European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

DIHYDROSTREPTOMYCIN
(Extrapolation to all ruminants)

SUMMARY REPORT (4)

1. Dihydrostreptomycin is an aminoglycoside antibiotic, which is usually used in veterinary medicine as the sulfate salt. It is used to treat bacterial diseases in cattle, pigs and sheep.

   Currently, dihydrostreptomycin is included 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
Dihydrostreptomycin	Dihydrostreptomycin	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	

2. A request was submitted to the EMEA for the extension of the existing entry in Annex I of Council Regulation (EEC) No. 2377/90 for bovine and ovine to goats. The scientific justification for this extension was assessed taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL). Based on the approach explained in this guideline the CVMP considered whether the extension to all ruminants would be possible.

3. In setting the ADI in the original assessment of dihydrostreptomycin, which was a combined assessment together with streptomycin as the chemical structure and biological activity of the substances are similar, the data summarised in the paragraphs below were considered:

4. In animals and humans dihydrostreptomycin and streptomycin 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.

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

6. 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).

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7. 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.

8. 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.

9. 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.

10. 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 the offspring. From this information it was possible to conclude that the use of dihydrostreptomycin in food producing animals treated in accordance with good veterinary practice in the use of veterinary drugs does not present a risk to peri and post natal development in these animals.

11. A literature review was presented on pregnancy outcomes in women receiving streptomycin or dihydrostreptomycin for 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.

12. 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.

13. No data on starter cultures were provided.

14. 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.

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

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

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

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

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.

16. 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 establish 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.

17. 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/day based on toxicological end-points was considered to be the most relevant ADI for assessing the risk to consumers.

18. For the extension to include all ruminant species in Annex I the information summarised in the paragraphs bellow was taken into account.

19. Information on the depletion of dihydrostreptomycin in cattle was available. Groups of 4 animals were treated by the intramuscular route with a combination product containing benzylpenicillin and dihydrostreptomycin. Animals received 10 mg dihydrostreptomycin/kg bw/day for 3 days. Animals were slaughtered 2, 14, 18 or 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 residues with antimicrobial activity 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 residues with antimicrobial activity were 1132, 6608, and 1700 µg equivalents 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 residues with antimicrobial activity in 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).

20. Information on the depletion of dihydrostreptomycin administered in combination with benzylpenicillin was available in sheep.

    Groups of 4 animals were treated by the intramuscular route with a combination 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 584 µg/kg at 18 days).

21. 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 a combination 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% respectively, of the residues with antimicrobial activity.

    Two additional sets of depletion data were obtained from groups of 4 pigs treated by the intramuscular route with a combination 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.

22. 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/ daily 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 quantification 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 to 265 µg/kg. The levels for dihydrostreptomycin ranged from 166 to 252 µg/kg. By the next sampling timepoint (24 hours post final administration) the levels for streptomycin ranged from 85.1 to 123 µg/kg and for dihydrostreptomycin the levels ranged from 74.5 to104 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 quantification in the range 51.6 to 61.7 µg/kg. At the same sampling timepoint, 5 samples had levels of dihydrostreptomycin below the limit of quantification (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 quantification 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 quantification 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 quantification 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 quantification of the assay.

Publications on residues of dihydrostreptomycin 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 microbiological assays depends on the formulation of the preparations. The times to reach levels below 200 µg/kg varied between 3 and 15 milkings.

23. 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 dihydrostreptomycin 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.

24. 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.

25. Analytical methods, using liquid chromatography with tandem mass spectrometric detection were available for the determination of dihydrostreptomycin in bovine and porcine tissues and in bovine milk. The methods were well described according to the ISO 78/2 format and validated according to Volume 8 of the Rules Governing Medicinal Products in the European Union. The limits of quantification were 250 µg/kg for bovine liver, muscle and fat and for porcine liver, muscle and fat+skin, 500 µg/kg for bovine and porcine kidney and 100 µg/kg for bovine milk.

An analytical method based on HPLC with fluorescence detection was also available for the determination of residues of dihydrostreptomycin in bovine, porcine and ovine edible tissues and in bovine and ovine milk. The limits of quantification were 250 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 8 of the Rules Governing Medicinal Products in the European Union. This method should be applicable to other ruminant species and therefore from this aspect extrapolation to the tissues and milk of other ruminants is possible.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 25 µg/kg bw (i.e. 1500 µg/person) for dihydrostreptomycin was previously established,
- the parent compound was identified as the marker residue;
- MRLs were previously established in bovine and ovine species; these MRLs are identical,
- an analytical method for the monitoring of residues in tissues and milk of bovine and ovine species was available which should be applicable to ruminants

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for dihydrostreptomycin for bovine and ovine species 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
Dihydrostreptomycin	Dihydrostreptomycin	All ruminants	500 µg/kg 500 µg/kg 500 µg/kg 1000 µg/kg 200 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRL values, the daily intake will represent 40% of the ADI.

MRLs for dihydrostreptomycin are also established for pigs, which are however, not part of this extrapolation and remain therefore unchanged.