The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Information Technology

EMEA/MRL/730/00-FINAL
March 2000

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NEOMYCIN

SUMMARY REPORT (2)

1. Neomycin is an aminoglycoside antibiotic consisting of 3 components, A, B and C. Component B is the largest component of commercial preparations of neomycin (over 90%). Framycetin (also known as soframycin) is largely component B. Component A is present only in traces (less than 1%). Neomycin is used to treat bacterial gastrointestinal infections of cattle, sheep, pigs, goats and poultry by oral route and to treat mastitis by intramammary administration. The therapeutic dosages are 10 to 20 mg/kg for cattle, 150 to 350 mg/infusion for intramammary use, 10 mg/kg for sheep, 10 to 15 mg/kg for porcine and 10 to 30 mg/kg for chickens, turkeys and ducks. The duration of treatment is 3 to 7 days for poultry and up to 14 days for larger animals.

   Currently, neomycin is included in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Neomycin (including framycetin)	Neomycin	Bovine, ovine caprine, porcine, chicken, turkey, duck	500 µg/kg 500 µg/kg 500 µg/kg 5000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2000
		Bovine, ovine, caprine	500 µg/kg	Milk	
		Chicken	500 µg/kg	Eggs	

   Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for neomycin for bovine, porcine and chicken, only.

2. Neomycin is poorly absorbed from the gastrointestinal tract of humans and animals, and it has low absorption from the udder. In healthy humans given therapeutic oral doses of neomycin sulphate (i.e. more than 1000 mg) neomycin absorption from the gastrointestinal tract is estimated to be less than 10% based on blood and urine analysis. A recent study in calves dosed orally with ^{14}C-neomycin has provided more direct evidence to support that high percentages of neomycin remain in the gastrointestinal tract. The absorption in this species was minimal (1 to 11%); about 90% were recovered in faeces and 70 to 80 was present as parent neomycin as indicated by mass spectrometric analysis.

   Neomycin undergoes negligible biotransformation after parenteral administration. It excreted after oral doses is in the faeces, but after parenteral administration it is excreted in the urine.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

3. Neomycin has low acute toxicity (LD_{50} values in excess of 2000 mg/kg bw) after oral administration but it is more toxic after intravenous dosing (LD_{50} in mice 100 mg/kg bw/day).

4. After repeated parenteral administration, nephrotoxic effects were noted in mice (30 to 300 mg/kg bw/day, subcutaneously), guinea pigs (10 to 60 mg/kg bw/day, subcutaneously) and in dogs (24 to 96 mg/kg bw/day, intramuscularly). Ototoxicity was noted in guinea pigs given repeated doses of neomycin. The lowest NOEL from these studies was 10 mg neomycin sulphate/kg bw/day equivalent to 6 mg/kg bw/day neomycin for ototoxicity in guinea pig.

5. Three old no-GLP genotoxicity studies were available and 2 of these (an *in vitro* chromosome aberration assay in human lymphocytes and an *in vivo* cytogenetics assay in mouse bone marrow) gave positive results.

   Three new GLP mutagenicity studies in compliance with OECD guidelines were provided.

   In a preincubation mutagenesis assay in bacteria (*Salmonella* microsomal assay) neomycin was judged to be non mutagenic when tested in four *Salmonella typhimurium* strains and in one *Escherichia coli* strain at doses ranging from 75 to 0.93 µg/plate, with and without metabolic activation.

   In the *in vivo* chromosome aberration assay in CD1 mouse bone marrow cells neomycin caused no significant increase in incidence of aberrant cells in any of the test groups when compared to controls. Doses tested ranged from 50 to 250 mg/kg in males and 40 to 200 mg/kg in females.

   Neomycin gave positive results in some old and poorly reported no GLP mutagenicity tests. A further battery of genotoxicity tests were performed under GLP conditions including a *Salmonella* microsomal assayt, a AS52/XPRT Chinese hamster ovary (CHO) cell mutation assay and an *in vivo* chromosome aberration assay in CD1 mouse bone marrow cells. All test gave negative results.

   Although neomycin gave positive results in 2 inadequate *in vivo* and *in vitro* mutagenicity tests, these findings could not be confirmed in a battery of well conducted battery of genotoxicity tests.

   In overall conclusion, it was concluded that neomycin is unlikely to be genotoxic.

6. There was no increased tumour incidence in a 2-year oral carcinogenicity study in rats treated with 0, 6.5, 12.5 and 25 mg neomycin sulphate/kg bw/day. However, hearing was impaired and the NOEL was 12.5 mg/kg bw/day.

7. In a multigeneration study in rats, no adverse effects on reproduction parameters were noted with doses of up to 25 mg/kg bw/day, the highest dose used. A teratogenicity study was conducted with the F_{2b} females. Neomycin was administered in feed at 0 to 25 mg/kg bw/day from days 0 to 6 and 16 to 20 of gestation. Doses were increased to 0 to 250 mg/kg bw/day in the day 16 to 20 period. There was no evidence of teratogenic effects in this study. The design of the study was not in accordance with the current requirements; a NOEL could not be derived from this study.

   A complete review of all the publications on the use of neomycin in humans was performed; the clinical documentation on the compound is very extensive, but no adverse effects on reproductive function are reported.

8. Several *in vitro* studies were conducted using various bacteria, most isolated from humans. The MIC_{50} of the most sensitive micro-organisms was 64 µg/ml, for *Lactobacillus* under conditions of high inoculum density. In a mouse study using animals with a human gut flora a NOEL of 125 mg/kg bw/day was identified. Effects on human gut flora in patients occurred at doses equal to or greater than 30 mg/kg bw/day.

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

$$\text{ADI} = \frac{\dfrac{\text{MIC}_{50} \text{ for the most sensitive organism x CF2}}{\text{CF1}} \text{ (µg/ml)} \quad \text{x} \quad \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for micro-organisms} \quad \text{x weight of human (60 kg)}}$$

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

$$\text{ADI} = \frac{\dfrac{64 \text{ x } 1}{1} \text{ x } 150}{1 \text{ x } 60} = 160 \text{ µg/kg bw i.e. } = 9600 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 because the MIC_{50} value for the most sensitive, relevant strain *Lactobacillus*;
- CF2 =1 because no data were available to correct for extrapolation from the *in vitro* to the in *vivo* situation.
- 150 g was the weight of the daily faecal bolus;

10. Neomycin was evaluated at the 47th Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA confirmed that the appropriate NOEL to establish an acceptable daily intake is the NOEL for the ototoxicity from the study in guinea pig. Applying a safety factor of 100, an ADI of 0-60 µg/kg bw was established. The JECFA also retained the same value for the microbiological ADI (160 µg/kg bw).

11. A toxicological ADI of 60 µg/kg bw (3.6 mg/person) was established based on the NOEL of 6 mg/kg bw/day in the guinea-pig and applying a safety factor of 100. This ADI is lower than the microbiological ADI (160 µg/kg bw) therefore the toxicological ADI was considered the relevant ADI for assessing the risk for the consumers.

12. The effect of neomycin in milk on bacterial starter cultures used in the production of fermented milk products was evaluated. Five bacterial starter culture types were used: a group of buttermilk/sour cream cultures containing *Lactococcus lactis spp. lactis* and *spp. cremoris* or a mixture of lactic acid producers and citric fermenters; a second group of Italian cheese cultures containing *S. thermophilus*; a third group of Italian cheese cultures containing *Lactobacillus helveticus* and a group of yoghurt cultures containing *S.thermophilus* and *Lactobacillus delbruckii spp bulgaricus*. Neomycin concentrations of 0.063, 0.125, 0.25, 0.50, 1.0, 2.0 and 4.0 µg neomycin/ml in milk were examined. The yoghurt starter cultures were the most sensitive. Results indicated that neomycin in milk at concentrations less than or equal to 2.0 µg/ml should not have an adverse effect on the growth of bacterial starter cultures used in the fermented milk products.

13. Only limited radiometric studies were carried out in cattle.

In calves of different ages (3 days to 60 days) orally dosing (via bottle or capsule) with approximately 30 mg/kg bw ^{14}C-neomycin (specific activity = 162 to 516 dpm/µg neomycin B), 96 hours after treatment, neomycin, in calves dosed at 3 days of age, at least 96% of the radioactivity in kidney was present as neomycin. Radioactivity was also in liver and muscle in all calves, with highest concentrations in tissues of 3 day old calves. For example, 96 hours after oral dosing of animals of 3 days of age, the following concentrations of total radioactivity wee measured: 55 000, 1930 and 91 µg/kg in kidney, liver and muscle, respectively, whereas in animals of 53 to 63 days of age, the levels of radioactivity were 7400, 330 and 64 µg equivalents neomycin/kg, respectively. Although this study showed that residues were highest in young calves confirming that there is a significant difference in absorption of neomycin in young calves versus older animals independent of whether the calf is ruminating or non-ruminating at the time of treatment, due to the large variation of residue measured in edible tissues of calves after oral administration with different formulations, no clear conclusion can be reached according to the amount susceptible to be found in edible tissues after administration of recommended dosage.

Therefore, the relevant ratio of the marker residue towards total residues could not be established. However, considering that the major part of neomycin 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, it was assumed that the parent compound represents the totality of the relevant metabolites assayed.

14. Residue data were available for cattle, sheep, goats, pigs, chickens, turkeys, ducks and milk. Residue levels in tissues, eggs and milk were low immediately after treatment, after oral administration.

Twenty cattle were daily dosed via medicated drinking water with about 20 mg neomycin sulphate/kg bw for 14 consecutive days. Animals were sampled at 0, 1, 3, 7 and 14 days after treatment. Liver, muscle, fat and kidney tissue samples were obtained from each sacrificed calf and analysed for neomycin residues by a microbiological method. No neomycin residues were found in muscle, liver, and fat tissues of any of the treated cattle at any sampling time. In kidney, neomycin concentrations were 2791 µg/kg immediately after treatment, 2899 µg/kg at 24 hours after treatment and 1685 µg/kg at 3 days after treatment. By 7 days after treatment, 2 of the 3 treated cattle that were sampled had detectable kidney neomycin residues below 500 µg/kg (limit of quantification of the microbiological method) and 1 animal had a level of 620 µg/kg. One of the 4 treated cattle sampled at 14 days after treatment showed residues at the limit of quantification and the other 3 animals did not have detectable residues.

Sixteen healthy cows received an intramammary infusion containing 330 mg lincomycin base, as lincomycin hydrochloride, and 100 mg neomycin base, as neomycin sulphate, in each of the 4 udder quarters, following each of 3 successive milkings at 12 hours intervals. Treated animals were slaughtered at 1, 7, 14 and 21 days after last treatment and the following tissues were harvested: liver, both kidneys, perineal fat, semitendinosus/semimembranosus muscle and one sample from each udder quarter. Neomycin was quantified in tissues using an HPLC method (limit of quantification: 100 µg/kg for all matrices). Measurable concentrations of neomycin residues were only present in kidney and udder. For kidney the mean concentrations were 700 µg/kg at day 1, 315 µg/kg at day 7, 205 µg/kg at day 14 and the concentrations were lower than limit of quantification or 107 µg/kg at day 21. For udder the mean concentrations were 1610 µg/kg at day 1 and 107 µg/kg at day 7 and the concentrations were lower than limit of quantification or 425 µg/kg and 106 µg/kg at 14 and 21 days, respectively. For the other tissues residues were all below the limit of quantification at all sampling times.

Twenty four healthy cows were divided in four groups and treated with an intramammary infusion containing 330 mg lincomycin base, as lincomycin hydrochloride and 100 mg neomycin base, as neomycin sulphate in each of the four udder quarters at a 12 hours interval, following each of 3 successive milkings. Neomycin was quantified in plasma, quarter milk and pooled milk samples using an HPLC method (limit of quantification: 100 µg/l for both matrices). Mean neomycin concentrations in quarter milk samples collected at 12 hours after each of the three infusions were 22 200, 29 900 and 28 000 µg/l, and it was 4900 µg/l at 24 hours after last infusion. For the pooled samples, the mean neomycin concentration at 12 hours after last infusion was 24000 µg/l. At 24 hours after last infusion, the mean concentration was 4800 µg/l. At 60, 72 and 84 hours after the last infusion, the mean (range) neomycin concentrations in pooled milk samples were estimated to be 240, 200 and 120 µg/l, respectively.

15. Twenty pigs were treated for 14 consecutive days with medicated water calculated to provide approximately 20 mg of neomycin sulphate/kg bw/day. At the end of the treatment, 4 animals were sacrificed for tissue collection and drug residue analyses at each of the withdrawal intervals of 0 hours and at 1, 3, 7 and 14 days. Tissues were assayed for neomycin residues by a microbiological method (limit of quantification: 500 g/kg). No neomycin residues were found in muscle, fat or liver tissues from any treated pig at any sampling time. For kidney mean neomycin levels were 2174 µg/kg immediately after treatment, 1920 µg/kg at 24 hours after treatment and 958 µg/kg at 3 days after treatment. At 14 days after treatment, 3 of the 4 animals had no detectable neomycin residues in kidney tissue while in one pig neomycin concentration in kidney was 906 µg/kg.

16. Twenty sheep were treated for 14 consecutive days with medicated water calculated to provide approximately 20 mg of neomycin sulphate/kg bw/day. At the end of the treatment, samples of tissues were collected for drug residue analyses at each of the withdrawal intervals of 1, 3, 7, 14 and 21 days. Tissues were assayed for neomycin residues by a microbiological method (limit of quantification: 500 µg/kg). No neomycin residues were found in muscle, fat or liver tissues from any treated sheep at any sampling time. In kidneys at 24 hours after treatment neomycin residue levels averaged 982 µg/kg. Of tissues collected at 3 days after treatment, only 1 of 4 animals sampled had a quantifiable kidney neomycin concentration (522 µg/kg). No detectable neomycin was measured in kidney at days 7, 14 and 21 after treatment.

    Twenty goats were treated for 14 consecutive days with medicated water calculated to provide approximately 20 mg of neomycin sulphate/kg bw/day. At the conclusion of the medicated period, the treated animals were sacrificed for tissue collection and drug residue analyses at each of the withdrawal intervals of 12, 24, 48 and 96 hours. Tissues were assayed for neomycin residues by a microbiological method (limit of quantification: 500 µg/kg). No neomycin residues were found in muscle, fat or liver tissues from any treated goat at any sampling time. In kidney neomycin residue levels averaged approximately 1000 µg/kg at 12 hours after treatment, 2100 µg/kg at 24 hours after treatment, 1700 µg/kg at 48 hours after treatment, 1100 µg/kg at 72 hours after treatment and 700 µg/kg at 96 hours after treatment.

17. A single dose of 36.7 mg neomycin base/kg bw given in the feed was administered to 150 broiler chickens for 7 consecutive days. At day 7 and for 5 consecutive days thereafter chickens were slaughtered per time point and their liver, muscle and kidneys analysed for the presence of neomycin residues, using a microbiological method (limit of detection: 500 µg/kg). Both liver and muscle were free of detectable neomycin residues at each time tested. In kidney, neomycin could be detected up to the third day after treatment cessation, with residue levels below 5 mg/kg at all time points.

    A single dose of 10 mg/kg or 30 mg of neomycin/kg bw, dissolved in drinking water, was given by intubation to broiler chickens for 7 consecutive days. With 0.5 mg/kg as the detectable limit of the microbiological assay, major edible visceral organs were examined for residues. In the 10 mg/kg/day group, the neomycin residue concentrations in kidney were 870 µg/kg at day 1 and 600 µg/kg at day 3. Neomycin was below the limit of detection in the kidney at the day 13. The neomycin 30 mg/kg group was comparable with the 10 mg/kg group in residue trend. The mean neomycin concentration in kidney was 3080 µg/kg at day 1 after treatment.

Fifty-four turkeys were treated for 5 consecutive days with medicated water calculated to provide approximately 20 mg of neomycin sulphate/kg bw/day. At the end of medication period, sample tissues of skin with adherent fat, abdominal fat, liver, kidney, white muscle (breast) and dark muscle (leg/thigh) were collected for residue analysis by microbiological assay (limit of quantification: 500 µg/kg) at withdrawal intervals of 12, 24, 48, 72, 120 and 240 hours. No neomycin residues were found in skin, liver, muscle or fat at any of the withdrawal intervals. Neomycin was found in measurable levels in the kidney at the 12 (727 µg/kg) and 24 hours (500 µg/kg) withdrawal intervals.

Fifty-four ducks were treated 21 consecutive days with medicated water calculated to provide approximately 10 mg of neomycin sulphate/kg bw/day. At the end of the treatment, sample tissues of skin with adherent fat, liver, kidney and muscle were collected from 6 animals selected random for residue analysis by microbiological assay (limit of detection: 500 µg/kg) at withdrawal intervals of 1, 2, 3, 4, 5, 7 and 14 days. No neomycin residues were found in skin + fat, liver or muscle at any of the withdrawal intervals. Neomycin was found in measurable levels in the kidney (mean residue concentration: 890 µg/kg) until 14 days after treatment.

One hundred and fifty laying hens were divided in three groups and were treated with different concentrations of neomycin: 40.25 mg neomycin base/kg bw for 5 days, 33.2 mg neomycin base/kg bw for 7 days and 40.25 mg neomycin base/kg bw for 7 days. Eggs were sampled from the treated groups 1, 2 and 3 days after treatment. In the third treatment group eggs were sampled also during treatment. Assay was performed according to an agar diffusion method (limit of detection: 500 µg/kg). No residue of neomycin were detected during or after drug administration in all 3 treatment groups.

18. The proposed routine analytical method was based on HPLC with fluorescence detection. The method detects precisely the B component of neomycin i.e. framycetin. The method had satisfactory specificity and it was shown that residues of other aminoglycosides did not interfere in the assay but were not validated according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community. The limits of quantification ranged from 100 µg/kg to 200 µg/kg for the edible tissues of bovine, including milk, and of chickens, including eggs, and porcine. No data were provided for edible tissues of ovine and caprine, including milk, and of ducks and turkeys.

    The Committee was aware that an analytical method published on behalf of the Commission is available for monitoring residues of neomycin. However, in absence of raw data, no conclusion could be given about the quality of the validation of this method.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 60 µg/kg bw (i.e. 3600 µg/person) was established;
- the ratio of marker to total residues was assumed to be equal to 1,
- an analytical method is available for bovine, porcine and chicken for monitoring purposes,
- the applicant has committed to address the outstanding issues concerning bovine, porcine and chicken;

The Committee for Veterinary Medicinal Products recommends, according to Article 4 of Council Regulation No 2377/90 as amended, a 2-year extension of the provisional MRL for neomycin in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Neomycin (including framycetin)	Neomycin B	Bovine, porcine, chicken	500 µg/kg 500 µg/kg 500 µg/kg 5000 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.6.2002
		Bovine	500 µg/kg	Milk	
		Chicken	500 µg/kg	Eggs	

Based on the MRLs, the daily intake will represent 35% of the toxicological ADI.

LIST OF QUESTIONS

1. The applicant should provide validated analytical methods in accordance with the requirements of Volume VI for all edible tissues of all the target species presented (per species) in a recognised format ISO 78/2.