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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NEOMYCIN

SUMMARY REPORT (3)

1. Neomycin (CAS: 1404-04-2) is an aminoglycoside antibiotic consisting of 3 components, A, B and C, and is used in veterinary medicine as the sulphate salt. 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 the oral route and to treat mastitis by intramammary administration. The therapeutic dosages are 10 to 20 mg/kg bw for cattle, 150 to 350 mg/infusion for intramammary use, 10 mg/kg bw for sheep, 10 to 15 mg/kg bw for porcine and 10 to 30 mg/kg bw 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 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	

   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.

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 the view that high percentages of neomycin remain in the gastrointestinal tract. The absorption in this species was minimal (1 to 11%); about 90% was 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 is excreted after oral doses in the faeces, but after parenteral administration it is excreted in the urine.

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} values in mice around 100 mg/kg bw/day).

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4. After repeated parenteral administration, nephrotoxic effects were noted in mice (administered 30 to 300 mg/kg bw/day, subcutaneously), guinea pigs (administered 10 to 60 mg/kg bw/day, subcutaneously) and in dogs (administered 24 to 96 mg/kg bw/day, intramuscularly). Ototoxicity was noted in guinea pigs given repeated parenteral doses of neomycin, but not following oral dosing. The lowest NOEL from these studies was 10 mg neomycin sulphate/kg bw/day (following oral administration for 90 days) equivalent to 6 mg/kg bw/day neomycin based on ototoxicity in the guinea pig.

5. Three old non-GLP-compliant 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-compliant mutagenicity studies in carried out in accordance 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 the incidence of aberrant cells in any of the test groups, when compared to controls. The doses tested ranged from 50 to 250 mg/kg bw in males and 40 to 200 mg/kg bw in females.

   Neomycin gave positive results in some old and poorly reported non-GLP-compliant mutagenicity tests. A further battery of genotoxicity tests were performed under GLP conditions including a *Salmonella* microsomal assay, a AS52/XPRT Chinese hamster ovary (CHO) cell mutation assay and an *in vivo* chromosome aberration assay in CD1 mouse bone marrow cells. All tests 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 genotoxicity tests.

   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 in several rats administered 25 mg/kg bw/day by the end of the study. The NOEL was 12.5 mg/kg bw/day.

7. In a multigeneration study in rats, no adverse effects on reproduction parameters were noted following administration of oral (dietary) 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 doses equivalent to 0, 6.25, 12.5 or 25 mg/kg bw/day from days 0 to 6 and 16 to 20 of gestation. The doses were increased to 0, 62.5, 125 or 250 mg/kg bw/day from days 16 to 20. 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 review of publications on the use of neomycin in humans was performed; the clinical documentation on this compound is extensive, and 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:

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

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

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

The following assumptions were made:

- CF1 = 1 because the MIC_{50} value was 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 43rd, 47th and 52nd meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA confirmed that the appropriate NOEL to establish an acceptable daily intake was the NOEL for the ototoxicity from the study in guinea pigs. 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 was 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 to 60 days old) orally dosing (via bottle or capsule) with approximately 30 mg/kg bw ^{14}C-neomycin (specific activity of 3 to 9 Bq/µg neomycin B), 96 hours after treatment, in calves dosed at 3 days of age, at least 96% of the radioactivity in kidney was present as neomycin. Residues were also found 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 were measured: 55 000, 1930 and 91 µg equivalents/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 regarding to the amount susceptible to be found in edible tissues after administration of the recommended dosage.

14. The ratio of marker to total residues had not been established in all relevant tissues of the target species. However, considering that the major part of neomycin administered to farm animals is excreted in an unchanged form in the urine and faeces, only a very small proportion of potential tissue residues in farm animals is likely to be in the form of a metabolite. 96 hours after oral dosing of calves with 30 mg/kg bw ^{14}C-neomycin, at least 96% of the residues in kidney was present as neomycin. Therefore, the available data suggest that, like the other aminoglycosides, neomycin is not significantly metabolised. Neomycin B was retained as the marker residue and a value of 1 was retained for the ratio of marker to total residues.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 slaughtered 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 into 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 12 hours intervals, 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 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 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 µg/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 bw group was comparable with the 10 mg/kg bw 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 hour (727 µg/kg) and 24 hour (500 µg/kg) withdrawal intervals.

    Fifty-four ducks were treated for 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 residues of neomycin were detected during or after drug administration in all 3 treatment groups.

18. For pigs, sheep, goats, turkeys, ducks and chickens, JECFA had recommended MRLs of 500 µg/kg for muscle, 500 µg/kg for fat, 500 µg/kg for liver and 10 000 µg/kg for kidney. It was considered that the JECFA MRLs for muscle, fat and liver could be supported. However the MRL recommended by JECFA for kidney was beyond the range of concentrations which had been validated in the routine analytical method. JECFA had also recommended an MRL of 500 µg/kg for hens' eggs and it was considered that this MRL could be supported. For cattle, JECFA had recommended the same MRLs for muscle and fat (500 µg/kg) but higher MRLs for liver (15 000 µg/kg) and kidney (20 000 µg/kg); it was considered that there was no scientific justification for such high MRLs. The analytical method for bovine kidney had been validated only up to a concentration of 5000 µg/kg and the residues distribution ratio between tissues would not be maintained if the MRL for liver alone was modified.

19. 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, which was 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.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 60 µg/kg bw (i.e. 3600 µg/person) was established for neomycin;
- the available data suggested that the ratio of marker to total residues was equal to 1,
- a validated analytical method based on HPLC with fluorescence detection for the determination of the marker residue, neomycin B, in the edible tissues of cattle, pigs and chickens plus cow's milk and chicken eggs was available; the method was considered to be applicable to the edible tissues, eggs and milk of other species,
- an MRL of 1500 µg/kg for bovine milk was supported by the data,
- the proposed MRLs for bovine, porcine and chicken tissues were identical; therefore extrapolation of these MRLs to all food producing species could be made in accordance with the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL);

the Committee for Veterinary Medicinal Products recommends the inclusion of neomycin into Annex I of Council Regulation (EEC) N° 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 B	All food producing species	500 µg/kg 500 µg/kg 500 µg/kg 5000 µg/kg 1500 µg/kg 500 µg/kg	Muscle* Fat ** Liver Kidney Milk Eggs	

*For fin fish this MRL relates to "muscle and skin in natural proportions".

**For porcine and poultry species this MRL relates to "skin and fat in natural proportions".

Based on these MRLs the daily intake of residue is 2775 µg which represents 77% of the toxicological ADI.