



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

STREPTOMYCIN AND DIHYDROSTREPTOMYCIN

SUMMARY REPORT (2)

1. Streptomycin and dihydrostreptomycin are aminoglycoside antibiotics which are closely related in structure. The pharmacokinetics, toxicological profile and spectrum of antimicrobial and biological activity are similar and therefore the two compounds were evaluated together to establish a single ADI. They are used to treat bacterial diseases in cattle, pigs, sheep and poultry. The recommended therapeutic regimen doses ranged from 10 to 20 mg/kg bw/day for 3 to 5 days by parenteral route or from 25 to 100 mg/kg bw/day for 3 to 5 days via drinking water. The dihydrostreptomycin is also recommended for intramammary use in combination with benzylpenicillins at a dose rate of 100 to 500 mg per quarter, three times at 12 hour apart.

Currently, streptomycin and dihydrostreptomycin are 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
Streptomycin	Streptomycin	Bovine, ovine, porcine, poultry	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2000
		Bovine, ovine	200 µg/kg	Milk	
Dihydrostreptomycin	Dihydro-streptomycin	Bovine, ovine, porcine, poultry	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2000
		Bovine, ovine	200 µg/kg	Milk	

Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for streptomycin and dihydrostreptomycin for bovine, ovine and porcine species only.

2. In animals and humans both drugs are poorly absorbed from the gastrointestinal tract and the majority of the oral dose is recovered in the faeces. After parenteral dosing, the drugs are excreted in the urine.
3. Both drugs have low toxicity after oral administration to rodents (LD₅₀ 9000 to 25000 mg/kg bw/day).

4. Parental administration of doses of 50 to 100 mg streptomycin/kg bw/day for 20 days to dogs resulted in renal damage. Ototoxicity was studied in guinea pigs and cats in 90-day studies. No hearing loss occurred in guinea-pigs treated orally with 40 mg dihydrostreptomycin/kg bw/day; no hearing loss or effects on vestibular function occurred in cats given 40 mg/kg bw/day. The NOELs for ototoxicity were 40 mg/kg bw/day from these studies. In the mouse studies, there was evidence of ototoxicity at the highest dose of streptomycin used (250 mg/kg bw/day).
5. There were no data available on the genotoxicity of these drugs, although it has been reported that streptomycin gave conflicting results in an *in vitro* study for chromosome aberrations.
6. In a 2-year chronic toxicity study, rats were given 1, 5 and 10 mg/kg bw/day of dihydrostreptomycin. There were no increases in the incidences of any tumour type and a NOEL of 5 mg/kg bw/day based on decreased body weights in males at the high dose was identified.
7. A number of teratology studies in mice were conducted with streptomycin with parenteral doses of up to 250 mg/kg bw/day on various days covering gestation days 9 to 16. No teratogenic effects were seen.

No teratogenic effects were noted in guinea-pigs given up to 200 mg/kg bw/day of dihydrostreptomycin or streptomycin by the intramuscular route.

No teratogenic effects occurred in rabbits given 5 or 10 mg dihydrostreptomycin/kg bw/day orally on days 6 to 18 of gestation. Streptomycin and dihydrostreptomycin are not teratogenic.
8. Panels of literature reviews and field data about the effects of streptomycin and of dihydrostreptomycin on reproduction of farm animals were provided. No adverse effects on reproduction have been reported. Streptomycin and dihydrostreptomycin did not affect the sperm quality, the fertility and the reproductive performances and induced no toxic effects on the development of offsprings. From this study it was possible to conclude that the consumption of residues of streptomycin and dihydrostreptomycin in food derived from animals treated in accordance with good practice in the use of veterinary drugs presents essentially no risk to peri and post natal human health.
9. Literature review was presented on pregnancy outcomes in women receiving streptomycin or dihydrostreptomycin for treatment of tuberculosis. Doses administered ranged from 15 to 30 mg/kg bw twice weekly for a long time. The only adverse effect observed in children was ear defects which consisted in vestibular dysfunction and varying degree of hearing loss. No adverse effects were noted in treated mothers.
10. An ADI of 25 µg/kg bw/day was calculated using the NOEL of 5 mg/kg bw/day derived from the 2-year rat study by applying a safety factor of 200, due to the limited data on reproductive toxicity study.
11. No data on starter cultures were provided.
12. The MICs of bacteria isolated from healthy human faeces were determined under aerobic and/or anaerobic conditions. The spectrum of antimicrobial effects is similar for streptomycin and dihydrostreptomycin. A range of isolates from human intestinal material was examined and the MIC₅₀ for the most sensitive species for dihydrostreptomycin (*Bifidobacterium*) was 32 µg/ml.

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

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

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

$$\text{ADI} = \frac{\frac{32 \times 1}{1} \times 150}{1 \times 60} = 80 \mu\text{g/kg bw i.e.} = 4800 \mu\text{g/person}$$

and the following assumptions were made:

- MIC₅₀ of the most sensitive micro-organism, *Bifidobacterium*, was retained: 32 µg/ml
 - CF1 = 1, because the MIC₅₀ of the most sensitive micro-organism was retained, and therefore no correction is warranted;
 - CF2 = 1, to cover variability between humans;
 - Fraction of an oral dose available for micro-organisms: as the absorption from the gut is low, it was assumed that a factor of 1.0 should be used to represent 100% availability to gut micro-organisms;
 - 150 g was the weight of the daily faecal bolus.
14. Dihydrostreptomycin and streptomycin were evaluated at the 43rd and 48th Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA Committee confirmed that the appropriate NOEL to established the acceptable daily intake is the NOEL derived from the 2-year study of toxicity in rats treated orally. Applying a safety factor of 100, a group ADI of 0 to 50 µg/kg bw for the combined residues of dihydrostreptomycin and streptomycin was established. At the 48th JECFA committee, the equation used by the 43rd JECFA Committee was modified by replacing the faecal bolus (150 g) with a value for colonic content of 220 g. This increases the ADI based on the microbiological activity of the combined residues of dihydrostreptomycin and streptomycin to 0-120 µg/kg bw.
- The Committee of Veterinary Products could not follow the JECFA approach for the determination of microbiological ADI as the parameters of the formula are different.
15. The lowest ADI based on toxicological end-points was considered to be the most relevant ADI for assessing the risk to consumers.
16. No radiometric studies were carried out. Therefore, the relevant ratio of the marker residue towards total residues could not be established. However, considering that the majority part of streptomycin and dihydrostreptomycin administered administered to farm animals is excreted in an unchanged form in the urine, only a very small proportion of potential tissue residues in farm animals is like to be in the form of a metabolite. Therefore, it was assumed that the parent compound represents the totality of the relevant metabolites assayed.
17. Information on the depletion of dihydrostreptomycin in cattle was available. Four set of depletion data were obtained from groups of 4 animals treated by intramuscular route with an association of benzylpenicillin and dihydrostreptomycin. Animals received 10 mg dihydrostreptomycin/kg bw/day for 3 days. Animals were slaughtered 2, 14, 18 and 21 days after the last injections.

Edible tissues from animals sacrificed 2 days after the final administration were collected and the concentrations of dihydrostreptomycin and of microbiologically active residues were simultaneously determined. The concentrations of residues in muscle and fat were below the limit of quantification of the analytical methods (lower than 300 and 400 µg/kg for the microbiological and HPLC assays, respectively). In liver, kidney and in the final injection site, the concentrations of antimicrobiologically active residues were 1132, 6608, and 1700 µg equivalents microbiologically residues expressed as dihydrostreptomycin, respectively and the corresponding concentrations of dihydrostreptomycin measured by HPLC were 1505, 5775, 1707 µg/kg, respectively. Dihydrostreptomycin represents nearly all the microbiologically activity of the bovine edible tissues.

In the animals slaughtered at 14, 18, and 21 days after treatment, the concentrations of dihydrostreptomycin were below 400 µg/kg in all edible tissues except in 3 of the 9 samples of the injection sites (982 and 954 µg/kg at 14 days and 1140 µg/kg at 18 days).

18. Information on the depletion of dihydrostreptomycin and streptomycin administered in combination with benzylpenicillin was available in sheep.

Two days after the end of repeated intramuscular administrations of streptomycin at a dose of 10 mg/kg bw/day for 3 days, the concentrations of streptomycin and of microbiologically active residues were simultaneously determined in edible tissues. The concentrations of residues in muscle and fat were below the limit of quantification of the analytical methods (lower than 300 and 200 µg/kg for the microbiological and HPLC assays, respectively). In liver, kidney and in the final injection site, the mean concentrations of microbiologically active residues were 655, 914 and 1373 µg equivalents microbiologically residues expressed as streptomycin, respectively, and the corresponding mean values of streptomycin measured by HPLC were 938, 886 and 1169 µg/kg, respectively. Streptomycin represents 97% and 85% of the microbiologically activity in ovine kidney and injection site whereas in liver the concentrations of streptomycin exceeded (+43%) the microbiologically ones.

Three sets of depletion data were obtained from groups of 4 animals treated by intramuscular route with an association of benzylpenicillin and dihydrostreptomycin. Animals received 10 mg dihydrostreptomycin/kg bw/day for 3 days. Animals were slaughtered 14, 18 and 28 days after the last injections. The concentrations of dihydrostreptomycin were below 400 µg/kg in all edible tissues except in samples of the injection sites (mean values of 634 µg/kg at 14 days and of 584 µg/kg at 18 days).

19. Information on the depletion of dihydrostreptomycin administered in combination with benzylpenicillin or streptomycin was available in pigs.

In a first study, a single group of 4 pigs received an association of streptomycin and dihydrostreptomycin sulphate (10 mg/kg bw of each active ingredient) by intramuscular route in the neck and rump muscles once daily for three days. The animals were sacrificed two days after the last injection. HPLC and microbiological assay simultaneously determined the residues.

The concentrations of residues in muscle and fat were below the limit of quantification of the analytical methods. In liver, kidney and in the final injection site, the mean concentrations of microbiologically active residues were 1193, 5660 and 1595 µg equivalents microbiologically residues expressed as the sum of streptomycin and dihydrostreptomycin, respectively. The corresponding mean values for streptomycin measured by HPLC were 472, 1756 and 525 µg/kg in liver, kidney and the injection site, respectively, and those of dihydrostreptomycin 620, 3363, 1184 µg/kg, respectively.

In this study, streptomycin and dihydrostreptomycin represents approximately 30% and 52 to 75% of the microbiologically active residues.

Two additional sets of depletion data were obtained from groups of 4 pigs treated by intramuscular route with an association of benzylpenicillin and dihydrostreptomycin. Animals received 10 mg dihydrostreptomycin/kg bw/day for 3 days. Animals were slaughtered 14 and 18 days after the last injection. The concentrations of dihydrostreptomycin were below 400 µg/kg in all edible tissues including the injection sites.

20. No additional data were provided for milk. Publications on residues of dihydrostreptomycin and streptomycin in the milk of cows treated with a variety of intramuscular and intramammary preparations are available. The persistence of the residues which were measured mainly by a microbiological assays depends on the formulation of the preparations. The times to reach levels below 200 µg/kg varied between 3 and 15 milkings.

21. No additional data were provided for chickens.

22. At the 48th JECFA, the JECFA experts considered that extrapolation from limited studies with other aminoglycosides in farm animals supports strong indication that both streptomycin and dihydrostreptomycin remain unmetabolised in food producing animals and humans and that additional studies may not yield substantial new information.

At its 52nd meeting the JECFA recommended definite MRLs edible tissues of cattle, pigs, sheep and chickens as follows: 600 µg/kg for muscle, fat and liver, 1000 µg/kg for kidney and a temporary MRL of 200 µg/kg for bovine milk. However, the marker residue is the sum of the concentrations of dihydrostreptomycin and streptomycin.

As an analytical method has been developed to assay separately dihydrostreptomycin and streptomycin, the CVMP considered that it was more appropriate to identify specific marker residue for each active ingredient and the parent compound was retained as marker residue.

23. Analytical methods for monitoring residues of streptomycin and dihydrostreptomycin in all edible tissues of bovine, ovine and porcine were available. These methods were based on HPLC with fluorescence detection after post-derivatisation. The limits of quantification were 400 µg/kg for dihydrostreptomycin and 200 µg/kg for streptomycin. The limits of detection ranged from 44 to 153 µg/kg and from 12.3 to 25.9 µg/kg for dihydrostreptomycin and for streptomycin, respectively. However, the analytical methods were not fully validated in accordance with the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community in terms of accuracy and precision.

A further method based on LC/MS was available for the detection of dihydrostreptomycin in bovine and porcine tissues, but it was not sufficiently validated.

Conclusion and recommendation

Having considered that:

- a toxicological ADI of 25 µg/kg bw (i.e. 1500 µg/person) was established,
- analytical methods are available for monitoring residues in edible tissues but not fully validated,
- the applicants have committed to address the outstanding issues concerning bovine, ovine and porcine species;

the Committee for Veterinary Medicinal Products recommends, in accordance with Article 4 of Council Regulation No 2377/90 as amended, a 2-year extension of the provisional MRLs for streptomycin and dihydrostreptomycin in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Streptomycin	Streptomycin	Bovine, ovine	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.6.2002
		Porcine	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg	Muscle Skin + fat Liver Kidney	
Dihydrostreptomycin	Dihydro-streptomycin	Bovine, ovine	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.6.2002
		Porcine	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg	Muscle Skin + fat Liver Kidney	

Based on these MRL values, the daily intake will represent approximately 20% of the toxicological ADI.

LIST OF QUESTIONS

1. Additional depletion data on streptomycin in edible tissues of cattle and in milk of ovine and on dihydrostreptomycin in milk of ovine in order to justify the MRLs allocated.
2. The analytical methods provided should be fully validated for accuracy and precision in all edible tissues of the target species including milk in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community.