The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

KANAMYCIN

SUMMARY REPORT (1)

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 and horses at a dose of 6 to 7.5 mg/kg bw, calves and foals at a dose of 7.5 to 12 mg/kg bw, piglets at a dose of 11 to 15 mg/kg bw, swine a the dose of 6 to 7.5 mg/kg bw, sheep and goats at a dose of 11 to 15 mg/kg bw, chicken and turkey at a dose of 15 mg/kg bw, rabbit 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 8000 to 12000 mg (120 to 200 mg/kg bw); recommended parenteral (intramuscular) daily doses range from 5 mg/kg bw for infants to 15 mg/kg bw for adults.

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 directed 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 aminoglysides.

   Like other aminoglysides, 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.

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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 LD_{50} in rats and mice were higher than 5000 mg/kg bw, whereas intravenous LD_{50} 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/kg bw/day of kanamycin 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 intramuscular with 0, 50 and 100 mg/kg bw/day of kanamycin for 30 days. Histopathological investigations were performed on both kidneys and ears. Dose-related effects were observed at both dose levels both 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/kg bw/day of kanamycin 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. Old, 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 aminoglysides, 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 midgestation. 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 *Slamonella 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, no carcinogenicity studies are 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, ten strains of *Escherichia coli* were tested under both aerobic and anaerobic conditions.

    MIC_{50}-values higher than 32 µg/ml were observed for *Bacteroides fragilis*, *Bifidobacterium spp.*, *Clostridium spp., Fusobacterium spp., Proteus spp.* and *Streptococcus spp.* The MIC_{50}-values for *Eubacterium spp., Lactobacillus spp.* and *Escherichia coli* (anaerobic conditions) were between 8 and 15 µg/ml. The MIC_{50} 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 old individuals, and in patients which 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 is sufficient ground to suspect a potential for fetotoxicity 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 parameters 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 cannot 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 aminoglycoside group of substances it is the microbiological activity which is the determinating factor on which to base the ADI.

    A microbiological ADI based on the MIC_{50} 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.

    The microbiological ADI was calculated as indicated below:

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

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

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

    The following assumptions were made:

- CF1 = 1 because the MIC_{50} 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 approximatly 1%;
- MIC_{50} most sensitive organism: 3.2 µg/ml

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/kg bw of kanamycin. 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. No metabolism studies were provided in the target species. 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.

18. Some new 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 provided. Residual concentrations were determined with a microbiological assay using *Bacillus subtilis* ATCC 6633 (limit of detection: 100 µg/kg or l).

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) ten 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 ten days and below the limit of detection 20 days after the last administration. Microbiologically active residual concentrations in liver were 3810, 1480, 200 and lower than 100 µg/kg at 10, 20, 30 and 40 days. The highest concentrations were detected in kidney: 16380, 5970, 710 and lower 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/kg bw, twice daily for five consecutive days, mean residual 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 kamamycin-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 4190, 820, 820 and lower than 100 µg/kg at 10, 20, 30 and 40 days, respectively, and kidney with 12210, 7420, 1020, 240 and lower 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 lower 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.

Although the residue depletion studies in edible tissues of cattle, pigs, chickens, rabbits and in cow's milk were carried out with a resticted number of animals the information provided was considered provisionally sufficient to reflect the depletion of kanamycin in these species; for sheep milk the data were considered satisfactory. No residue data were provided for turkeys, goats and horses.

19. An HPLC method with UV detection for the determination of kanamycin A and B in bovine edible tissues including milk and kanamycin A in sheep milk was proposed as routine analytical method. However, as the experimental design of the validation was not clearly presented and as all the parameters of validation were not determined according to the recommendations of Volume VI of The Rules Governing Medicinal Products in the European Community, the method could not be considered as fully validated. In addition in absence of information concerning the proportion of kanamycin A and B in edible tissues, it was concluded that the analytical method proposed could not be accepted, at present, for monitoring purposes. The limits of quantification of each compound in this method are 50 µg/kg for fat, muscle and milk, 100 µg/kg for liver and 200 µg/kg kidney.

    A microbiological method using *Bacillus subtilis* ATCC 6633 with a limit of detection of 100 µg/kg is available; the method is not validated according to the requirements of Volume VI. Considering that kanamycin is not metabolised and the analytical method reveals the microbiologically active compounds the method can be proviosionally accepted for monitoring purposes.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 8 µg/kg (i.e 480 µg/person) was established,
- kanamycin is the microbiological active residue,
- data on the residue distribution of kanamycin in cattle, sheep, pigs, chickens and rabbits is available,
- a microbiological analytical method is available; however a validated routine analytical method in accordance with the requirements of Volume VI is not available,
- no residues data were available for turkeys, goats and horses;

the Committee recommends the inclusion of kanamycin in Annex III 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	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 1.1.2002
		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	

Based on these MRLs values, the daily intake will represent about 96% of the ADI.

Before the Committee can consider the inclusion of kanamycin in Annex I to Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should provide additional data in order to identify the marker residue and the ratio of marker residue to total residues in the major target species and in cow's milk.
2. The applicant should provide further residue depletion studies in edible tissues of cattle, sheep, pigs, chicken, rabbits and in cow's milk in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community, with particular attention to the following points:
   - an adequate number of animals should be sampled at each time point,
   - residue depletion values should be provided for skin plus fat in natural proportions in pigs and poultry.
3. In order to establish Maximum Residues Limits for turkeys, goats and horses additional residue depletion studies in accordance with Volume VI, taking also into account the agreed CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (Doc. EMEA/CVMP/153a/97-FINAL), should be provided.
4. The applicant should provide a fully validated physico-chemical analytical method for monitoring of residues in all edible tissues of sheep, pigs, chicken, rabbits, turkeys, goats and horses in accordance with Volume VI of the "Rules Governing Medicinal Products in the European Community", taking also into account the agreed CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (Doc. EMEA/CVMP/153a/97-FINAL). The method should be described in an internationally recognised format (e.g. ISO 78/2). Particular attention should be paid to the specificity of the method in comparison to other aminoglycosides.
5. The applicant should provide further validation data concerning the physico-chemical analytical method for cattle tissues including milk and sheep milk in accordance with Volume VI, taking also into account the agreed CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (Doc. EMEA/CVMP/153a/97-FINAL). Particular attention should be paid to the specificity of the method in comparison to other aminoglycosides.