NEOMYCIN

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ADDENDUM

To the monographs prepared by the 43rd, 47th and 52nd meetings of the Committee published in the FAO Food and Nutrition Paper 41/7, Rome 1995, 41/9, Rome 1997 and 41/12, Rome 1999, respectively.

INTRODUCTION

The Committee has previously considered neomycin at the 43rd, 47th, 52nd and 58th meetings. The 43rd meeting of the Committee (WHO 1995) established a temporary ADI of 0–30 μg per kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in a 90-day study on the guinea pig and a safety factor of 200. The ADI was made temporary in view of deficiencies in the genotoxicity data. Gene mutation studies and an *in vivo* study on chromosome aberrations were requested for evaluation in 1996. Temporary MRLs of 5,000 μg/kg for kidney and 500 μg/kg for muscle, liver, and fat, expressed as the parent drug, were recommended for cattle, sheep, goats, pigs, turkeys, ducks and chickens. Temporary MRLs of 500 μg/kg and 500 μg/L, also expressed as the parent drug, were recommended for chickens' eggs and cows' milk, respectively.

The 47th meeting of the Committee (WHO 1998) considered new genotoxicity data for neomycin and, based on these data, established a full ADI of 0 - 60 μg/kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in the guinea pig and a safety factor of 100. Subsequently, the Committee recommended that the MRL for kidney for all species should be increased to 10,000 μg/kg. The higher MRL permitted the oral administration of neomycin sulphate, equivalent to 7.7 mg of neomycin base, per kg bodyweight per day on five consecutive days to non-ruminating calves. The Committee recommended also that the temporary status of all MRLs for neomycin be withdrawn.

The 52nd meeting of the Committee (WHO 2000) considered two new residue depletion studies. One study compared tissue residues following oral and intramuscular administration of neomycin to calves; the second study assessed tissue residue depletion in cattle after intramuscular administration of neomycin. The Committee concluded that although the MRLs for liver and kidney for cattle established at the 47th meeting were appropriate for oral formulations, the MRLs did not accommodate the use of injectable formulations of neomycin. Accordingly, MRLs for liver and kidney were increased to 15,000 μg/kg and 20,000 μg/kg, respectively, and MRLs of 500 μg/kg for muscle and fat, and 500 μg/L for milk were confirmed for cattle. The Committee confirmed also the MRLs of 10,000 μg/kg for kidney and 500 μg/kg for muscle, fat and liver for chickens, ducks, goats, pigs, sheep, and turkeys, and 500 μg/kg for chickens' eggs.

The 58th meeting of the Committee (WHO Technical Report Series, No 911, 2002) considered information on the registration of injectable neomycin products as well as how they were used in relation to good practice in the use of veterinary drugs. This followed a request by the Codex Committee on Residues of Veterinary Drugs in Foods at its 12th Session (ALINORM 01/31, paragraph 90). The information indicated that use of parenteral formulations is not regarded as good practice in the use of veterinary drugs, and few such products were found to be authorised. The Committee also considered information about the toxicity of neomycin in calves and concluded that the information was relevant only to target animal welfare, which falls outside of the mandate of JECFA. Finally, the sponsor provided data in support of a proposal to increase the MRL of neomycin for milk, contending that an increase in the MRL was necessary to support practical withdrawal times for neomycin-containing intramammary products. In addition, the sponsor provided data to allow the MRLs for liver and kidney to be reconsidered. However, in the light of a request from the 13th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (ALINORM 03/31, paragraph 18) to evaluate new safety data, the Committee recommended maintaining the MRLs that had been recommended at the 43rd, 47th and 52nd meetings and deferring the review of the MRLs until such time as the toxicology of neomycin was re-evaluated.

The 60th meeting of the Committee evaluated new safety data, which comprised information on microbiological aspects of consumer safety of neomycin and the evidence for a link between the presence of a specific mutation to mitochondrial DNA in humans and increased susceptibility to aminoglycoside ototoxicity. The Committee confirmed the ADI of 0 – 60 μg per kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in a 90-day study on the guinea pig and a safety factor of 100. The 60th meeting of the Committee also evaluated the residues depletion data submitted by the sponsor to the 58th meeting.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Neomycin is an aminoglycoside antibiotic produced by *Streptomyces fradiae* and is a mixture of neomycin A (<1% present in commercial preparations), neomycin B (>90% present in commercial preparations) and neomycin C. Other aminoglycoside antibiotics include streptomycin, kanamycin, gentamicin, tobramycin and amikacin. Aminoglycosides enter susceptible bacteria by oxygen-dependent active transport and by passive diffusion, and then bind irreversibly to the 30S bacterial ribosomes (Brown, 1988). This blocks the formation of a complex that includes mRNA, formylmethionine, and tRNA, and induces the misreading of the genetic code on the mRNA template. As a result, the tRNA is translated incorrectly, producing a non-functional protein. Aminoglycosides have additional effects on microorganisms such as interference with the cellular electron transport system, induction of RNA breakdown, inhibition of translation, effects on DNA metabolism, and damage to cell membranes. The bactericidal effect is through the formation of abnormal cell membrane channels by misread proteins (Prescott, Baggot and Walker, 2000).

Aminoglycosides are utilized primarily in the treatment of infections caused by aerobic Gram-negative microorganisms. They can be effective in the treatment of some Gram-positive organisms such as *Staphylococcus aureus*, some mycobacteria, some Mycoplasma strains, and some spirochetes. Aminoglycosides are not active against anaerobic organisms. Neomycin demonstrates bactericidal activity against most Gram-positive and Gram-negative rods, many Gram-positive cocci, and such acid-fast pathogens as *Mycobacterium tuberculosis*. Acidic or purulent conditions at the site of infection can limit the efficacy of aminoglycosides, as can the presence of cations (Prescott, Baggot and Walker, 2000).

Neomycin is formulated, either alone or in combination with other antimicrobials such as lincomycin, penicillin, cephalosporins and some sulphonamides, for oral (including in-feed and medicated drinking water) administration and for injection, intramammary infusion and topical (including ocular) application. In aquaculture, neomycin is administered as a bath solution.

Neomycin has a long history of use. It is indicated in the treatment of intestinal and respiratory infections, wound and skin infections, and mastitis (Prescott, Baggot and Walker, 2000). Orally it is used to treat enteric infections, including salmonellosis and enterotoxigenic *Escherichia coli* diarrhoea in calves. Neomycin is also administered to cattle by intramuscular injection to treat respiratory tract infections, and by intramammary infusion, most commonly in combination with other antibiotics, to treat mastitis in lactating and non-lactating dairy cows. Other clinical applications of neomycin include intrauterine administration for uterine infections, the oral treatment of pigs with coliform diarrhoea, and of chickens and turkeys with salmonellosis, and topical administration of infectious conditions of the eye and external ear, as well as in contaminated wounds. Neomycin is administered by intramuscular or intravenous injection to foals with *Rhodococcus equi* pneumonia.

Because the use of injectable formulations of neomycin is associated with ototoxicity (deafness in cattle) and nephrotoxicity, including at doses indicated, such use is generally limited to the treatment of serious Gram-negative infections resistant to less toxic medications or as an alternative to costly medications . In some countries such as the USA, Canada and South Africa, injectable neomycin products are not authorised for use in food animals on account of such use being associated with a high risk of toxicity.

Dosage

Table 1. Maximum daily doses of neomycin reported in studies submitted to the 43rd, 47th, 52nd and 58th meetings of JECFA

Species	Oral	Parenteral	Intramammary
Poultry	20 mg/kg		
Pigs	15 mg/kg		
Cattle	15 mg/kg	12 mg/kg intramuscular	
Sheep	15 mg/kg		
Goats	15 mg/kg		
Lactating dairy cows			100 mg/quarter every 12 hours

METABOLISM

Pharmacokinetics

General

The pharmacokinetic properties of neomycin are largely attributed to it being a polar organic base (Prescott, Baggot and Walker, 2000). Neomycin is generally not significantly absorbed from the gastrointestinal tract, with very young calves being an exception. Aschbacher and Feil (1994) reported that 11 % of an oral neomycin dose of 30 mg/kg bodyweight was absorbed in 3-day-old calves and 1 to 2 % of the dose was absorbed by 2-month-old calves, regardless of the status of ruminal development. Damage to the gastrointestinal mucosa can also lead to increased aminoglycoside absorption (Thomson et al, 1991; Brown and Riviere, 1991). The binding of neomycin (at concentrations of 5 – 10 μg/mL) to plasma proteins is reportedly 45 % in cows and 50 % in ewes (Ziv and Sulman, 1972). The poor diffusion of neomycin across biological membranes can be attributed to its poor lipid solubility. Selective binding to tissues, including kidney cortex, occurs, resulting in residues that persist in animals for prolonged periods. As a result, a dose-dependent, slow elimination phase (gamma-phase), many times longer than the initial elimination phase has been described (Brown et al, 1985). The aminoglycosides, as a class, undergo negligible metabolism after parenteral administration (Bevan and Thompson, 1983). Neomycin is excreted in the faeces after oral doses and in the urine (glomerular filtration) after parenteral administration (Prescott, Baggot and Walker, 2000; WHO 1995).

Lactating dairy cattle

Eight healthy lactating dairy cows received intramammary infusions of Lincocin Forte® Sterile containing 100 mg neomycin base and 330 mg lincomycin base into each mammary quarter at 12-hour intervals following three successive milkings (Deluyker et al, 1996). Heparinised blood samples were collected prior to treatment and at 0.5, 1, 2, 4, 8, 12, 24 and 36 hours after the first infusion. Plasma separated from the blood samples was assayed for neomycin using solid phase extraction and HPLC. Neomycin was not detected (<0.024 μg/mL) in any of the plasma samples. Milk excretion of neomycin estimated from the total amount of neomycin recovered in pooled milk up to 120 hours post-treatment, based on measured milk production, was 55.7 ± 9 % of the total dose administered.

MILK RESIDUE DEPLETION STUDIES

The Committee evaluated one new residues depletion study in milk, in which unlabelled neomycin was administered by intramammary infusion to lactating dairy cows. Eight cows were common to the pharmacokinetic study (see previous section). The milk residue study was GLP-compliant.

Lactating dairy cattle

Twenty-four healthy lactating cows were assigned to four blocks of 6 cows each according to parity and milk yield. Two lactating cows from each block were randomly re-assigned to a pharmacokinetic group (n = 8). All 24 animals were used in the milk residue depletion study. The cows all received intramammary infusions of Lincocin Forte® Sterile containing 100 mg neomycin base and 330 mg lincomycin base in each mammary quarter at 12-hour intervals after three successive milkings (Deluyker et al, 1996).

In the pharmacokinetic group of cows, quarter milk samples were collected before each infusion and continued until the second milking after the last infusion. Quarter milk production, starting from 6 milkings before treatment up to and including 10 milkings after the last infusion, was also measured. Milk samples were assayed for neomycin concentration using solid phase extraction and HPLC. Neomycin was not detected (<0.0327 μg/mL) in milk prior to the first infusion, whereas the mean neomycin concentration in milk collected before the second and third infusions was 22.2 μg/mL and 29.7 μg/mL, respectively. At 12 hours and 24 hours after the last infusion, the mean neomycin concentrations were 28.0 μg/mL and 4.92 μg/mL, respectively.

The depletion of neomycin residues in milk from all 24 cows, including the pharmacokinetic group, was determined from pooled milk samples collected before every infusion and until the tenth milking after the last infusion. Pooled milk samples were assayed for neomycin concentration using solid phase extraction and HPLC. The LOQ of the analytical method was 0.1 μg/mL. The results for the individual cows are shown in Table 2. The mean neomycin concentrations at 12 and 24 hours after the last infusion were 24 μg/mL and 4.8 μg/mL, respectively. At 60, 72 and 84 hours after the last infusion, the mean (range) neomycin concentrations in pooled milk samples were estimated to be 0.26 (<LOQ – 1.05), 0.21 (<LOQ – 0.65) and 0.16 (<LOQ – 0.51) μg/mL, respectively. Statistical tolerance limits for the neomycin milk residue concentration versus time depletion curve were determined by linear regression of the logarithmic concentrations of neomycin in milk versus time, and then estimating the upper one-sided 95% confidence interval for the 95th percentile of a population receiving the described treatment. Upper limits of 1,800 μg/kg, 1,500 μg/kg and 1,000 μg/kg were determined for neomycin concentrations in milk samples at 72 hours, 76 hours and 84 hours, respectively.

Table 2. Neomycin residues (μg/mL) in milk after three successive intramammary infusions of 100 mg neomycin base into each quarter of the udder at 12-hour intervals

Cow No	Hours after last infusion									
	12	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**
4	8.67	3.14	1.17	0.49	0.15	0.11	0.12	<LOQ	<LOQ	<LOQ
9	18.0	8.78	3.74	1.59	0.63	0.48	0.17	0.13	0.15	<LOQ
11	17.4	4.85	1.14	0.42	0.16	0.12	0.12	<LOQ	<LOQ	<LOQ
16	20.3	2.03	0.40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
18	27.2	4.82	0.74	0.14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	20.3	3.36	2.67	1.49	0.37	0.45	0.51	0.18	0.26	0.19
27	20.8	3.64	1.41	0.52	0.20	0.13	<LOQ	<LOQ	<LOQ	<LOQ
28	20.3	3.90	0.75	0.27	0.10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	29.4	6.30	1.10	0.28	0.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	16.0	2.96	0.94	0.25	0.12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	15.3	1.63	0.94	0.36	<LOQ	0.11	<LOQ	<LOQ	<LOQ	<LOQ
41	29.9	3.41	0.43	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	18.9	6.15	5.51	1.78	0.76	0.65	0.30	0.52	0.48	0.11
47	34.7	13.7	7.12	2.06	0.56	0.55	0.21	0.14	0.12	<LOQ
48	7.71	1.59	0.28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	16.7	5.23	2.67	0.81	0.26	0.24	0.19	0.084	<LOQ	<LOQ
53	25.2	2.36	0.43	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
57	33.7	4.41	1.63	0.57	0.17	0.21	0.18	<LOQ	<LOQ	<LOQ
60	44.6	6.19	1.59	0.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
61	19.5	3.53	1.26	0.48	0.11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
63	30.7	6.58	2.70	0.86	0.46	0.22	<LOQ	<LOQ	<LOQ	<LOQ
65	20.6	3.72	1.54	0.44	0.20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
71	49.5	9.97	6.49	1.46	1.05	0.58	0.47	0.24	0.20	0.16
73	31.3	2.88	0.65	0.10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mean	24.0	4.80	1.97	0.63	0.26	0.21	0.16			
SD	10.1	2.83	1.92	0.59	0.25	0.18	0.11			
Min.	7.71	1.59	0.28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max.	49.5	13.7	7.12	2.06	1.05	0.65	0.51	0.52	0.48	0.19

LOQ = 0.1 μg/mL in milk

MILK PRODUCTION AND SOMATIC CELL COUNTS

Milk production and somatic cell counts were monitored prior to and throughout the milk residue depletion study. The milk production data confirmed that low and high yielding cows had been selected for the trial. Milk production was not affected by treatment and remained at pre-treatment levels for at least 84 hours after the last infusion. Decreased milk production was reported at 96 hours or later following the last infusion for cows 31, 46, 53 and 73. Somatic cell counts remained at pre-treatment levels throughout both the infusion and post-infusion periods, except for cows 31 and 46 which had elevated somatic cell counts commencing at 72 hours after the last infusion. The cows with decreased milk production and/or increased somatic cell counts were investigated further, and shown to have acquired yeast mastitis (*Candida kefir*) unrelated to the intramammary formulation.

EFFECT OF NEOMYCIN ON STARTER CULTURES IN MILK PROCESSING

The effect of neomycin on bacterial starter cultures used in the production of Italian cheese, yogurt, buttermilk and sour cream was assessed based on "time to clot" ratios (Hallberg et al, 1994). Neomycin concentrations in milk of less than 2 μg/mL were shown to have no effect on the growth of the bacteria in any of the starter cultures.

TISSUE RESIDUE DEPLETION STUDIES

The Committee evaluated one GLP-compliant tissue residue depletion study, which involved the oral administration of neomycin to calves. An additional study of tissue residues in heifers, which did not comply with Good Laboratory Practice,

was not considered as the animals received less than half the total dose administered in the first study and less than the dose recommended by the sponsor.

Calves

Sixteen non-ruminating Holstein bull calves of approximately 35 kg bodyweight were treated orally for 14 consecutive days with neomycin sulphate, equivalent to 15 mg of neomycin base, per kg bodyweight (Arnold et al, 1991). Groups of four calves were sacrificed at 7, 14, 21, or 28 days after the last treatment. Livers and kidneys were analysed for neomycin residues using a microbiological assay with a LOQ of 0.92 μg/g (Stahl, 1991). The bacterial test strain was *Staphylococcus epidermidis* UC 719 (Official Method of Analysis, 1984). The results are presented in Table 3.

Table 3. Neomycin residues (μg/g) in the kidneys and livers of calves

Animal No.	Days after last treatment	Kidney	Liver
150	7	71.3	2.5
158	7	55.3	<LOQ
160	7	30.4	2.16
168	7	36.8	2.18
149	14	10.0	<LOQ
161	14	7.3	<LOQ
163	14	10.6	<LOQ
167	14	8.4	<LOQ
156	21	11.5	<LOQ
157	21	13.1	2.22
164	21	3.5	<LOQ
166	21	5.0	<LOQ
152	28	6.8	<LOQ
159	28	3.9	<LOQ
162	28	4.6	<LOQ
165	28	5.0	1.17

The study was initially designed for determination of residues in kidneys only; however, liver samples collected at the same time were analysed for neomycin residues 16 months later. These liver data were not validated by data on stability during storage. The statistical tolerance for kidney was greater than 10'000 μg/kg at all times. The concentrations in liver were below the limit of quantification from day 14 through day 28, except for individual animals on days 21 and 28, in which concentrations of 2,200 μg/kg and 1,200 μg/kg were found, respectively. Due to the paucity and quality of the data for both kidney and liver, no conclusions were drawn from the study.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Chemical Methods

The 43rd meeting of the Committee considered an HPLC method of analysis for neomycin residues in milk with a limit of detection of 0.050 μg/g (Agarwal, 1990).

A method reported by Deluyker et al (1996) for the quantification of neomycin in milk of cattle was considered by the present Committee. A trichloroacetic acid extract of milk was prepared, neutralised with sodium sulphate/sodium monohydrogen phosphate and centrifuged. The supernatant fraction was retained, adjusted to pH 6.8 with NaOH, and applied to a carboxylic acid cartridge that had been preconditioned sequentially with methanol and 0.1 M Na_2SO_4/Na_2HPO_4, pH 6.8. Following cartridge clean-up using water, the neomycin residue was eluted with citrate/phosphate buffer pH 2.3.

HPLC determination of neomycin was performed on a C_{18} column with post-column derivatisation with o-phthalaldehyde and fluorescence detection (340 nm excitation, 435 nm emission). Under these conditions, neomycin chromatographed at approximately 12 minutes.

Validation in bovine milk

Accuracy of the method was evaluated with fortified quality control (QC) samples at concentrations of 0.100 and 0.103 μg/mL (QC-low samples), and 4.008 and 4.115 μg/mL (QC-high samples). Results ranged from 95.3 – 95.9 % for QC-low samples and from 102 – 103 % for QC-high samples. The acceptance range for QC-low samples was 80 – 120 % and for QC-high samples was 80 – 115 %. Runs were accepted if more than 75 % of the determined QC sample concentrations were within the acceptance range.

Recovery determinations were conducted at fortification concentrations of 0.1, 0.25, 0.5, 1, 2 and 5 μg/mL in blank solid phase extracts. The mean recoveries at the respective concentrations were 56.5, 53.3, 59.5, 60.7, 60.2 and 60.4 %; the overall recovery (mean ± SD) was 58.7 ± 4.4 % (n = 16).

Linearity of calibration curves (r > 0.9962) was demonstrated for concentrations of neomycin between 0.05 and 5 μg/mL.

Limit of detection (LOD) for the method was defined as the mean plus three standard deviations of noise concentration in blank chromatograms at the location where neomycin eluted. In this study, blank milk samples from 24 cows were prepared, extracted and chromatographed, and the LOD for the method was calculated to be 0.03 μg/mL.

Limit of quantitation (LOQ) for the method was defined as the lowest concentration that can be determined with a precision (CV %) of <20 %. Milk was fortified, prepared, extracted and nine replicates chromatographed over three runs. The LOQ for the method was established as 0.1 μg/mL with a precision of 12.7 %.

Repeatability, expressed as the mean intra-day precision (CV %), was 15.9 % (n = 14) and 6.7 % (n = 6) for QC-low samples, 6.5 % (n = 14) and 4.6 % (n = 6) for QC-high samples, and 4.7 % (n = 22) for replicate analyses of incurred samples.

Reproducibility, expressed as the mean between-day precision (CV %), was 0.0 % (n = 14) and 8.1 % (n = 6) for the QC-low samples, 3.0 % (n = 14) and 3.9 % (n = 6) for QC-high samples, and 7.7 % (n = 17) for replicate analyses of incurred samples.

Specificity from matrix components and from lincomycin (at a concentration of 8.92 μg/mL) was assessed and no interference was demonstrated in either situation. Specificity of the method in the presence of other veterinary drugs was not described.

Stability of neomycin in milk during storage was investigated. Milk was fortified at 46.1 μg/mL and stored at ≤-20°C for 163 days or 210 days. These storage times exceed the maximum storage time for the experimental milk samples of 119 days. The mean neomycin concentrations were 103 % and 135 % of the fortified levels following storage at 163 days and 210 days, respectively. Overall, the stability of neomycin in milk upon storage is acceptable. It is noted, however, that when fortified milk samples were stored for 210 days, recoveries significantly exceeded 100 %. The sponsor suggested that an incorrect dilution might have explained this high value.

Microbiological Methods

Quarter and pooled milk samples were collected from eight cows immediately prior to the second and third intramammary infusions and assayed for neomycin by a microbiological method and HPLC. The bacterial test strain used in the microbiological assay was *Escherichia coli*. It was claimed that the microbiological assay had been validated in study 94.087.3 (a copy of which was not provided) and only limited validation data were provided in the present submission. The latter did not include either the LOD or the LOQ for the method but reported the recoveries from fortified milk to be 89 % and 88 % at 2.51 μg/mL and 101 μg/mL, respectively.

Comparison of Neomycin Concentrations in Milk Measured by HPLC and the Microbiological Method

Concentrations of neomycin determined by HPLC and a microbiological method in selected quarter and pooled milk samples were compared. Good agreement was demonstrated (Table 4).

Table 4. Comparison of neomycin residues (μg/mL) in milk measured by HPLC and a microbiological method (MB).

	Before the second infusion				Before the third infusion			
Cow No.	Quarter milk		Pooled milk		Quarter milk		Pooled milk	
	HPLC	MB	HPLC	MB	HPLC	MB	HPLC	MB
16	41.8	39.1	14.2	9.79	57.5	29.4	14.9	10.0
	41.1	32.7			53.9	27.1		
	23.9	21.2			36.5	11.7		
	28.7	21.7			39.7	24.4		
18	52.2	65.7	23.2	21.9	54.3	51.2	30.1	13.4
	41.8	41.9			42.1	47.6		
	33.5	29.4			48.7	46.6		
	36.3	31.6			52.5	29.1		
22	30.6	36.0	12.5	18.2	32.4	34.8	15.4	13.7
	31.2	33.1			43.9	48.3		
	15.0	17.3			21.8	22.9		
	18.8	18.8			15.3	18.5		

Cow No.	Before the second infusion				Before the third infusion			
	Quarter milk		Pooled milk		Quarter milk		Pooled milk	
	HPLC	MB	HPLC	MB	HPLC	MB	HPLC	MB
27	27.1	25.4	12.8	12.4	50.7	46.1	13.7	9.79
	24.7	26.0			46.4	21.6		
	16.5	19.7			30.4	25.1		
	18.0	14.7			27.3	20.4		
28	38.8	32.5	15.0	17.4	25.2	26.0	18.4	12.1
	9.19	23.4			36.0	38.2		
	23.5	26.3			27.3	21.8		
	41.0	40.1			34.4	32.9		
37	7.58	9.65	7.85	6.13	14.8	11.1	10.4	12.4
	3.19	5.07			10.5	9.77		
	7.70	7.82			15.3	31.9		
	18.6	20.0			25.4	29.4		
46	11.6	19.0	8.80	14.6	12.9	15.6	16.4	35.0
	8.72	15.6			14.3	19.7		
	11.7	16.0			16.2	19.0		
	5.13	15.1			13.0	15.8		
50	13.7	16.7	7.68	11.3	17.3	20.4	9.90	8.51
	13.1	13.1			13.2	16.0		
	8.55	12.8			11.4	11.0		
	7.34	10.2			16.8	14.4		

Qualitative tests for milk

A report by Deluyker et al (1996) that investigated the suitability of five qualitative tests for detecting neomycin residues at concentrations approximating the milk MRL was assessed. The tests considered were Delvotest® SP, Penzym, Valio T101, Brilliant Black Reduction, and Dutch Tube Diffusion. Blank milk samples fortified with neomycin were used to establish the detection levels of each of the five tests. The results are shown in Table 5.

Table 5. Detection levels of neomycin (μg/mL) in milk for qualitative screening tests

Qualitative Screening Test	Neomycin (μg/mL)
Delvotest® SP	0.20
Penzym	> 2.0
Valio T101	> 2.0
Brilliant Black Reduction	2.0
Dutch Tube Diffusion	0.10

Pooled milk samples from twenty-four dairy cows involved in the residues depletion trial (Deluyker et al, 1996) taken from 60 hours to 120 hours after the third infusion of Lincocin Forte® Sterile were subjected to the five qualitative tests. The results are presented in Table 6.

Notwithstanding the non-specific nature of qualitative tests, the results generally reflect the status of neomycin residues in milk since data provided by the sponsor demonstrated that residue depletion for neomycin was slower than for lincomycin following intramammary infusion with Lincocin Forte® Sterile. Large variations in the duration and number of positive tests were observed, however, no positive results occurred with the Brilliant Black Reduction test in samples taken 84 hours after the last infusion or later. False-positive results for mastitic milk were observed with the Penzym, Valio T101, and Dutch Tube Diffusion tests.

Table 6. Number of negative (N), uncertain (U), or positive (P) results for qualitative tests on milk

Test	Hours after last infusion						
	Result	60	72	84	96	108	120
Delvotest® SP	N	7	8	11	16	19	21
	U	9	9	9	5	4	2
	P	8	7	4	3	1	1

Test	Hours after last infusion						
	Result	60	72	84	96	108	120
Penzym test	N	18	14	18	18	19	21
	U	6	9	6	6	5	2
	P	0	1	0	0	0	1
Valio T101 test	N	6	17	24	24	23	23
	U	0	0	0	0	0	0
	P	18	7	0	0	1	1
Brilliant Black Reduction test	N	12	18	20	23	24	23
	U	10	5	4	1	0	1
	P	2	1	0	0	0	0
Dutch Tube Diffusion test	N	6	6	7	9	11	16
	U	7	8	11	9	10	4
	P	11	10	6	6	3	4

APPRAISAL

The clinical pharmacology of the aminoglycosides has been reviewed recently by Prescott, Baggot and Walker (2000). Neomycin is used for the local treatment of intestinal infections, of wound or skin infections, and of mastitis. For these clinical conditions, neomycin is formulated either alone or in combination with other antibiotics for oral, topical or intramammary administration. The toxic side effects of neomycin are generally not evident following such use. By contrast, the systemic use of neomycin is limited by a relatively high risk of nephrotoxicity and ototoxicity (deafness). Neomycin is considered the most nephrotoxic aminoglycoside (Riviere, Craigmill and Sundlof, 1991). Aminoglycosides cause nephrotoxicity by accumulating in the proximal tubular cells, where they interfere with cellular metabolism and transport processes. The initial tubular changes can progress to proximal tubular necrosis, followed by perturbations in glomerular filtration, and azotemia. The auditory ototoxicity associated with the systemic use of neomycin may be due to the drug's distribution characteristics and its ability to accumulate in the cochlear, causing severe cochlear toxicity (Kitasato et al, 1990). Vestibular, in addition to auditory, ototoxicity can occur with parenteral neomycin, but damage of cranial nerve VIII is usually not seen unless parenteral therapy is extended past 5 days (Bowen and Crawford, 1976). The neuromuscular blocking effects of neomycin that have been demonstrated during pentobarbital anaesthesia in nonhuman primates (Adams, 1973) are considered rare compared to its nephrotoxic and ototoxic effects. Because of these toxicity concerns, neomycin is not recommended for systemic use on animals. Importantly, alternate drugs that are safer and demonstrate equal or better efficacy than neomycin are readily available for parenteral use. Despite this, neomycin is approved for parenteral administration to food animals in some countries, and is regarded as an inexpensive "alternative" to gentamicin.

In respect to good practice in the use of veterinary drugs, injectable formulations of aminoglycosides are generally dosed to achieve a high peak blood concentration (typically 8 to 10 times higher than the minimum inhibitory concentration for the microorganism), followed by a low trough concentration, significantly below therapeutic blood concentrations. This strategy is justified since aminoglycosides kill bacteria by a concentration-dependent mechanism (Campbell et al, 1996), with the length of time the organism is exposed to the antibiotic being of lesser importance (Xiong et al, 1997). Because the occurrence of nephrotoxicity due to aminoglycosides is more influenced by the trough than the peak blood concentrations, a dosing strategy is applied whereby the interval between treatment is extended to ensure that the trough drug concentrations drops low enough, a concentration specific for each aminoglycoside, to minimise toxicity. This phenomenon has been extensively studied with gentamicin (Cummings et al, 1990; Grauer, 1996) and to some extent with amikacin (Brown and Riviere, 1991). By comparison, scant information on this approach is available for neomycin, most likely due to its toxicity at therapeutic dose rates, which is well documented.

Presently, the available information pertaining to the registration of injectable neomycin products and how they are used with respect to Good Veterinary Practices is incomplete. Injectable neomycin products are not approved for use in food animals in the USA, Canada or South Africa whereas a very small number of such products are approved in Australia, the Czech Republic and Thailand. From the preceding discussion, it would appear that: (i) compelling evidence exists on the toxicity of neomycin when administered by parenteral injection; (ii) an approach to parenteral dosing with neomycin that both provides therapeutic concentrations and overcomes toxicity concerns has not been proposed; and (iii) safe alternatives, with equal or better efficacy than neomycin, are approved for parenteral administration to food animals.

From a residues perspective, neomycin residues are characterised by persistence in kidney, and to a lesser degree in liver and at injection sites. The bioavailability of neomycin markedly influences the magnitude of the incurred residues and, in turn, the time required for residues to deplete. In calves, for example, the bioavailability of oral doses of neomycin ranges from 1 to 11 %, depending on the age of the calves (Aschbacher and Feil, 1994). In this respect, it was noted by the 47th meeting of the Committee that increasing the (temporary) MRL for kidney from 5,000 μg/kg to 10,000 μg/kg permitted the practical use of formulations administered orally to very young calves. It should be noted that neomycin is not approved for use in veal calves in some countries; this overcomes the concerns relating to the occurrence of neomycin residues in very young calves. The 52nd

meeting of the Committee noted that its recommendations to increase the MRL for kidney from 10,000 μg/kg to 20,000 μg/kg, and to increase the MRL for liver from 500 μg/kg to 15,000 μg/kg, allowed practical withdrawal times to be established for injectable formulations of neomycin. The latter reflects the fact that neomycin is readily bioavailable when injected.

One study in the literature of particular relevance to the injectable use of neomycin investigated both neomycin toxicity and kidney residues in four heifer calves weighing 150 – 190 kg bodyweight (Crowell et al, 1981). Results from the study are shown in Table 7 and indicate that toxic manifestations occur as early as 5 days after the initiation of parenteral dosing regimens. The study demonstrates that nephrotoxicity and deafness in cattle occur at sub-maximal dose rates. It was noted also that these toxic effects occurred when residue concentrations in the kidneys were less than 500 μg/kg.

Table 7. Neomycin toxicity and kidney residues in calves that were administered neomycin by intramuscular injection.

Calf No.	Neomycin Treatment Regimen	Days after first neomycin injection when symptom first occurred			Days after the last injection	Kidney Residues (μg/kg)
		Renal casts	Azotemia	Deafness		
1	**4.5 mg/kg IM twice daily for 12 days**	5	10	None*	0.25	300
2	**4.5 mg/kg IM twice daily for 12 days**	5	12	14	1	226
3	**2.25 mg/kg IM twice daily for 13 days**	12	12	19	6	210
4	**2.25 mg/kg IM twice daily for 13 days**	10	12	None	11	430

* Calf 1 was euthanised at 12 days after the first injection of neomycin; it may have become deaf had it survived longer.

The data suggest that the use of injectable formulations of neomycin in food animals does not represent good practice in the use of veterinary drugs, and that injectable neomycin formulations should be excluded from consideration when recommending MRLs. The sponsor who submitted data in support of the injectable uses of neomycin has confirmed that they do not wish to defend the injectable use patterns. Furthermore, information on the registered use patterns for injectable formulations of neomycin in food-producing animals provided by Member Governments in response to a request of the 12th Session of CCRVDF indicated that use of parenteral formulations is not regarded as good practice in the use of veterinary drugs, and few such products were approved. The MRLs for kidney of 20,000 μg/kg and for liver of 15,000 μg/kg recommended by the 52nd meeting of the Committee were therefore considered by the present Committee to be unnecessary.

One GLP-compliant milk residue depletion study, which used unlabelled compound, was considered. The recommended label rate of 100 mg of neomycin base was infused into each quarter of the udder at 12-hour intervals following three successive milkings. The formulation was well tolerated. Unrelated to the formulation was the development of yeast mastitis in four of twenty-four cows; the causative microorganism was *Candida kefir*. The four cases of mastitis occurred late in the study and did not compromise the findings.

Toxicity associated with systemic uptake of neomycin following the intramammary infusion of Lincocin Forte® Sterile was not manifested in the studies considered by the Committee. Indeed, neomycin was not detected (<0.024 μg/mL) in any of the plasma samples in the pharmacokinetic study on lactating dairy cows that received intramammary infusions of Lincocin Forte® Sterile into each mammary quarter at 12-hour intervals following three successive milkings. Although the study does not provide supporting evidence of systemic uptake of neomycin following intramammary infusion, neither does it rule out the possibility of some systemic absorption occurring. For example, the average daily intramammary dose administered to the lactating cows in the pharmacokinetic study was 0.65 mg/kg, considerably less than the recommended oral and parenteral doses of neomycin, which may explain why neomycin could not be detected in plasma even in the presence of intramammary absorption. The recovery of neomycin in pooled milk up to 120 hours post-treatment based on measured milk production was 55.7 ± 9 % (mean ± SD) of the total dose administered which may suggest that some absorption could have occurred. In a study reported by the EMEA (2000), absorption following intramammary administration of neomycin was confirmed in 16 healthy cows that received an intramammary infusion containing 300 mg lincomycin and 100 mg neomycin base, as neomycin sulphate, in each of 4 udder quarters, following each of 3 successive milkings at 12 hour intervals. In that study, measured concentrations of neomycin residues were only present in the kidney and udder. For the kidney, mean concentrations were 700 μg/kg (day 1), 315 μg/kg (day 7), and 205 μg/kg (day 14). The mean concentrations were below the limit of quantification (107μg/kg) at day 21. Despite this evidence for some systemic uptake of neomycin following intramammary infusion, there is no evidence for toxicity, possibly because residues do not persist in the kidneys at toxic concentrations for long enough. It is concluded that intramammary infusions of neomycin reflect good practice in the use of veterinary drugs.

The sponsor's HPLC method for quantifying neomycin in milk is suitable for regulatory purposes. Moreover, there was good agreement between the results of the HPLC and microbiological methods, demonstrating that microbiological assays would be suitable for preliminary analyses of large numbers of milk samples in regulatory programs. Five qualitative tests were assessed for their suitability for screening commercial milk supplies for neomycin residues approaching the MRL. The Brilliant Black Reduction, Penzym and Valio T101 tests were negative within 84 hours of the last intramammary infusion whereas the Dutch Tube Diffusion and Delvotest® SP tests were positive at 120 hours after the last treatment. False-positive results with mastitic milk were observed with the Penzym, Valio T101, and Dutch Tube Diffusion tests. It appeared from the study that the Brilliant Black Reduction test would be suitable for screening commercial milk supplies for neomycin exceeding the MRL.

The study into the depletion of neomycin residues from kidneys was conducted in non-ruminating bull calves weighing about 35 kg, given neomycin sulphate orally at a dose equivalent to 15 mg/kg bw as neomycin base for 14 consecutive days. Although this study was initially designed for the determination of residues in kidneys only, liver samples were also analysed for neomycin residues albeit 16 months later. Stability data for liver residues during storage were not provided. No conclusions could be drawn from the study on account of the paucity and quality of the data generated.

Maximum Residue Limits

The Committee considered the following factors in recommending MRLs

- An ADI of 0 – 60 μg/kg bodyweight based on a toxicological endpoint, which results in a maximum daily intake of 3,600 μg for a 60 kg person.
- Neomycin undergoes negligible metabolism following parenteral administration to animals and the parent drug represents the total of the residues present.
- Neomycin is the marker residue for tissues, milk and eggs.
- A validated HPLC method with a LOQ of 0.1 μg/mL for neomycin in cows' milk is available that could be used routinely in many laboratories.
- Concentrations of neomycin up to 2 mg/L had no effect on bacterial starter cultures used in the production of fermented milk products.
- Data on residues in milk supported an MRL for cows' milk of 1,500 μg/kg.
- Information on the registered use patterns for injectable formulations of neomycin in food-producing animals was requested from Governments and considered. The information indicated that use of parenteral formulations is not regarded as good practice in the use of veterinary drugs, and few such products were found to be approved.
- The MRLs for kidney of 20,000 μg/kg and for liver of 15,000 μg/kg recommended by the Committee at its 52nd Meeting to accommodate use of parenteral formulations are therefore unnecessary.

The Committee, having considered the database submitted since its 43rd meeting, decided to revert to the MRLs for cattle kidney and liver that it had recommended at its 47th meeting.

On the basis of the above considerations, the Committee recommended the following MRLs: cattle kidney, 10,000 μg/kg; cattle liver, 500 μg/kg; and cows' milk, 1,500 μg/kg. The MRLs of 500 μg/kg for cattle muscle and fat were maintained.

Based on the consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, 1.5 kg of milk and 100 g of eggs, the theoretical maximum daily intake of neomycin residues is 3,025 μg (Table 8). This accounts for 84 % of the ADI of 3,600 μg for a person of 60 kg bodyweight.

Table 8. Theoretical maximum daily intake (TMDI) of neomycin residues

Tissue	Food Basket (kg)	MRL (μg/kg)	Intake (μg)
Muscle	0.300	500	150
Liver	0.100	500	50
Kidney	0.050	10,000	500
Fat	0.050	500	25
Milk	1.500	1,500	2,250
Eggs	0.100	500	50
Total			3,025

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