Date of Approval: _____

FREEDOM OF INFORMATION SUMMARY

NADA 011-315

NEOMIX® 325 Soluble Powder
NEOMIX® AG 325 Soluble Powder

(neomycin sulfate)

"...for the control of mortality associated with *Escherichia coli* organisms susceptible to neomycin sulfate in growing turkeys"

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

Sponsored by:

Pharmacia & Upjohn Company

I. GENERAL INFORMATION

NADA Number:	011-315
Sponsor:	Pharmacia & Upjohn Company 7000 Portage Road Kalamazoo, Michigan 49001-0199
Established Name:	neomycin sulfate
Proprietary Name:	NEOMIX® 325 Soluble Powder; NEOMIX® AG 325 Soluble Powder
Marketing Status:	OTC
Supplemental Effect:	Provides for the use of neomycin sulfate (NEOMIX® 325 Soluble Powder/NEOMIX® AG 325 Soluble Powder) in growing turkeys for the control of mortality associated with *Escherichia coli* organisms susceptible to neomycin sulfate.

II. INDICATIONS FOR USE

NEOMIX® 325/NEOMIX® AG 325 Soluble Powder (neomycin sulfate) is indicated for the control of mortality associated with *Escherichia coli* organisms susceptible to neomycin sulfate in growing turkeys.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION AND RECOMMENDED DOSAGE

A. Dosage Form

NEOMIX® 325/NEOMIX® AG 325 Soluble Powder is currently marketed in foil packets and cardboard drums. Each 3.5 oz foil packet contains 71.5 gm neomycin sulfate (commercial grade) equivalent to 50 gm neomycin. Each drum contains 50 lb (22.6 kg) Neomix® 325/Neomix® AG 325 Soluble Powder with each pound containing 325 gm neomycin sulfate equivalent to 227.5 gm neomycin.

B. Route of Administration

NEOMIX® 325/NEOMIX® AG 325 Soluble Powder is administered orally in water.

C. Recommended Dosage

NEOMIX® 325/NEOMIX® AG 325 Soluble Powder should be administered in the drinking water at a dosage of 10 mg neomycin sulfate per pound (22 mg/kg) of body weight per day for five consecutive days.

IV. EFFECTIVENESS

A. Field Study

1. Type of Study: clinical effectiveness

2. Investigator: Terry TerHune D.V.M., Ph.D.
Health Management Services
Tulare, California 93275

3. General Design:

a. Purpose: To evaluate the effectiveness of neomycin sulfate in the drinking water of growing turkeys for the control of mortality associated with colibacillosis (*E. coli*) compared to non-medicated controls.

b. Animals: Male and female Nicholas broad-breasted white turkeys; 21 days of age; obtained from a local hatchery at one day of age.

c. Control: Non-medicated water was available *ad libitum* from waterers for the duration of the study.

d. Challenge: The poults were challenged at 21 days of age with infected litter obtained from a research flock that had recently experienced colibacillosis. Three days after challenge, when *E. coli* associated mortality was in excess of 0.5%, the poults were randomly allotted to pens containing litter from the same source as the challenge litter. The treatment groups are shown in Table 4.1.

Table 4.1. Treatment Groups

Treatment	Dose	Sex	Pens/ treatment	Poults/ pen	Poults/ treatment
Control	0	M	12	40	480
Control	0	F	12	40	480
Neomycin sulfate	10 mg/lb	M	24	40	960
Neomycin sulfate	10 mg/lb	F	24	40	960

e. Dosage Form: Soluble powder for use in drinking water

f. Route of Administration and Dose: In the drinking water at a dosage of 10 mg/lb (22 mg/kg) of body weight per day for five consecutive days.

g. Test Duration: Fifteen days (a five-day treatment period followed by a ten-day post-treatment period)

h. Lesion scoring: All poults that died during the 15-day study period were necropsied. Lesions were scored as follows:

0 = no lesions
1 = minimal airsacculitis (slight cloudiness)
2 = moderate airsacculitis (non-coalesced fibrinous material) and/or mild pericarditis and/or perihepatitis
3 = severe airsacculitis (coalesced fibrinous material) and/or moderate to severe pericarditis and/or perihepatitis.

i. Pertinent Parameters Measured: The pivotal response parameter was poult mortality associated with *E. coli*. Poults with a gross necropsy diagnosis of colibacillosis, a lesion score of ≥ 2, and a positive *E. coli* culture from air sac, perihepatic, and/or pericardial tissues were considered to have died due to colibacillosis.

j. Results: Table 4.2 shows the percent mortality that occurred in each treatment group for the 15-day period (including five medication days and a 10-day post-medication observation period). There was a statistically significant difference ($p<0.001$) in the number of *E. coli* associated mortalities in the neomycin treated birds as compared to the untreated control birds.

Table 4.2. Percent *E. coli** associated mortality from initiation of medication to 10 days post-medication

Dose Neomycin	*E. coli* Associated Mortality		
(mg/kg)	female	male	combined
0	3.96 (19/480)	4.17 (20/480)	4.06 (39/960)
22	0.31 (3/960)	0.52 (5/960)	0.42 (8/1920)

*Diagnosis supported by lesions and culture

k. Statistical Analysis: The pivotal decision variable was *E. coli* associated mortality during the 15-day test period. A model of two factors, sex and treatment, plus their interaction was analyzed by the General Linear Model. There was no SEX x TREATMENT interaction.

4. Conclusion: Neomycin sulfate administered in the drinking water at a dosage of 10 mg/lb (22 mg/kg) for five consecutive days is effective for the control of mortality associated with *E. coli* in growing turkeys.

B. Minimum Inhibitory Concentration (MIC) Study with *E. coli* isolated from young growing turkeys

1. Type of Study: MIC Study

2. Investigators: S. A. Salmon, J. L. Watts, R. Goodenough, C. A. Case, and L. E. Marrett
The Upjohn Company
Kalamazoo, Michigan 49001

3. General Design: The purpose of the study was to determine the *in vitro* activity of neomycin and comparator antimicrobial agents with bacteria isolated from tissues of diseased turkeys. Bacterial isolates were obtained from diseased turkeys from five study sites at four geographic locations.

4. Test Method: Neomycin MIC determinations were performed using panels prepared in-house and a semi-automatic inoculation system (Sensititre, Westlake, OH). This method conforms to NCCLS guidelines for microdilution broth MIC determination. In addition to the clinical isolates, the following quality control organisms were included in the study: *S. aureus* ATCC 29213; *E. coli* ATCC 25922; *P. aeruginosa* ATCC 27853; and *E. faecalis* ATCC 29212.

5. Results: For the 429 *E. coli* isolates tested, MIC_{50} and MIC_{90} for neomycin were 16.0 and 512.0 μg/mL, respectively. The distribution of MICs for neomycin against *E. coli* was bimodal in that 59.2% of the isolates had MIC values ranging from 8.0 to 32 μg/mL and 34% of the isolates had MIC values of > 256.0 μg/mL. While this distribution was observed at two locations, the majority of the isolates from the remaining three sites had MIC values ranging from 8.0 to 32.0 μg/mL.

6. Conclusion: Neomycin demonstrated good *in vitro* activity against the majority of *E. coli* isolates obtained from turkeys in this study.

V. ANIMAL SAFETY

Target Animal Safety Study

A. Report Number and Title: Report No. 7919-95-050; U-4567 (Neomycin Sulfate; Neomix® 325 Soluble Powder): 15 Day Oral Target Animal Safety/Toxicity Study in Turkeys with Neomycin Sulfate Administered in Drinking Water

B. Investigator: Rodney K. Frank, D.V.M.
The Upjohn Company
301 Henrietta Street
Kalamazoo, Michigan 49001

C. General Design:

1. Purpose: To establish the safety/toxicity of neomycin sulfate (formulated commercially as NEOMIX® 325 Soluble Powder) administered orally in drinking water daily for 15 days at 3 concentration levels to deliver doses of 66, 110, and 220 mg/kg/day in male and female broad-breasted white turkey poults.

2. Animals: Male and female broad-breasted white turkey poults; approximately four weeks of age at the start of the dosing period. The treatment groups are shown in Table 5.1.

Table 5.1. Treatment Groups

Group	Dose Neo Sulfate (mg/kg/day)	Poults/pen	No. of pens*	Total birds
1	0	6	20	120
2	66	6	20	120
3	110	6	20	120
4	220	6	20	120

*10 pens of males, 10 pens of females per treatment group

3. Controls: Untreated drinking water

4. Dosage Form: Neomycin sulfate (NEOMIX® 325 Soluble Powder) in drinking water

5. Route of Administration and Doses: Orally in drinking water at doses of 0, 66 (3X), 110 (5X), and 220 (10X) mg/kg/day for fifteen days (3X).

6. Study Duration: Fifteen days

7. Parameters Studied: Daily clinical observations, body weights, food consumption, and water consumption (all by pen); gross necropsy observations, absolute and relative organ weights, and histologic evaluation of tissues and organs.

8. Results: No clinical signs of toxicity were observed and no deaths occurred in any of the treated turkeys. Treatment-related findings were limited to a significant dose-related reduction in daily water consumption in mid-dose males and high-dose male and female turkeys. The no-observed-effect-level (NOEL) was 66 mg/kg/day.

9. Statistical analysis: Water consumption data were analyzed by a repeated measures analysis of variance, using the average of the pretest values as covariates. If the TREATMENT x TIME interaction was not significant, then treatment groups were compared to the control group using the overall means. If the interaction was significant ($p<0.10$), then the data were analyzed at each time point. Statistical calculations were made using PROC GLM and PROC MIXED of SAS, and group comparisons were made at the $p=0.10$ level.

   Body weight changes were analyzed using a two-way analysis of variance and food consumption data were analyzed by analysis of covariance using pretest values as covariates.

   Differences in water consumption from controls were statistically significant ($p<0.10$) for mid- (110 mg/kg/day) and high-dose (220 mg/kg/day) males and for high-dose females. No other statistically significant differences were noted.

10. Conclusion: Neomycin sulfate is safe for use in turkey poults at the label dose (10 mg/lb/day or 22 mg/kg/day) and duration (five consecutive days) for control of mortality associated with *E. coli*.

VI. HUMAN SAFETY

A. Toxicity Tests

Toxicity testing of neomycin sulfate is documented in the FOI summary found in Veterinary Master File No. 3640, submitted on December 8, 1987. Information relative to tissue residues in the target species can be found in the FOI summary in Veterinary Master File No. 5154.

B. Safe Concentration of Total Residues and Tolerances

1. No-Observed-Effect-Level (NOEL): The safe concentration of total residue was determined from the lowest NOEL in the most sensitive species from the various toxicology studies conducted. The 90-day oral ototoxicity study in the guinea pig was selected as the most appropriate study, since it had the lowest NOEL for determining the acceptable daily intake (ADI). The NOEL was 10 mg neomycin sulfate/kg/day, which was equivalent to 6 ppm (free base) of neomycin. This information is referenced in the FOI summary of Veterinary Master File No. 3640.

2. Calculation of the Acceptable Daily Intake (ADI) and the Safe Concentration for Neomycin

a. Acceptable Daily Intake (ADI)

$$ADI = \frac{NOEL}{Safety\ Factor} = \frac{6\ mg/kg/day}{1000} = 0.006\ mg/kg/day\ or\ 6.0\ \mu g/kg/day$$

b. Safe Concentration (SC): The calculation of the SC is based on the *General Principles for the Evaluation of the Safety of Compounds Used in Food-Producing Animals* (FDA/CVM, revised July 1994). For calculations of the SC, the average human weight is approximated at 60 kg. The daily consumption values of edible tissues of turkeys are approximated as 300 g muscle, 100 g liver, and 50 g skin/fat. The SC for the edible tissues of turkeys are calculated as follows:

$$SC = ADI\ x\ \frac{60\ kg}{Consumption\ Factor}$$

$$SC\ (muscle) = 6\ \mu g/kg/day\ x\ \frac{60\ kg}{0.3\ kg} = 1.2\ \mu g/g = 1.2\ ppm$$

$$SC\ (liver) = 6\ \mu g/kg/day\ x\ \frac{60\ kg}{0.1\ kg} = 3.6\ \mu g/g = 3.6\ ppm$$

$$SC\ (skin/fat) = 6\ \mu g/kg/day\ x\ \frac{60\ kg}{0.05\ kg} = 7.2\ \mu g/g = 7.2\ ppm$$

Table 6.1 shows the safe concentrations for total residues.

Table 6.1. Safe Concentrations (SC) for total residues of neomycin in edible tissues of turkeys using the revised food consumption factors

Edible Tissue	Amount Consumed/Day	Safe Concentration (SC)
Muscle	300 g	1.2 ppm
Liver	100 g	3.6 ppm
Skin/Fat	50 g	7.2 ppm

Because neomycin undergoes only limited metabolic changes, tolerances for the edible tissues are established at the same values as the safe concentrations.

C. Residue Depletion Study to Establish a Withdrawal Period

Title: Tissue Residue Levels in Turkeys Medicated with Neomycin Sulfate by Drinking Water Continuously for Five Days

1. Report No. 802-9690-95-002, Care C-9412, Author: L. E. Marrett

2. Investigator: Dr. Diane Fagerberg
Colorado Animal Research Enterprises, Inc.
6200 East County Road 56
Fort Collins, Colorado 80524

3. Summary of the Total Residue Depletion Study in Turkeys:

Neomycin sulfate was administered to 36 (18 males and 18 females) 12 week old turkeys in the drinking water for five consecutive days. Neomycin was present in the water at a concentration of approximately 22 µg/mL to supply a final daily oral dose of 22 mg/kg (10 mg/lb) BW. In addition, a negative

control group of six turkeys (3 males and 3 females) received unmedicated water. Following the five-day medication period, six turkeys (3 males and 3 females) were exsanguinated at 12, 24, 48, 72, 120, and 240 hours after completion of the five-day dosing period to obtain the tissues for residue analysis. The entire control group of turkeys was exsanguinated at the 120 hour time point.

Tissues collected for this study were skin with adherent fat, abdominal fat, liver, kidney, white muscle (breast), and dark muscle (leg/thigh). The neomycin assay used for this study to analyze both the drinking water and the tissue was conducted using the cylinder plate microbiologic method as outlined in 21 CFR part 436.105, USP XXII<81>, and methodology developed by The Upjohn Company as adapted by validated CARE standard operating procedures. *Staphylococcus epidermidis* was used as the test organism and antibiotic medium #11 was the media used to prepare inoculated assay plates. This method has a limit of quantitation of 0.5 μg/g for all tissues. Table 6.2 summarizes the data from the turkey residue study.

Table 6.2. Mean tissue neomycin concentrations at various withdrawal periods following administration of neomycin sulfate at 22 mg/kg body weight for 5 consecutive days

Withdrawal period (hr)	Skin (μg/g)	Liver (μg/g)	Muscle (μg/g)	Fat (μg/g)
12	NDT	NDT	NDT	NDT*
24	NDT	NDT	NDT	NDT*
48	NDT	NDT	NDT	NDT*
72	NDT	NDT	NDT	ND*/NDT
120	NDT	NDT	NDT	NDT
120 (Control)	NDT	NDT	NDT	NDT
240	ND	ND	ND	ND

Limit of Quantitation (LOQ) for assay = 0.5 μg neomycin/g tissue
NDT = No detectable zone diameter
NDT* = Fat samples were inadvertently not collected from original treated birds. Additional birds (6 per sacrifice interval) were treated and sacrificed to permit collection of fat samples.
ND = Sample not assayed
ND* = Sample not assayed since abdominal fat was less than 10 g (males only)
For averaging: values of < 0.5 were included as 0.5; NDT were included as 0.

4. Conclusion: No neomycin residue were detected in skin, liver, muscle, or fat at any of the withdrawal periods. The data from this study support the assignment of a zero withdrawal period for turkeys given medicated water containing neomycin sulfate at 10 mg/lb for five consecutive days.

D. Regulatory Method: The regulatory analytical method for detection of neomycin is a microbiological test using *Staphylococcus epidermidis* suspension. The method is as published by the Food and Drug Administration, *Antibiotic Residues in Milk, Dairy Products and Animal Tissues: Methods, Reports and Protocols*, revised October 1968, reprinted December 1974.

VII. AGENCY CONCLUSIONS

The data submitted in support of this NADA supplement satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that neomycin sulfate, when administered in the drinking water of growing turkeys at a dosage of 10 mg/lb (22 mg/kg) for five consecutive days, is safe and effective for the control of mortality associated with *Escherichia coli* organisms susceptible to neomycin sulfate.

Based on a battery of toxicology tests, an acceptable daily intake of 6.0 μg/kg body weight/day was calculated, which further yielded safe concentrations for total neomycin-related residues of 1.2 ppm in muscle, 3.6 ppm in liver, and 7.2 ppm in skin/fat. Because neomycin undergoes only limited metabolic changes, tolerances for the edible tissues are established at the same values as the safe concentrations.

A zero withdrawal period is assigned for turkeys given medicated water containing neomycin sulfate at 10 mg/lb for five consecutive days. The zero withdrawal period is based on the depletion data which showed that no neomycin residues were detected in skin, liver, muscle, or fat at any of the withdrawal periods.

Proper use by lay persons can be expected because the directions are clearly written and there is reasonable certainty that the conditions of use, including mixing directions on the label, can and will be followed by the producer. The agency has concluded that this product can be approved for over-the-counter use.

The agency has considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Dockets Management Branch (HFA-305), Park Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20857, between 9 a.m. and 4 p.m., Monday through Friday.

Under the Center's supplemental approval policy (21 CFR 514.106(b)(2)(vii)), this is a Category II change. The approval of this change is not expected to have any adverse effect on the safety or effectiveness of this new animal drug and, therefore, did not require a reevaluation of the human food or target animal safety data in the parent application.

Under Section 512(c)(2)(F)(iii) of the FFDCA, this approval for food-producing animals qualifies for THREE (3) years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the applicant. The three years of marketing exclusivity applies only to the addition of the new species and indication, control of mortality associated with *Escherichia coli* organisms susceptible to neomycin sulfate in growing turkeys, for which the supplemental application is approved.

VIII. APPROVED LABELING

A copy of the draft facsimile labeling is attached to this document.

A. NEOMIX® AG 325 Soluble Powder – 50 Lb Package Label

B. NEOMIX® 325 Soluble Powder – 50 Lb Package Label

C. NEOMIX® 325 Soluble Powder – Packet Package Label

D. NEOMIX® 325 Soluble Powder – Packet Label