The limits of quantification were 5000 µg/kg for kidney and liver and 500 µg/kg for muscle, fat and skin/fat. The limits of detection were in the range of 23 µg/kg (porcine fat) to 394 µg/kg (ovine kidney). The stability of residues in bovine and ovine edible tissues and in rabbit kidney was shown to be satisfactory following storage at -20°C for several weeks.

28. Details of a microbiological method with a limit of detection of 100 µg/kg for all tissues was also provided. However the method was not specific and so was not suitable as the routine analytical method.

29. The residue depletion data on sheep and chickens were obtained using only a semi-quantitative non-specific microbiological assay. For sheep, there was an interval of 3 weeks between the first and second time-points. The data indicated that residues did not occur in chicken or sheep muscle nor in sheep liver and that residues were found only occasionally in chicken skin and fat and sheep fat. The results were at variance with the results for the bovine. It was concluded that no reliance could be placed on these results and therefore no MRLs could be elaborated for sheep and chickens.

30. Kidney was the appropriate target tissue for monitoring purposes in both cattle and pigs. Residues of apramycin were not detectable in porcine muscle and skin/fat samples at any time point; therefore it was decided to propose arbitrary MRLs for these tissues of 1000 µg/kg, i.e. at twice the limit of quantification of the HPLC assay. Residues of apramycin were not detectable in porcine liver samples at any time point; it was decided to propose the same arbitrary MRL of 1000 µg/kg for this tissue also.

Conclusions and recommendation

Having considered :

- the microbiological ADI of 40 µg/kg (2400 µg/person) for apramycin,
- apramycin as the marker residue,
- that an physico-chemical analytical method is available to detect residues of apramycin in target tissues in cattle and pigs,
- the pattern of residues depletion, which varied between the different species;

The Committee recommends the inclusion of apramycin in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Apramycin	Apramycin	Bovine	1 000 µg/kg 10 000 µg/kg 20 000 µg/kg	Muscle, fat Liver Kidney	For use in non-lactating cattle only. Provisional MRLs expire on 01.07.1999
		Porcine	1 000 µg/kg 1 000 µg/kg 5 000 µg/kg	Muscle, fat + skin Liver Kidney	Provisional MRLs expire on 01.07.1999

Based on these MRL values, the daily intake will not exceed 97% of the microbiological ADI.

LIST OF QUESTIONS

1. The analytical method should be improved to obtain limits of quantification which were no greater than half of the provisional MRLs for cattle and pigs. Validation data should be provided in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community .

2. To support MRLs for chickens and sheep, satisfactory residues depletion data to demonstrate the distribution of residues in these species should be provided and the routine analytical method should be validated in accordance with the requirements of Volume VI for all edible tissues of these species.

3. To support MRLs for rabbits, data should be provided in accordance with the agreed policy on minor species.