COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PAROMOMYCIN

SUMMARY REPORT (2)

1. Paromomycin (synonym: aminosidine, CAS-No 7542-37-2) is an aminoglycoside antibiotic intended for treatment of a number of bacterial infections (coli bacillosis, salmonellosis) either by parenteral administration (calves, pigs, dry cows) or by oral administration in feed or drinking water (calves, piglets, broiler chickens). Paromomycin is not intended for use in milk producing animals or laying hens. The sulphate salt is normally used in veterinary medicine.

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

Paromomycin is currently included under its synonym aminosidine in Annex III of Council Regulation (EEC) No 2377/90, in accordance with the following table:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Marker residue</th>
<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminosidine</td>
<td>Aminosidine</td>
<td>Bovine, porcine, rabbits, chicken</td>
<td>500 µg/kg</td>
<td>Muscle</td>
<td>Provisional MRLs expire on 1.7.2000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1500 µg/kg</td>
<td>Liver</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1500 µg/kg</td>
<td>Kidney</td>
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</tbody>
</table>

Further data have now been provided to support the establishment of final MRLs.

2. In human therapy, paromomycin is used to treat some intestinal parasites. For intestinal amoebiasis, the recommended dose for adults and children is 25 to 35 mg/kg bw for 5 to 10 days. Higher doses (45 to 67 mg/kg bw) for shorter periods (1 to 7 days) are used to treat tapeworm infections. Moreover paromomycin is used to treat giardiasis in children, adults and pregnant women (25 to 30 mg/kg bw/day in 3 doses for 5 to 10 days).

3. After oral administration paromomycin sulphate is poorly absorbed from the gastrointestinal tract and most of the dose is eliminated unchanged in the faeces.

After parenteral administration accumulation occurs in the renal cortex and the cochlea and excretion is almost exclusively in the urine as unchanged drug. Pharmacokinetics studies, fully complied with OECD GLP guidelines, were performed in rabbits, poultry, bovine and porcine. No substantial differences between species were observed. Like other aminoglycosides, paromomycin is poorly absorbed from the gastro-intestinal tract.
Twenty six calves of different age were treated orally with 50 mg paromomycin sulphate/kg bw. Blood samples were taken before treatment at day 0 and subsequently 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. Concentration of paromomycin was measured in blood with a microbiological assay. Results clearly indicated that paromomycin sulphate was better absorbed in newborn than in older calves. After oral administration in calves treated during the first hour of life 24% of the paromomycin sulphate was absorbed; the adsorption rate decreased constantly and was less than 1% at 30 days of age. Plasma peak concentrations were achieved between 2 and 6 hours irrespective of the age.

The pharmacokinetics, including renal clearance and urinary recovery, of paromomycin sulphate were studied in 6 pre-ruminant calves (aged 3 to 4 weeks) after multiple intravenous and intramuscular injections at a dose of 21 mg/kg bw/day, referred to the base. After multiple intravenous injection no drug accumulation occurs in plasma. Pharmacokinetic analysis demonstrated a relatively long distribution half-life (about 1 hour) and an elimination half-life of about 5 hours. Following intramuscular administration paromomycin was rapidly absorbed; the absorption half-life was shorter than 7 minutes. C\text{max} was achieved within 0.5 hours after injection and ranged from 22.0 to 28.4 µg/ml. The urinary drug recovery ranged from 80.2 to 107.9%. Quantitative analysis of kidney demonstrated the longest persistence of residues in kidney up to 30 days. No paromomycin could be detected in the inner ear and membranous cochlea tissue.

A total of 26 pigs were treated by oral route with paromomycin sulphate, either in the diet or in drinking water at a concentration of 1000 mg/kg or 2000 mg/kg. Serum concentrations were below the level of quantification of the bioassay (limit of detection: 0.2 µg/ml) for the animals treated with 1000 mg/kg in feed, slightly above 0.2 µg/ml for the animals treated with 1000 mg/kg in drinking water and for the animals treated with 2000 mg/kg in feed.

A total of 250 young chickens received via gastric probe paromomycin sulphate, equivalent to 43 mg/kg bw of paromomycin at 1, 3, 5, 8 and 15 days of life. Blood samples were obtained after 1, 2, 3, 4, 5, 6, 12 and 24 hours after treatment and analysed by a microbiological assay (limit of detection: 0.125 µg/ml). Results demonstrated that paromomycin was absorbed to a small extent; the concentration was higher in younger chickens with measurable concentrations up to 24 hours after treatment in 1 day old chickens compared to measurable concentrations up to 6 hours after administration in older chickens only.

Nine rabbits orally received 80 mg paromomycin sulphate/day for 7 consecutive days. At 48 hours after the last administration, levels near the detection limit of the microbiological method (limit of detection: 0.16 µg/ml) were measured in serum of 1 rabbit only. More than 80% of the administered dose was excreted in faeces.

4. Paromomycin sulphate shows a low acute oral toxicity with a minimal lethal dose in rats of 10000 mg/kg bw, when compared to parental treatment, with a minimal lethal dose in the rat of 670 mg/kg bw by intramuscular and of 620 mg/kg bw by intravenous injection; this reflects a low oral bioavailability which is typical of aminoglycoside antibiotics.

5. Parenteral 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 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 studies using the oral route were available.

6. The data available on the reproductive system were obtained in mice, rats and rabbits. Paromomycin was administered to ICR mice (20 animals per group) at dose levels of 100, 200 and 400 mg/kg bw/day by intramuscular injection on days 7 to 13 of pregnancy. Teratogenic effects of paromomycin in foetuses enucleated on the 19th day of pregnancy and neonates born normally was compared to the control group. No adverse effects were observed up to the highest dose.
In rats 2 different experiments were carried out. In the first, paromomycin was administered subcutaneously to SD rats (15 animals per group) at dose levels of 100 and 200 mg/kg bw/day on days 0 to 19 of pregnancy. Ten additional dams treated at the highest dose were allowed to deliver. The neonates were followed up to 3 months of age and subjected to a complete haematological and biochemical examination. The animals were then sacrificed and subjected to a complete morphological and histological examination. All laboratory parameters and histological findings were normal, indicating no effects of paromomycin on any system, including the reproductive organs of F1 generation.

In the second study, paromomycin was administered at dose levels of 100, 200 and 300 mg/kg bw/day intramuscularly to Donryu rats (20 animals per group) on days 7 to 13 of pregnancy. Neonates from 5 pregnant rats were bred for 6 weeks; no abnormalities were found in the genital organs of 4-week-old males and 5-week-old females.

Paromomycin was administered subcutaneously at dose levels of 12.5 and 25 mg/kg bw/day to New Zealand White rabbits (25/30 animals per group) on days 0 to 28 of pregnancy. Treatment with paromomycin sulphate at a dose of 12.5 mg/kg bw did not cause mortality in the mothers, as in the control animals; also with a dose of 25 mg/kg bw the incidence of mortality did not differ from the control. The frequency of abortions and conceptions and the percentage of normal pregnancies were not affected by the treatment. The average number of corpora lutea and implantations and the percentage of normal foetuses did not differ from the values obtained in the control animals. The weight and length of the foetuses of the treated mothers were normal and similar to those of the foetuses of the control animals.

7. Prenatal toxicity studies were carried out in rats with doses up to 400 mg/kg bw, by intramuscular injection during gestation from day 7 to 13, and up to 200 mg/kg bw, by subcutaneous injection throughout pregnancy. No developmental toxicity was observed even at the higher dose levels.

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 paromomycin toxicity in rodents as compared to the ear.

8. A battery of genotoxicity tests were performed under GLP conditions, including an \textit{in vitro} bacterial mutagenicity assay (Ames test), an \textit{in vitro} assay for gene mutation in mammalian cells (CHO), an \textit{in vivo} mouse micronucleus test. All tests gave negative results.

9. Two GLP-compliant 2-year studies were available, a combined chronic toxicity-carcinogenity study in Sprague-Dawley rats and a chronic toxicity study in Beagle dogs. In both trials animals were exposed through the diet to 0, 100, 2000 and 50 000 mg paromomycin sulphate/kg feed (approximately 3.9, 78.5 and 1950 mg/kg bw in rats and 3.4, 6.8 and 1700 mg/kg bw in dogs).

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 were observed. The NOEL was 2000 mg/kg feed (approximately 78.5 mg/kg bw).

The dog appears markedly more sensitive to paromomycin toxicity than the rat. Dose-related increases of cataracts and renal tubular lesions were observed from 2000 mg/kg feed (approximately 68 mg/kg bw). The NOEL was 100 mg/kg feed (approximately 3.4 mg/kg bw).

10. No specific studies on immune function were presented, other than information on the lack of effect on the response to fowl cholera vaccine in chicken given 1000 mg paromomycin/kg feed for 2 months. No effects indicative of toxicity to the immune response were observed in any of the standard toxicity studies.

11. The influence of paromomycin residues on the human gut flora was studied. \textit{Escherichia coli}, \textit{Proteus} and \textit{Lactobacilli} proved particularly sensitive, while \textit{Klebsiella}, \textit{Clostridia} and \textit{Enterococci} showed a relative tendency to increase. Adequate \textit{in vitro} studies confirmed the high sensitivity of \textit{Escherichia coli} and \textit{Lactobacilli}: the MICs for these most sensitive organisms was 10 µg/ml.
12. As paromomycin is not intended for use in lactating animals, the influence of its residues on starter cultures was not studied.

13. A complete review of all the publications on the use of paromomycin in humans was performed; the clinical documentation on the compound is very extensive, but no adverse effects on reproductive function are reported. The general consensus that paromomycin is free from side effects on reproductive system is supported by the extensive clinical use in pregnant women, in children and infants and in young adult men with different pathological conditions.

Paromomycin is considered an elective drug for the treatment of giardiasis in pregnancy. Because the compound is practically unabsorbed from the gastrointestinal tract, very high doses can be administered in pregnancy. The extensive documentation on the clinical use of paromomycin in children and infants clearly shows the safe use of paromomycin in young population. All results show clearly that paromomycin can be used during lactation without adverse effects on mothers and infants. The lack of adverse effects on reproductive function in males is documented by the clinical use of paromomycin in the treatment of blenorragic urethritis in young and adult men.

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.

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

14. A toxicological ADI of 0.034 mg/kg (i.e. 2 mg/person) bw was established based on the NOEL of 3.4 mg/kg bw/day derived from the oral chronic toxicity study in dogs and applying a safety factor of 100.

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

$$\text{ADI} = \frac{\text{MIC}_{50} \text{ for the most sensitive organism} \times \text{CF2}}{\text{CF1}} \times (\mu g/ml) \times \text{daily faecal bolus (150 ml)}$$

$$\text{ADI} = \frac{\text{fraction of an oral dose available for microorganisms}}{\text{weight of human (60 kg)}}$$

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

$$\text{ADI} = \frac{10 \times 1}{0.95 \times 60} \times 150 = 25 \mu g/kg \text{ bw i.e. } 1500 \mu g/\text{person}$$

The following assumptions were made:
- CF1 = 1 because the MIC$_{50}$ for the most sensitive microorganism was used;
- CF2 = 1 because no information was available to allow for correction from in vitro to in vivo conditions;
- 150 g was the weight of the daily faecal bolus;
- fraction of the oral dose available for intestinal gut flora = 0.95, because systemic bioavailability upon oral exposure appears to be poor and in absence of a precise estimate, a conservative assumption is made that 95% of the dose remains unabsorbed.

16. The microbiological ADI, rounded to 25 µg/kg bw, is lower than the toxicological ADI of 34 µg/kg bw, therefore the microbiological ADI was considered for the safety assessment of paromomycin.
17. In a residues depletion study, 24 calves were treated with 5 consecutive daily intramuscular administrations of 21 mg/kg bw of paromomycin sulphate; 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 7 but not 15 days after the end of the treatment. In kidneys and liver, residues could be quantified until 30 days after the last injection at concentrations of 0.45 mg/kg and 0.4 mg/kg, respectively (sensitivity of the method 0.3 mg/kg). In kidneys, the highest concentration of 26 mg/kg was observed 7 days after treatment. In liver, the highest concentration of 4.7 mg/kg was also observed 7 days after treatment. No residue concentrations were detectable in fat 15 days after the end of the treatment.

18. Thirty-two pigs were treated with 5 consecutive daily intramuscular administrations of 21 mg paromomycin sulphate/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, paromomycin persisted 7 days. In kidneys, residues could be quantified until 20 days with residue concentration of 0.45 mg/kg 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. No detectable residue concentrations were found in fat at 15 days after the end of the treatment.

19. In chickens sacrificed at 0, 2, 4, 7, 14 and 21 days after a dietary administration of paromomycin sulphate at the recommended therapeutic low levels (280 mg/kg of feed), administered for 5 days. Samples of tissues were assayed by a microbiological method (sensitivity of the method 100 μg/kg). No residues of paromomycin were detected in muscle and liver at any sampling time. In skin and fat, residues were decreasing from 1400 μg/kg at day 0 to 500 μg/kg at day 7, and were no longer detectable at day 14. In kidneys, the highest concentration of 2600 μg/kg was observed at day 0 after the end of the treatment, whilst residues were no longer detectable at day 4.

20. In rabbits sacrified 2 hours, 48 hours and 7 days, respectively after oral administration of a daily dose of 80 mg of paromomycin sulphate for 7 consecutive days, no residues were detected in muscle and liver (sensitivity of the microbiological method 0.16 mg/kg) 2 hours after the last dosing. In kidneys, residues disappeared within 48 hours after the oral treatment.

21. After oral and parenteral administration paromomycin was poorly metabolised, therefore the parent compound could be considered a suitable marker residue.

22. The proposed routine analytical methods for the determination of residues of paromomycin in muscle, liver and kidney of cattle, pigs, rabbits and chickens were based on HPLC with ECD-detection. The specificity of the method was satisfactory and residues of the other aminoglycosides did not interfere with the paromomycin quantification. The limits of quantification were 750 μg/kg for kidney and liver, and 250 μg/kg for muscle in all species. The limits of detection ranged from 40 to 101 μg/kg according to the edible tissues and the target species. The methods were described in the ISO 78/2 format.
Conclusions and recommendation

Having considered that:

- a microbiological ADI of 25 µg/kg bw (i.e. 1500 µg/person) was established for paromomycin,
- paromomycin was considered to be the marker residue,
- validated analytical methods for the determination of residues of paromomycin in kidney, liver and muscle of cattle, pigs, rabbits and chickens are available;

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

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
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<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paromomycin</td>
<td>Paromomycin</td>
<td>Bovine</td>
<td>500 µg/kg</td>
<td>Muscle</td>
<td>Not for use in animals from which milk is produced for human consumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1500 µg/kg</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1500 µg/kg</td>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Porcine, rabbits</td>
<td></td>
<td></td>
<td>500 µg/kg</td>
<td>Muscle</td>
<td></td>
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<tr>
<td></td>
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<td>1500 µg/kg</td>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td>500 µg/kg</td>
<td>Muscle</td>
<td>Not for use in animals from which eggs are produced for human consumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1500 µg/kg</td>
<td>Liver</td>
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Based on these MRLs values, the daily intake of biologically significant residues of paromomycin will represent about 26% of the microbiological ADI.