Date of Approval: January 4, 2011

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-321

TRIFEXIS

Spinosad and Milbemycin oxime
Chewable Tablets
Dogs

TRIFEXIS Chewable Tablets are indicated for the prevention of heartworm disease (*Dirofilaria immitis*). TRIFEXIS Chewable Tablets kill fleas and are indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*), and the treatment and control of adult hookworm (*Ancylostoma caninum*), adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and adult whipworm (*Trichuris vulpis*) infections in dogs and puppies 8 weeks of age or older and 5 pounds of body weight or greater.

Sponsored by:

Elanco Animal Health
A Division of Eli Lilly & Co.

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I. GENERAL INFORMATION:

A. File Number: NADA 141-321

B. Sponsor:

Elanco Animal Health
A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285

Drug Labeler Code: 000986

C. Proprietary Name: TRIFEXIS

D. Established Name: Spinosad and milbemycin oxime

E. Pharmacological Category: Antiparasitic

F. Dosage Form: Chewable tablet

G. Amount of Active Ingredients: Each tablet contains:

140 mg spinosad and 2.3 mg milbemycin oxime
270 mg spinosad and 4.5 mg milbemycin oxime
560 mg spinosad and 9.3 mg milbemycin oxime
810 mg spinosad and 13.5 mg milbemycin oxime
1620 mg spinosad and 27 mg milbemycin oxime

H. How Supplied: The product is available in five tablet strengths in color-coded packages of 6 tablets each, according to the weight of the dog, as listed below:

140 mg spinosad and 2.3 mg milbemycin oxime
270 mg spinosad and 4.5 mg milbemycin oxime
560 mg spinosad and 9.3 mg milbemycin oxime
810 mg spinosad and 13.5 mg milbemycin oxime
1620 mg spinosad and 27 mg milbemycin oxime

I. How Dispensed: Rx

J. Dosage:

TRIFEXIS Chewable Tablets are given orally, once a month at the minimum dosage of 13.5 mg/lb (30 mg/kg) spinosad and 0.2 mg/lb (0.5 mg/kg) milbemycin oxime body weight. For heartworm prevention, give once monthly for at least 3 months after exposure to mosquitoes.

Body Weight (lbs)	Spinosad per Tablet (mg)	Milbemycin oxime per Tablet (mg)	Tablets Administered
5 to 10	140	2.3	One
10.1 to 20	270	4.5	One
20.1 to 40	560	9.3	One
40.1 to 60	810	13.5	One
60.1 to 120	1620	27	One
Over 120.1	Administer the appropriate combination of tablets		

K. Route of Administration:

Oral

L. Species:

Dogs

M. Indications:

TRIFEXIS Chewable Tablets are indicated for the prevention of heartworm disease (*Dirofilaria immitis*). TRIFEXIS Chewable Tablets kill fleas and are indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*), and the treatment and control of adult hookworm (*Ancylostoma caninum*), adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and adult whipworm (*Trichuris vulpis*) infections in dogs and puppies 8 weeks of age or older and 5 pounds of body weight or greater.

II. EFFECTIVENESS:

A. Dosage Characterization:

For the Prevention of Heartworm Disease:

Laboratory studies were conducted to confirm the dose of milbemycin oxime in the combination tablet against induced infections using two recent *Dirofilaria immitis* field isolates. Against one recent isolate (Study T3A260805), a single dose achieved 100% effectiveness.

In a study using a recent isolate (Study T3A130804), a single dose did not provide 100% effectiveness.

In another study with the second isolate (Study T3A260812), three consecutive monthly doses achieved 100% effectiveness, but two doses, when administered at 30 and 60 days after infection with heartworm larvae, did not provide 100% effectiveness.

For the Prevention and Treatment and of Flea Infestations:

The effectiveness of TRIFEXIS Chewable Tablets administered at a minimum effective dose of 30 mg/kg spinosad against adult cat fleas (*Ctenocephalides felis*) was demonstrated by data contained in the Freedom of Information summary for COMFORTIS Chewable Tablets under NADA 141-277 (Elanco Animal Health, a Division of Eli Lilly & Co).

For the Treatment and Control of Gastrointestinal Nematodes:

The minimum effective dose of 0.5 mg/kg milbemycin oxime for the dose-limiting nematode parasite, *Ancylostoma caninum* (dog hookworm), was established by published data.[1]

B. Substantial Evidence:

Statistical Methods

Each laboratory effectiveness study used a similar analysis for effectiveness. Drug effectiveness was calculated as:

$$\% \text{ Effectiveness} = (P2 - P1)/P2 \times 100$$

P1 = Geometric mean parasite count for the treatment group
P2 = Geometric mean parasite count for the control group

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[1] Blagburn BL, Hendrix CM, Lindsay DS, et al. Efficacy of Milbemycin oxime against naturally acquired or experimentally induced *Ancylostoma* spp and *Trichuris vulpis* infections in dogs. Am J Vet Res 1992; 53: 513-516.

Log-transformed parasite counts for the treatment and control groups were analyzed using a general linear model (GLM) with a fixed effect treatment. If the normality assumption was not met for residuals from the GLM, the non-parametric Wilcoxon rank sum test was used to evaluate the data. Results are presented as calculated in the non-parametric tests of the data unless otherwise noted with "GLM." In the flea study, contrasts were formed on the treatment by study day interaction to allow comparison at different time points. The nematode studies were assessed at a single time point only. All statistical tests were two-tailed and conducted at alpha = 0.05.

For the Prevention of Heartworm Disease:

1. **Laboratory Dose Confirmation Study T3A260812**
   a. Title: Clinical Study (Good Clinical Practices, (GCP)): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Heartworms (*Dirofilaria immitis*)

   b. Investigator: Craig Reinemeyer, DVM, PhD
      Stanwood, MI

   c. Study Design:
      1) Objective: Confirm the dose of TRIFEXIS Chewable Tablets and evaluate the adult heartworm (*Dirofilaria immitis*) prevention in dogs experimentally infected with larval heartworms.

      2) Study Animals: 40 dogs

3) Treatment Groups:

Table 1. Study T3A260812 Treatment Groups

Group	Infection Day	Treatment Days*	Treatment	Dose	Number and Gender of Dogs
1	Day -30	Days 0,15, 30, 45, 60	Control (final oral dosage form without actives)	0 mg/kg BW**	10 (5 M, 5 F)
2	Day -30	Days 0, 30	TRIFEXIS Chewable Tablets	Spinosad: 30-45 mg/kg BW Milbemycin oxime: 0.5-0.75 mg/kg BW	10 (5 M, 5 F)
		Days 15, 45, 60	Control (final oral dosage form without actives)	0 mg/kg BW	
3	Day -30	Day 0, 30, 60	Control (final oral dosage form without actives)	0 mg/kg BW	10 (5 M, 5 F)
		Days 15, 45	TRIFEXIS Chewable Tablets	Spinosad: 30-45 mg/kg BW Milbemycin oxime: 0.5-0.75 mg/kg BW	
4	Day -30	Days 0, 30, 60	TRIFEXIS Chewable Tablets	Spinosad: 30-45 mg/kg BW Milbemycin oxime: 0.5-0.75 mg/kg BW	10 (5 M, 5 F)
		Days 15, 45	Control (final oral dosage form without actives)	0 mg/kg BW	

*Treatment Groups 2, 3, and 4 received control on days that they did not receive spinosad and milbemycin oxime, to ensure that animals were handled similarly and to maintain masking.
**BW=body weight

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On designated Treatment Days, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: Physical examinations were conducted on Day -32 and Knott's and antigen tests were conducted on Study Day -31 on all dogs. All dogs were inoculated with 40 ± 2 third-stage infective *Dirofilaria immitis* larvae once on Study Day -30. On Study Day -1, dogs were weighed, blocked by gender and ranked by body weight, and allocated to one of the four treatment groups. Body weights were also collected on Study Days 14, 29, 44 and 59. Dogs were treated on Study Days 0, 15, 30, 45 and 60 based on Study Days -1, 14, 29, 44 and 59 body weights, respectively. A heartworm antigen test was performed on all study dogs on Study Day 90. On Study Day 120, necropsy was performed on all study dogs and *Dirofilaria immitis* heartworms in the heart and/or lungs were collected, sexed and counted.

d. Results: The post-treatment geometric mean (GM) heartworm (HW) count in Treatment Group 1 was 19.7 (range 13 to 30) and all 10 dogs were infected, demonstrating an adequate infection in the vehicle control group. There were no heartworms recovered in Treatment Group 4, demonstrating 100% effectiveness against heartworm infection when spinosad and milbemycin oxime was administered once a month for three consecutive months after infection. There were also no heartworms recovered from Treatment Group 3. One heartworm was recovered from one of the dogs in Treatment Group 2, demonstrating less than 100% effectiveness against heartworm infection when spinosad and milbemycin oxime was administered once a month for two consecutive months after infection.

e. Adverse Reactions: Adverse reactions for Treatment Group 2 were one dog with two occurrences of vomiting. Adverse reactions for Treatment Group 3 were one dog with one occurrence of vomiting. Adverse reactions for Treatment Group 4 were two dogs with a single occurrence of vomiting for each dog. The vomiting in each case occurred within two hours after dosing with TRIFEXIS Chewable Tablets.

f. Conclusions: TRIFEXIS Chewable Tablets are 100% effective against experimental infection with *Dirofilaria immitis* when it was administered once a month for three consecutive months after infection. It demonstrated less than 100% effectiveness when administered once a month for two consecutive months starting 30 days after infection.

2. **Laboratory Dose Confirmation Study T3A260805**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Heartworms (*Dirofilaria immitis*)

b. Investigator: Dwight D. Bowman, MS, PhD
Stanwood, MI

c. Study Design:

1) Objective: Confirm the dose of TRIFEXIS Chewable Tablets and evaluate the adult heartworm (*Dirofilaria immitis*) prevention in dogs experimentally infected with larval heartworms.

2) Study Animals: 30 dogs

3) Treatment Groups:

Table 2. Study T3A260805 Treatment Groups

Group	Infection Day	Treatment Days*	Treatment	Dose	Number and Gender of Dogs
1	Day -30	Days 0, 15	Control (final oral dosage form without actives)	0 mg/kg BW**	10 (5 M, 5 F)
2	Day -30	Day 0	TRIFEXIS Chewable Tablets	Spinosad: 30-45 mg/kg BW Milbemycin oxime: 0.5-0.75 mg/kg BW	10 (5 M, 5 F)
		Day 15	Control (final oral dosage form without actives)	0 mg/kg BW	
3	Day -30	Day 0	Control (final oral dosage form without actives)	0 mg/kg BW	10 (5 M, 5 F)
		Day 15	TRIFEXIS Chewable Tablets	Spinosad: 30-45 mg/kg BW Milbemycin oxime: 0.5-0.75 mg/kg BW	

*Treatment Groups 2 and 3 received control on Study Days 15 and 0, respectively, to ensure animals were handled similarly and to maintain masking.
**BW=body weight

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: Physical examinations and Knott's and antigen tests were conducted on Study Day -32. All dogs were inoculated with 50 ± 5 third-stage infective *Dirofilaria immitis* larvae once on Study Day -30. On Study Day -1, dogs were weighed, blocked by gender and ranked by body weight, and allocated to one of the three treatment groups. Body weights were also collected on Study Day 14.

Dogs were treated on either Study Day 0 or Day 15 based on the Study Day -1 or Study Day 14 body weights, respectively. A heartworm antigen test was performed on all dogs on Study Day 91. On Study Day 120, necropsy was performed on all dogs and *Dirofilaria immitis* heartworms in the heart and/or lungs were collected, sexed and counted.

d. Results: The post-treatment geometric mean (GM) heartworm (HW) count in the control group was 22.7 (range 14 to 39), demonstrating an adequate infection. There were no heartworms recovered in Treatment Groups 2 or 3, demonstrating 100% effectiveness against experimental infections of *Dirofilaria immitis*.

e. Adverse Reactions: One dog in Treatment Group 3 had one occurrence of vomiting within 1 hour of being dosed with TRIFEXIS Chewable Tablets.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets is 100% effective against experimental infections with *Dirofilaria immitis* in dogs.

For the Prevention and Treatment and of Flea Infestations:

1. **Laboratory Dose Confirmation and Non-Interference Study T3A060803**
   a. Title: Clinical Study (GCP): Dose Confirmation and Non-Interference Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infested with Fleas (*Ctenocephalides felis*).

   b. Investigator: David R. Young, DVM, PhD
   Turlock, CA

   c. Study Design:
      1) Objective: Confirm the dose and non-interference of TRIFEXIS Chewable Tablets and to evaluate the effectiveness in dogs experimentally infested with adult fleas (*Ctenocephalides felis*).

      2) Study Animals: 40 purebred or crossbred dogs (20 dogs per gender)

3) Treatment Groups:

Table 3. Study T3A060803 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	30-45 mg/kg spinosad 0.5-0.75 mg/kg milbemycin oxime	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)
3	30-60 mg/kg	Spinosad	Once on Study Day 0	10 (5 M, 5 F)
4	0.5-1 mg/kg	Milbemycin oxime	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: On Study Days -1, 5, 12, 19, 28, and 35 each dog was infested with approximately 100 unfed adult fleas. The Study Day -1 flea infestations were used to determine the immediate knockdown effectiveness of the test articles based on the 24-hour (Study Day 1) post-treatment flea comb counts. The 48 hour post-infestation flea comb counts conducted on Study Days 7, 14, 21, 30, and 37 were used to determine the residual activity of the single treatment administered on Study Day 0. Dogs were combed on Study Days 1, 7, 14, 21, 30 and 37, respectively, to evaluate the knockdown and residual effectiveness in each of the treated groups compared to the control group ~48 hours after each infestation. Dogs were observed prior to dosing on Study Day 0 and at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing for adverse events.

The primary variable evaluated was the post-treatment reduction of adult *Ctenocephalides felis* based on flea counts in the groups treated with TRIFEXIS Chewable Tablets, spinosad only, milbemycin oxime only, and the control groups through Study Day 30. Effectiveness ≥

90% against *Ctenocephalides felis* with the combination tablet, < 90% for the milbemycin oxime only group, and a statistically significant difference ($p < 0.05$, two-sided) between the control group and the group treated with TRIFEXIS Chewable Tablets was needed to demonstrate non-interference.

d. Results: The combination tablets demonstrated 100% knockdown on Study Day 1 post-treatment, and 100% residual effectiveness at Study Day 30 post-treatment. See Table 4 below.

Table 4. Geometric Mean Post-Treatment Counts (% Reduction) of Adult *Ctenocephalides felis* on Dogs by Treatment Group.

Treatment Group	**Study Day 1**	**Study Day 7**	**Study Day 14**	**Study Day 21**	**Study Day 30**	**Study Day 37**
Group 1: Control	72.1 (--)	65.3 (--)	70.1 (--)	69.6 (--)	68.2 (--)	70.9 (--)
Group 2: TRIFEXIS Chewable Tablets	0.0 (100.0)	0.0 (100.0)	0.1 (99.9)	0.0 (100.0)	0.0 (100.0)	0.9 (98.8)
Group 3: Spinosad only	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	0.0 (100.0)	1.6 (97.7)
Group 4: Milbemycin oxime only	78.9 (0.0)	65.9 (0.0)	80.9 (0.0)	62.6 (10.0)	75.6 (0.0)	78.3 (0.0)

e. Adverse Reactions: Adverse reactions in the TRIFEXIS Chewable Tablet group consisted of loose stool in one dog and regurgitation of food in a second dog. These adverse reactions were likely treatment related.

f. Conclusions: Effectiveness of spinosad either alone or in combination with milbemycin oxime demonstrated 100% knockdown and residual effectiveness against adult flea infestations of 97.7% to 98.8% on Study Day 37 post-treatment. Milbemycin oxime alone did not demonstrate any flea effectiveness at any post-treatment time point. TRIFEXIS Chewable Tablets are as effective as spinosad alone against adult flea infestations. Milbemycin oxime did not interfere with the activity of spinosad against adult flea infestations.

For the Treatment and Control of Gastrointestinal Nematodes:

1. **Laboratory Dose Confirmation Study T3ADIE0603**
   a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Naturally Infected with Whipworms (*Trichuris vulpis*)

   b. Investigator: Padraig Doherty
   Glenamoy, Ireland

   c. Study Design:
      1) Objective: Confirm the dose of a spinosad and milbemycin oxime combination and evaluate the effectiveness in dogs naturally infected with adult whipworms (*Trichuris vulpis*).

      2) Study Animals: 22 purebred and/or mongrel dogs (6 male, 16 female)

      3) Treatment Groups:

Table 5. Study T3ADIE0603 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	11 (3 M, 8 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	11 (3 M, 8 F)

      4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

      5) Measurements and Observations: In the pre-treatment phase, all dogs having a positive *Trichuris vulpis* egg count continued in the study (> 5 eggs per gram average). The primary variable evaluated was effectiveness against adult *Trichuris vulpis* in naturally infected populations, based on comparing post-treatment worm count reduction

between the treated and control groups. Animal health observations made twice daily and treatment day animal observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (±30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment.

d. Results: All 22 dogs completed the study and were euthanized and necropsied on Study Day 8. Seven of the eleven dogs in the control group had at least five adult *Trichuris vulpis* worms present at necropsy (range 0 to 41), confirming adequacy of infection. There was a significant reduction in the number of *Trichuris vulpis* adults ($p = 0.0002$) when compared to the control group. Dogs in the control group had a geometric mean adult worm count of 8.0 compared to zero in the spinosad and milbemycin oxime treatment group. The combination product demonstrated 100% post-treatment effectiveness against *Trichuris vulpis*. See Table 6 below.

Table 6. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Trichuris vulpis*

Group	**Treatment**	**Geometric Mean Number of *Trichuris vulpis***	**Percent Effectiveness**
1	Control	8.0	N/A
2	TRIFEXIS Chewable Tablets	0	100

e. Adverse Reactions: There were no treatment-related adverse reactions during this study.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets was 100% effective against natural infections with adult *Trichuris vulpis* in dogs.

2. **Laboratory Dose Confirmation Study T3A370811**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Whipworms (*Trichuris vulpis*)

b. Investigator: Larry R. Cruthers, PhD
Corapeake, NC

c. Study Design:

1) Objective: Confirm the dose of a spinosad and milbemycin oxime combination and evaluate the effectiveness in dogs experimentally infected with adult whipworms (*Trichuris vulpis*).

2) Study Animals: 20 purebred dogs (10 per gender)

3) Treatment Groups:

Table 7. Study T3A370811 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: On Day -86, all dogs were inoculated orally with approximately 700 ± 50 larvated eggs of *Trichuris vulpis*. No dogs vomited post-inoculation. All dogs having a positive average *Trichuris vulpis* egg count (> 0 eggs per gram) over Days -3 to -2 continued in the study.

The primary variable evaluated was effectiveness against adult *Trichuris vulpis* in experimentally infected populations based on comparing post-treatment worm count reduction between the treated and control groups. Animal health observations made twice daily and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment.

d. Results: All dogs completed the study and were euthanized and necropsied on Study Day 7. Nine of ten dogs in the control group had at least five

adult *Trichuris vulpis* worms present at necropsy (range 1 to 712), confirming adequacy of infection. There was a significant reduction in the number of *Trichuris vulpis* adults ($p = 0.0009$, GLM) when compared to the control group. Dogs in the control group had a geometric mean adult worm count of 177.5 compared to 6.3 in the TRIFEXIS Chewable Tablet treatment group. The combination product demonstrated 96.5% post-treatment effectiveness against *Trichuris vulpis*. See Table 8 below.

Table 8. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Trichuris vulpis*

Group	**Treatment**	**Geometric Mean Number of *Trichuris vulpis***	**Percent Effectiveness**
1	Control	177.5	N/A
2	TRIFEXIS Chewable Tablets	6.3	96.5

e. Adverse Reactions: There were no adverse reactions in either treatment group.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets was 96.5% effective against experimental infections with adult *Trichuris vulpis* in dogs.

3. **Laboratory Dose Confirmation Study T3A370806**

a. Title: Clinical Study (GCP): Dose Confirmation and Non-Interference Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Hookworms (*Ancylostoma caninum*)

b. Investigator: Larry R. Cruthers, PhD
Corapeake, NC

c. Study Design:

1) Objective: Confirm the dose and non-interference of a spinosad and milbemycin oxime combination and evaluate the effectiveness in dogs experimentally infected with adult hookworms (*Ancylostoma caninum*).

2) Study Animals: 40 purebred dogs (20 per gender)

3) Treatment Groups:

Table 9. Study T3A370806 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)
3	30-60 mg/kg	Spinosad	Once on Study Day 0	10 (5 M, 5 F)
4	0.5-1 mg/kg	Milbemycin oxime	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: On Day -27, all dogs were inoculated orally with 100 third-stage infective larvae of *Ancylostoma caninum*. No dogs vomited after inoculation. All dogs having a positive average *Ancylostoma caninum* egg count (> 0 eggs per gram) over Days -3 to -2 continued in the study. Animal health observations made twice daily and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment.

The primary variable evaluated was effectiveness against adult *Ancylostoma caninum* in experimentally infected populations, based on comparing post-treatment worm count reduction between the treated and control groups. Effectiveness ≥ 90% against *Ancylostoma caninum* with the combination tablet, < 90% for the spinosad only group, and a statistically significant difference ($p < 0.05$, two-sided) between the control group and the combination treated group was needed to demonstrate non-interference.

d. Results: All dogs completed the study and were euthanized and necropsied on Study Day 8. All dogs in the control group had at least five adult *Ancylostoma caninum* worms present at necropsy (range 21 to 53), confirming adequacy of infection. There was a significant reduction in the number of *Ancylostoma caninum* adults ($p < 0.0001$) when compared to the control group. Dogs in the control group had a geometric mean adult worm count of 32.9 compared to 0.1 in the TRIFEXIS Chewable Tablets treatment group. The combination product demonstrated 99.8% post-treatment effectiveness against *Ancylostoma caninum*. See Table 10 below.

Table 10. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Ancylostoma caninum*

Group	**Treatment**	**Geometric Mean Number of *Ancylostoma caninum***	**Percent Effectiveness**
1	Control	32.9	N/A
2	TRIFEXIS Chewable Tablets	0.1	99.8
3	Spinosad	29.7	9.6
4	Milbemycin oxime	0.1	99.5

e. Adverse Reactions: There were three adverse reactions in this study. No reactions were reported in the milbemycin oxime or spinosad/milbemycin oxime treatment groups. Two dogs in the spinosad only group had one event each of blood in the feces. One dog in the control group had a single incident of diarrhea containing blood. All animals recovered without therapy.

f. Conclusions: Effectiveness of milbemycin either alone or in combination with spinosad demonstrated ≥ 99.5% effectiveness against adult *Ancylostoma caninum.* Spinosad alone did not demonstrate effectiveness against *Ancylostoma caninum*. The combination of spinosad and milbemycin oxime was as effective as milbemycin oxime alone against adult *Ancylostoma caninum* infections. Spinosad did not interfere with the activity of milbemycin oxime against adult *Ancylostoma caninum.*

4. **Laboratory Dose Confirmation Study T3AZA0807**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Naturally Infected with Hookworms (*Ancylostoma caninum*).

b. Investigator: Larry R. Cruthers, PhD
Bloemfontein, South Africa

c. Study Design:

1) Objective: Confirm the dose of TRIFEXIS Chewable Tablets and evaluate the effectiveness in dogs naturally infected with adult hookworms (*Ancylostoma caninum*).

2) Study Animals: 20 purebred or crossbred dogs (10 dogs per gender)

3) Treatment Groups:

Table 11. Study T3AZA0807 Treatment Groups

Treatment Group	**Dose**	**Treatment**	**Frequency/ Duration**	**Number and Gender of Dogs**
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: In the pre-treatment phase, all dogs having a positive average *Ancylostoma caninum* egg count of > 40 eggs per gram continued in the study. The primary variable evaluated was effectiveness against adult *Ancylostoma caninum* in naturally infected populations based on comparing post-treatment worm count reduction in both the treated and control groups. Animal observations were conducted twice daily to assess dogs for abnormal observations or adverse reactions. All enrolled dogs completed the study and were euthanized and necropsied on Study Day 7.

d. Results: All ten dogs in the control group had at least five adult *Ancylostoma caninum* worms (range 13 to 157) present at necropsy, confirming adequacy of infection. The effectiveness against adult *Ancylostoma caninum* was 99.8%. The post-treatment geometric mean *Ancylostoma caninum* count was 0.1. Only one of the ten treated dogs had adult *Ancylostoma caninum* present at necropsy. There was a statistically significant difference ($p < 0.0001$) in post-treatment *Ancylostoma caninum*

counts between the treatment groups using both parametric and non-parametric statistical methods. See Table 12 below.

Table 12. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Ancylostoma caninum*

Group	**Treatment**	**Geometric Mean Number of *Ancylostoma caninum* adult worms**	**Percent Effectiveness**
1	Control	47.2	NA
2	TRIFEXIS Chewable Tablets	0.1	99.8

e. Adverse Reactions: Adverse reactions, consisting of loose stool, were observed in three dogs treated with TRIFEXIS Chewable Tablets.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets was 99.8% effective against natural infections with adult *Ancylostoma caninum* in dogs.

5. **Laboratory Dose Confirmation Study T3A260809**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Roundworms (*Toxocara canis*)

b. Investigator: Dwight D. Bowman, MS, PhD
Stanwood, MI

c. Study Design:

1) Objective: Confirm the dose of a spinosad and milbemycin oxime combination and evaluate the effectiveness in dogs experimentally infected with adult roundworms (*Toxocara canis*).

2) Study Animals: 20 purebred dogs (10 per gender)

3) Treatment Groups:

Table 13. Study T3A260809 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: On Day -51, all dogs were inoculated with 150 embryonated eggs of *Toxocara canis*. No dogs vomited post-inoculation. All dogs having a positive average *Toxocara canis* egg count (> 0 eggs per gram) over Days -3 to -2 continued in the study.

The primary variable evaluated was effectiveness against adult *Toxocara canis* in experimentally infected populations based on comparing post-treatment worm count reduction between the treated and control groups. Animal health observations made twice daily and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment.

d. Results: All dogs completed the study and were euthanized and necropsied on Study Day 7. Nine of ten dogs in the control group had at least five adult *Toxocara canis* worms present at necropsy (range 3 to 38), confirming adequacy of infection. There was a significant reduction in the number of *Toxocara canis* adults ($p < 0.0001$) when compared to the control group. Dogs in the control group had a geometric mean adult worm count of 16.2 compared to 0.1 in the TRIFEXIS Chewable Tablets treatment group. The combination product demonstrated 99.6% post-treatment effectiveness against *Toxocara canis*. See Table 14 below.

Table 14. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Toxocara canis*

Group	Treatment	Geometric Mean Number of *Toxocara canis*	Percent Effectiveness
1	Control	16.2	N/A
2	TRIFEXIS Chewable Tablets	0.1	99.6

e. Adverse Reactions: There were no adverse reactions reported during the study.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets was 99.6% effective against experimental infections of adult *Toxocara canis* in dogs.

6. **Laboratory Dose Confirmation Study T3ADIE0601**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Naturally Infected with Roundworms (*Toxocara canis*)

b. Investigator: Padraig Doherty
Glenamoy, Ireland

c. Study Design:

1) Objective: Confirm the dose of TRIFEXIS Chewable Tablets and evaluate the effectiveness in dogs naturally infected with adult ascarids (*Toxocara canis*).

2) Study Animals: 20 purebred and/or mongrel dogs (10 per gender)

3) Treatment Groups:

Table 15. Study T3ADIE0601 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: In the pre-treatment phase, all dogs having a positive *Toxocara canis* egg count continued in the study. The primary variable evaluated was effectiveness against adult *Toxocara canis* in naturally occurring populations based on comparing post-treatment worm count reduction between the treated and control groups. Daily observations made twice daily, and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment. All enrolled dogs completed the study and were euthanized and necropsied on Study Day 7 or Study Day 8.

d. Results: All dogs completed the study and were euthanized and necropsied on Study Day 8. Eight of ten dogs in the control group had at least five adult *Toxocara canis* present at necropsy, confirming adequacy of infection. There was a significant reduction in the number of *Toxocara canis* adults ($p < 0.0001$) when compared to the control group. Dogs in the control group had a geometric mean of 6.1 adults compared to zero in the TRIFEXIS Chewable Tablets treatment group. TRIFEXIS Chewable Tablets demonstrated 100% post-treatment effectiveness against *Toxocara canis*. See Table 16 below.

Table 16. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Toxocara canis*

Group	Treatment	Geometric Mean Number of *Toxocara canis*	Percent Effectiveness
1	Control	6.1	NA
2	TRIFEXIS Chewable Tablets	0	100

e. Adverse Reactions: There were no adverse reactions in either treatment group.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets is 100% effective against natural infections with adult *Toxocara canis* in dogs.

7. **Laboratory Dose Confirmation Study T3A370810**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Experimentally Infected with Roundworms (*Toxascaris leonina*)

b. Investigator: Larry R. Cruthers, PhD
Corapeake, NC

c. Study Design:

1) Objective: Confirm the dose of a spinosad and milbemycin oxime combination and evaluate the effectiveness in dogs experimentally infected with adult roundworms (*Toxascaris leonina*).

2) Study Animals: 20 purebred dogs (10 per gender)

3) Treatment Groups:

Table 17. Study T3A370810 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: On Study Day -76, all dogs were inoculated orally with approximately 700 embryonated eggs of *Toxascaris leonina*. No dogs vomited post-inoculation. All dogs having a positive average *Toxascaris leonina* egg count (> 0 eggs per gram) over Study Days -3 to -2 continued in the study.

The primary variable evaluated was effectiveness against adult *Toxascaris leonina* in experimentally infected populations based on comparing post-treatment worm count reduction between the treated and control groups. Animal health observations made twice daily and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment.

d. Results: All 20 dogs completed the study and were euthanized and necropsied on Study Day 7. All 10 dogs in the control group had at least 5 adult *Toxascaris leonina* worms present at necropsy, confirming adequacy of infection. There was a significant reduction in the number of *Toxascaris leonina* adults ($p < 0.0001$, GLM) when compared to the control group. Dogs in the control group had a geometric mean adult worm count of 18.6 compared to 1.2 in the spinosad + milbemycin oxime treatment group. The combination product demonstrated 93.4% post-treatment effectiveness against *Toxascaris leonina*. See Table 18 below.

Table 18. Effectiveness of Spinosad and Milbemycin oxime Against Adult *Toxascaris leonina*

Group	Treatment	Geometric Mean Number of *Toxascaris leonina*	Percent Effectiveness
1	Control	18.6	N/A
2	TRIFEXIS Chewable Tablets	1.2	93.4

e. Adverse Reactions: There were no adverse reactions in either treatment group.

f. Conclusions: A single dose of spinosad + milbemycin oxime was 93.4% effective against experimental infections with adult *Toxascaris leonina* in dogs.

8. **Laboratory Dose Confirmation Study T3ADIE0602**

a. Title: Clinical Study (GCP): Dose Confirmation Efficacy Laboratory Study of a Flavored Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Naturally Infected with Roundworms (*Toxascaris leonina*)

b. Investigator: Padraig Doherty
Glenamoy, Ireland

c. Study Design:

1) Objective: Confirm the dose of a TRIFEXIS Chewable Tablets and to evaluate the effectiveness in dogs naturally infected with adult ascarids (*Toxascaris leonina*)

2) Study Animals: 20 mongrel or purebred dogs

3) Treatment Groups:

Table 19. Study T3ADIE0602 Treatment Groups

Treatment Group	Dose	Treatment	Frequency/ Duration	Number and Gender of Dogs
1	0 mg/kg	Control (final oral dosage form without actives)	Once on Study Day 0	10 (5 M, 5 F)
2	Spinosad: 30-45 mg/kg + Milbemycin oxime: 0.5-0.75 mg/kg	TRIFEXIS Chewable Tablets	Once on Study Day 0	10 (5 M, 5 F)

4) Drug Administration: All treatments were administered orally. Food was removed overnight on the day prior to dosing. On Study Day 0, dogs were offered approximately 25% of their ration and given 30 minutes to eat. Dogs were dosed after consuming their food.

5) Measurements and Observations: In the pre-treatment phase, all dogs having a positive average *Toxascaris leonina* egg count of > 40 eggs per gram continued in the study. The primary variable evaluated was effectiveness against adult *Toxascaris leonina* in naturally infected populations based on comparing post-treatment worm count reduction in both the treated and control groups. Daily observations made twice daily, and treatment day observations at 1 and 2 hours (± 15 min) and at 4 and 8 hours (± 30 min) post-dosing were used to evaluate the occurrence of adverse reactions associated with treatment. All enrolled dogs completed the study and were euthanized and necropsied on Study Day 7 or Study Day 8.

d. Results: All dogs in the control group had at least five adult *Toxascaris leonina* worms present at necropsy, confirming adequacy of infection. The treated group showed a significant reduction in the number of *Toxascaris leonina* adults ($p = 0.0005$) compared to the control group. Dogs in the control group had a geometric mean of 14.5 adults compared to one in the spinosad + milbemycin oxime treatment group. TRIFEXIS Chewable Tablets demonstrated 93.3% post-treatment effectiveness against *Toxascaris leonina*. See Table 20 below.

Table 20. Effectiveness of TRIFEXIS Chewable Tablets Against Adult *Toxascaris leonina*

Group	Treatment	Geometric Mean Number of *Toxascaris leonina*	Percent Effectiveness
1	Control	14.5	NA
2	TRIFEXIS Chewable Tablets	1.0	93.3

e. Adverse Reactions: There were no adverse reactions in either treatment group.

f. Conclusions: A single dose of TRIFEXIS Chewable Tablets is 93.3% effective against natural infections with adult *Toxascaris leonina* in dogs.

III. TARGET ANIMAL SAFETY:

1. **Field Safety Study T3AAM0801**

a. Title: Clinical Study (GCP): Field Safety Evaluation of a Flavored Spinosad and Milbemycin Oxime Combination Tablet in Client-Owned Dogs

b. Investigators:

Gary Brotze, DVM
New Braunfels, TX

Robin Downing, DVM
Windsor, CO

Roger Sifferman, DVM
Springfield, MO

Bill Campaigne, DVM
Seguin, TX

Kevin McGinn, DVM
Summerville, SC

Casey Thomas, DVM
Junction City, KS

Terry Clekis, DVM
Bradenton, FL

c. Study Design:

1) Objectives: To evaluate the safety of TRIFEXIS Chewable Tablets compared to an active control product in client-owned dogs under field conditions when administered monthly for six consecutive months. To assess the palatability of TRIFEXIS Chewable Tablets and an active control tablet in client-owned dogs.

2) Study Animals: 352 client-owned, single-dog households (176 treated with TRIFEXIS Chewable Tablets; 176 dogs treated with an active control, SENTINEL (lufenuron and milbemycin oxime) as per approved label.

3) Treatment Groups:

Table 21. Study T3AAM0801 Treatment Groups

Treatment Group No.	Treatment Dose	Treatment Group Description
1	TRIFEXIS Chewable Tablets [Spinosad (30-60 mg/kg BW) and Milbemycin Oxime (0.5-1 mg/kg BW)] Orally	Once monthly for 6 consecutive months
2	Lufenuron (10-20 mg/kg BW) and Milbemycin Oxime (0.5-1 mg/kg BW) Combination, Orally	Once monthly for 6 consecutive months

4) Drug Administration: Every thirty days for six consecutive treatments, dogs were offered TRIFEXIS Chewable Tablets or active control by their owner at home prior to offering food.

5) Measurements and Observations: Physical examinations, body weight measurement, heartworm antigen test, qualitative modified Knott's test, hematology and serum chemistry were performed during the pre-enrollment phase. Physical examinations and body weight measurements were performed during the enrollment visit. Physical examinations, body weight measurements, and animal observations obtained from the owner were performed once monthly during the treatment phase. Heartworm antigen and modified Knott's tests were performed on Study Day 60. Heartworm antigen tests were also performed on Study Day 180 and 3 months after Study Completion. Hematology and serum chemistry were collected upon Study Completion on Study Day 180. The change from pre- to post-treatment hematology and serum chemistry values was analyzed using a mixed model, with a treatment as the main effect and site and site-by-treatment interaction as random effects. Palatability of the drug, by free choice or in food, was summarized at each visit. Overall palatability is the average of the palatability from Study Days 0 through 150.

d. Results: Changes in hematology and clinical chemistry values were compared from the enrollment visit (Study Day -1) and the exit visit (Study Day 180) and were unremarkable, showing no consistent clinically significant trends. Mean weight changes during the course of the study were within normal expectations for both growing and adult dogs. Heartworm testing at the exit visit and again three months later showed none of the dogs treated with TRIFEXIS Chewable Tablets were positive for heartworm disease. In a field study of client-owned dogs where 175 dogs were each offered TRIFEXIS Chewable Tablets once a month for 6 months, dogs voluntarily consumed 54.1% of the doses when offered plain as if a treat and 33.2% of the doses when offered in or on food. The remaining 12.7% of doses were administered by pilling like other tablet medications.

e. Adverse Reactions: The most frequently reported adverse reactions for TRIFEXIS Chewable Tablets were vomiting, pruritus, lethargy, diarrhea, dermatitis, reddening of the skin, decreased appetite, and reddening of the ears. The most frequently reported adverse reactions for lufenuron + milbemycin oxime were vomiting, pruritus, lethargy, diarrhea, dermatitis, reddening of the skin, and decreased appetite. See Table 22 below.

The rate of vomiting decreased monthly from 10.8 to 2.9% up to the first four months of treatment then increased slightly to 6.0% and dropped again to 3.0% at the end of the study.

Table 22. Average Monthly Rate (%) of Dogs With Adverse Reactions

Adverse Reaction	TRIFEXIS Chewable Tablets[a]	Lufenuron and Milbemycin Oxime Tablets[a]
Vomiting	6.13	3.08
Pruritus	4.00	4.91
Lethargy	2.63	1.54
Diarrhea	2.25	1.54
Dermatitis	1.47	1.45
Skin Reddening	1.37	1.26
Decreased appetite	1.27	1.35
Pinnal Reddening	1.18	0.87

[a]n=176 dogs

One dog administered TRIFEXIS Chewable Tablets experienced a single mild seizure 2 ½ hours after receiving the second dose. The dog remained enrolled after the event and completed the study without further incident.

f. Conclusions: Monthly use of TRIFEXIS Chewable Tablets administered orally under field conditions in the United States is safe for dogs at a minimum dose of 30 mg/kg (range 30 to 60 mg/kg) of spinosad and 0.5 mg/kg (range 0.5 to 1.0 mg/kg) of milbemycin oxime.

2. **Avermectin Sensitive Collie Study T3A260802**

a. Title: Clinical Laboratory Study (GCP): Clinical Safety of a Spinosad and Milbemycin Oxime Combination Administered Orally to Avermectin-Sensitive Collies

b. Investigator: Allan Paul, DVM, MS
Stanwood, MI

c. Study Design:

1) Objective: Evaluate the safety of TRIFEXIS Chewable Tablets when administered to avermectin-sensitive Collie dogs at 1X, 3X and 5X the upper end of the recommended therapeutic dose band evaluated primarily by using an avermectin sensitivity scoring system.

2) Number of Animals: 24 Collie dogs demonstrated to be avermectin-sensitive (pre-study).

3) Treatment Groups:
Table 23. Study T3A260802 Treatment Groups

	Tablet Dosed (Control or TRIFEXIS Chewable Tablets)[a]				
Treatment Group	**Study Day 0[b]**	**Study Day 1**	**Study Day 2**	**Study Day 3**	**Study Day 4**
Control	C	C	C	C	C
1X	C	C	C	C	T
3X	C	C	T	T	T
5X	T	T	T	T	T

[a] C=control (final oral dosage form without actives) tablet dosed, T=TRIFEXIS Chewable Tablets dosed at 1X the upper half of the therapeutic dose band (45-60 mg/kg Spinosad/ 0.75-1.0 mg/kg Milbemycin Oxime).

[b] Dose interval 1 is shown. The same schedule was followed in 28 day intervals for the second and third dose sequences.

4) Drug Administration: All treatments were administered orally with food. Animals were re-dosed if they vomited within one hour.

5) Measurements and Observations: Avermectin sensitivity scores collected on Study Day -30 were used to rank the dogs within gender and allocate them to one of four treatment groups. Complete physical examinations were performed, and blood samples and body weights were collected for hematology and chemistry pre-dosing to confirm good health. Knott's and antigen tests were conducted pre-dosing to confirm that the animals were negative for heartworms. The average avermectin sensitivity scores were collected pre-dosing and on all dosing days. On all observation days, the animals were observed for any signs of avermectin toxicity (i.e. depression, ataxia, mydriasis, and/or salivation/drooling). A physical examination was also conducted on Study Days 26 and 54. Body weights, physical exams and blood collection (hematology and chemistry) were conducted on Study Day 63 following the final dosing interval.

d. Results: The avermectin sensitivity scores for all animals in all groups were zero on all scoring days from Study Day 0 through the final avermectin sensitivity assessment. Mean body weights in all groups remained consistent throughout dosing. Pre-dosing hematology and chemistry results showed that all dogs were considered healthy prior to allocation to treatment groups. Hematology and chemistry results from blood samples collected on Study Day 63 demonstrated that there were no clinically relevant changes attributable to treatment and that all dogs were considered healthy.

e. Adverse Reactions: There were ten adverse reactions during this study. One dog in the control group had diarrhea and two dogs in the 1X group experienced vomiting. In the 3X group, one dog had two events of vomiting, and one dog had two events of vomiting and one event of diarrhea. In the 5X group, one dog vomited once and one dog had one event of diarrhea. All animals recovered without therapy.

f. Conclusions: No signs of avermectin sensitivity were observed after administration of TRIFEXIS Chewable Tablets throughout the entire study in avermectin-sensitive Collie dogs. Vomiting and diarrhea were likely associated with treatment with TRIFEXIS Chewable Tablets.

3. **Margin of Safety Study T9A260802**

a. Title: Non-Clinical Laboratory Study (GLP): A Margin of Safety Study of a Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Starting at 8 Weeks of Age

b. Study Director: Edwin Goldenthal, PhD, ATS
Mattawan, MI

c. Study Design:

1) Objective: Evaluate the safety of TRIFEXIS Chewable Tablets when first administered to 8-week old dogs at 1X, 3X, and 5X the upper half of the recommended therapeutic dose based for six dosing periods.

2) Number of Animals: 32 Beagle dogs

3) Treatment Groups:

Table 24. Study T9A260802 Treatment Groups

	Tablet Dosed (Control or TRIFEXIS Chewable Tablets)[a]				
Treatment Group	**Study Day 0[b]**	**Study Day 1**	**Study Day 2**	**Study Day 3**	**Study Day 4**
Control	C	C	C	C	C
1X	C	C	C	C	T
3X	C	C	T	T	T
5X	T	T	T	T	T

[a] C=control (final oral dosage form without actives) tablet dosed, T= TRIFEXIS Chewable Tablets dosed at 1X the upper half of the therapeutic dose band (45-60 mg/kg Spinosad/ 0.75 – 1.0 mg/kg Milbemycin Oxime).

[b] Dose interval 1 is shown. The same schedule was followed in 28 day intervals for the second through the sixth dose interval.

4) Drug Administration: All treatments were administered orally with food. Animals were re-dosed if they vomited within one hour.

5) Measurements and Observations: All animals were observed at least twice daily throughout the duration of the study. On all observation days, the animals were observed for any signs of avermectin toxicosis (i.e. depression, ataxia, mydriasis, and/or salivation/drooling). Body weights for all animals were measured and recorded on specified days during the study. Fecal examinations were performed on Study Days -5, 83 and 167. A complete physical examination was conducted on all animals by a staff veterinarian on Study Days -7, -3, and 167. Clinical pathology (hematology and clinical chemistry) evaluations were conducted on all animals on Study Days -5, 83, and 167. Blood samples were collected for determination of plasma spinosyn A, spinosyn D, milbemycin A_3 5-oxime and milbemycin A_4 5-oxime concentrations on days -5, 27, 55, 83, 111, 139 and 167. Necropsy examinations and organ weight determination were performed on Study Day 168. Histopathological examination was performed on tissues from 0X and 5X dogs only.

d. Statistical methods: Repeated measurement endpoints were analyzed using a repeated measures mixed-effects model. The fixed effects in the model included Treatment, Sex and Time and interactions between and among the three factors. A covariance matrix which minimized the Akaike's Information Criterion (AIC) was used. Single post-dose measurement endpoints were analyzed using a

mixed-effect linear analysis of variance (ANOVA) model with fixed effects for Treatment, Sex and the Treatment by Sex interaction.

e. Results: All dogs survived to their scheduled termination at the end of six 28-day dosing periods. Emesis was observed in all groups including the control group with similar frequency. Clinical findings that were considered treatment-related were salivation in one dog in the 1X group, and two dogs in the 3X group, tremors in one dog in the 3X group and one dog in the 5X group, decreased activity in one 1X dog and two 5X dogs, vocalization in one 5X dog, and coughing in one 3X dog.

   Body weights and body weight changes were similar between control and treated male and female groups throughout the study. No test article-related alterations in hematology or clinical chemistry values were seen at any of the intervals of analysis. The mean values for all of the hematology and clinical chemistry parameters were comparable between control and treated animals.

   Plasma spinosyn A, spinosyn D, milbemycin A_3 5-oxime, and milbemycin A_4 5-oxime concentrations increased throughout the study. At each dosing period, plasma spinosyn A and spinosyn D concentrations were greater than dose proportional across the dose range 1 to 5X. Plasma milbemycin A_4 5-oxime concentrations appeared to be dose proportional across range 1 to 5X by the end of the study. A conclusion about dose proportionality for plasma milbemycin A_3 5-oxime could not be made because of a limited number of quantifiable concentration measurements.

   One dog in the 1X group had a red ovarian cyst found at necropsy. The few additional macroscopic observations were considered incidental and unrelated to treatment. There was a decrease in mean kidney/brain weights for the 3X dogs and an increase in mean kidney/body weight values in the 5X group both when compared to controls. The relevance of the changes in the 3X group is unknown as that group was not histologically examined. One 5X dog had minimal glomerular lipidosis observed microscopically. The clinical relevance of this finding is unknown.

f. Conclusions: The oral administration of TRIFEXIS Chewable Tablets at 1X, 3X, and 5X the upper half of the therapeutic dose band once monthly for six dosing periods in dogs was well tolerated in this study. Plasma concentrations of spinosad and milbemycin oxime indicate that expected systemic exposures were achieved throughout the study. Clinical signs related to treatment were salivation, tremors, decreased activity, cough and vocalization. Vomiting was seen in all groups, including the control group.

4. **Female Reproduction Study T9A260803**

a. Title: Non-clinical laboratory study (GLP): A Reproductive Safety Study of a Spinosad and Milbemycin Oxime Combination Administered Orally to Female Beagle Dogs

b. Study Director: Edwin Goldenthal, PhD, ATS
Mattawan, MI

c. Study Design
Objective: Evaluate the safety of TRIFEXIS Chewable Tablets administered to breeding female dogs at 1X and 3X the upper half of the recommended therapeutic dose band.

1) Number of Animals: 39 adult female Beagles were randomized to treatment groups and received study treatment. The first 30 females confirmed to be pregnant were selected to continue on study for evaluation of reproductive variables. The remaining nine adult female dogs received study treatment until all treatment groups had ten pregnant females. The 18 breeding males and the pups did not receive study treatment. Females and males had to be healthy and to have had at least four pups in each of their previous two litters, with no congenital malformations in any pup within either litter.

2) Treatment Groups:

Table 25. Study T9A260803 Treatment Groups

Treatment Group	**Tablet Dosed** (Control or TRIFEXIS Chewable Tablets)[a]			**Number and Gender of Dogs**
	Day 0[b]	**Day 7**	**Day 14**	
0X	C	C	C	13 females
1X	T	C	C	13 females
3X	T	T	T	13 females

[a] C=control (final oral dosage form without actives) tablet dosed, T=TRIFEXIS Chewable Tablets dosed at 1X the upper half of the therapeutic dose band (45-60 mg/kg Spinosad/ 0.75-1.0 mg/kg Milbemycin Oxime).

[b] This table shows the dosing scheme for the first 28-day dosing period. This dosing scheme was repeated every 28 days.

3) Drug Administration: All treatments were administered orally with food. Animals were re-dosed if they vomited within one hour. The 30 adult female dogs selected for evaluation of reproductive variables continued to receive study treatment, until their pups were 6 weeks old.

4) Variables Measured: Adult clinical pathology at baseline, clinical observations, physical examinations, body weight, litter size at birth and

weaning, stillbirths, pup mortality, gross pathology, and histopathology

d. Statistical Methods: Repeated measurement endpoints, body weights of female adult dogs at each phase (gestation or lactation) and litter average body weight were analyzed using a linear mixed model with the baseline as a covariate and the fixed effects for treatment, time, and the treatment by time interaction. A covariance matrix which minimizes the Akaike's Information Criterion (AIC) was used. Weight gain of female adult dogs between Study Days -2 and 40 was analyzed using a linear mixed effect model with treatment as fixed effect and block and treatment by block as random effects.

   For numerical parameters such as number of pups at birth, a linear one-way analysis of variance (ANOVA) was used to test for treatment differences. For binary outcomes such as proportion of pups with malformation, a generalized linear model with fixed effect treatment, binomial error distribution and logit link function was used to test treatment effect.

e. Results:
   1) Clinical Findings in Adult Females: No treatment-related adverse reactions or signs of avermectin toxicosis were noted for adult females.

   2) Body Weights in Adult Females: Between Study Day -2 (prior to the first study treatment on Study Day 0) and Study Day 40 (prior to the first mating on Study Day 42) there was a significant difference ($p = 0.0406$) in weight gain between the 3X group and the 0X group. The 0X group gained weight and the 3X group lost weight. The body weights of the treated groups were comparable to the control group during the gestation and post-parturition phases of the study.

   3) Selection of Pregnant Females: The first ten confirmed pregnant dogs in each treatment group remained in the study. One 3X and one 1X group dog did not become pregnant.

   4) Gestation and Litter Variables: Gestation length, litter size, stillborn pups, pup survival, and litter average body weight were comparable between treated and control dam groups.

   5) Pups with Malformations: The proportion of pups with malformations was comparable between groups, with the types of malformations differing between groups. Malformations in the 1X group included a pup with cleft palate and a littermate with anophthalmia, fused single nares, misshapen palate, hydrocephalus, omphalocele, and small testes malpositioned cranial to kidneys; a pup with a malformation of the anterior tip of the urinary bladder and umbilical blood vessel; and a 4-day-old pup with patent ductus arteriosus (PDA). Malformations in the 3X group included three 2- to 3-day-old littermates with PDA (a fourth 2-day-old littermate had a very

slightly patent ductus considered to be normal). Malformations in the control group included a pup with a malformed sternum (pectus excavatum), and a 2-day-old pup with PDA and a malpositioned superior vena cava. The incidence of cleft palate is not unexpected based on the historical data collected at the breeding site.

6) Pup Clinical Findings: No treatment-related effects were noted, with the exception of one 1X group pup. From three weeks of age on, one 1X group pup was smaller in size and less coordinated than its littermates, and received daily food and subcutaneous fluid supplements. It had tremors when excited.

f. Conclusions: With the exception of weight loss in the first six weeks at the 3X dose, spinosad and milbemycin oxime administration throughout the female reproductive cycle was clinically well-tolerated in adult Beagle dogs. The relationship between spinosad and milbemycin oxime treatment and the 1X and 3X dogs that did not become pregnant, the specific pup malformations, and the unthrifty 1X group pup are unknown.

5. **Heartworm Positive Study T3A1260603**

a. Title: Clinical Laboratory Study (GCP): Clinical Safety of a Spinosad and Milbemycin Oxime Combination Administered Orally to Dogs Infected with Adult Heartworms (*Dirofilaria immitis*)

b. Investigator: Larry Cruthers, PhD
Corapeake, NC

c. Study Design:

1) Objective: To evaluate the safety of TRIFEXIS Chewable Tablets in heartworm positive dogs with established adult heartworm infections and circulating microfilariae administered at 1X, 3X, and 5X the upper half of the therapeutic dose band.

2) Number of Animals: 32 Beagle dogs (4 dogs per gender per treatment group). These dogs were previously inoculated with 50 ± 5 third-stage infective *Dirofilaria immitis* larvae once approximately 270 days prior to dosing. Modified Knott's and antigen tests occurred 182 and 203 days post-inoculation. Additional antigen tests were conducted 120 and 141 days post-inoculation.

3) Treatment Groups:.
Table 26. Study T3A1260603 Treatment Groups

	Treatment Groups & Dosing (Control or TRIFEXIS Chewable Tablets)[a]				
Treatment Group	**Study Day 0[b]**	**Study Day 1**	**Study Day 2**	**Study Day 3**	**Study Day 4**
Control	C	C	C	C	C
1X	C	C	C	C	T
3X	C	C	T	T	T
5X	T	T	T	T	T

[a] C=control (final oral dosage form without actives) tablet dosed, T= TRIFEXIS Chewable Tablets dosed at 1X the upper half of the therapeutic dose band (45-60 mg/kg Spinosad/ 0.75-1.0 mg/kg Milbemycin Oxime).

[b] This table shows the dosing scheme for the first 28-day dosing period. This dosing scheme was repeated every 28 days for a total of three treatment intervals. Under this scheme, dogs were treated on Study Days 0-4, 28-32, and 56-60.

4) Drug Administration: All treatments were administered orally with food. Dogs were re-dosed if they vomited within one hour.

5) Measurements and Observations: Dogs had a complete physical examination (Study Day -6), hematology and chemistry testing pre-treatment (Study Day -7) to confirm they were in good health, with the exception of a patent heartworm infection. An antigen test and modified Knott's test were conducted on Study Day -14 and Study Day -12, respectively, on all dogs pre-dose to confirm a patent heartworm infection was present. On treatment days, dogs were observed at 1, 2, 4, and 8 hours post treatment for signs of avermectin toxicosis or hypersensitivity reactions. On all other Study Days, dogs were observed twice daily. Body weights were collected on Study Days 25 and 53, prior to the second and third dosing periods, respectively. Antigen tests and microfilaria counts were conducted on Study Days 7, 25, 53, and 63 to confirm the continued presence of a patent heartworm infection in all study dogs. Adverse events were recorded throughout the study. A physical examination was conducted on Study Day 64, prior to necropsy on Study Day 65. *Dirofilaria immitis* heartworms in the heart and/or lungs were collected at necropsy.

d. Results: Pre-treatment Knott's test results showed adequate levels of heartworm infection across all four treatment groups. The antigen test and microfilaria count results were positive for all dogs at each assessment throughout the study. At necropsy, a similar number of adult heartworms were collected across all treatment groups, based on the following ranges: control

group (range 14 to 37); 1X group (range 13 to 34); 3X group (range 17 to 35) and 5X group (range 17 to 35). Microfilariae counts decreased over time in a dose-dependent manner.

Body weights in the four groups followed a similar pattern across the study and remained comparable between the groups at each of the three assessment time points.

There were a total of eight adverse reactions observed during the course of the study. Six of these were incidents of vomiting; affecting one dog in the 1X group (a single incident), three dogs in the 3X group (four incidents in total) and one dog in the 5X group. The remaining adverse reactions were single incidents of decreased appetite and lethargy affecting one dog in the control group. That control dog subsequently died, and gross pathology results revealed that the death was attributed to complications associated with the patent infection of *Dirofilaria immitis*.

e. Conclusions: TRIFEXIS Chewable Tablets did not cause adverse reactions associated with heartworm and microfilariae status, when administered to laboratory dogs with patent adult heartworm infections at 1X, 3X, and 5X the upper half of the therapeutic dose. Although microfilariae counts decreased following treatment, hypersensitivity reactions were not observed. Adverse reactions in treated dogs were limited to vomiting.

IV. HUMAN FOOD SAFETY:

This drug is intended for use in dogs, which are non-food animals. Because this new animal drug is not intended for use in food producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to TRIFEXIS Chewable Tablets:

Warnings: Not for human use. Keep this and all drugs out of the reach of children.

VI. 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. The data demonstrate that TRIFEXIS Chewable Tablets, when used according to the label, are safe and effective for the prevention of heartworm disease (*Dirofilaria immitis*). TRIFEXIS Chewable Tablets kill fleas and are indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*), and the treatment and control of adult hookworm (*Ancylostoma caninum*), adult roundworm (*Toxocara canis* and *Toxascaris leonina*) and

adult whipworm (*Trichuris vulpis*) infections in dogs and puppies 8 weeks of age or older and 5 pounds of body weight or greater.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because the product is indicated for the prevention of heartworm infections (*Dirofilaria immitis*) in dogs, which requires veterinary examination and testing to ensure dogs are negative for adult heartworm disease prior to administration of the product to dogs.

B. Exclusivity:

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval.

C. Patent Information:

TRIFEXIS Chewable Tablets are under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
US 6,664,237	August 10, 2020
US 5,496,931	March 5, 2013
US 7,772,194	September 19, 2021

For current information on patents, see the Animal Drugs @ FDA database (formerly the Green Book) on the FDA CVM internet website.

VII. ATTACHMENTS:

Facsimile Labeling:
Package Insert
Client Information Sheet

Blister Packs:	5 to 10 lbs
	10.1 to 20 lbs
	20.1 to 40 lbs
	40.1 to 60 lbs
	60.1 to 120 lbs
Product Carton:	5 to 10 lbs
	10.1 to 20 lbs
	20.1 to 40 lbs
	40.1 to 60 lbs
	60.1 to 120 lbs

Display Carton: 5 to 10 lbs
10.1 to 20 lbs
20.1 to 40 lbs
40.1 to 60 lbs
60.1 to 120 lbs

Reminder Stickers