Date of Approval: October 17, 2005

FREEDOM OF INFORMATION SUMMARY

Supplemental New Animal Drug Application

NADA 013-076

TYLAN (tylosin tartrate) Soluble

"...for the control of American foulbrood *(Paenibacillus larvae)* in honey bees."

Sponsored by:

Elanco Animal Health

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1. GENERAL INFORMATION

a.	File Number:	NADA 013-076
b.	Sponsor:	Elanco Animal Health A Division of Eli Lilly & Co. Lilly Corporate Center Indianapolis, IN 46285 Drug labeler code: 000986
c.	Established Name:	Tylosin tartrate
d.	Proprietary Name:	TYLAN Soluble
e.	Dosage Form:	Water soluble powder
f.	How Supplied:	In bottles containing 100 grams of tylosin tartrate powder
g.	How Dispensed:	Over-the-counter (OTC)
h.	Amount of Active Ingredients:	Each gram contains 1 g tylosin tartrate powder
i.	Route of Administration:	Oral - mixed with confectioners/powdered sugar and dusted over the top bars of the brood chamber
j.	Species/Class:	Honey bees
k.	Recommended Dosage:	The 200 mg dose is applied (dusted) over the top bars of the brood chamber once weekly for 3 weeks.
l.	Pharmacological Category:	Antimicrobial
m.	Indications:	For the control of American foulbrood *(Paenibacillus larvae)*.
n.	Effect of Supplement:	To add the indication for the control of American foulbrood *(Paenibacillus larvae)* in honey bees.

2. EFFECTIVENESS

The data summarized in this section are publicly available data contained in Public Master File 005783 (69 FR 46553 dated August 3, 2004) which were compiled under National Research Support Project-7, a national agricultural research program for obtaining clearances for use of new drugs in minor species and for special uses.

a. Dosage Characterization

The rationale for the targeted dose of 200 mg tylosin tartrate and the treatment regimen was based upon the targeted dose and treatment regimens used in the tylosin target animal safety and human safety (residue depletion) studies, which in turn were based on comparable zones of inhibition to oxytetracycline (OTC) in the microbiological assay [see Kochansky et al., 2001. Screening alternative antibiotics against oxytetracycline-susceptible and -resistant *Paenibacillus larvae.* Apidologie 32: 215-222].

The doses tested are also supported by the work of Dr. Christine Peng [see Peng et. al., Laboratory and Field studies on the Effects of the Antibiotic Tylosin on Honey bee *Apis Mellifera L.* (Hymenoptera: Apidae). Development and Prevention of American Foulbrood Disease. Journal of Invertebrate Pathology 67, 65-71. (1996) Article No. 0010].

An effectiveness study was conducted with 100 mg and 200 mg tylosin tartrate (see summary below). Both the 100 mg and the 200 mg tylosin tartrate significantly ($p < 0.001$) reduced disease when compared to controls. For this study the 200 mg tylosin tartrate dose eliminated active disease in all treated colonies while the 100 mg dose did not.

b. Substantial Evidence

1. Efficacy of Tylosin Tartrate Against American Foulbrood Disease of Honey bees. May 2002 to August 2002.

a. Type of Study**:** Effectiveness Study

b. Investigators:

Dr. Mark Feldlaufer, USDA ARS, Bee Research Laboratory, Beltsville, MD

Dr. Patti Elzen & Mr. James Baxter, Honey bee Research Unit, Weslaco, TX

c. Study Design**:**

1) *Objective*: To demonstrate the effectiveness of tylosin tartrate in controlling American foulbrood disease (AFB) associated with *Paenibacillus larvae* in honey bees when applied in a dust of confectioners sugar to honey bee colonies.

2) *Animals*: Thirty honey bee (*Apis mellifera*) colonies were established in two isolated apiaries in Beltsville, MD. These colonies were inoculated with a suspension of *P. larvae* bacterial spores to initiate disease. The colonies all contained various larval instars, pupae, and variable age adults, mostly females (worker bees and queen). There were approximately 40,000 adult bees per colony. All honey bee colonies used were the property of the USDA.

3) *Dosage form*: The drug preparation used was a water soluble powder formulation of tylosin tartrate. The drug was mixed with confectioners sugar. The preparation was dusted across the tops of the frames in the hive. Treatments were administered weekly for a total of three treatments.

4) *Route of administration*: Oral. The bees ingested the sugar mixture to clean up the hive. The worker bees then fed the larvae, thus treating them.

5) *Treatment Groups*: Colonies were numbered and randomly divided into three treatment groups after all colonies were determined to have active AFB infections. The three groups were treated as follows:

- Sugar treated control: 0 mg tylosin tartrate in 20 g confectioners sugar
- Full dose: 200 mg tylosin tartrate in 20 g confectioners sugar
- Half dose: 100 mg tylosin tartrate in 20 g confectioners sugar

6) *Measurements and Observations*:

- Colonies were rated for AFB severity after infection but before treatment
- Colonies were rated 45 days post the final treatment (Day 119 of the study)

Each frame within a colony was rated on a 0 to 3 scale: "0" represented no visible signs of disease; "1" represented less than 10 individual cells/frame with disease; "2" represented 11 to 100 cells/frame with disease; and "3" represented more than 100 cells/frame with disease. The average single colony score was calculated by dividing the total frame scores of each colony by the number of frames in that colony. The average colony scores in each treatment group were added and the total was divided by ten (the number of colonies in each treatment group) to yield a mean treatment score for that group.

d. Statistical analysis:

The average colony scores were analyzed using an analysis of variance.

e. Results:

Both the 100 mg and the 200 mg doses of tylosin tartrate significantly ($p < 0.001$) reduced disease when compared to controls. At the end of the

study, AFB in the untreated (placebo) group had increased considerably and the colony scores had risen to a mean treatment group rating of 0.84 ± 0.11 standard error of the mean (SEM). By comparison, only a single colony in the 100 mg treatment group had active disease (mean treatment group rating of 0.025 ± 0.025 SEM) and all signs of disease

were eliminated in the 200 mg treatment group. Individual ratings by colony are listed in Table 2.1.

Table 2.1: Disease ratings of AFB-infected honey bee colonies treated with tylosin

Colony #	**Treatment Group**	**Pre-Treatment Average colony Score**	**Day 119 (45 days after 3rd treatment) Average colony Score**
3	100 mg	0.18	0.00
7	100 mg	0.60	0.00
9	100 mg	0.27	0.00
13	100 mg	0.50	0.00
24	100 mg	0.15	0.00
27	100 mg	0.78	0.00
33	100 mg	0.22	0.00
37	100 mg	0.22	0.00
46	100 mg	0.60	0.00
50	100 mg	0.50	0.25
	Mean Treatment Score ± SEM	**0.40 ± 0.07**	**0.025 ± 0.025**

Colony #	**Treatment Group**	**Pre-Treatment Average colony Score**	**Day 119 (45 days after 3rd treatment) Average colony Score**
4	200 mg	0.38	0.00
8	200 mg	0.20	0.00
15	200 mg	0.54	0.00
17	200 mg	0.23	0.00
23	200 mg	0.31	0.00
26	200 mg	0.20	0.00
28	200 mg	0.36	0.00
30	200 mg	0.83	0.00
31	200 mg	0.10	0.00
36	200 mg	0.40	0.00
	Mean Treatment Score ± SEM	**0.35 ± 0.07**	NA

Colony #	**Treatment Group**	**Pre-Treatment Average colony Score**	**Day 119 (45 days after 3rd treatment) Average colony Score**
1	Placebo	0.67	0.56
2	Placebo	1.00	1.00
5	Placebo	0.36	1.00
11	Placebo	0.22	0.56
18	Placebo	0.25	0.63
32	Placebo	0.25	1.00
38	Placebo	0.33	0.56
39	Placebo	0.50	1.63
42	Placebo	0.11	0.67

45	Placebo	0.55	0.81
	Mean Treatment Score ± SEM	**0.42 ± 0.08**	**0.84 ± 0.11**

e. Adverse Reactions: None

f. Conclusion**:** The data demonstrate that tylosin tartrate is effective in controlling American foulbrood disease when applied to infected honey bee colonies as a dust in confectioners sugar, three times, one week apart. These results are in general agreement with previous published data. Both the 100 mg and the 200 mg doses of tylosin tartrate significantly ($p < 0.001$) reduced disease when compared to controls and in this study the 200 mg dose eliminated the disease in all treated colonies. Thus, it was determined that the target dose (200 mg) cannot be reduced and still provide adequate control.

3. TARGET ANIMAL SAFETY

The data summarized in this section are publicly available data contained in Public Master File 005783 (69 FR 46553 dated August 3, 2004) which were compiled under National Research Support Project-7, a national agricultural research program for obtaining clearances for use of new drugs in minor species and for special uses.

Toxicity of Tylosin Tartrate to Immature and Adult Honey bees.
September 2000 to December 2000.

1. Type of Study: Target Animal Safety Study

2. Investigators:

   Dr. Mark Feldlaufer, USDA ARS, Bee Research Laboratory, Beltsville, MD

   Dr. Patti Elzen and Mr. James Baxter, Honey bee Research Unit, Weslaco, TX

3. Experimental Design:

   a) *Objective*: To demonstrate the safety of tylosin tartrate treatments to honey bee colonies; including adults, larvae, and the queen.

   b) *Test animals*: Twenty colonies of honey bees *(Apis mellifera).* All colonies were examined prior to inclusion in the study and only healthy hives were used. Colonies were all determined to be 'queenright', to contain at least 40,000 adult workers, to have uncapped brood (larval stages), to have no visible signs of disease, and to contain no surplus honey.

   c) *Dosage form*: The drug preparation used was a water soluble powder formulation of tylosin tartrate. The drug was mixed with confectioners sugar. The preparation was

dusted across the tops of the frames in the hive.

d) *Route of administration*: Oral. The bees ingested the sugar mixture to clean up the hive. The worker bees then fed the larvae, thus treating them.

e) *Treatment Groups*: The twenty colonies were randomly divided into five treatment groups. All groups were treated weekly for nine weeks (3X duration). The treatment groups were as follows:

1) untreated control (no drug or sugar)

2) 0 mg tylosin (no drug) with 20 grams of sugar

3) 200 mg tylosin mixed with 20 grams of sugar (1X)

4) 600 mg tylosin mixed with 20 grams of sugar (3X)

5) 1000 mg tylosin mixed with 20 grams of sugar (5X)

f) *Parameters*:

- adult bee mortality;
- presence of sealed brood (healthy larvae);
- queen health.

4. Results:

a) *Adult bees*

Adult bees were assessed for mortality. Dead adult bees were removed from the hive by the remaining live bees. The dead bees were collected in a plastic fabric that was placed in front of each hive. The number of dead bees was recorded one, four, and seven days after each weekly treatment for a total of nine weeks. The total recorded in Table 3.1 represents the sum of the counts for the three days for the four colonies for each treatment group for each week of the study. After the counts were made, the dead bees were removed and the fabric replaced for the next count.

Table 3.1: Daily Dead Bee Count Totals - Adult Bees

Treatment Groups	*Wk 1**	*Wk 2*	*Wk 3*	*Wk 4*	*Wk 5*	*Wk 6*	*Wk 7*	*Wk 8*	*Wk 9*
No tx	154	1017	216	927	195	290	306	1004	65
Sugar	370	2509	303	561	180	563	788	737	82
1X	34	1358	82	399	112	392	584	974	91
3X	276	836	117	278	132	246	246	697	75
5X	130	1045	198	360	160	372	241	785	129

*Totals for this week include counts from only two observation days. The remaining weekly totals are for three observation days.

The counts of the dead bees (Y) were transformed using log (Y+1) and analyzed using repeated measures analysis of variance

The interaction effect between treatment groups and treatments (weeks of treatment) was included in the model. Models were run with temperature effects, linear alone or linear and quadratic, as covariates. The same daily temperature, provided by a local weather station, was recorded for each colony.

For both models, the interaction of treatment groups and treatments (weeks of treatment) ($P \leq 0.01$), the effect of weeks of treatment ($P < 0.0001$) and the effect of days of recording ($P < 0.04$) were all significant, indicating that bee deaths across treatment groups, weeks, and observation days were not consistent. However, averaging across weeks, there were no mortality differences among treatment groups ($P = 0.68$).

b) *Larvae*

The presence of sealed brood, indicating healthy larvae, was assessed three times during the course of the study. In each colony, two areas (100 cells each) on a frame containing larval bees were marked prior to the first, fourth, and eighth treatment application. Observations were made at the start (first observation), middle (second observation), and end (third observation) of the study, one week after treatment. For each observation, the presence of sealed brood in each marked area was recorded. The proportion of sealed brood (larvae) cells was defined as the number of sealed cells divided by the number of initial cells containing larvae.

The proportion of sealed brood (larvae) cells (Y) was transformed using sine^{-1} (square root of Y) to stabilize the variance. The transformed data was analyzed using repeated measures analysis of variance.

The results showed that there were marginally significant proportion differences of sealed larvae among treatment groups ($P = 0.1017$). There were significant proportion differences of sealed larvae among collection days ($P < 0.0001$), but there was no significant interaction between treatment groups and days of treatment. Table 3.2 illustrates the differences in percent of sealed brood observed in each of the five treatment groups at the first, second, and third observations.

Table 3.2: Percent* of Sealed Brood Cells: Averages of all hives per treatment group

Treatment Group	% Sealed Brood 1st Observation		% Sealed Brood 2nd Observation		% Sealed Brood 3rd Observation	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
No Tx	92.00	92.50	87.00	87.00	89.00	81.50
Sugar Tx	92.25	94.50	87.50	83.50	82.50	76.00

1X Tx	92.00	92.50	89.50	93.00	82.25	85.33
3X Tx	95.50	95.50	95.75	94.25	87.66	90.25
5X Tx	93.75	92.00	92.25	94.25	90.33	86.00

* percent = proportion x 100

c) *Emerging Adults*

The two areas of 100 cells on each frame containing larval bees were also checked for emerging adults at the beginning, middle, and end of the study, or 18 days after the first, fourth, and eighth treatment application. In some parts of the study, there were no sealed brood to mark and therefore no emerging adults to count. No abnormalities were observed in any of the emerging adults. Table 3.3 illustrates the differences in percentages of emerging adults observed in each of the five treatment groups at the first, second, and third observations.

Table 3.3: Percent of Emerging Adults: Averages of all hives per treatment group

Treatment Group	**% Emerging 1st Observation**		**% Emerging 2nd Observation**		**% Emerging 3rd Observation**	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
No Tx	99.75	99.25	97.50	79.75	78.75	75.00
Sugar Tx	94.75	96.50	78.00	63.25	100.00	100.00
1X Tx	95.25	98.25	83.75	88.75	96.25	75.00
3X Tx	96.25	97.25	95.50	94.50	93.75	92.50
5X Tx	95.25	98.50	97.25	98.50	95.00	85.00

d) *Queens*

Queens were marked at the beginning of the study for quick identification. At the end of the study queens were visually found and observed. During the course of the study two of the queens died (one queen from a hive in the 3X group and one queen from a hive in the 5X group) and the hives failed to produce new queens. Commercial queens were introduced into those hives and the colonies survived. On Day 64 of the study, all queens were accounted for, including the two replacements.

5. Conclusion: Tylosin tartrate is safe when administered to honey bees at a level of 200 mg per hive once per week for three consecutive weeks. No drug-related adverse effects were observed during the study.

4. HUMAN FOOD SAFETY

a. Toxicology: Toxicology studies supporting the human food safety of tylosin tartrate are available under NADA 013-076. An FOI Summary was not written for the original approval.

b. Residue Chemistry:

The data summarized in this section are publicly available data contained in Public Master File 005783 (69 FR 46553 dated August 3, 2004) which were compiled under National Research Support Project-7, a national agricultural research program for obtaining clearances for use of new drugs in minor species and for special uses.

1. Residues of Tylosin Tartrate in Honey

a. Type of study: Residues of tylosin tartrate in honey

b. Investigator: Mark F. Feldlaufer, Ph.D., Bee Research Laboratory, Beltsville, MD

c. Test animals: Honey bee, *Apis mellifera*.

d. Number of animals: 40,000 workers/colony; 12 colonies

e. Route of administration: Dusting of hives with tylosin-containing confectioners sugar

f. Treatment groups:

1) Untreated controls (4 colonies)

2) 200 mg tylosin in 20 g confectioners sugar (1X; 4 colonies)

3) 1000 mg tylosin in 20 g confectioners sugar (5X; 4 colonies)

g. Duration of treatment: Once every seven days for a total of three treatments (21 days).

h. Sampling: Honey was sampled from the honey supers (surplus honey) between the first and second treatments and from the honey supers and the brood chamber weekly for three weeks following the final treatment.

i. Results:

Table 4.1: Mean concentrations of tylosin (ppm) in brood chamber honey (lower, upper 95% confidence limits)

Treatment	Withdrawal		
	7 Days	**14 Days**	**21 Days**
200 mg (1X)	1.45 (0.66, 3.46)	0.47 (0.21, 1.04)	0.40 (0.17, 0.88)
1000 mg (5X)	5.55 (2.20, 17.46)	4.52 (1.85, 13.39)	1.98 (0.87, 4.90)
0 mg (control)	0.12 (0.03, 0.31)	0.00 (0.00, 0.06)	0.00 (0.00, 0.03)

Table 4.2: Mean concentrations of tylosin (ppm) in surplus honey (lower, upper 95% confidence limits)

Treatment	Withdrawal			
	0 Days	**7 Days**	**14 Days**	**21 Days**
200 mg (1X)	1.30 (0.59, 3.06)	0.39 (0.17, 0.85)	0.33 (0.14, 0.73)	0.16 (0.05, 0.38)
1000 mg (5X)	8.73 (3.21, 34.27)	3.57 (1.50, 9.90)	2.46 (1.07, 6.33)	1.61 (0.72, 3.85)
0 mg (control)	00.05 (0.0, 0.16)	0.00 (0.00, 0.06)	0.0 (0.00, 0.07)	0.05 (0.00, 0.17)

2. Target Tissue and Marker Residue

The target tissue is honey. The marker residue is parent tylosin.

3. Tolerances

Tolerances for residues of tylosin in honey are not required because residues of tylosin in honey collected from hives treated with tylosin tartrate are very low. Consistent with the labeling for other products approved for use in honey bees, tylosin tartrate "should be fed early in the spring or fall and consumed by the bees before the main honey flow begins to avoid contamination of production honey."

4. Withdrawal period

Treatments should be completed at least four weeks prior to the main honey flow.

c. Microbial Food Safety

The impact of the proposed use of tylosin tartrate in honey bees (for the treatment of American foulbrood - 200 mg tylosin tartrate per hive for three treatments, seven days between treatments) on microbial food safety was carefully considered by the Agency. The Agency thinks that under the labeled conditions of use, selection and emergence of antimicrobial-resistant bacteria of human health concern will not be significantly impacted by this particular use of tylosin tartrate in honey bees, and therefore should not significantly impact public health.

d. Analytical Methods for Residues

The analytical method for the detection of residues of tylosin in honey used in the residue study is a microbiological assay using an oxytetracycline-resistant strain of *Paenibacillus larvae* (the causative agent of American foulbrood disease of honey bees). This method is found in "Diagnosis of Honeybee Diseases," Shimanuki, H, and Knox, DA. 2000. US Department of Agriculture, Agricultural Handbook N$^{o.}$ AH-690. A copy

of the method is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

e. User Safety

Human warnings are provided on the product labeling as follows:

Avoid contact with human skin. Exposure to tylosin may cause a rash.

To report adverse effects, access medical information, or obtain additional product information, call 1-800-428-4441.

5. AGENCY CONCLUSIONS:

The data submitted in support of this NADA 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 TYLAN (tylosin tartrate) Soluble, when administered at a dose of 200 mg/hive in 20 g confectioners sugar once a week for 3 weeks, is safe and effective for the control of American foulbrood (*Paenibacillus larvae*) in honey bees.

Tolerances for residues of tylosin in honey are not needed because the residues of tylosin in honey collected from hives treated with tylosin tartrate during the honey flow are very low. Additionally, the consumption value for honey is very low. Therefore, the exposure resulting from consumption of honey from treated hives does not represent a human food safety concern.

TYLAN (tylosin tartrate) Soluble is labeled for over-the-counter (OTC) use. Routine treatment (dusting) of honey bee hives is a widely accepted and recommended practice performed by the lay person for this product. Additionally, adequate directions for use have been written for the lay person and the conditions for use prescribed on the label are likely to be followed in practice.

This approval does not qualify for exclusivity under section 512(c)(F)(iii) of the Federal Food, Drug, and Cosmetic Act.

In accordance with 21 CFR 514.106(b)(2)(vii), this is a Category II change involving the addition of a species and a new claim. The safety and effectiveness data in the parent application did not need to be reevaluated.

6. ATTACHMENTS:

Facsimile labeling is attached as indicated below:

TYLAN Soluble – 100 g bottle label5