NEOMYCIN

First draft prepared by

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Addendum
to the Neomycin residue monographs prepared by the 43rd meeting and the 47th meeting of the Committee published in the
FAO Food and Nutrition Paper 41/7, Rome 1995 and 41/9, Rome 1997, respectively

At the 43rd meeting of the Committee, a temporary ADI of 0 - 30 µg per kg of body weight was recommended for neomycin. The Committee evaluated both toxicological and microbiological data and concluded that the toxicological data provided the most appropriate end-point. A safety factor of 200 was used to compensate for the lack of genotoxicity data. Temporary MRLs of 5000 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. The temporary MRLs recommended for chicken eggs and cow's milk were 500 µg/kg and 500 µg/L, respectively, also expressed as the parent drug.

After evaluating new genotoxicity data, the 47th meeting of the Committee recommended an ADI of 0 - 60 µg/kg BW using a safety factor of 100. Although no new residue depletion studies were submitted, the Committee concluded that the MRL for kidney should be doubled (to 10000 µg/kg) in order to permit practical withdrawal times to be established for all target animal species. The Committee had information indicating that adsorption of neomycin in calves that are just a few days old is higher than in older calves. The neomycin residue levels in the kidneys of these younger calves require approximately thirty days to deplete below 10,000 µg/kg.

RESIDUES IN FOOD AND THEIR EVALUATION

The present Committee evaluated two new residue depletion studies. Previous studies assessed tissue residue depletion following oral dosing. The first of the two new studies compared tissue residues after oral dosing at 10 mg/kg, twice a day for 5 days and intramuscular dosing at 12 mg/kg once a day for 5 days. This study used only four animals per treatment group and one sacrifice time. Results provide a temporal comparison of the residue levels of neomycin in edible tissues resulting from an injectable and an oral route of administration. The second study assessed tissue residue depletion after intramuscular dosing at 12 mg/kg daily for 5 days. This study utilized 4 animals per slaughter point and met contemporary standards for a residue depletion study. The two studies also used a new HPLC method with fluorimetric detection that had a limit of quantification 5-fold lower than the microbiological method used in many of the earlier studies.

TISSUE RESIDUE DEPLETION STUDIES

Cattle

Two groups (2 male and 2 female each) of 3 week old, non-ruminating calves were dosed with neomycin sulfate. Calves in group 1 were given 12 mg neomycin/kg by intramuscular injection for 5 consecutive days (Birckel, 1999a). Those in group 2 were given neomycin,10 mg/kg, orally, twice a day for 5 days. Blood was collected at day 3 (pre-dose) and day 5 (post-dose). The animals were slaughtered 8 days after the first treatment, i.e., 4 days after the last treatment. Tissue samples (muscle, liver, kidney and fat) were collected at slaughter.

Concentrations of neomycin were determined in plasma and tissues by HPLC using fluorimetric detection. For plasma, the assay method was validated in terms of specificity, extraction recovery and linearity. The limit of quantification was 100 µg/l. In each tissue, the method was validated in terms of specificity, extraction recovery, linearity, precision and accuracy. A limit of quantification of 100 µg/kg was achieved for muscle, liver, kidney and fat.

Neomycin was not detected in plasma sampled at day 3, pretreatment. At day 5 (one day after the last treatment), neomycin was detected in plasma samples from all animals (range; 530 to 49 μg/L). Neomycin was detected in kidney, liver, fat and muscle from calves in Group 1 and in kidney and liver of those in Group 2. Residues of neomycin were highest in kidney, followed by liver, fat and muscle. Neomycin concentrations found in all tissues during this study are presented in Table 1. The residue concentrations in the edible tissues from calves in Group 2 are indicative of the relatively low absorption of orally administered neomycin. The injectable formulation was a four-way combination of neomycin (12 g/100 ml), procaine benzylpenicillin (21 g/100 ml), methylprednisolone (0.4 g/ 100 ml) and procaine hydrochloride (3 g/100 ml).

Table 1. Concentration (μg/kg) of neomycin in tissues of individual calves administered 12 mg/kg BW of neomycin by injection (Group 1) and 10 mg/kg BW orally (Group 2).

Group	Day after the last treatment	Fat	Muscle	Liver	Kidney
1	4	546	207	13100	145000
		830	214	9880	106000
		956	277	11800	109000
		987	200	9020	132000
2	4	<LOQ	<LOQ	380	25100
		<LOQ	<LOQ	141	7730
		<LOQ	<LOQ	363	18700
		<LOQ	<LOQ	361	17000

<LOQ = below the limit of quantification (100 μg/kg).

Tissue residue depletion of neomycin was assessed in 24 (12 males and 12 females) ruminating beef cattle, approximately 6 months old, following intramuscular injection of 12 mg/kg BW of neomycin for 5 consecutive days (Birckel, 1999b). The injection formulation was the same as that used in the previous study. Calves were randomly allocated to 6 groups of 4, two males and two females (Group 2 to 7). Two additional calves, 1 male and 1 female, (Group 1) were identified as untreated controls.

Blood was collected immediately prior to dosing and at day 5, post-dose. The calves (4 per slaughter time) were slaughtered 7, 14, 21, 30, 45 and 60 days after the last injection. Calves from group 1 (untreated controls) were slaughtered 7 days after the first injection. Tissues (muscle, liver, kidney, fat and the injection site of the last injection) were collected at each slaughter time.

Concentrations of neomycin were determined in plasma and tissues by HPLC with fluorimetric detection, as in the previous study. In this study, concentrations of benzylpenicillin and methylprednisolone were also determined in plasma and tissues by HPLC with UV detection. The limit of quantification (LOQ) of benzylpenicillin in tissues and plasma was 25 μg/L and the LOQ for methylprednisolone was 10 μg/L.

Benzylpenicillin was detected only in animals from Group 2, slaughtered 7 days after the fifth treatment. Residues were highest in injection site, followed by liver, kidney and fat. Benzylpenicillin was not detected in muscle. Methylprednisolone was detected only in the injection site in animals from Groups 2 and 3, slaughtered 7 and 14 days after the fifth treatment. Neomycin was detected in all tissues (kidney, liver, fat, muscle and injection site). Residues of neomycin were highest in kidney followed by liver, injection site, fat and muscle. Neomycin concentrations in the four tissues and the injection site are presented in Table 2. Mean residue concentrations are presented in Table 3.

Table 2. Concentrations (µg/kg) of neomycin in tissues of individual calves administered 12 mg/kg of neomycin by intramuscular injection for 5 consecutive days.

Days after last treatment	Group	Fat	Muscle	Injection Site*	Liver	Kidney
		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
7	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		323	109	791	4900	39300
	2	468	126	1920	8770	45200
		615	169	2060	5540	71400
		663	144	871	7290	40800
		348	<LOQ	432	11700	25300
14	3	500	<LOQ	188	25400	29000
		208	<LOQ	358	9710	22000
		270	<LOQ	146	13400	19500
		189	<LOQ	584	6650	21900
21	4	302	<LOQ	669	11700	16300
		128	<LOQ	822	7880	26200
		147	<LOQ	365	6990	18200
		<LOQ	<LOQ	479	8180	17000
30	5	154	<LOQ	225	11000	10600
		<LOQ	<LOQ	285	6360	13800
		<LOQ	<LOQ	414	2330	14800
		<LOQ	<LOQ	370	5050	7900
45	6	161	-	180	5390	9220
		<LOQ	-	388	5740	13700
		<LOQ	-	401	9360	8790
		<LOQ	-	264	3190	5510
60	7	<LOQ	-	<LOQ	1760	3770
		<LOQ	-	<LOQ	5840	4810
		<LOQ	-	360	1140	3860

<LOQ = below the limit of quantification (100 µg/kg).

Table 3. Mean concentrations (μg/kg) of neomycin residues treated by intramuscular injection for 5 consecutive days.

Days after the last treatment	Fat	Muscle	Injection Site	Liver	Kidney
Control	BLQ	BLQ	BLQ	BLQ	BLQ
7	517	137	1410	6620	49200
14	332	BLQ	381	1510	24000
21	192	BLQ	610	8320	20700
30	114	BLQ	351	6960	14100
45	115	BLQ	335	6380	9920
60	BLQ	BLQ	206	2980	4500

BLQ: below the limit of quantification (100 μg/kg). Except for the control group, when an individual animal value was BLQ, 100 μg/kg was used in calculating the mean.

METHODS OF ANALYSIS

The HPLC method with fluorimetric detection used to determine the concentration of neomycin in tissues consists of four stages: homogenisation, precipitation of proteins with 5% trichloracetic acid, clean-up on ion-exchange column using CM-Sephadex and post-column derivatisation (Decolin and Nicolas, 1998). The absolute recoveries for muscle, liver, kidney and fat were 66%, 57%, 59% and 72%. The concentrations in Tables 1 and 2 were corrected for recoveries by the use of a standard curve prepared in the respective tissue matrix.

APPRAISAL

An ADI of 0 – 60 μg/kg is equivalent to 3600 μg/day. The previously recommended MRLs for eggs and milk of 500 μg/kg and 500 μg/l would use up 800 μg of the acceptable daily intake, leaving up to 2800 μg to be distributed between the four edible tissues. Using the residue depletion data in Table 2, the dietary residue intake (DRI) can be calculated for each of the 24 animals by summing the individual tissues for that animal according to the market basket approach. Figure 1 demonstrates the relationship between the natural log of the dietary residue intake and the days after treatment. The intercept of the 95% Confidence Limit on the 95th Percentile (the 95/95 residue limit) and the natural log of 2800 μg is 30 days. A similar plot of the 95/99 residue limit intercepts the natural log of 2800 μg at 38 days. At a withdrawal period of 30 - 38 days, only the residue concentrations in kidney and liver contribute significantly to the daily residue intake. Therefore, the residue concentrations in liver and kidney at 30 - 38 days withdrawal can be used to estimate MRLs that would be necessary to permit the use of an injectable formulation of neomycin. The MRLs for liver and kidney also can be estimated to be 15,000 and 20,000 μg/kg, respectively, by using the mean concentrations ± three standard deviations for either the 30 or 45 day sacrifice times

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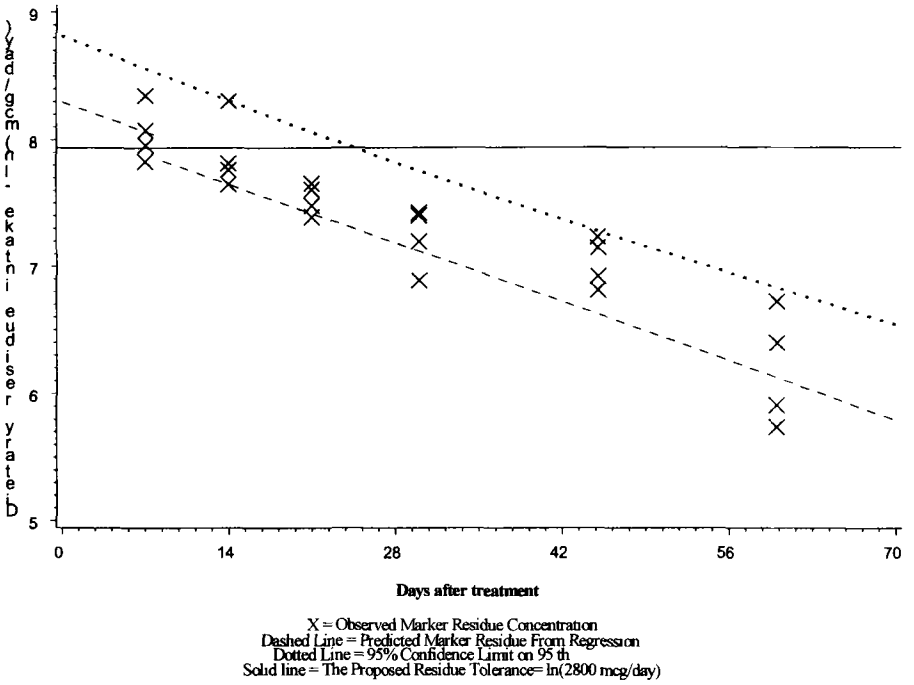
Figure 1. **Depletion of the dietary residue intake (DRI) of neomycin from cattle administered 12 mg neomycin/kg by intramuscular injection.**



Maximum Residue Limits

The Committee recommends MRLs for sheep, goat, pig, turkey, duck and chicken of 0.5 mg/kg in muscle, liver, kidney, fat and eggs. Using the upper 99% confidence limits for cattle, the Committee recommends MRLs of 0.5 mg/kg in muscle, fat and cattle milk; for liver, 15 mg/kg and kidney, 20 mg/kg, espressed as equivalents of parent drug.

Using the MRL values for cattle and 0.5 mg/kg for eggs, the calculated theoretical maximum daily intake of neomycin residues is 3475 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.5 kg of milk (Annex 1, reference yy).

REFERENCES

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