DIHYDROSTREPTOMYCIN
STREPTOMYCIN

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IDENTITY

Chemical names:

Streptomycin:

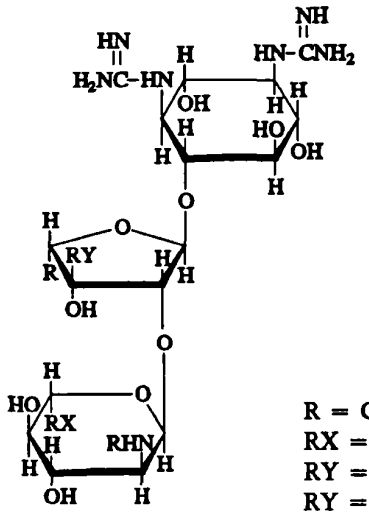
O-2-deoxy-(methylamino)-α-L-glucopyranosyl-(1→2)-O-5-deoxy-3C-formyl-α-L-lyxofuranosyl-(1→4)-N,N'-bis(aminoiminomethyl)-D-streptamine

Dihydrostreptomycin:

O-2-deoxy-(methylamino)-α-L-glucopyranosyl-(1→2)-O-5-deoxy-3C-(hydroxymethyl)-α-L-lyxofuranosyl-(1→4)-N,N'-bis(aminoiminomethyl)-D-streptamine

Synonyms: Many trade names

Structural formula:



R = CH_3
RX = CH_2OH
RY = CHO (Streptomycin)
RY = CH_2OH (Dihydrostreptomycin)

Streptomycin:

Molecular formula: $C_{21}H_{39}N_7O_{12}$ (Streptomycin A)

Molecular weight: 581.58

Molecular formula: $C_{42}H_{84}N_{14}O_{36}S_3$ (Streptomycin sesquisulphate)

Molecular weight: 1457.4

Dihydrostreptomycin:

Molecular formula: $C_{21}H_{41}N_7O_{12}$

Molecular weight: 583.62

Molecular formula: $C_{42}H_{88}N_{14}O_{36}S_3$ (Dihydrostreptomycin sesquisulphate)

Molecular weight: 1461.4

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:	Streptomycin or dihydrostreptomycin
Appearance:	White to grey or pale buff powder (streptomycin)
Melting point:	Dihydrostreptomycin sulphate, dec. at 250°C
Solubility:	Sesquisulphates are very soluble in water. Slightly soluble in alcohols.
Optical rotation:	$[\alpha]^{25}_{D}$ -88.5° (1% soln of dihydrostreptomycin sulphate)
UV_{max}:	Streptomycin sulphate - 200 nm Dihydrostreptomycin sulphate - 202 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Streptomycin and dihydrostreptomycin are active against a wide range of Gram-negative organisms and some Gram-positive pathogens in cattle, pigs and sheep. The combination of penicillin and streptomycin is especially useful in the treatment of mixed infections involving both Gram negative and Gram-positive organisms. Preparations of dihydrostreptomycin in combination with a penicillin derivative are used for the treatment and prevention of mastitis. The drugs may be prescribed for humans in certain second line treatments of infections.

Dosages

The preparations under consideration are administered as daily intramuscular injections (i.m.i.) at a dose rate of approximately 10 mg per kg body weight (BW) of streptomycin/Dihydrostreptomycin. They contain streptomycin or streptomycin plus dihydrostreptomycin or dihydrostreptomycin combined with a penicillin for horses, cattle, pigs or sheep. The length of treatment depends on the resolution of the infection and in the case of the procaine penicillin-G and dihydrostreptomycin preparations treatment should not exceed three to five days. There are licensed preparations containing dihydrostreptomycin usually combined with a penicillin which are administered into the mammary glands of cattle and sheep for the treatment or prevention of mastitis.

Preparations/Formulations submitted for evaluation

The sponsors (Norbrook) have submitted data for three preparations containing streptomycin or dihydrostreptomycin.

1. An aqueous solution of 200,000 units streptomycin sulphate per ml.
2. An aqueous solution of 120,000 units streptomycin sulphate and 120,000 units of dihydrostreptomycin sulphate per ml.
3. An aqueous suspension of 250 mg dihydrostreptomycin sulphate and 200 mg procaine penicillin per ml.

The sponsors (Pitman-Moore) have submitted data for their intramuscular injection preparation which is an aqueous suspension of 250 mg dihydrostreptomycin and 200 mg procaine penicillin-G per ml. They also license three preparations for intramammary infusion which contain both dihydrostreptomycin and procaine penicillin-G.

METABOLISM

Pharmacokinetics

Ten cattle (mean wt 467 ± 23 kg), six sheep (mean wt 44.8 ± 11.3 kg) and six pigs (mean wt 18.7 ± 6 kg) received daily for three days i.m.i. of streptomycin sulphate. The cattle and pigs received a dose of approximately 10,000 units streptomycin per kg BW and the sheep a dose of 8000 - 9500 units per kg BW. Blood samples were collected at frequent intervals after the first and third injection and assayed for streptomycin using a microbiological method (LOD 0.1 μg/ml). The results for plasma concentrations during the 24 hour period after the last (third) injection are shown in Figure 1 and the pharmacokinetic parameters are summarised

in Table 1. There is a more rapid uptake of streptomycin and also a shorter half-life of streptomycin in pigs than in cattle and sheep. In all three species the concentration of streptomycin is less than 1 μg/ml at 24 hours after the last (third) injection.

In a second study six calves (mean wt 136 ± 29 kg), six sheep (mean wt 39.2 ± 6.6 kg) and six pigs (mean wt 26.8 ± 1.8 kg) received daily for three days i.m.i. of streptomycin sulphate and dihydrostreptomycin sulphate. The animals received a dose of approximately 5,000 units streptomycin per kg and 5000 units dihydrostreptomycin per kg. Blood samples were collected at frequent intervals after the first and third injection and assayed for total streptomycin plus dihydrostreptomycin activity using a microbiological method (LOD 0.1 μg/ml). The results for plasma concentrations during the 24 hour period after the last (third) injection are shown in Figure 1 and the pharmacokinetic parameters are summarised in Table 1. In this study there were similar results for plasma levels and pharmacokinetic parameters for each of the species. The half-life activity of the combined preparation appears longer than for the streptomycin alone preparation. This is difficult to explain as being related to the dihydrostreptomycin component as the half life of dihydrostreptomycin in the combined preparation with procaine penicillin G is much shorter (see below).

Table 1. **Pharmacokinetic parameters of streptomycin in the plasma of farm animals after intramuscular injection of streptomycin sulphate or streptomycin sulphate plus dihydrostreptomycin sulphate.**

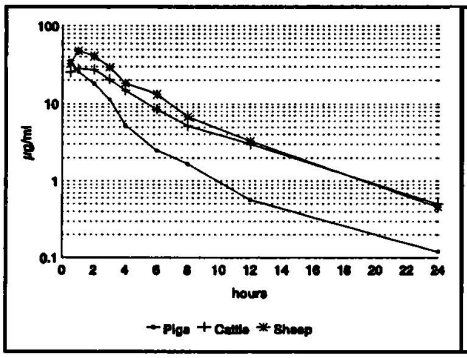
Species i.m.i.		Cmax (mg/l)	Tmax (hours)	AUC (mg·h/l)	$t_{½}$ (hours)
STREPTOMYCIN					
Cattle	1st	31 ± 9	1.4 ± 0.5	159 ± 26	2.5 ± 0.6
	3rd	32 ± 10	1.2 ± 0.6	163 ± 28	3.1 ± 0.6
Sheep	1st	54 ± 6	1.0 ± 0	222 ± 21	2.6 ± 0.5
	3rd	49 ± 5	1.2 ± 0.4	225 ± 23	2.9 ± 0.5
Pigs	1st	32 ± 4	0.75 ± 0.3	112 ± 18	1.9 ± 0.2
	3rd	36 ± 10	0.5 ± 0	90 ± 17	1.8 ± 0.2
STREP+DIHYDROSTREP					
Calves	1st				
	3rd	47 ± 15	1.25 ± 0.6	167 ± 17	4.0 ± 0.7
		56 ± 18	1.08 ± 0.5	157 ± 36	3.7 ± 0.3
Sheep	1st	45 ± 12	0.83 ± 0.3	138 ± 25	4.4 ± 1.2
	3rd	52 ± 13	1.08 ± 0.5	162 ± 33	3.8 ± 0.6
Pigs	1st	39 ± 10	1.25 ± 0.6	179 ± 63	4.0 ± 0.7
	3rd	50 ± 15	1.22 ± 0.52	220 ± 68	4.4 ± 0.7
DIHYDROSTREP+ PROCAINE PENICILLIN G					
Cattle	1st	23.0 ± 5.7	1.4 ± 0.8	104 ± 21	2.7 ± 0.6
	3rd	23.8 ± 3.2	1.5 ± 0.5	110 ± 28	2.6 ± 0.4
Sheep	1st	23.0 ± 6.4	1.7 ± 0.5	80 ± 18	1.6 ± 0.3
	3rd	23.2 ± 5.0	1.0 ± 0	64 ± 16	1.8 ± 0.1
Pigs	1st	27.1 ± 9.3	1.2 ± 0.4	86 ± 13	2.3 ± 0.2
	3rd	23.2 ± 6.1	1.3 ± 0.5	87 ± 18	2.3 ± 0.3

A comparison of levels of streptomycin in plasma and tissue cage fluid (TCF) was carried out in five calves (mean wt 147 ± 12 kg) implanted with polythene practice golf balls. The calves were administered by i.m.i. 10,000 units streptomycin sulphate per kg BW daily for three days. Plasma and TCF was collected at 6, 12 and 24 hours after each injection and assayed for streptomycin by a microbiological method. The concentration of streptomycin in the TCF was between 1 - 2.8 times higher than in the peripheral plasma which may indicate

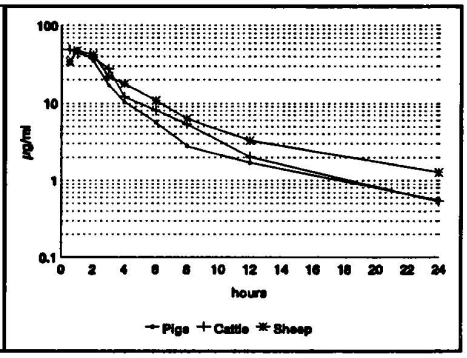
a high degree of penetration of streptomycin into tissue.

Figure 1. **Plasma concentrations of streptomycin/dihydrostreptomycin in farm animals after a third daily intramuscular injection. Time 0 is at the time of the third injection and 48 hours after the first daily injection.**

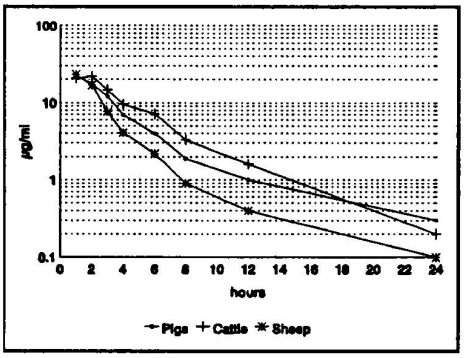
Streptomycin



Streptomycin + Dihydrostreptomycin



Dihydrostreptomycin + Proc. Penicillin G



Metabolism in Food Animals

No data was provided by the sponsors on the metabolism of streptomycin.

Metabolism in Toxicological Test Species

No data was provided by the sponsors on the metabolism of streptomycin.

In a review of comparative metabolism in food-producing animals Juskevitch (1987) stated that "It appears that there is minimal metabolism of the tetracyclines and aminoglycosides in ruminant species."

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Streptomycin

No data was provided by the sponsors for the radiolabeled depletion of the residues of streptomycin.

Dihydrostreptomycin

Dihydrostreptomycin, radiolabeled uniformly with tritium, was administered to cattle and pigs by i.m.i. at 25 mg/kg BW (Stalheim, 1970). The work did not identify the metabolites or the extent of metabolism but concentrated on the determination of the total activity and biological activity in blood and urine. The work showed that there was an exponential decrease in the concentration of radioactivity and antibacterial activity in plasma of both species. Most of the activity was excreted at an exponential rate into the urine within 12 hours and was 72% of dose in the case of the pigs. Nevertheless residues were still detected in porcine urine at 86 hours (3.1 mg/l) and in bovine urine at 96 hours (1.7 mg/l). There was good agreement between the measurements for the concentrations of total radioactivity and the antibacterial activity measured by bioassay. The LOD for the bioassay was 0.4 mg/l and was estimated as 8 μg/l for the radioassay. No activity was detected in bovine saliva or lacrimal fluid. The values of apparent volume of distribution (21% and 23%) agree with estimates for extracellular water. These results together with the lack of activity in both saliva and lacrimal fluids indicate that dihydrostreptomycin neither equilibrates with intracellular fluids nor accumulates intracellularly.

Other residue depletion studies (with unlabeled drug)

A series of residue studies in the edible tissues were carried out as outlined in Table 2.

Table 2. Studies for the determination of residues in edible tissues.

Species	Mean BW ± SD (kg)	n per time point	Dose i.m.i. & no. days[ ] (units/kg BW or mg/kg BW)	Tissue	Sampling times after last injection (days)	LOD (mg/kg mg/l)
STREP						
Cattle	157 ± 52	4	10,000 [3]	edible	7, 14	1
	181 ± 64	4	25,000 [1]	edible	14, 21	1
Sheep	40 ± 2.6	4	10,000 [3]	edible	7, 14	1
Pigs	24.9 ± 4.3	4	10,000 [3]	edible	7, 14	1
STREP +	**DIHYDROSTREP**					
Calves	130 ± 27	4	10,000 [3]	edible	7, 14, 21	1
	156 ± 33	4	25,000 [1]	edible	21, 28	1
Sheep	42 ± 10	4	10,000 [3]	edible	7, 14, 21	1
Pigs	24 ± 4	4	10,000 [3]	edible	7, 14, 21	1
DISTREP	**PROC. PEN G**					
+	184 ± 81	4/3	10,000 [3]	edible	7, 18	1
Cattle	100 ± 13	4	10,000 [3]	edible	28	0.1
Calves	100 - 165	3/4	10 mg [5]	M,IS,K	6, 10, 14	0.05
Calves[P]	180 - 230	4	10 mg [5]	M,IS,K	21, 28	0.05
Calves[P]		2-5	10 mg [1]	edible	2h - 68h	0.3
Cows[M]						
Sheep	51 ± 17	4/3	10,000 [3]	edible	7, 18	1
Sheep	29 ± 2	4	10,000 [3]	edible	28	0.1
Sheep[P]	27 - 112	3/4	10 mg [5]	M,IS,K	5, 7, 10, 14	0.05
Sheep[P]	34 - 84	4	10 mg [5]	M,IS,K	21 - 28	0.05
Pigs	50 ± 38	4/3	10,000 [3]	edible	7, 18	1
Pigs	44 ± 2	4	10,000 [3]	edible	28	0.1
Pigs[P]	23 - 27	3/4	10 mg [5]	M,IS,K	6, 10, 14	0.05
Pigs[P]	19 - 155	4	10 mg [5]	M,IS,K	21, 28	0.05

[P] are studies by Pitman-Moore, [M] uses a Mycopharm preparation (Nouws & Ziv, 1977), the others are by Norbrook; M = muscle, IS = injection site muscle and K = kidney

Edible tissues were heart, liver, muscle, injection site, kidney, kidney fat and subcutaneous fat. The residues were assayed by microbiological methods or in the Pitman-Moore studies, EIA was used.

In the Norbrook studies residues were detected in the edible tissue samples especially for the liver and kidney tissues collected at 7 days (and 14 days for the combined streptomycin and dihydrostreptomycin preparation) after the last injection. The residues in liver and kidney are shown in Table 3.

Table 3. **Residues of antimicrobial activity (streptomycin - Strep and dihydrostreptomycin - DiStrep) (Mean values for 4 animals in mg/kg) in liver and kidney sampled at 7 and 14 days after the last injection (see Table 2 for doses).**

Tissue	Withdrawal time (days)	Cattle	Sheep	Pigs
Liver				
Streptomycin	7	1.17	1.04	1.38
Strep + DiStrep	7	1.2	2.2	3.4
Strep + DiStrep	14	1.1	1.4	<1
DiStrep + Proc.Pen G	7	2.25	8.38	3.87
Kidney				
Streptomycin	7	1.07	1.12	1.84
Strep + DiStrep	7	4.6	13.0	16.5
Strep + DiStrep	14	3.5	2.5	5.9
DiStrep + Proc.Pen G	7	10.5	15.8	12.6

Streptomycin preparation:

There were NO residues in tissues other than liver and kidney at 7 days post injection and there were NO residues detected in any edible tissue at time points 14 days or later (see Table 2 for times).

Streptomycin/Dihydrostreptomycin preparation:

In **cattle** there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection. In **sheep** there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection **except** residues were detected at the injection site at 7 days in one sheep (2.8 mg/kg) and one sheep (2.8 mg/kg) at 14 days. One sheep muscle sample taken at 7 days was also positive (1.3 mg/kg).

In **pigs** there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection **except** residues were detected at the injection site at 14 days in one pig (1.4 mg/kg). One porcine subcutaneous fat sample taken at 7 days was positive (3.3 mg/kg) and one porcine kidney fat sample was positive (1.4 mg/kg) at 14 days.

Dihydrostreptomycin/Procaine penicillin G preparation:

Residues were present in most tissues at 7 days after three daily injections (see Table 4). The next sampling point was 18 days post injection and NO residues were detected in any edible tissue at this time (Norbrook studies).

In the Pitman-Moore studies residues of dihydrostreptomycin in cattle, sheep and pigs were measured only in kidney, muscle and injection site muscle. Residues were present in the muscle at day 7 and were <0.07 mg/kg at day 14. Residues were found in kidney and at injection site on days 21 and 28 although they were <1 mg/kg on day 28. The results are shown in Table 5.

Table 4. **Samples with positive (⊕) and no (-) microbiological activity (LOD ≥1 mg/kg) for dihydrostreptomycin 7 days after last injection of dihydrostreptomycin / procaine penicillin G (see Table 2 for doses).**

Tissue	Cattle	Sheep	Pigs
Muscle	----	⊕---	⊕---
Inj. site	⊕---	⊕⊕⊕⊕	----
Heart	----	⊕⊕⊕⊕	⊕⊕--
Liver	⊕⊕⊕⊕	⊕⊕--	⊕⊕--
Kidney	⊕⊕⊕⊕	⊕⊕⊕⊕	⊕⊕⊕⊕
Kidney fat	----	⊕⊕⊕*	⊕---
Subcut. fat	----	⊕⊕⊕*	⊕---

* Only three samples.

Table 5. **Residues (mg/kg) of dihydrostreptomycin in tissues of farm animals after 5 daily intramuscular injections of dihydrostreptomycin combined with procaine penicillin-G (see Table 2 for doses).**

Species	No.	Days after dosing	Muscle	Injection Site	Kidney
Cattle	4	6	0.28 - 1.25	0.56 - 3.52	10.8 - 56.3
	2	10	<0.05, 0.65	0.07, 2.08	7.04, 12.80
	4	14	<0.05 - 0.05	0.80 - 1.60	5.12 - 19.2
	4	21	n.m.	<0.88 - 2.28	1.75 - 1.96
	4	28	n.m.	<0.28 - <0.8	<0.40 - 0.46
Sheep	4	5	0.07 - 0.18	1.52 - 19.2	6.72 - 16.6
	2	7	0.12, 0.13	1.04, 2.08	4.00, 4.16
	4	10	0.08 - 0.10	0.30 - 2.24	3.04 - 5.44
	4	14	<0.05 - 0.07	0.88 - 2.40	1.00 - 1.52
	4	21	n.m.	1.16 - 5.64	0.05 - 0.93
	4	28	n.m.	<0.1 - <0.2	<0.3 - <0.8
Pigs		6	0.06 - 2.32	0.10 - 1.28	8.96 - 26.2
		10	0.05, 15.7	0.84, 1.04	3.84, 4.16
		14	<0.5 - 0.5	0.21 - 0.56	1.40 - 4.32
		21	n.m.	<0.8 - 0.94	0.96 - 1.92
		28	n.m.	<0.1 - <0.4	<0.1 - <0.4

Data from Pitman-Moore; n.m. not measured

Nouws and Ziv (1977) reported residues of dihydrostreptomycin in kidney and urine 68 hours after i.m. injection of dihydrostreptomycin/Procaine penicillin-G but NO residues were detected (LOD 0.2 - 0.6 mg/kg) in muscle, liver, serum or bile.

Milk

Residues of dihydrostreptomycin and streptomycin in the milk of cows treated with a variety of intramuscular and intramammary preparations containing dihydrostreptomycin were measured by EIA (Pitman-Moore (AnH89/R/87); Dõtsch, 1993; Usleber et al., 1993, 1994) or bioassay (Norbrook file; Moretain and Boisseau, (1993). The times to reach levels below the MRL (200 μg/l, 12th JECFA) and the limits of detection for each study are shown in table 6. The persistence of the residues depends on the formulation of the preparation.

Table 6. Depletion of dihydrostreptomycin and streptomycin in milk of treated cows

Route Study	Dose mg/kg BW * g total	No. cows	Milkings to reach MRL Mean	 200 μg/l Max	Milkings to reach LOD Mean	 Max	LOD (μg/l)
Intramuscular Pitman-Moore	10^{G} x 5	10	1	3	>14	>14	5
Dõtsch	12.5^{G}	9	5	6	12	21	19
Dõtsch	12.5^{GDC}	12	7	8	13	19	19
Norbrook	10^{S} x 3	10	3 - 4	>4	3 - 4	>4	200
Norbrook	25^{S}	5	3	3	4	4	100
Norbrook	10^{M} x 3	5	2	3	4	4	100
Norbrook	25^{M}	5	3	4	4	4	100
Norbrook	10^{G} x 3	6	2	3	3	4	100
Intramammary Moretain$	0.35 g^{G*}	5	14	15	14	15	150
Moretain$	0.1 g^{G*}	5	5	7	8	11	150
Usleber@	0.5 g^{GK*}	6	5	6	16	24	19
Usleber@	2 g^{GK*}	1	13	-	>26	-	19
Usleber@	0.5 g^{G*}	6	5	6	16	24	19

$ data from Moretain & Boisseau (1993), @ data from Usleber et al., (1994) and Dõtsch (1993).
DS is dihydrostreptomycin; S is streptomycin; M is DS combined with streptomycin; G is DS combined with penicillin G; GDC is DS combined with penicillin G, dexamethasone and chlorpheniramin; GK is DS combined with penicillin G, kanamycin, diaphenylsulphone and prednisolone.
LOD is Limit of Detection.

Poultry

Nakamura et al (1967) studied the distribution of dihydrostreptomycin (in combination with potassium penicillin-G) in chickens for periods ranging from 10 min up to 24 h following oral dosing. They assayed the residues by bioassay and showed that the maximum levels were recorded during the first two hours after administration and that the highest concentrations were in the bile with the levels in the tissues in the following decreasing order; pancreas > kidney > serum > spleen > muscle. No residues were found in the brain, heart or testes.

Eggs

The depletion of dihydrostreptomycin in eggs was reported and showed that after a single i.m. injection of 100 mg/kg BW, residues were above 500 μg/kg in whole egg for at least 8 days after injection (Roudaut, 1989). Laying hens were also administered the drug in the drinking water at level of 1 g/l, equivalent to 100 mg/kg BW, for five consecutive days. No antimicrobial residues in eggs were detected (LOD 300 μg/kg albumin, 3000 μg/kg yolk) suggesting a very low absorption of the drug from the gastrointestinal tract.

Bound Residues and Bioavailability

No data was submitted by the sponsors.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Overview

Several types of analytical methods are used for the assay of the aminoglycosides, especially in the analysis of body fluids. Immunochemical and microbiological assays are the best suited for rapid screening and work most successfully with milk samples. Chromatographic methods using either thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) are suitable for the analysis of streptomycin and dihydrostreptomycin but have not been adequately developed for the analysis of residues in edible tissues.

The sponsors have used specific microbiological assays for measuring both streptomycin and dihydrostreptomycin. The methods do not distinguish between the two compounds. The older method from Norbrook laboratories (1988 et seq) uses the organism *Bacillus subtilis* NCIB 8054 and has a limit of detection of 1 mg/kg. A newer method uses *Bacillus subtilis* ATCC 6633 with a limit of detection of 0.1 mg/kg. There is no interference from penicillins. The methods are partially validated for the measurement of recovery from spiked samples of muscle, liver and kidney for both methods. Although results using both methods are given for residues in heart, injection site, subcutaneous fat, kidney fat and skin only the newer method was validated for fat and skin. The recoveries using both methods vary with both the type of tissue and the mass of spike. Although not given the limit of quantification must be considerably greater than the limit of detection. No repeatability data is provided for incurred samples. The results are not compared with other non-microbiological assays.

Outline of Methods

Assay using *Bacillus subtilis* NCIB 8054 and streptomycin, (Sponsor). Five grams of tissue are homogenised in phosphate buffer, pH 1.5. Spikes of streptomycin equivalent to 1-5 mg/kg tissue were added at this point for the validation of the assay in muscle, liver and kidney. After an equilibration period of 30-45 min the mixture is adjusted to pH 8.0 with 20% sodium hydroxide and equilibrated for a further 30 min. The mixture is centrifuged to remove the tissue debris and denatured proteins and the supernatant is collected and used for the microbiological assay. The assay uses *Bacillus subtilis* NCIB 8054 as the test medium and incorporates penicillinase to destroy any penicillins present in the extract. A calibration curve was prepared using a pure standard of streptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using a recovery factor of 60% for all tissues. The percentage recoveries $\pm$ SD at the 1 mg/kg level were, muscle 56 $\pm$ 18, liver 65 $\pm$ 10 and kidney 54 $\pm$ 13 for at least 10 replicates per tissue.

Assay using *Bacillus subtilis* ATCC 6633 and dihydrostreptomycin, (Sponsor). Ten grams of tissue are homogenised in phosphate buffer, pH 2.0 . Spikes of dihydrostreptomycin equivalent to 0.1, 0.2 and 0.5 mg/kg tissue were added after centrifugation for the validation of the assay in muscle, liver, kidney, fat and skin. After an equilibration period of about 45 min. the mixture is adjusted to pH 8.0 with ethanolamine and equilibrated for a further 30 min. The mixture is centrifuged to remove the tissue debris and denatured proteins and the supernatant is collected and used for the microbiological assay. The assay uses *Bacillus subtilis* ATCC 6633 as the test medium and incorporates penicillinase to destroy any penicillins present in the extract.
A calibration curve was prepared using a pure standard of dihydrostreptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using recovery factors of 78, 75, 82, 85 and 76% respectively for muscle, liver, kidney, fat and skin tissues. These recovery factors are the mean value for the recoveries of the three different levels of spikes. The percentage recoveries $\pm$ SD at the 0.1 mg/kg level were, muscle 70 $\pm$ 12, liver 70 $\pm$ 9, kidney 69 $\pm$ 1, fat 73 $\pm$ 3 and skin 79 $\pm$ 2 for an unstated number of replicates per tissue.

Assay using *Bacillus pumilis* and dihydrostreptomycin, (Pitman-Moore). Tissues are incubated for 30 min at 37°C with phosphate buffer, pH 8.0 containing lipase. The sample is centrifuged and the supernatant used for bioassay. Spikes of dihydrostreptomycin were added for the validation of the assay in muscle, injection site muscle, kidney and milk. The LOD in muscle, liver and fat is 0.31 mg/kg, in kidney 0.62 mg/kg and for milk it is 5 μg/l.

A calibration curve was prepared using a pure standard of dihydrostreptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using recovery factors of 78, 75, 82, 85 and 76% respectively for muscle, liver, kidney, fat and skin tissues. These recovery factors are the mean value for the recoveries of the three different levels of spikes. The percentage recoveries ± SD at the 0.1 mg/kg level were, muscle 70 ± 12, liver 70 ± 9, kidney 69 ± 1, fat 73 ± 3 and skin 79 ± 2 for an unstated number of replicates per tissue.

Immunochemical Method for Residues in Milk using Enzyme Immunoassay (EIA)

Pitman-Moore method

1 gram of tissue was homogenised in phosphate buffered saline (PBS), pH 7.0. After centrifugation an aliquot of the supernatant was used for an EIA using polyclonal antibodies specific for streptomycin and dihydrostreptomycin. There was no cross-reaction with other antibacterial drugs including other aminoglycosides. Because of matrix effects standard curves were prepared by spiking tissues prior to extraction. No measurements of residues were made for liver, fat or skin. Milk was assayed after dilution with PBS. The LOD for milk was 5 μg/l and varied for tissues from 140-800 μg/kg. The method was not done to GLP or accredited.

In a method from Germany (Usleber et al, 1994) defatted milk was diluted in phosphate buffered saline and directly assayed by EIA using rabbit antibodies and horse-radish peroxidase conjugates. The measuring range of the standard curves was 0.2 to 50 μg/l with 50% inhibition about 5 μg/l, CVs were <7.5% and the LOD was 20 μg/l. The method is suitable for monitoring the current MRL of 200 μg/l.

APPRAISAL

The aminoglycosides streptomycin and dihydrostreptomycin were evaluated together because their pharmacokinetic and residue patterns were similar and also they were not distinguished from each other in the measurement of residues by either microbial inhibition assays or enzyme immuno assay (EIA) methods.

Both drugs were absorbed rapidly from the site of intramuscular injection in cattle, sheep and pigs and were thought to be distributed in the extracellular fluids. They were cleared rapidly from the blood.

There was very little information on the metabolism in farm animals of either compound although probably the drugs are not substantially metabolised.

There were no radiodepletion studies reported to determine the total residues in the tissues.

Numerous residues studies using unlabeled drug(s) were presented in a variety of preparations. The residues in these studies were measured in cattle, sheep and pigs following intramuscular injections at the recommended doses. The residues were measured by either microbial inhibition assays or by EIA methods which do not distinguish between the two compounds and may indeed cross-react with possible metabolites. The highest and most persistent residues were found in the kidney. Residue levels in muscle, liver and fat depleted fairly rapidly to below the level of detection (0.03 to 1 mg/kg varying with tissue and study). There was wide variability in the disappearance of the residues depending mainly on the formulation of the preparation. Dihydrostreptomycin was retained in the injection sites of cattle, sheep and pigs in low amounts (<0.8 - 5.6 mg/kg) for three weeks but was <0.8 mg/kg at four weeks. Residues of dihydrostreptomycin and streptomycin in the milk of cows treated with a variety of intramuscular and intramammary preparations containing dihydrostreptomycin were measured by EIA, a receptor binding assay or microbial inhibition assays. The times to reach levels below the MRL (200 μg/kg, 12th JECFA) for each study varied between 3 and 15 milkings after drug administration. Again the persistence of the residues depends on the formulation of the preparation. Kidney, muscle and milk are recommended as target tissues. The depletion of dihydrostreptomycin in eggs was reported and showed that after a single i.m. injection of 100 mg/kg BW residues were above 500 μg/kg in whole egg for at least 8 days after injection. Laying hens were also administered the drug in the drinking water at level of 1 g/l, equivalent to 100 mg/kg BW, for five consecutive days. No antimicrobial residues in eggs were detected (LOD 300 μg/kg albumin, 3000 μg/kg yolk) suggesting a very low absorption of the drug from the gastrointestinal tract.

No data were presented on bioavailability of bound residues.

The analytical microbial inhibition assays and EIA methods for measuring residues are adequate for screening purposes at sensitivities well below 1 mg/kg for tissues. In milk the EIA methods have claimed limits of detection (LOD) of 5-20 μg/l whereas in the microbial inhibition assays the LOD are 100-200 μg/l. Although much data was presented neither method was fully validated nor in the case of the EIA done to Good Laboratory Practice (GLP) standards. Chemical methods for measuring aminoglycosides based on chromatography were reported in the literature for assaying residues in animal tissues. LOQs were 20 μg/kg for streptomycin and 40 μg/kg for dihydrostreptomycin for muscle and kidney (Gerhardt et al., 1994a) and half of these values for milk (Gerhardt et al., 1994b). An LC/MS/MS method was reported for measuring residues in bovine kidney with LOQs of 440 μg/kg and 320 μg/kg for streptomycin and dihydrostreptomycin, respectively.

Maximum Residue Limits

Based on the temporary ADI of 0 - 30 μg/kg for the parent drugs established by the Committee, the permitted daily intake of the parent drugs and/or their equivalents is 1800 μg for a 60 kg person per day.

The following factors were considered in estimating the MRLs:

1. The temporary ADI which is established on a toxicological basis.
2. The limits of detection or quantitation of the methods.
3. The marker residue is the sum of streptomycin and dihydrostreptomycin for edible tissues and milk.
4. Insufficient data was presented for residues in eggs to maintain the MRL set at the 12th JECFA meeting.
5. There is an uncertain ratio of the marker residues to the total residues although it is predicted that there is little metabolism of either drug and the ratio may be close to unity.
6. MRLs established following the 12th JECFA meeting were; meat 1000 μg/kg, milk 200 μg/kg and eggs 500 μg/kg.

The Committee recommends temporary MRLs of 500 μg/kg for muscle, liver and fat and 1000 μg/kg for kidney of cattle, sheep, pigs and chickens and 200 μg/l for cattle milk expressed as the sum of streptomycin and dihydrostreptomycin. Using these values for the MRLs and taking into account the factors above, the ingested residue of parent drug are muscle (300 g) 150 μg, liver (100 g) 50 μg, kidney (50 g) 50 μg, fat (50 g) 25 μg and milk (1.5 liters) 300 μg . The total is 575 μg/day.

The Committee requests further information, either experimentally or as an expert report, on the metabolism of the drugs and the total residues in farm animals. Additional residue data is required for eggs. The relationship between the antimicrobial activity of the residues and the measurement of residues by specific chemical methods is requested.

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