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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

STREPTOMYCIN

SUMMARY REPORT (3)

1. Streptomycin is an aminoglycoside antibiotic, which is usually used in veterinary medicine as the sulfate salt. It is has been used to treat bacterial diseases in cattle, pigs, sheep and poultry. The recommended therapeutic doses range from 5 to 10 mg/kg bw/day for 3 to 5 days by the parenteral route or from 25 to 100 mg/kg bw/day for 3 to 5 days via the drinking water.

   Currently, streptomycin 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
Streptomycin	Streptomycin	Bovine, ovine, porcine	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2002
		Bovine, ovine	200 µg/kg	Milk	

   Additional data, as response to the List of Questions adopted at the time of recommending the extension of the expiry date of the provisional MRLs, were provided, intended to enable the inclusion of streptomycin for bovine, ovine and porcine species in Annex I of Council Regulation (EEC) No 2377/90. Further to the assessment of the response, a CVMP Opinion on streptomycin was adopted in November 2001. The Opinion concluded that the establishment of maximum residue limits, as referred to in Article 2 of the aforementioned Council Regulation for streptomycin bovine, ovine and porcine species, could not be recommended.

   An intention to appeal against the Opinion and grounds for appeal were subsequently submitted to the EMEA.

   The chemical structure and biological activity of streptomycin and dihydrostreptomycin are similar and therefore the safety evaluation of the two substances was carried out together.

2. In animals and humans streptomycin and dihydrostreptomycin are poorly absorbed from the gastrointestinal tract and the majority of the oral dose is recovered in the faeces. After parenteral administration, the drugs are excreted in the urine.

3. Dihydrostreptomycin and streptomycin have low toxicity after oral administration to rodents (LD_{50} values 9 000 to 25 000 mg/kg bw).

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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 administered (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 or 10 mg/kg bw/day of dihydrostreptomycin *via* the diet. 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. Literature reviews and field data concerning 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 or the reproductive performance and induced no toxic effects on the development of offspring. From this information, it was possible to conclude that the use of streptomycin in food producing animals treated in accordance with good practice in the use of veterinary drugs does not present a risk to peri and post natal development in these animals.

9. A literature review was presented on pregnancy outcomes in women receiving streptomycin or dihydrostreptomycin for the treatment of tuberculosis. The doses administered ranged from 15 to 30 mg/kg bw twice weekly for all or part of their pregnancy. The only adverse effects observed in children were ear defects which consisted of vestibular dysfunction and varying degree of hearing loss. No adverse effects were noted in treated mothers.

10. For both streptomycin and dihydrostreptomycin an ADI of 25 µg/kg bw was calculated using the NOEL of 5 mg/kg bw/day derived from the 2-year rat study with dihydrostreptomycin by applying a safety factor of 200, due to the limited data on reproductive toxicity.

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 MIC_{50} 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:

$$\text{ADI} = (\mu g/kg\ bw) = \frac{\dfrac{\text{geometric mean } MIC_{50} \text{ x CF2}}{\text{CF1}} (\mu g/ml) \quad \text{x} \quad \text{daily faecal bolus (150 ml)}}{\text{Fraction of an oral dose Available for microorganisms} \quad \text{x weight of human (60 kg)}}$$

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

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

and the following assumptions were made:

- MIC_{50} of the most sensitive micro-organism, *Bifidobacterium,* was retained: 32 µg/ml
- CF1 = 1, because the MIC_{50} 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 for Veterinary Medicinal Products could not follow the JECFA approach for the determination of microbiological ADI as the parameters of the formula are different.

15. The pharmacokinetics, pharmacodynamics and toxicological profile of streptomycin and dihydrostreptomycin are similar and therefore a single ADI was established for both substances. The lowest ADI of 25 µg/kg bw based on toxicological end-points was considered to be the most relevant ADI for assessing the risk to consumers.

16. Residue data were obtained from twelve cattle, 6 males and 6 females, treated with streptomycin sulfate by deep intramuscular injection at a dose of 10 mg/kg bw/day for 3 consecutive days. The animals were slaughtered (4 per time point) at 3, 5 or 7 days after the final dose.

    The tissues sampled were fat, muscle, liver, kidney and injection site and were assayed for residues of streptomycin using an HPLC assay with fluorescence detection. The limit of quantitation for the streptomycin assay was 250 µg/kg.

Residues of streptomycin below the limit of quantitation for the assay (250 μg/kg) were recorded in the muscle and fat samples for all four animals, 5 days after the final administration. Quantifiable residues of streptomycin were recorded in 3 liver samples with values ranging from 258 to 401 μg/kg, in all kidney samples with values ranging from 1020 to 1670 μg/kg and in 3 injection site samples with values ranging from 595 to 2130 μg/kg. Residues of streptomycin below the limit of quantification for the assay (250 μg/kg) were recorded in the muscle, liver, fat and injection site samples for all four animals, 7 days after final administration. Quantifiable levels were recorded in all kidney samples with values ranging from 978 to 2800 μg/kg.

17. Information on the depletion of streptomycin administered in combination with benzylpenicillin was available in sheep.

    Two days after the last of repeated intramuscular administrations of streptomycin at a dose of 10 mg/kg bw/day for 3 days, the concentrations of streptomycin and of residues with antimicrobial activity 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 residues with antimicrobial activity were 655, 914 and 1373 μg equivalents 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 residues with antimicrobial activity in ovine kidney and injection site whereas in liver the concentrations of streptomycin exceeded (more than 43%) the residues with antimicrobial activity.

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

    A single group of 4 pigs received a combination product 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 residues with antimicrobial activity were 1193, 5660 and 1595 μg equivalents 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, the concentrations of streptomycin and dihydrostreptomycin represent approximately 30% and 52 to 75% of the residues with antimicrobial activity, respectively.

19. Additional residue data were provided for bovine and ovine milk.

    Eight lactating cows were treated by intramuscular injection with a combination of streptomycin sulphate and dihydrostreptomycin sulphate at a dose of 10 mg/kg bw/day for 3 consecutive days.

    Milk samples (taken from the total milk yield) were taken from each animal for streptomycin and dihydrostreptomycin determinations immediately prior to first administration and at the following timepoints (± 1 hr) after final administration: 12, 24, 36, 48, 60, 72, 84 and 96 hours.

    Streptomycin and dihydrostreptomycin were determined by HPLC using fluorescence detection. The limit of quantitation for the assay was 50 μg/kg for both active substances.

    Twelve hours post final administration, all milk samples had detectable levels of streptomycin and dihydrostreptomycin. The levels for streptomycin ranged from 173-265 μg/kg. The levels for dihydrostreptomycin ranged from 166-252 μg/kg. By the next sampling timepoint (24 hours post final administration) the levels for streptomycin ranged from 85.1-123 μg/kg and for dihydrostreptomycin the levels ranged from 74.5-104 μg/kg.

At 36 hours post final administration, 3 samples had levels of streptomycin below the limit of detection (below 50 µg/kg), with the levels in the remaining 5 samples being just above the limit of quantitation in the range 51.6-61.7 µg/kg. At the same sampling timepoint, 5 samples had levels of dihydrostreptomycin below the limit of quantitation (below 50 µg/kg), with the levels in the remaining 3 samples being just above the limit of quantitation in the range 50.6- 66.7 µg/kg.

At each of the subsequent sampling timepoint (48, 60, 72, 84 and 96 hours post final administration), the levels of streptomycin and dihydrostreptomycin in all samples were below the limit of quantitation of the assay (below 50 µg/kg) for both active substances.

Eight lactating sheep were treated by intramuscular injection with a combination of streptomycin sulphate and dihydrostreptomycin sulphate at a dose of 10 mg/kg bw/day for 3 consecutive days.

Bulk milk samples were taken from each animal for streptomycin and dihydrostreptomycin determination prior to first administration and at approximately the following timepoints after final administration: 12, 24, 36, 48, 60, 72, 84 and 96 hours.

Streptomycin and dihydrostreptomycin were determined by HPLC using fluorescence detection The limit of quantitation for the assay was 50 µg/kg for both active substances.

By 12 hours after final administration, the mean streptomycin concentration was 241.6 µg/kg. At 36 hours after final administration, the mean concentration of streptomycin had decreased to 82.3 µg/kg (mean of 7 quantifiable results). By 48 hours after final administration the mean concentration had decreased to 72.4 µg/kg (mean of 6 quantifiable results). At the remaining sampling timepoints, 60, 72, 84 and 96 hours after final administration, the streptomycin concentrations in the samples for all animals were below the limit of quantitation of the assay.

By 12 hours after final administration, the mean dihydrostreptomycin concentration was 244.3 µg/kg. At 36 hours after final administration, the mean concentration of dihydrostreptomycin had decreased to 112.9 µg/kg (mean of 7 quantifiable results). By 48 hours after final administration the mean concentration had decreased to 60.2 µg/kg (mean of 3 quantifiable results). At the remaining sampling timepoints, 60, 72, 84 and 96 hours after final administration, the dihydrostreptomycin concentrations in the samples for all animals were below the limit of quantitation of the assay.

Publications on residues of streptomycin in the milk of cows treated with a variety of intramuscular preparations are available. The persistence of the residues which were measured mainly by microbiological assays depends on the formulation of the preparations. The times to reach levels below 200 µg/kg varied between 3 and 15 milkings.

20. 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 of streptomycin 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 likely to be in the form of a metabolite. Therefore, the parent compound was identified as the marker residue.

21. At the 48th JECFA, the JECFA experts considered that extrapolation from limited studies with other aminoglycosides in farm animals provided 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 definitive MRLs for the 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 was identified as the sum of the concentrations of dihydrostreptomycin and streptomycin.

22. An analytical method based on HPLC with fluorescence detection was available for the determination of residues of streptomycin in bovine, porcine and ovine edible tissues and in bovine and ovine milk. The limits of quantification were 200 µg/kg for edible tissues and 50 µg/kg for milk. The method was described according to the ISO 78/2 format and validated according to Volume VI of the Rules Governing Medicinal Products in the European Union.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 25 µg/kg bw (i.e. 1500 µg/person) has previously been established,
- the parent compound was previously identified as the marker residue,
- a validated analytical method is available for monitoring residues of streptomycin;

the Committee for Veterinary Medicinal Products, further to the consideration of the grounds for appeal, recommends the inclusion of streptomycin in Annex I 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	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	
		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 40% of the toxicological ADI.