



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AMINOSIDINE

SUMMARY REPORT (1)

1. Aminosidine (Paromomycin) is an aminoglycoside antibiotic intended for treatment of a number of bacterial infections (colibacillosis, salmonellosis) either by parental administration (calves, pigs, dry cows) or by oral administration through feed or drinking water (calves, piglets, broiler chickens). Aminosidine is not intended for use in milk producing animals or laying hens. The sulphate salt is normally used in veterinary medicine.

The recommended dosage regimen is comprise between 10 and 50 mg/kg bw for bovine, pigs, poultry and rabbits, once or twice daily, for 3 to 5 days.

2. Pharmacokinetics studies were performed in rabbits, poultry, bovine and porcine. No substantial differences between species were observed. Like other aminoglycosides, aminosidine is poorly absorbed from the gastro-intestinal tract. Absorption from the intramuscular injection site is rapid and nearly complete.

Following intramuscular administration peak plasmatic levels are observed within 1 hour.

The plasmatic profile indicates that aminosidine does not accumulate during treatment, except for the renal cortex and cochlea. Pharmacokinetics analysis shows also a relatively long distribution half-life (about 1 hour) and an elimination half-life of about 5 hours.

Aminosidine undergoes negligible biotransformation when administered parenterally. The parent compound is consistently the main component of the total residue in both urine and feces.

Excretion occurs mostly in the feces following oral administration and almost exclusively in the urine following parental treatment. The urinary drug recovery ranges from 80 to 100 % of the total dose administrated.

3. Aminosidine shows a low acute oral toxicity (the rat minimal lethal dose is 10.000 mg/kg bw), when compared to parental treatment were the rat minimal lethal dose is 670 mg/kg bw by intramuscular and 620 mg/kg bw by intravenous; this reflects a low oral bioavailability which is typical of aminoglycoside antibiotics.
4. Parental repeated dose toxicity studies were carried out in the rat and mouse (2 months, subcutaneous), rabbit (1 month, intramuscular) and cat (1 month, subcutaneous). Typical tubular nephropathy lesions were elicited in rodents and rabbits at 400, 200 and 60 mg/kg bw in the mouse, rat and rabbit, respectively. No NOEL was determined. In cats, vestibular and neurological alterations were induced at 50 mg/kg bw.

No oral studies were available.

5. Prenatal toxicity studies were carried out in rats (up to 400 mg/kg bw, by intramuscular injection during gestation from day 7 to 13; up to 200 mg/kg bw, by subcutaneous injection throughout pregnancy).

No developmental toxicity was observed even at the higher dose levels tested.

In the subcutaneous study, the general health and development of auditory function were also examined in the offspring : no effects were seen. The potential for impaired renal development was not investigated, although the kidney appears to be a more sensitive target of aminosidine toxicity in rodents as compared to the ear. No other studies on the reproductive function were available.

6. Two 2-year studies were available, a combined chronic toxicity-carcinogenicity study on Sprague-Dawley rats and a chronic toxicity study on beagle dogs. Both were performed under GLP conditions. In both trials the animals were exposed through the diet to 0, 100, 2.000 and 50.000 mg of aminosidine per kg of food.

The dog appears markedly more sensitive to aminosidine toxicity than the rat.

In rats, at the highest exposure level, body weight gain and food utilisation were reduced, and the urinary pH was consistently lower; occasional lowering of urinary pH was observed also in the other treated groups. No evidence of dose-related increases in neoplastic or non-neoplastic alterations was observed. The NOEL was 2.000 mg/kg (approx. 78.5 mg/kg bw).

In dogs, dose-related increases of cataracts and renal tubular lesions were observed from 2.000 mg/kg (approx. 68 mg/kg bw). The NOEL was 100 mg/kg (approx. 3.4 mg/kg bw).

7. A battery of genotoxicity tests were performed under GLP conditions, including an *in vitro* bacterial mutagenicity assay (Ames test), an *in vitro* assay for gene mutation in mammalian cells (CHO), an *in vivo* mouse micronucleus test. All tests gave negative results.
8. No specific studies on immune function were presented, besides information on the lack of effects on the response to fowl cholera vaccine in chicken given 1.000 mg of aminosidine per kg of food for 2 months.

No effects indicative of toxicity to the immune response were observed in any of the standard toxicity studies.

9. In human therapy, aminosidine has been used to treat some intestinal parasites. For intestinal Amoebiasis, the recommended dose for both adults and children is 25-35 mg/kg bw for 5-10 days. Higher doses (45-67 mg/kg bw) for shorter periods (1-7 days) are recommended for the treatment of tapeworm infections.

The reported adverse effects are analogous to those induced by other aminoglycosides, including hypersensitivity reactions and tubular nephrotoxicity. However, no detailed data have been provided to determine a level without adverse effects in humans.

10. Although there are deficiencies in the available data, a provisional toxicological ADI for aminosidine can be established on the basis of the NOEL derived from the oral chronic toxicity study in dogs.

Based on this NOEL of 3.4 mg/kg bw/day and on a safety factor of 200 to cover the lack of an oral reproduction study and of a detailed appraisal of human data, the provisional toxicological ADI is calculated to be 0.017 mg/kg bw.

11. Since the ADI should be low enough to eliminate the possibility of any pharmacological, microbiological or other biological effect, in the case of aminosidine the microbiological risk of residues for the human gut flora was considered.

In a study on human volunteers, an effect on gut flora was observed even at the lower dose tested (0.75 g/day, i.e., 12.5 mg/kg, resulting in an aminosidine concentration in the feces of at least 300 mg/l).

E. coli, *Proteus* and *Lactobacilli* proved particularly sensitive, while *Klebsiella*, *Clostridia* and *Enterococci* showed a relative tendency to increase.

Adequate *in vitro* studies confirmed the high sensitivity of *E. coli* and *Lactobacilli* : the MIC₅₀ for these most sensitive organisms was 10 mg/l.

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

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} \times \text{daily faecal bolus (0.15 l)}}{\frac{\text{fraction of an oral dose available for microorganisms}}{\text{weight of human (60 kg)}}} \quad (\mu\text{g/kg bw})$$

As the MIC₅₀ of the most sensitive organisms (*E. coli* and *Lactobacillus* spp.) was retained (10 mg/l) no correction factor is needed and CF1 is equal to 1. A conservative CF2 safety factor, equal to 1, was retained.

The systemic bioavailability upon oral exposure appears to be poor; in the absence of a precise assessment a conservative assumption is made that 95% of the dose remains unabsorbed.

Based on the above, the microbiological ADI can be established as:

$$\text{ADI} = \frac{10 \times 0.15}{0.95 \times 60} = 0.025 \text{ mg/kg bw} = 1.5 \text{ mg for a 60 kg person}$$

As the microbiological ADI is greater than the provisional toxicologically derived ADI of 1 mg/person, the provisional toxicological ADI will be retained to establish the MRLs values.

12. In a residues depletion study, calves were treated with 5 consecutive daily intramuscular administrations of 21 mg/kg bw; residue analysis were carried out by means of microbiological assay.

No detectable residues were observed in the muscle from day 7 onwards (sensitivity of the method 0.2 mg/kg). At the injection site, residues below 1 mg/kg were detected at 7 but not at 15 days after the end of the treatment).

In kidneys and liver, residues could be quantified till 30 days after the last injection (sensitivity of the method 0.3 mg/kg). In kidneys, the highest concentration of 26 mg/kg being observed at 7 days after the end of the treatment. In liver, the highest concentration of 4.7 mg/kg being also observed at 7 days after the end of the treatment.

13. Pigs were treated with 5 consecutive daily intramuscular administrations of 21 mg/kg bw; residue analysis were carried out by means of microbiological assay.

No detectable residues were observed in the liver (sensitivity of the method 0.16 mg/kg) and in the muscle from day 7 onwards. At the injection sites, aminosidine persisted 7 days.

In kidneys, residues could be quantified till 20 days after the last injection (sensitivity of the method 0.32 mg/kg), the highest concentration of 2 mg/kg being observed at 7 days after the end of the treatment.

14. In chickens sacrificed at 0,2,4,7,14 and 21 days after a dietary administration at the recommended therapeutic low levels (280 mg/kg of feed), administered during 5 days, no residues of aminosidine in tissues were detected in muscle and liver (sensitivity of the method 0.1 mg/kg). In skin and fat, residues were decreasing from 1.4 mg/kg at day 0 to 0.5 at day 7, and were no longer detectable at day 14. In kidneys, the highest concentration of 2.6 mg/kg was observed at day 0 after the end of the treatment, whilst residues were no longer detectable at day 4.
15. In rabbits sacrificed at 2h, 48h and 7 days respectively after the end of an oral administration of a daily dose of 80 mg of aminosidine for 7 consecutive days, no residues were detected in muscle and liver (sensitivity of the method 0.16 mg/kg) 2 hours after the last dosing. In kidneys, residues disappear within 48 hours after the oral treatment.
16. The parent compound was the main component for the total residue in both urine and feces of treated animals; thus, aminosidine could be retained as marker residue.

17. A screening microbiological method for the determination of residues in the tissues of calves, pigs and chickens utilises *B. subtilis* ATCC 6633. The sensitivity of the method is 0.3 mg/kg.
18. A HPLC method is currently under development for detection and quantification of residues in kidneys, liver, fat and muscle from bovine and pigs.

Conclusions and recommendation

Having considered that

- a provisional toxicological ADI can be set at 0.017 mg/kg bw (1 mg/person) for aminosidine;
- a screening microbiological method is available;
- no validated physico-chemical analytical method is available to detect residues of aminosidine in bovine, porcine, chicken and rabbits tissues;

The Committee recommends the inclusion of aminosidine into Annex III of Council Regulation (EEC) No 2377/90 for bovine, porcine chicken and rabbits in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Aminosidine	Aminosidine	Bovine, porcine, rabbits, chicken	500 µg/kg 1500 µg/kg 1500 µg/kg	Muscle Liver Kidney	Provisional MRLs expire on 1.7.1998

Based on these MRLs, the predicted total daily intake of biologically significant residues of aminosidine resulting from consumption of the complete meat package is about 0.4 mg/person, which accounts for 40% of the provisional toxicological ADI of 1 mg/person.

LIST OF QUESTIONS

Safety File

1. In line with the recommendations made by the JEFCA with regard to other aminoglycosides, the Applicant should discuss the potential of aminosidine for eliciting effects on reproduction and postnatal development upon oral exposure.

Residue File

2. In accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community, the applicant should provide a fully validated method for the analysis of aminosidine residues in the tissues of bovine, chicken, rabbits and porcine species for which MRLs are required and this method should be described in an internationally recognised standard layout (e.g. ISO 78/2).