



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

KANAMYCIN

SUMMARY REPORT (2)

1. Kanamycin is an aminoglycoside antibiotic produced by the growth of *Streptomyces kanamyceticus*. Kanamycin comprised three components, kanamycin A, the major component usually designated as kanamycin, and kanamycins B and C, two minor congeners. It has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria.

Kanamycin (generally as sulphate form) is intended for intramuscular, intramammary or subcutaneous administration in adult cattle, horses and swine at a dose of 6 to 7.5 mg/kg bw, in calves and foals at a dose of 7.5 to 12 mg/kg bw, in piglets, sheep and goats at a dose of 11 to 15 mg/kg bw and chicken, turkey and rabbits at a dose of 15 mg/kg bw.

Kanamycin has a 30-year long history of human use. The recommended daily oral dose in adults is 8 000 to 1 2000 mg/person (120 to 200 mg/kg bw) and the recommended parenteral (intramuscular) daily doses range from 5 mg/kg bw for infants to 15 mg/kg bw for adults.

Currently, kanamycin is included 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
Kanamycin	Kanamycin	Bovine, ovine	100 µg/kg 100 µg/kg 600 µg/kg 2500 µg/kg 150 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.1.2004
		Porcine, chicken	100 µg/kg 100 µg/kg 600 µg/kg 2500 µg/kg	Muscle Skin + fat Liver Kidney	
		Rabbit	100 µg/kg 100 µg/kg 600 µg/kg 2500 µg/kg	Muscle Fat Liver Kidney	

In response to the list of questions, further to the establishment of provisional MRLs for kanamycin, additional data were provided for bovine, porcine and chicken only. No additional data were provided for sheep, goats, rabbits, turkeys and horses.

2. Like other aminoglycosides, kanamycin exerts a bactericidal action through inhibition of bacterial protein synthesis and reduction of translation fidelity at ribosomal level. The drug is primarily active against Gram-negative aerobic bacteria, whereas it shows a limited activity against most Gram-positives as well as anaerobes in general.

Cross-resistance occurs frequently between kanamycin, neomycin and paromomycin. Resistance can be either chromosomal or plasmid-mediated.

3. A number of studies exist on kanamycin pharmacokinetics, including old, pre-GLP tests in laboratory animals and clinical pharmacokinetics trials in humans. There is no evidence of any major difference in pharmacokinetics between laboratory species and humans, nor of any major difference between kanamycin and other aminoglycosides.

Like other aminoglycosides, kanamycin is a highly polar, cationic compound; therefore it has a very low oral bioavailability (approximately 1%) in all species, including humans. On the other hand, after parenteral administration the compound is rapidly and completely absorbed in all species. In human patients treated with kanamycin, the plasma concentration after intramuscular injection is adequately described by a one-compartment model, with an elimination half-life of 2 to 3 hours. Following absorption, kanamycin is primarily distributed into the extracellular fluid; in fact, the volume of distribution is equal to approximately 40% of body water. However, the half-life is longer in infants, in relation to birthweight and age, likely reflecting the ongoing maturation of the renal function. Moreover, in patients with severe renal dysfunction the plasma elimination half-life can be 20 to 40 times longer.

Plasma protein binding and erythrocyte binding are both below 10%.

Concentration in the bile approximates 30% of that in the plasma; only minor enterohepatic recirculation occurs.

4. In both laboratory species and humans a small portion of each kanamycin dose accumulates in body tissues and is tightly bound intracellularly. Such persistence occurs mainly in the selective target sites for aminoglycoside toxicity, i.e., the endolymph and perilymph of the inner ear and the renal cortex. Binding sites in the tissues become progressively saturated with the compound over the course of therapy. The portion of drug retained is then slowly released. Due to the accumulation of this small portion, complete recovery of a single parenteral dose in the urine may require up to 20 days in human patients with normal renal function.

Kanamycin is essentially unmetabolized in both laboratory species and humans. Following parenteral administration the parent compound is excreted unchanged in the urine. In patients with normal renal function between 80% and 90% of a single intramuscular dose is excreted within 24 hours.

Kanamycin is not inactivated in the gut. Following oral administration, unchanged kanamycin is excreted through the faeces.

5. The oral LD₅₀ in rats and mice were higher than 5 000 mg/kg bw, whereas intravenous LD₅₀ ranged from 200 to 600 mg/kg bw.
6. The available repeated-dose toxicity studies on kanamycin are scientific publications investigating the mechanism and pathogenesis of the selective target organ toxicity. No oral repeated toxicity studies were provided.

Guinea pigs were dosed intramuscularly with 0, 100, 200 and 400 mg kanamycin/kg bw/day for 4 weeks. Ototoxicity was assessed by means of pinna reflex response and histological examination of cochlea cells. Marked reduction of pinna reflex and slight loss of cochlea cells were observed at 200 and 400 mg/kg bw. No effect was seen at 100 mg/kg bw.

Male rabbits were dosed intramuscularly with 0, 50 and 100 mg kanamycin/kg bw/day for 30 days. Histopathological investigations were performed on both kidneys and ears. Dose-related effects were observed at both dose levels in kidneys and ears. At 50 mg/kg bw effects included slight outer hair cell loss and slight proximal tubular nephropathy.

Clear evidence of functional hearing loss was observed in female rats were given daily subcutaneous doses of 225 mg/kg bw for 6 weeks.

Male CD rats were dosed subcutaneously with 0, 50 and 150 mg kanamycin/kg bw/day for 4 weeks. Investigations concerned primarily the kidney; auditory function was measured by startle response. At 50 mg/kg bw the only effects were small increases in the number of epithelial cells in the urine and pale renal cortices, without apparent histological changes. Tubular nephropathy with mild necrosis of tubular cells was seen at 150 mg/kg bw. No changes in auditory function were observed.

Due to the presence of minor ototoxic effects in rabbits and nephrotoxic effects in rats and rabbits even at the lower parenteral dose tested of 50 mg/kg bw, no NOEL could be retained from these studies.

7. No reproduction studies were carried out.

Kanamycin concentrations up to 1000 µg/ml did not affect the motility of bull's spermatozoa upon exposure for 2 to 4 days.

No evidence for impaired fertility has been recorded for human patients treated with kanamycin.

8. Pre-GLP studies provide clear evidence that kanamycin, like other aminoglycosides, does not elicit gross abnormalities when administered parenterally to pregnant laboratory animals. Supportive evidence of a lack of teratogenicity is provided by a screening *in vitro* test with rat embryo midbrain and limb bud cell cultures, as well as by the lack of increase in birth defects observed in the clinical use of the drug.

Like other aminoglycosides, kanamycin may impair hearing and/or renal function of foetuses exposed *in utero*.

Loss of Corti's organ hair cells was observed in newborn guinea pigs exposed *in utero* during late pregnancy (gestation days 55 to 62) to dose levels as low as 200 mg/kg bw.

Significant damage of the cochlea epithelium was observed in newborn mice following exposure *in utero* to 400 mg/kg bw intraperitoneally during mid-gestation. In rats damage to cochlea epithelium occurred in pups treated with 400 mg/kg bw intraperitoneally during the second postnatal week.

No conclusion can be reached about a level without effect on auditory or renal development in laboratory animals.

9. Kanamycin has been tested for mutagenicity in a battery of assays. Three *Salmonella*/microsome assays have been performed in the presence and absence of metabolic activation with negative results being obtained in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1536, TA1537 and TA1538. However, kanamycin caused mutation of the non-standard strains TA104 and TA2638. Kanamycin was also tested for mitotic recombination in *Escherichia coli* and *Bacillus subtilis* in the presence and absence of metabolic activation; in a mouse lymphoma assay and an *in vivo* micronucleus test in mouse bone marrow (intraperitoneal administration), and all of these other tests gave negative results for mutagenicity. The weight of evidence indicates that kanamycin is not an *in vivo* mutagen.
10. No carcinogenicity studies were provided. Due to the absence of structural alerts for carcinogenicity, the absence of *in vivo* genotoxicity, the long history of safe therapeutic use in humans, carcinogenicity studies were not required.
11. Kanamycin sulphate did not elicit skin sensitisation in guinea pigs or in human volunteers administered kanamycin in ointment and cream. No significant effects on haematological parameters or target organs potentially relevant to immune function were detected in repeated dose toxicity studies.
12. The effect of kanamycin on relevant microorganisms of the human gut flora was determined in an *in vitro* assay. Ten strains each of the following bacterial species were tested: *Bacteroides fragilis*, *Bifidobacterium spp.*, *Clostridium spp.*, *Eubacterium spp.*, *Fusobacterium spp.*, *Lactobacillus spp.*, *Peptostreptococcus spp.*, *Proteus spp.*, *Streptococcus spp.* Moreover, 10 strains of *Escherichia coli* were tested under both aerobic and anaerobic conditions.

MIC₅₀-values higher than 32 µg/ml were observed for *Bacteroides fragilis*, *Bifidobacterium spp.*, *Clostridium spp.*, *Fusobacterium spp.*, *Proteus spp.* and *Streptococcus spp.* The MIC₅₀-values for *Eubacterium spp.*, *Lactobacillus spp.* and *Escherichia coli* (anaerobic conditions) were between 8 and 15 µg/ml. The MIC₅₀ for the most sensitive anaerobic species, *Peptostreptococcus*, was 3.2 µg/ml.

13. In an *in vitro* study the MIC-values of kanamycin against lactic acid bacteria used in the transformation of milk was evaluated under micro-aerophilic conditions. Strains of the following bacterial species were assayed: *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus casei*, *Pediococcus pentasaceus* and *Streptococcus thermophilus*.

MIC-values higher than 32 µg/ml were observed for *Lactobacillus casei* and *Pediococcus pentasaceus*; *Lactobacillus bulgaricus* showed a MIC of 4 µg/ml; *Lactobacillus lactis* and *Streptococcus thermophilus* are the most sensitive bacteria with a MIC of 2 µg/ml. No effect on any tested strain was observed at the concentration of 1 µg/ml, which can be retained as a No-Effect Concentration on microorganisms relevant to dairy industries.

14. Ototoxicity and nephrotoxicity are the most important clinical side effects of aminoglycosides, including kanamycin, in humans; these effects are related to duration and dose of the treatment and most likely occur in older individuals, and in patients who are dehydrated or suffer from renal diseases.

Ototoxicity derives from damage of the eighth cranial nerve with progressive loss of vestibular or cochlear cells. The form of ototoxicity induced by kanamycin is characterized by hearing impairment and more rarely by vestibular signs. A rare form of progressive deafness may occur after discontinuing the aminoglycoside therapy.

Induction of ototoxicity by kanamycin is associated with prolonged peak serum concentrations equal to or higher than 30 µg/ml. The incidence of either ototoxicity or nephrotoxicity in aminoglycoside-treated patients may be as high as 25%.

Aminoglycosides have been shown to readily cross the human placenta. Treatment late in pregnancy may lead to the accumulation of the drug in foetal serum and amniotic fluid; concentrations in human foetal serum are 16 to 50% of maternal serum concentrations. While irreversible bilateral hearing loss has been reported only for newborns exposed *in utero* to streptomycin, there are sufficient grounds to suspect a potential for foetotoxicity for all aminoglycosides. It was estimated that a breast-fed infant of a mother receiving kanamycin sulphate would ingest 0.95% of the usual therapeutic dose for an infant.

Clinical pharmacokinetics studies indicate that the daily dose and C_{max} may be the most relevant parameter as regards ototoxicity, whereas total dose, and AUC may be most relevant to nephrotoxicity.

Nevertheless, the abundant clinical literature indicates that a daily intramuscular dose of 10 mg/kg bw of kanamycin does not lead to any appreciable risk of ototoxicity or nephrotoxicity either in adults or in infants.

15. The studies on laboratory animals do not allow the determination of a toxicological ADI due to the lack of reproduction studies, of a NOEL for induction of ototoxicity or nephrotoxicity either in adult or in newborn animals. However, it is recognised that for the substances of the aminoglycoside group it is the microbiological activity, which is the determining factor on which to base the ADI.

A microbiological ADI based on the MIC₅₀ of 3.2 µg/ml against the most sensitive anaerobic species, *Peptostreptococcus*, reflecting the strong predominance of anaerobes among the intestinal bacterial flora is proposed.

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

$$\text{ADI} = \frac{\text{MIC}_{50} \text{ most sensitive organism} \times \text{CF2}}{\text{CF1}} \frac{(\mu\text{g/ml}) \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}} \quad (\mu\text{g/kg bw})$$

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

$$\text{ADI} = \frac{\frac{3.2 \times 1}{1} \times 150}{1 \times 60} = 8 \text{ } \mu\text{g/kg bw i.e.} = 480 \text{ } \mu\text{g/person}$$

The following assumptions were made:

- MIC₅₀ most sensitive organism (*Peptostreptococcus*): 3.2 µg/ml
 - CF1 = 1 because the MIC₅₀ for the most sensitive strain was used;
 - CF2 = 1 since no data were provided on the influence of pH and inoculum size;
 - 150 g was the weight of the daily faecal bolus;
 - fraction of an oral dose available for microorganisms: 1 because the oral bioavailability of kanamycin in humans is approximately 1%;
16. Kinetic studies were conducted in cattle, swine, sheep, treated intramuscularly and chicken treated subcutaneously with one single dose of kanamycin sulphate. Bleeding was carried out prior to, half, 1, 2, 3, 4, 6, 9, 12 and 14 hours after treatment. The antibiotic determination was carried out with a microbiological method using *Bacillus subtilis* ATCC 6633 strains with a detection limit of 0.3 to 0.4 µg/ml (equivalent to 300 to 400 µg/l).
- In cattle after intramuscular administration, peak plasma concentrations occurred between 0.5 and 1 hour after injection and were dose related: 30 µg/ml after administration of 10 mg/kg bw and 65 µg/ml after administration of 50 mg/kg bw.
- In chickens, as in cattle, maximum plasma concentrations of about 30 µg/ml were detected between 1 and 2 hours after subcutaneous administration of 15 mg kanamycin/kg bw. Concentration decreased rapidly, down to 1 µg/ml after 12 hours.
- In pigs maximum plasma concentrations of about 30 and 50 µg/ml occurred 1 hour after intramuscular administration of 10 and 20 mg/kg bw, respectively.
- Studies in sheep confirmed the same trend: maximum plasma concentrations of about 30 and 50 µg/ml occurred 1 hour after administration of 10 and 20 mg/kg bw, respectively.
17. In humans and in laboratory animals kanamycin is not metabolised and is eliminated as parent compound in the urine after parenteral administration or in the faeces after oral administration.

Metabolism studies have been conducted in target species

Three healthy milking cows received a single intramuscular administration of 5 ml of an injectable aqueous solution containing kanamycin equivalent to 7.5 mg kanamycin/kg bw. Urine, faeces and milk were collected from each animal at approximately 12 hour intervals during the 72 hours following administration. At the end of the sampling period, liver, kidneys and samples of muscle tissue, injection site and fat were collected.

A total of 87.3 to 104.2% of the administered dose was recovered by chemical (HPLC method, limits of quantification: 100 µg/kg for liver; 200 µg/kg for kidney and 50 µg/kg for muscle, fat and milk) and microbiological analyses. The highest amount of kanamycin A was recovered from urine (78.7 to 95.1% of the dose). Elimination was rapid, up to 86.9% of the total dose administered was eliminated during the first 12 hours after injection. Elimination via faeces ranged from 8.1 to 8.6% of the administered dose, approximately 6.3 to 7.2% were eliminated during the first 12-hour interval after treatment.

Excretion in milk was negligible, only 0.03 to 0.05% of the kanamycin dose were recovered within 12 hours after treatment.

Distribution into tissues was low. At 72 hours after treatment, only 0.5 to 0.6% of the administered kanamycin was found as total tissue residues. The highest amount was detected in kidneys (0.3 to 0.4%), residues in liver, injection site and abdominal fat were negligible. No kanamycin residues were detected in muscle. Residue concentrations of kanamycin A were practically equal to the total amount of residues with antimicrobial activity in the tissues investigated.

Three healthy male pigs received a single intramuscular administration of 1 ml of an injectable aqueous solution containing kanamycin equivalent to 15 mg kanamycin/kg bw. At approximately 12 hour intervals during the 72 hours following treatment, urine and faeces were collected from each animal. At the end of the sampling period, liver, kidneys and samples of muscle, injection site and skin with adhering fat were collected.

Chemical analyses were performed by HPLC method (limits of quantification: 100 µg/kg for liver; 200 µg/kg for kidney and 50 µg/kg for muscle and fat+skin) on the excreta and tissue/organ samples to detect concentrations of kanamycin. A total of 74.9 to 91.3% of the administered dose was recovered. The highest amount of kanamycin was recovered from urine (72.6 to 90.2% of the dose). Elimination was rapid, up to 86.4% of the total dose administered was eliminated during the first 12 hours after injection. Elimination via faeces ranged from 0.7 to 2.2% of the administered dose, approximately 0.4 to 1.6% were eliminated during the first 12 hours interval after treatment. Distribution into tissues was low. At 72 hours after treatment, only 0.6 to 0.7% of the administered kanamycin was found as total tissue residues. The highest amount was detected in kidneys (0.4 to 0.6%), residues in liver and injection site were negligible. No kanamycin residues were detected in muscle and skin with adhering fat samples. Residue concentrations of kanamycin A were practically equal to the total amount of residues with antimicrobial activity in the tissues investigated.

Six healthy chickens (3 males and 3 females) received a single subcutaneous administration of 1 ml/10 kg bw of an injectable aqueous solution (equivalent to 15 mg kanamycin/kg bw). At approximately 12 hour intervals, during the 72 hours following dosing, urine/faeces were collected from each animal. At the end of the sampling period, liver, kidneys and samples of muscle, injection site and skin+fat were collected.

Chemical analyses were performed by HPLC method (limits of quantification: 100 µg/kg for liver; 200 µg/kg for kidney and 50 µg/kg for muscle and fat+skin), on the excreta and tissue/organ samples to detect concentrations of kanamycin.

A total of 67.4 to 83.9% of the administered dose was recovered during 72 hours after treatment. The highest amount of kanamycin was recovered from urine/faeces (66.4 to 82.9% of the dose). Elimination was rapid, up to 60.4% of the total dose administered was eliminated during the first 12 hours after injection. Distribution into tissues was low. At 72 hours after treatment, only 0.7 to 1.1% of the administered kanamycin was found as total tissue residues. The recovered amount was nearly evenly distributed to kidneys, liver, muscle and injection site. Negligible kanamycin residues were detected in skin+fat samples. Residue concentrations of kanamycin A were practically equal to the total amount of residues with antimicrobial activity in the tissues investigated.

The ratio of marker to total residues with antimicrobial activity was not established in all the target species. However, considering that the major part of kanamycin administered to farm animals is excreted in an unchanged form in the urine and faeces, only a very small proportion of potential tissue residues in farm animals is likely to be in the form of a metabolite. Therefore, the available data suggest that, like the other aminoglycosides, kanamycin is not significantly metabolised. Therefore, kanamycin A was retained as the marker residue. Considering the data available the ratio of marker residue to total residues with antimicrobial activity was assumed to be equal to 1.

18. Residue depletion studies in the target species conducted with a commercial injectable product containing 200 mg of kanamycin sulphate (equivalent to 150 mg kanamycin) were performed. Residual concentrations were determined with a microbiological assay using *Bacillus subtilis* ATCC 6633 (limit of detection: 100 µg/kg).

In 10 calves, after intramuscular administration of 12 mg/kg bw kanamycin twice daily, 12 hours apart for 5 consecutive days, the microbiologically active residues in muscle were close the limit of detection of the analytical method (100 µg/kg) 10 days after the last administration and below the limit of detection at the other sampling times. At the injection site the microbiologically active residue values were 650 ± 220 µg/kg at 10 days and below the limit of detection 20 days after the last administration. Microbiologically active residue concentrations in liver were 3810, 1480, 200 and less than 100 µg/kg at 10, 20, 30 and 40 days, respectively. The highest concentrations were detected in kidney: 16380, 5970, 710 and less than 100 µg/kg at 10, 20, 30, 40 and 50 days, respectively. In fat concentrations were always below the limit of detection of the analytical method.

After intramuscular administration in 5 lactating cows of 7.5 mg kanamycin/kg bw, twice daily for 5 consecutive days, mean residue concentrations were 1400, 840, 150 and below the limit of detection of the microbiological analytical method (100 µg/kg) at the first, second, third and after the fourth milking, respectively.

Kanamycin residue concentrations were determined in milk samples from sheep with different milk productivity (4 high yielding and 4 low yielding ewes), following the intramuscular administration of an injectable kanamycin-based solution given at the maximum recommended dose (15 mg/kg bw every 12 hour for 5 consecutive days). The milk samples were collected from the 8 animals at 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours after administration. Kanamycin residue concentrations in the sheep milk samples were determined by HPLC (limit of quantification: 43 µg/l). Milk concentrations were below the limit of quantification after the fourth milking.

In 10 piglets, after intramuscular administration of 15 mg/kg bw twice daily for 5 consecutive days, tissue residues as well as tissue depletion kinetics were very similar to those obtained in calves. In muscle and at the injection site microbiologically active residues were below the limit of detection at 20 days after the last administration. Microbiologically active residues were more persistent in liver with 4 190, 820, 820 and less than 100 µg/kg at 10, 20, 30 and 40 days, respectively, and kidney with 12 210, 7 420, 1 020, 240 and less than 100 µg/kg at 10, 20, 30, 40 and 50 days, respectively. In fat concentrations were always below the limit of detection.

In 25 chickens, after subcutaneous administration of 15 mg/kg bw twice daily for 5 consecutive days, microbiologically active residues concentrations were below the limit of detection in muscle, fat and injection site at any time. In liver, microbiologically active residue concentrations were low (170 µg/kg) 10 days after the last administration. In kidneys microbiologically active residues concentrations were 2980, 190 and less than 100 µg/kg at 10, 20 and 30 days, respectively.

Twenty-five rabbits were administered kanamycin subcutaneously at 15 mg/kg bw twice daily, 12 hours apart, for 5 consecutive days. Microbiologically active residue concentrations in kidneys were 10160, 3470, 210 and lower than 100 µg/kg at 10, 20, 30, 40 and 50 days, respectively, and in liver 960, 470, 170 and lower than 100 µg/kg at 0, 20, 30 and 40 days, respectively. In muscle and fat microbiologically active residues were always below the limit of detection. The depletion kinetics of kanamycin were studied in milk following two intramammary infusions at 12 hours interval of 12 cows with a commercial product containing cephalexin 200 mg and kanamycin sulphate 100 000 I.U. Milk samples were collected every 12 hours until 144 hours. The analyses of the milk samples were performed using an HPLC analytical method (limit of detection: 7 µg/kg, limit of quantification: 100 µg/kg). The milk kanamycin residue levels were 668 µg/kg at 36 hours, 329 µg/kg at 48 hours, 154 µg/kg at 60 hours and 99 µg/kg at 72 hours.

19. Residue depletion studies according to the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Union have been conducted in cattle, pigs and chickens.

Four groups of calves, each comprising 2 males and 2 females, received intramuscular administrations of a commercial injectable product containing 200 mg of kanamycin sulphate (equivalent to 150 mg kanamycin) for 5 consecutive days (total of 10 injections). Animals were killed after withdrawal periods of approximately 7, 28, 35 and 49 days.

Samples of kidneys, liver, muscle, fat and injection site were collected. The analyses of the tissue samples were performed using an HPLC analytical method (limit of quantification: liver 100 µg/kg, kidney 200 µg/kg, muscle 50 µg/kg, fat 50 µg/kg). In muscle kanamycin concentrations were 71 µg/kg, 53 µg/kg at 7 and 28 days after treatment. At 35 days residue concentrations above the limit of quantification were detected in 2 animals only (mean 57 µg/kg). At 49 days residue concentrations were below the limit of quantification in all animals. In kidney residue concentrations were 719 µg/kg, 4 706 µg/kg, 4 327 µg/kg, 3 417 µg/kg at 7, 28, 35 and 49 days after the last treatment, respectively; in fat 202 µg/kg and less than the limit of quantification at 7, 28, 35 and 49 days after the last treatment, respectively; in liver 2611 µg/kg, 1 671 µg/kg, 1 057 µg/kg, 1 013 µg/kg at 7, 28, 35 and 49 days after the last treatment; at the injection site 3 133 µg/kg, 2 237 µg/kg, 2 875 µg/kg and 1 631 µg/kg at 7, 28, 35 and 49 days after the last treatment, respectively.

The milk residue depletion profile of kanamycin was investigated in 20 lactating cows treated twice daily (at approximately 12 hours intervals) with an intramuscular administration of 5 ml/100 kg bw of an injectable aqueous solution containing a dose equivalent to 7.5 mg kanamycin/kg bw for 5 consecutive days (total of 10 injections per cow). From 36 hours after treatment all the values of kanamycin A concentrations were below the limit of quantification (50 µg/l) or below the limit of detection (17 µg/l).

Four groups of pigs, each comprising 2 males and 2 females, received intramuscular administrations of a commercial injectable product containing 200 mg of kanamycin sulphate (equivalent to 150 mg kanamycin) for 5 consecutive days (total of 10 injections). The animals were killed after withdrawal periods of approximately 7, 28, 35 and 49 days. Samples of kidneys, liver, muscle, muscle at the injection site and skin+fat were collected. The analyses of the tissue samples were performed using an HPLC method (limit of quantification: 50 µg/kg for skin+fat and muscle, 100 µg/kg for liver and 200 µg/kg for kidney).

Kanamycin concentrations were in muscle 366 µg/kg at 7, 28, 35 and 49 days after the last treatment, respectively. At 28 days residue concentrations above the limit of quantification were detected in 2 animals only (52 µg/kg). At 35 and 49 days residue concentrations were below the limit of quantification in all animals. In liver 4 378 µg/kg, 1 157 µg/kg, 917 µg/kg, 429 µg/kg at 7, 28, 35 and 49 days after the last treatment, respectively; in kidney 22 745 µg/kg, 1 508 µg/kg, 1 040 µg/kg, 1 613 µg/kg at 7, 28, 35 and 49 days after the last treatment, respectively; in skin+fat 163 µg/kg at 7 days after the last treatment and less than the limit of quantification at the other sampling times; at the injection site 8 679 µg/kg, 339 µg/kg, 3 811 µg/kg, 1 419 µg/kg at 7, 28, 35 and 49 days after the last treatment.

Four groups of chickens, each comprising 3 males and 3 females, received a single intramuscular administration of a commercial injectable product containing 200 mg of kanamycin sulphate (equivalent to 150 mg kanamycin), at a dose volume of 0.1 ml/kg (equivalent to 15 mg/kg of active ingredient). Animals were killed after withdrawal periods of 5, 15, 20 and 30 days.

Samples of kidneys, liver, muscle, muscle at the injection site and skin + fat were collected.

The analyses of the tissue samples were performed using an HPLC method (limit of quantification: 50 µg/kg for skin/fat and muscle, 100 µg/g for liver, 200 µg/g for kidney).

Kanamycin concentrations were 50 µg/kg in muscle at 5 days after the treatment and less than the limit of quantification at the other sampling times; in kidney 1600 µg/kg and 600 µg/kg at 5 and 15 days respectively. At day 20 residue concentrations above the limit of quantification were detected in one animal only (249 µg/kg). At day 30 residue concentrations were below the limit of quantification in all animals; in liver 1890 µg/kg, 490 µg/kg, 120 µg/kg and less than the limit of quantification at 5, 15, 20 and 30 days after the treatment, respectively; in skin+fat 70 µg/kg at 5 days after the treatment and less than the limit of quantification at the other sampling times; at the injection site 1150 µg/kg, 40 µg/kg, 261 µg/kg, 130 µg/kg at 5, 15, 20 and 30 days after the treatment, respectively.

20. The proposed routine analytical method for bovine including milk, porcine and chicken was based on HPLC with spectrofluorimetric detection. The method detects the A and B components of kanamycin. The method was validated according to the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Union. Residues of other aminoglycosides did not interfere in the assay. The limits of quantification for kanamycin A were 100 µg/kg for liver; 200 µg/kg for kidney and 50 µg/kg for muscle, fat for the edible tissues of bovine, chicken and porcine. The limit of quantification for bovine milk was 50 µg/kg. Applicability of this method should not be problematic and therefore from this aspect extrapolation to the tissues and milk of other species would be possible.
21. In application of the guideline “Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL)” it was considered possible to extrapolate the proposed MRLs for bovine, porcine and chicken to further food producing species. As no information was available on residue data allowing to confirm the marker residue in fish tissues, fish species had to be exempted from the extrapolation.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 8 µg/kg (i.e. 480 µg/person) was established,
- kanamycin A was retained as the marker residue for all food producing species, but could not be confirmed for fish;
- the ratio of marker residue to total residues with antimicrobial activity was assumed to be equal to 1,
- a validated analytical method for monitoring residues for porcine, chicken and bovine including milk, is available and that the method is also considered to be applicable to all food producing species except fish;

the Committee recommends the inclusion of kanamycin in Annex I to 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
Kanamycin	Kanamycin A	All food producing species except fish	100 µg/kg 100 µg/kg 600 µg/kg 2500 µg/kg 150 µg/kg	Muscle Fat* Liver Kidney Milk	Not for use in animals from which eggs are produced for human consumption

*For porcine and poultry species this MRL relates to “skin and fat in natural proportions”

Based on these MRLs values, the daily intake will represent about 93% of the ADI.