Date of Approval: September 25, 2007

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-277

COMFORTIS

Spinosad
Chewable tablets
Dogs

Kill fleas and are indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*) on dogs for one month.

Sponsored by:

Elanco Animal Health
A Division of Eli Lilly & Co.

TABLE OF CONTENTS

I. GENERAL INFORMATION: 1

II. EFFECTIVENESS: 2

A. Dosage Characterization: 2
1. Study T9CAM0006 2
2. Study T9C370103 3
B. Substantial Evidence: 6
1. Study T9C370225 6
2. Study T9C060232 7
3. Study T9C180104 8
4. Study T9C010313 13
5. Study T9C060330 14
6. Study T9C010508 17

III. TARGET ANIMAL SAFETY: 18

A. Study D00203 18
B. Study T9C420226 20
C. Study T9C420230 23
D. Study T9C750101 25

IV. HUMAN FOOD SAFETY: 26

V. USER SAFETY: 26

VI. AGENCY CONCLUSIONS: 26

A. Marketing Status: 26
B. Exclusivity: 26
C. Patent Information: 26

VII. ATTACHMENTS: 27

I. GENERAL INFORMATION:

A. File Number:	NADA 141-277
B. Sponsor:	Elanco Animal Health A Division of Eli Lilly & Co. Lilly Corporate Center Indianapolis, IN 46285 Drug Labeler Code: 000986
C. Proprietary Name(s):	COMFORTIS
D. Established Name(s):	Spinosad
E. Pharmacological Category:	Flea adulticide
F. Dosage Form(s):	Chewable tablets
G. Amount of Active Ingredient(s):	Each tablet contains 140, 270, 560, 810, or 1620 mg spinosad.
H. How Supplied:	The product is available in five strengths of tablets (140, 270, 560, 810, or 1620 mg), formulated according to the weight of the dog. Each tablet size is available in color-coded packages of 6 tablets each.
I. How Dispensed:	Rx
J. Dosage(s):	COMFORTIS chewable tablets are given orally, once a month at the recommended minimum dosage of 13.5 mg/lb (30 mg/kg) body weight as specified in the following table (Table 1):

Table 1: Recommended Dosage Schedule

	Recommended Dosage Schedule	
Body Weight	**Spinosad Per Tablet (mg)**	**Tablets Administered**
5 to 10 lbs	140	One
10.1 to 20 lbs	270	One
20.1 to 40 lbs	560	One
40.1 to 60 lbs	810	One
60.1 to 120 lbs*	1620	One

*Dogs over 120 lbs should be administered the appropriate combination of tablets.

K. Route(s) of Administration: Oral

L. Species/Class(es): Dogs

M. Indication(s): Kill fleas and are indicated for the prevention and treatment of flea infestations (*Ctenocephalides felis*) on dogs for one month.

II. EFFECTIVENESS:

A. Dosage Characterization:

1. Study T9CAM0006

Title: Efficacy Evaluation of Spinosad Administered at Different Dosages *per os* to Dogs for the Treatment and Control of Fleas (*Ctenocephalides felis*)

Type of Study: Laboratory effectiveness

Purpose: To determine the effectiveness of different dosages of spinosad given orally every 30 days for 3 consecutive treatments against the cat flea, *Ctenocephalides felis*, on experimentally infested dogs in a controlled laboratory setting.

Investigator: Robyn Slone, BS, Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 48 dogs in 6 groups of 8 dogs per group comprised of male and female, young and adult mixed breed and purpose-bred dogs

Dosage Form: Spinosad technical active in gelatin capsules

Route of Administration: Oral

Dosage Groups: Treatment group 1, the negative control group, received empty gelatin capsules (0 mg/kg). Treatment groups 2-5 received spinosad technical active in gelatin capsules at doses of 15, 20, 30, or 40 mg/kg, respectively. Treatment

group 6, the active control group, was treated topically with imidacloprid at 10 mg/kg.

Study Duration: 91 days

Study Design: Animals in groups 1-5 were dosed on days 0, 30, and 60. Animals in group 6 were dosed with the active control, on day 0 only. On post-treatment days 5, 12, 19, and 28, all dogs were infested with ~100 newly emerged unfed adult fleas (*Ctenocephalides felis*). On post-treatment days 33, 47, 58, 61, 75, and 89, each dog in groups 1 through 5 was infested with ~100 newly emerged unfed adult fleas (*Ctenocephalides felis*). Flea counts were conducted (~48 hours post-infestation) on days 7, 14, 21, 30, 35, 49, 60, 63, 77, and 91. The active control group was assessed only during the first 30 days of the study.

Parameters Measured: The numbers of live, dead, and moribund fleas were counted. The number of live adult fleas counted in each treatment group was compared to the number found on the negative control group.

Results: Effectiveness in the groups treated with 15 and 20 mg/kg dropped below 90% at some time point over the course of 91 days. Effectiveness in the groups treated with 30 and 40 mg/kg ranged between 97.2 and 100% throughout the study.

Table 2: Calculated Post-treatment Percent Reduction (Based on Geometric Means) in Adult Flea counts compared to Negative Control Group

Treatment Group	Day 30	Day 60	Day 91
15 mg/kg spinosad	89	68.6	66.1
20 mg/kg spinosad	95.5	88.2	83.1
30 mg/kg spinosad	98.4	97.2	99.0
40 mg/kg spinosad	100	100	97.7

Conclusions: Because the 30 mg/kg dose consistently eliminated fleas with $\geq$90% effectiveness, it was selected as the minimum dose for future studies.

Adverse Reactions: There were no drug-related adverse reactions noted during the study period.

2. Study T9C370103

Title: Evaluation of the Efficacy of Spinosad Administered Orally to Fed Versus Fasted Dogs for the Treatment and Control of Adult Cat Fleas (*Ctenocephalides felis*)

Type of Study: Laboratory effectiveness

Purpose:

1) Confirm the effectiveness of spinosad, dosed orally at 30 mg/kg, in experimentally infested dogs fed or fasted at the time of treatment for the knockdown and residual control of the adult cat flea, *Ctenocephalides felis*, under laboratory conditions.

2) Determine the concentrations of spinosad in plasma from blood samples collected at different time points post-treatment.

Investigator: Larry Cruthers, PhD, Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 32 dogs in 4 treatment groups of 8 dogs per group comprised of male and female, adult, mixed breed and purebred dogs

Dosage Form: Spinosad technical active in gelatin capsules

Route of Administration: Oral

Dosage Groups:

Table 3: Treatment Groups for Study T9C370103

Treatment Group No.	Treatment Dose Rate	Treatment Group Description
1	0 mg/kg oral spinosad dose	Control (empty gelatin capsule) dosed; then immediately fed wet food
2	30 mg/kg oral spinosad dose	Spinosad in gelatin capsules dosed; then immediately fed wet food
3	30 mg/kg oral spinosad dose	Spinosad in gelatin capsules dosed; then immediately fed dry dog chow
4	30 mg/kg oral spinosad dose	Spinosad in gelatin capsules dosed to fasted dogs; no dry food until 4 hours post-dosing

Study Duration: 37 days

Study Design: Dogs were infested on study days -1, 5, 12, 19, 28, and 35 with approximately 100 unfed adult fleas. Dogs were dosed orally, once on study day 0. Adult live flea counts were conducted on all dogs in each group, 48 hours post-treatment or post-infestation, on days 2, 7, 14, 21, 30, and 37 to assess effectiveness. Blood samples were collected from all dogs in the three spinosad treatment groups on day 0 at 1, 2, 4, and 8 hours post-treatment. Additional blood samples were collected on days 1 (~24 hours), 2 (~48 hours), 7, 14, 21, 30, and 37 post-treatment.

Parameters Measured: Percent effectiveness of spinosad was calculated based on 48-hour post-infestation or post-treatment geometric mean flea counts. Blood samples were analyzed for detection of spinosyns A and D in dog plasma.

Statistical Methods: Wilcoxon exact tests were used to compare flea counts in each treatment group to the control group at each time point.

Results: Summaries of flea effectiveness can be seen in Table 4. Table 5 is a summary of pharmacokinetic parameters for spinosyns A and D by treatment group.

Table 4: Summary of Spinosad Effectiveness (Calculated Percent Reduction in Geometric Mean Live Flea Counts Compared to the Control Group)

Group	Day 2	Day 7	Day 14	Day 21	Day 30	Day 37

1	-	-	-	-	-	-
2	100	100	99.6	100	97.8	91.1
3	100	99.8	99.3	95.3	89.1[a]	40.9[b]
4	100	97.6	97.1	94.6	85.3	61.4 [b]

[a]Four dogs were removed from this group because they did not consume any of the dry food when their food bowls were examined at 4 hours post-dosing; the effectiveness for the four dogs that did eat dry dog food becomes 89.1%.

[b] Not significantly different from Group 1. All other comparisons to Group 1 were significant ($p < 0.01$).

Table 5: Bioavailability Summary (Mean[a] ± 1SD) of Spinosyn A and Spinosyn D in Plasma from Dogs administered Spinosad Technical Powder 30 mg/kg in Gelatin Capsules and Fed (wet or dry dog food) immediately or 4 hours post-dose

	Spinosyn A			**Spinosyn D**		
	Treatment Group			**Treatment Group**		
	2	**3[b]**	**4**	**2**	**3[b]**	**4**
Parameter						
C_{max}, ng/mL	2502 (851)	2743 (393)	947 (925)	467 (154)	527 (84)	150 (154)
T_{max}, day	0.08[a] [0.08, 0.33]	0.17 [0.08, 0.17]	0.13 [0.08, 1.00]	0.13 [0.08, 0.33]	0.17 [0.08, 0.17]	0.13 [0.08, 1.00]
$AUC_{0-\infty}$, day*ng/mL	4992 (899)	4986 (1845)	2364 (2241)	889 (111)	873 (267)	662 (323)
Half-Life, days	10.6 (2.7)	6.8 (3.0)	10.6 (2.6)	11.0 (2.7)	6.9 (2.6)	10.6 (3.4)
Ratio (A/D)[c]						
C_{max}	5.4 (0.3)	5.2 (0.2)	7.2 (2.0)			
$AUC_{0-\infty}$	5.6 (0.4)	5.6 (0.4)	5.9 (0.5)			

[a]Mean ± 1SD reported for all values except T_{max}. Median and range [Minimum value, Maximum value] reported for T_{max}.
[b]Group 3 represents data from only four dogs who consumed spinosad with food immediately post-dose.
[c]Ratio of parameter values for Spinosyn A and Spinosyn D within dogs.

Conclusions: The results of this study confirm that spinosad, at a dose of 30 mg/kg, should be dosed in conjunction with food to achieve adequate 30-day residual effectiveness for the treatment of adult cat flea infestations on dogs. Dosing in a fasted state results in a reduction in the duration of effectiveness presumably due to reduced systemic absorption and exposure of spinosad. Additionally, this study demonstrates that there is a positive correlation between flea effectiveness and spinosyn A and D (spinosad) plasma concentrations.

Adverse Reactions: Two spinosad-treated dogs vomited within two hours of dosing. These vomiting episodes, which the investigator assessed as mild, were considered probably related to treatment with spinosad, but neither had an impact on flea knockdown or 30-day residual flea effectiveness. One spinosad-treated dog vomited 14 days after treatment. The relationship to the spinosad administration is unclear.

B. Substantial Evidence:

1. Study T9C370225

Title: Dose Confirmation of the Efficacy of Flavored Spinosad Tablets Administered Orally to Dogs for the Treatment of Adult Cat Fleas (*Ctenocephalides felis*)

Type: Laboratory effectiveness

Purpose:

1) Confirm the 30-day residual effectiveness of flavored spinosad tablets (dosed once orally at a dose range of 30-40 mg/kg) for the treatment of adult cat flea, *Ctenocephalides felis*, infestations on dogs under laboratory conditions.

2) Determine the *in vitro* viability of moribund fleas, if found, recovered from dogs treated with flavored spinosad tablets.

Investigator: Larry Cruthers, PhD, Professional Laboratory and Research Services, Inc., Corapeake, NC

Animals: 24 dogs in 2 treatment groups of 12 dogs per group comprised of male and female, adult, mixed and purebred dogs

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups:

1) Control (0 mg/kg delivered in the flavored tablet formulation without the active ingredient)

2) Spinosad (30-40 mg/kg)

Study Duration: 37 days

Study Design: Masked, parallel arm, controlled, laboratory study, utilizing a randomized, complete block design. Each dog was infested with approximately 100 newly emerged, unfed adult fleas, *Ctenocephalides felis*, on test days -1, 5, 12, 19, 28, and 35. Dogs were dosed once on test day 0 and were fed at the time of dosing. Adult flea counts, at approximately 48 hours post-treatment or post-infestation, were conducted on test days 2, 7, 14, 21, 30, and 37 to assess effectiveness.

Parameters Measured: Individual animal flea counts were performed approximately 48 hours post-treatment or post-infestation. Percent effectiveness was based on geometric means of live flea counts on treated and control dogs at 2, 7, 14, 21, 30, and 37 days after dosing or infestation.

Statistical Methods: Wilcoxon exact tests were used to compare flea counts in the treated group to the control group at each time point.

Results: The percent effectiveness ranged from 96.4 to 100% against the cat flea through test day 30 (Table 6). Flea counts in the treated group were significantly different from the control group at each time point ($p < 0.001$).

No moribund fleas were recovered during any of the flea counts.

Table 6: Geometric Mean Percent Effectiveness of Flavored Spinosad Tablets against *Ctenocephalides felis* Infestations of Dogs

Study Day	Day 2	Day 7	Day 14	Day 21	Day 30	Day 37
Percent Effectiveness	99.9	100.0	99.6	99.3	96.4	87.4

Conclusions: This study confirmed the 30-day residual effectiveness of spinosad, dosed once orally at a dose range of 30-40 mg/kg, for the treatment of adult cat flea infestations on dogs.

Adverse Reactions: One spinosad-treated dog experienced one incident of vomiting two days after dosing. Because of the proximity between dosing and the vomiting episode, it is likely drug-related.

2. Study T9C060232

Title: Dose Confirmation of the Efficacy of Flavored Spinosad Tablets Administered Orally to Dogs for the Treatment of Adult Cat Fleas (*Ctenocephalides felis*)

Type: Laboratory effectiveness

Purpose:

1) Confirm the 30-day residual effectiveness of spinosad tablets (dosed once orally at a dose range of 30-40 mg/kg) for the treatment of adult cat flea, *Ctenocephalides felis*, infestations on dogs under laboratory conditions.

2) Determine the *in vitro* viability of moribund fleas, if found, that were recovered from dogs treated with spinosad tablets.

Investigator: David R. Young, DVM, PhD, Young Veterinary Research Service, Turlock, CA

Animals: 24 dogs in two treatment groups of 12 dogs per group comprised of male and female, adult, mixed and purebred dogs

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups:

1) Control (0 mg/kg delivered in the chewable tablet formulation without the active ingredient)

2) Spinosad (30-40 mg/kg)

Study Duration: 37 days

Study Design: Masked, parallel arm, controlled, laboratory study, utilizing a randomized, complete block design. Each dog was infested with approximately 100 newly emerged, unfed fleas, *Ctenocephalides felis*, on test days -1, 5, 12, 19, 28, and 35. Dogs were dosed once on test day 0. To assess effectiveness, flea counts were

conducted at approximately 48 hours post-treatment or post-infestation, on test days 2, 7, 14, 21, 30, and 37.

Parameters Measured: Individual animal flea counts were performed approximately 48 hours post-treatment or post-infestation. Percent effectiveness was based on geometric means of live plus moribund flea counts on treated and control dogs at 2, 7, 14, 21, 30, and 37 days after dosing or infestation.

Statistical Methods: Wilcoxon exact tests were used to compare flea counts in the treated group to the control group at each time point.

Results: The percent effectiveness ranged from 100% to 99.9% against the cat flea through test day 30 (Table 7). On test days 30 and 37, two and one moribund fleas, respectively, were added to the total flea count in the treated group. Flea counts in the treated group were significantly different from the control group at each time point ($p < 0.001$).

Table 7: Geometric Mean Percent Effectiveness of Flavored Spinosad Tablets against *Ctenocephalides felis* Infestations of Dogs

Study Day	Day 2	Day 7	Day 14	Day 21	Day 30	Day 37
Percent Effectiveness	100	100	100	100	99.9	97.7

Conclusions: This study confirmed the 30-day residual effectiveness of spinosad, dosed once orally at a dose range of 30-40 mg/kg, for the treatment of adult cat flea infestations on dogs.

Adverse Reactions: Six spinosad-treated dogs experienced pruritus on test day 1. The investigator assessed these as mild, and possibly related to treatment. In all cases, the pruritus was self-limiting, with complete resolution observed within 24 hours without veterinary medical therapy.

3. Study T9C180104

Title: Evaluation of the Clinical Efficacy of Flavored Spinosad Tablets against Natural Infestations of *Ctenocephalides felis* on Client-owned Dogs

Type: Field Study

Purpose: The primary study objectives were to assess clinical effectiveness of a flavored tablet formulation of spinosad against natural infestations of the cat flea (*Ctenocephalides felis*) on dogs when used monthly for three consecutive months, and to assess the safety of spinosad when used in a diverse group of dogs under field conditions. Secondary objectives were to assess the palatability of the product in client-owned dogs, and to evaluate the effect of treatment with spinosad on signs of flea allergy dermatitis (FAD) in the treated dogs.

Investigators:

Joanna Bender, DVM Pat Tolchin, DVM Rochester, NY	Bill Campaigne, DVM David O'Brien, DVM Seguin, TX	Kevin McGinn, DVM D'ara Klein, DVM Summerville, SC	Roger Sifferman, DVM Doe Keen, DVM Springfield, MO
Gary Brotze, DVM Michael Doherty, DVM New Braunfels, TX	Mary Grabow, DVM Indianapolis, IN	Ann Parker, DVM Fayetteville, NC	Mark Spiegle, DVM Ian Sandler, DVM Toronto, ON Canada
Jay Butan, DVM Lake Worth, FL	Nigel Gumley, DVM Lianne Titcombe, DVM Gloucester, ON Canada	Andrew Pickering, DVM Terre Haute, IN	Casey Thomas, DVM Junction City, KS
Lynn Buzhardt, DVM Gwen Ryan, DVM Zachary, LA	Richard Johnson, DVM Nancy Hampel, DVM El Cajon, CA	Dean Rund, DVM Springfield, MO	Terry Clekis, DVM Bradenton, FL

Animals: 330 spinosad-treated dogs and 140 selamectin-treated dogs completed the study and were evaluated for safety. The effectiveness analysis was performed on 91 spinosad-treated dogs and 38 selamectin-treated dogs.

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups: Dogs in the analysis were dosed with COMFORTIS chewable tablets (spinosad) at approximately 30 to 60 mg/kg. Dogs in the active control group were treated with REVOLUTION (selamectin), as per approved label.

Study Duration: Three months

Study Design: Conducted at 16 sites throughout the United States (14 sites) and Canada (2 sites), this was a multi-center, parallel-arm, randomized, treatment-masked study comparing spinosad to an active control. Four United States sites and both Canadian sites did not enroll enough dogs (< eight effectiveness-evaluable cases) and these dogs were excluded from the effectiveness evaluation. Dogs with existing flea infestations were enrolled and fleas on dogs were counted at 2, 4, 8, and 12 weeks (visits 2, 3, 4, and 5) to measure effectiveness. The study utilized a randomized block design, with type of household [one dog only (no cats), multiple dogs (no cats), or mixed dog/cat household], and time of entry into the study as blocking factors. The experimental unit for effectiveness assessment was the household, with each household represented by one dog. All dogs in a household received the same treatment.

Parameters Measured: Serial flea counts on one dog from each household (the primary dog) were used to evaluate effectiveness. To determine the product's effect on the clinical signs of FAD, papules, erythema, alopecia, scaling, dermatitis/pyodermatitis, and pruritus were scored for severity at each study visit. Owners reported whether their dogs consumed the COMFORTIS chewable tablets free choice.

Statistical Methods: A mixed model repeated measures analysis was used to analyze the log of the flea counts + 1.

Results: Both spinosad-treated dogs and selamectin-treated dogs showed a statistically significant reduction in fleas from pre-treatment (visit 1) to the end of the study ($p < 0.001$) and both showed greater than 90% effectiveness. For the spinosad-treated dogs, the effectiveness population showed a 98.8% reduction (based on geometric mean) two weeks (15 ± 5 days) after initial treatment. After three months of treatment, the reduction was 99.8%. The selamectin-treated group demonstrated a reduction (treatment differences not statistically compared) of 92.4% after two weeks and achieved 99.1% reduction after three months (Table 8).

Table 8: Flea Count Data Summary

	Visit 1 (Pre-treatment)	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
Geometric Mean Flea Count					
Spinosad	63.4	0.8	1.3	0.2	0.1
Selamectin	61.7	4.7	4.9	1.2	0.6
Percent Reduction, Geometric Mean					
Spinosad	N/A	98.8%	98.0%	99.6%	99.8%
Selamectin	N/A	92.4%	92.1%	98.0%	99.1%

Twenty-four spinosad-treated and seven selamectin-treated primary dogs were included in the FAD analysis. Table 9 illustrates the percent of dogs that improved for each FAD sign comparing visit 1 to visit 5 evaluations. Improvements in signs were seen in 86-100% of dogs treated with spinosad and in 57-100% of dogs treated with selamectin.

Table 9: Comparison of FAD signs at Visits 1 and 5

FAD Signs	**Number of Dogs with Score > 0 at Visit 1[a]**	**Number of Dogs with Improved Score at Visit 5[b]**	**Percent Improved[c]**
Spinosad Primary Dogs (24 dogs in FAD population)			

Erythema	22	19	86%
Papules	18	18	100%
Scaling	19	17	89%
Alopecia	19	19	100%
Dermatitis/ Pyodermatitis	18	18	100%
Pruritus	24	23	96%
Selamectin Primary Dogs (7 dogs in FAD population)			
Erythema	5	5	100%
Papules	4	3	75%
Scaling	5	3	60%
Alopecia	4	4	100%
Dermatitis/ Pyodermatitis	5	5	100%
Pruritus	7	4	57%

[a] Among the FAD population, the number of dogs with a score > 0 at visit 1
[b] Among the dogs with the indicated sign at Visit 1, the number of dogs with an improved score at visit 5.
[c] The percent of dogs that had an improved score for the indicated sign at visit 5 among all dogs that were positive for the indicated sign at visit 1 (pre-treatment).

Prior to offering food, COMFORTIS chewable tablets were consumed free choice (by hand, from the floor or in a bowl) by 81% of dogs at Dose 1, 72% of dogs at Dose 2, and 79% of the dogs at Dose 3. Averaged over all three doses, 77% of the dogs in which palatability was assessed accepted COMFORTIS chewable tablets free choice.

Conclusions: This field study demonstrated that monthly use of COMFORTIS chewable tablets, at a minimum dose of 30 mg/kg, is safe and effective in dogs for the treatment of fleas.

Monthly treatment with COMFORTIS chewable tablets resulted in effective reduction of fleas on the dogs maintained in their home environment. A 98.8% reduction in fleas was achieved approximately 15 days after the first dose. After three consecutive monthly doses, approximately 90 days, spinosad provided 99.8% elimination of fleas on dogs in their home environment.

Treatment with COMFORTIS chewable tablets resulted in improvement in all signs of FAD, consistent with effective flea control. All signs were improved at least 86% and most signs were improved 96-100%.

COMFORTIS chewable tablets were palatable, accepted by free choice in 77% of the dogs.

Adverse Reactions: COMFORTIS chewable tablets, administered once a month, were not associated with any serious adverse reactions. The safety of COMFORTIS chewable tablets was evaluated through review of adverse reactions, laboratory value (complete blood count, serum chemistry profile, and urinalysis) changes, and body weight changes during the study. Both primary and secondary spinosad-treated dogs (n=330) were evaluated. Over the 90-day study period, all observations of potential

adverse reactions were recorded. Adverse reactions that occurred at an incidence > 1% within any of the 3 months of observation are presented in the following table (Table 10). The most frequently reported adverse reaction in dogs, based on owner diaries, in the COMFORTIS chewable tablets and selamectin groups was vomiting. The occurrence of vomiting, most commonly within 48 hours after treatment, decreased with repeated doses of COMFORTIS chewable tablets.

Table 10: Adverse Reactions That Occurred at an Incidence > 1% Within Any of the 3 months of Observation

	Month 1		Month 2		Month 3	
	COMFORTIS Chewable Tablets (N=330	**Selamectin (N=139[a])**	**COMFORTIS Chewable Tablets (N= 282)**	**Selamectin (N= 124)**	**COMFORTIS Chewable Tablets (N= 260)**	**Selamectin (N=125)**
Vomiting	12.7	12.2	7.8	3.2	5.8	4.8
Decreased Appetite	9.1	5.0	2.8	1.6	1.9	0.8
Lethargy	7.6	5.0	3.5	4.0	1.2	0.8
Diarrhea	6.7	5.0	4.3	0.8	1.2	0.0
Cough	3.9	5.0	0.4	2.4	0.0	0.0
Polydipsia	2.4	1.4	0.7	0.0	0.4	0.0
Vocalization	1.8	0.0	0.4	0.0	0.4	0.0
Increased Appetite	1.5	0.0	0.4	0.8	0.4	0.0
Erythema	1.5	0.0	0.4	0.0	0.4	0.0
Hyperactivity	1.2	1.4	0.0	0.0	0.4	0.0
Excessive Salivation	1.2	0.0	0.4	0.0	0.0	0.0

[a] This number (n=139) is less than the total number of dogs in the safety population for selamectin (n=140) because one dog joined the study late and was only dosed at Month 3.

Two dogs with pre-existing seizure conditions in the COMFORTIS chewable tablet group experienced at least one seizure within one week of dosing. These two dogs were dosed above the labeled 30-60 mg/kg dose range. Four dogs with pre-existing seizure conditions, dosed within the labeled range of COMFORTIS chewable tablets, did not experience any seizure activity during the study.

More spinosad-treated dogs (1.5%) experienced substantial weight loss (>15% body weight change) during the course of the study than selamectin-treated dogs (0%). Additionally, spinosad-treated puppies gained less weight than selamectin-treated puppies. Fourteen of the 26 (53.8%) spinosad-treated puppies gained greater than 10% of their initial body weight compared with 25 of the 31 (80.6%) selamectin-treated puppies. A portion of this difference in the percentage of weight gain may be attributed to a higher number of selamectin-treated puppies in the five months or less age group as compared to the spinosad-treated puppies. These findings were not associated with any clinical or clinical pathology abnormalities.

Laboratory parameter changes from pre-treatment to post-treatment were within expectations for normal dog populations.

4. Study T9C010313

Title: Clinical Study: Confirmation of the Efficacy of Flavored Spinosad Tablets Administered Orally to Dogs for the Prevention of the Pre-Adult Stages of the Cat Flea (*Ctenocephalides felis*)

Type: Laboratory effectiveness

Purpose: Confirm the effectiveness of flavored spinosad tablets, administered orally to experimentally infested dogs, for the prevention of the pre-adult stages of the cat flea, *Ctenocephalides felis*, under laboratory conditions.

Investigator: Byron Blagburn, PhD, Auburn University, College of Veterinary Medicine, Auburn, AL

Animals: 12 dogs in 2 treatment groups of 6 dogs per group which included male and female, adult mongrel dogs

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups:

1) Control (0 mg/kg delivered in the chewable tablet formulation without the active ingredient)

2) Spinosad (30-90 mg/kg) (six dogs received the labeled dose of 30-60 mg/kg, six dogs received 60-90 mg/kg)

Study Duration: 33 days

Study Design: A randomized, complete block design, with 6 dogs in the treatment group and 12 dogs in the control group, was used. Dogs were dosed on test day 0, and infested with approximately 600 unfed fleas on test days 0, 4, 11, 18, 27, and 30. Debris (dander, flea feces, hair, and scales) from each dog was collected overnight after each infestation for three consecutive nights. Flea eggs were collected approximately 3 days after each infestation (test days 3, 7, 14, 21, 30, and 33). When possible, up to 250 eggs were collected from each dog and cultured with the collected debris to determine flea egg hatch rate and subsequent larval development when eggs were exposed to the debris from treated animals.

Parameters Measured: Effectiveness of spinosad against flea egg production and hatchability, larval survival, and emergence of new adult fleas was evaluated. Also, shed debris (dander, flea feces, hair, and scales) from spinosad-treated dogs was evaluated for its effect on normal (untreated) flea eggs and larvae.

Statistical Methods: A mixed model was used to compare log count + 1 of flea eggs produced in the treated group to the control group at each time point.

Results: Spinosad-treated dogs had fewer total flea eggs, hatched eggs, viable larvae, and emerged adults than the control group. The flea egg yield from spinosad-treated dogs was so low that a cumulative developmental success rate could not be calculated. Results of the analysis of flea egg production indicated that spinosad reduced the number of flea eggs produced, with a percent effectiveness greater than 96.2% (Table 11).

Table 11: Mixed Model Analysis of Transformed Post Treatment Counts of Flea Eggs Collected from Study Animals

	Spinosad		Control			
Study Day	**N[a]**	**Geometric Mean**	**N**	**Geometric Mean**	**Percent Effectiveness**	**Spinosad vs. Control P-Value**
Across 30 Study Days		0.6		250.0	99.8%	< 0.0001
3	6	0.0	12	250.0	100.0%	< 0.0001
7	6	0.0	12	250.0	100.0%	< 0.0001
14	6	0.0	12	250.0	100.0%	< 0.0001
21	6	0.0	12	243.8	100.0%	< 0.0001
30	6	9.6	12	250.0	96.2%	< 0.0001

[a] This group only contained the six animals dosed within the intended 30-60 mg/kg range.

Conclusions: The effectiveness of spinosad in the prevention of the development of pre-adult stages of the cat flea could not be determined because flea eggs could not be recovered in sufficient numbers from spinosad-treated dogs. However, spinosad tablets administered orally to dogs were effective in reducing flea egg counts.

Adverse Reactions: One dog received a spinosad dose of 85.3 mg/kg, which is above the maximum labeled dose of 60.0 mg/kg. This dog was not eating well and experienced 11% weight loss by one month post-dose. Physical examination was unremarkable and a biochemical panel revealed hypoproteinemia and hypoglobulinemia. The veterinarian presumptively diagnosed protein losing enteropathy or malnutrition and recommended nutritional support. The relationship between the spinosad overdose and this dog's condition is unclear.

Another dog received a spinosad overdose of 75 mg/kg and vomited immediately post-dose, which is likely drug-related.

5. Study T9C060330

Title: Clinical Study: Knockdown and Speed of Kill Effectiveness of Spinosad Flavored Tablets Administered for the Treatment of Adult Cat Fleas (*Ctenocephalides felis*)

Type: Laboratory effectiveness

Purpose: Confirm the knockdown and speed of kill effectiveness of spinosad flavored tablets administered orally to experimentally infested dogs, at a minimum dosage of 30 mg/kg, for the treatment of the adult cat flea, *Ctenocephalides felis*, under laboratory conditions.

Investigator: David R. Young, DVM, PhD, Young Veterinary Research Service, Turlock, CA

Animals: 60 dogs in 15 treatment groups of 4 dogs per group which included male and female, young and adult, mixed and purebred dogs

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups:

1) Untreated control
2) Negative control (0 mg/kg delivered in the chewable tablet formulation without the active ingredient)
3) Spinosad (30-90 mg/kg, 7 groups)

Study Duration: 16 days

Study Design: On test day -1, all dogs were infested with approximately 100 adult cat fleas, followed by a single treatment with a negative control (7 groups) or spinosad (7 groups) on test day 0. Fleas were counted at 0, 0.5, 1, 2, 4, 8, 24, and 48 hours post-treatment as indicated in Table 12.

Table 12: Dosage Groups Defined by Schedule for Flea Counts

Hours Post-treatment	Untreated Control	Negative Control	Spinosad
0	Group 1	N/A	N/A
0.5	--	Group 2	Group 9
1	--	Group 3	Group 10
2	--	Group 4	Group 11
4	--	Group 5	Group 12
8	--	Group 6	Group 13
24	--	Group 7	Group 14
48	--	Group 8	Group 15

Parameters Measured: Fleas were counted and percent effectiveness of spinosad was calculated based on geometric mean flea counts at 0.5, 1, 2, 4, 8, 24, and 48 hours post-treatment.

Statistical Methods: A linear model of log flea count + 1 was used to compare the fifteen treatment groups.

Results: The geometric mean live flea count in group 1 at time 0 was 97.5. All spinosad-treated groups and negative control group 2 were statistically different from group 1 ($p < 0.005$ for spinosad-treated groups and $p < 0.05$ for negative control group 2). Based on the mean geometric flea counts at 0.5 hour post treatment, spinosad started to kill fleas with a calculated effectiveness of 53.7 percent. The first time at which the geometric mean flea count for spinosad was significantly less than the negative control geometric mean live flea count was at 1 hour post-treatment. At 1 and 2 hours post-treatment, the geometric mean flea counts from dogs treated with spinosad flavored tablets were significantly less than the negative control counts, with percent effectiveness of 64.3 and 85.8%, respectively (Table 13). Effectiveness increased to 100% by 4 hours post-treatment and remained at that level through the duration of the study.

Table 13: Speed of Kill of Adult Cat Fleas (*Ctenocephalides felis*) on Dogs During the First 48 Hours after Administration of Spinosad Flavored Tablets

	Geometric Mean Live Flea Counts at Times (hours) Post-treatment						
Treatment	**0.5**	**1**	**2**	**4**	**8**	**24**	**48**
Negative Control	36.2	68.4	69.4	46.6	60.3	87.7	50.4
Spinosad	16.7	24.4[a]	9.8[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Percent Effectiveness							
% Effectiveness	53.7	64.3	85.8	100	100	100	100

[a] The spinosad groups were significantly different ($p < 0.025$) from their corresponding negative control at every time point but 0.5 hours.

Conclusions: Following a one-time oral dose, flea count reductions in spinosad-treated dogs were 57% within 30 minutes and 100% within four hours after treatment.

Adverse Reactions: One dog that received a dose of spinosad (62.3 mg/kg) higher than the maximum labeled dose (60.0 mg/kg), regurgitated undigested food approximately one hour after dosing. Regurgitation did not result in decreased effectiveness. This event is likely drug-related.

6. Study T9C010508

Title: Efficacy of Flavored Spinosad Tablets Administered Orally to Dogs, in a Simulated Home Environment, for the Prevention of Cat Flea (*Ctenocephalides felis*) Infestations

Type: Laboratory effectiveness

Purpose: To confirm the 30-day residual effectiveness of spinosad flavored tablets, administered at the proposed label dose, for the prevention of cat flea, *Ctenocephalides felis*, infestations on dogs under simulated home environment conditions.

Investigator: Byron Blagburn, Ph.D., Auburn University, College of Veterinary Medicine, Auburn, AL

Animals: 24 dogs in two treatment groups of 12 dogs per group which included male and female, adult, mongrel dogs

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups:

1) Control (0 mg/kg delivered in the chewable tablet formulation without the active ingredient)

2) Spinosad (30-60 mg/kg)

Study Duration: 125 days

Study Design: Masked, parallel arm, controlled, laboratory study utilizing a randomized complete block design. Each dog was infested with approximately 100 newly emerged, unfed adult fleas, *Ctenocephalides felis*, on test days -7, 1, 7, 14, 62, 66, and 73. Dogs were dosed according to their treatment group once on test days 0, 30, 60, and 90 and were fed at the time of dosing.

Parameters Measured: Individual animal adult flea counts, at approximately 48 hours post-infestation, were conducted on test days 3, 9, 16, 21, 28, 35, 42, 49, 66, 70, 77, 84, 91, 98, 105, 112, 119, and 125 to assess effectiveness. After counting, up to 300 fleas were replaced onto the dog. General health observations were made twice daily with additional observations at 1, 2, 4, 8, and 24 hours after each treatment administration.

Statistical Methods: Two-sided exact Wilcoxon Rank Sum tests were used to compare flea counts at each observation time. Group geometric means were calculated from individual animal live flea counts conducted from test days 3 to 125 and were used to determine percent effectiveness.

Results: The percent effectiveness ranged from 96.0 to 100% against the cat flea through test day 125 (Table 14).

Table 14: Geometric Mean Percent Effectiveness of Flavored Spinosad Tablets against *Ctenocephalides felis* Infestations of Dogs

	Study Day								
	3	**9**	**16**	**21**	**28**	**35**	**42**	**49**	**66**
Percent Effectiveness	99.7	99.7	99.5	100.0	100.0	100.0	100.0	100.0	99.7

	Study Day								
	70	**77**	**84**	**91**	**98**	**105**	**112**	**119**	**125**
Percent Effectiveness	100.0	99.8	100.0	100.0	96.0	97.4	100.0	100.0	100.0

Conclusions: This study confirmed the 30-day residual effectiveness of spinosad, dosed once orally at a dose range of 30-60 mg/kg, for the prevention of adult cat flea infestations on dogs under simulated home environment conditions.

Adverse Reactions: Two incidents of vomiting were observed in the same spinosad-treated dog 1 and 2 hours after dosing, which was attributed to spinosad. Two spinosad-treated dogs experienced diarrhea. One dog experienced bloody diarrhea on days 7 and 14. The other dog had one episode of loose stools on day 79. The relationship of these two events to the spinosad administration is unclear.

III. TARGET ANIMAL SAFETY:

A. Study D00203

Title: A Non-Clinical Laboratory Study: The Tolerance of Spinosad in Dogs at 10X the Therapeutic Dose

Type of Study: Target animal safety study conducted in accordance with Good Laboratory Practice (GLP) for non-clinical laboratory studies.

Purpose: To provide information on the toxic effects of spinosad orally administered to Beagle dogs following 10 consecutive daily doses at the proposed therapeutic high-dose range.

Study Director: Charles D. Miller DVM, PhD, Eli Lilly and Company, Greenfield, IN

Animals: Twelve Beagle dogs (6 male and 6 female) between 9 and 11 months of age and weighing between 8.2 and 10 kg. Dogs were allocated to 2 treatment groups of 6 dogs per group (3 per sex).

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups: Treatment group 1 received 810 mg spinosad daily for 10 days (up to 16.7X the maximum recommended monthly dose (60 mg/kg)). Treatment group 2

received the control article [0 mg/kg (0X)] delivered in the chewable tablet formulation without the active ingredient once daily for 10 days.

Study Duration: 24 days

Study Design: Masked, controlled, laboratory study with dogs randomized to two treatment groups by body weight. Animals were dosed either with the control or spinosad for 10 consecutive days, with a 14-day, post-dosing observation period.

Parameters Measured: Clinical observations conducted a minimum twice daily, neurological examination prior to study start, days 11, and 22, body weight twice pre-test and on days 5, 10, 17, 21, and 24, food intake twice daily, hematology and clinical chemistry twice pre-test and on days 3, 10, and 24, urinalysis once pre-test and near study termination, gross pathological exam and organ weights day 24, and histopathological exam of all major organ tissues.

Statistical Methods: Parameters were analyzed at each time point using a two-factor analysis of variance with factors treatment, sex, and their interaction with α=0.10. When available, a pre-test average was used as a covariate. Also, mean, standard deviation, and sample size for each treatment group were reported for each sex and for both combined at each time point.

Results: No treatment-related findings for physical or neurological exams were noted. Vomiting was observed at least once in 5 of 6 spinosad-treated dogs, the majority occurring within the first 6 days of the treatment period. All treated females had either weight loss or reduced weight gain during the study, but by the end of the study those animals' weights were similar to the control females. No changes in food consumption occurred when treated dogs were compared to control dogs. However, food consumption seemed to be inconsistent throughout the study for both the control and treated dogs. Moderate liver glycogen depletion was observed in treated females.

No clinically significant changes in hematology, coagulation, or urinalysis parameters occurred. Mild elevations (as high as 2.5X the day -7 values) in alanine transaminase (ALT) occurred in all spinosad-treated dogs on study days 3 and 10. These elevations were resolving by day 24; however, all ALT values remained higher than the pre-study ALT values. No subsequent follow up blood work was reported.

The primary histopathological finding was phospholipidosis, evident as multifocal cytoplasmic vacuolation, observed in lymphoid tissues of all spinosad-treated dogs. As reported in the literature, investigations have been done to determine if phospholipidosis poses a safety risk. The general consensus is that there is no evidence that the presence of phospholipidosis is deleterious to the animal[1]. It is thought that this condition resolves once the phospholipidosis-inducing drug is discontinued[2].

---

[1] Reasor, MJ and Kacew S. Drug-Induced Phospholipidosis: Are There Functional Consequences? *Society for Experimental Biology and Medicine* 2001; 226(9): 825-830.

[2] Rudman, DG. et al. Epididymal and Systemic Phospholipidosis in Rats and Dogs Treated with the Dopamine D3 Selective Antagonist PNU-1177864. *Toxicologic Pathology* 2004;32:326-332.

Conclusions: Treatment-related effects resulting from 10 consecutive daily doses of 810 mg spinosad to dogs with a 14-day post-dosing observation period were vomiting, ALT elevation, weight loss, and glycogen depletion. The long term clinical relevance of phospholipidosis is unknown.

B. Study T9C420226

Title: Non-Clinical Laboratory Study: A Margin of Safety Study of Orally Administered Spinosad to Dogs Starting at Six Weeks of Age

Type of Study: Target animal safety study conducted in accordance with GLPs for non-clinical laboratory studies

Purpose: To evaluate the safety of spinosad when administered orally to dogs every 28 days at 0X and up to 1.5, 4.4, and 7.4X the maximum recommended therapeutic dose (60 mg/kg) for six consecutive treatment intervals over 168 days.

Study Director: Michael A. Schnell, DVM, MBA, White Eagle Laboratories, Inc., Doylestown, PA

Animals: Forty-eight purpose-bred Beagle dogs (24 males and 24 females), 42 days of age at study initiation (study day 0 and puppy day 42), weighing at least 1.0 kg at 40 days of age. Dogs were allocated to 4 treatment groups of 12 puppies per group (6 per sex).

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups: Treatment group A received the control article (0 mg/kg (0X) delivered in the chewable tablet formulation without the active ingredient), treatment groups B through D received up to 1.5, 4.4, or 7.4X the maximum recommended therapeutic dose (60 mg/kg). Doses ranged from approximately 46-90 mg/kg for the 1.5X treatment group, 143-269 mg/kg for the 4.4X treatment group and 226-449 mg/kg for the 7.4X treatment group.

Study Duration: 168 days

Study Design: Masked, controlled, randomized incomplete block design with blocking factors room, sex, and litter. Two male and two female puppies from each of 12 litters were assigned to one of four treatment groups. Starting at 6 weeks of age (study day 0 and puppy day 42), puppies were administered spinosad or control tablets for the first consecutive 5 days of each of the six, 28-day treatment intervals according to Table 15 below.

Table 15: Treatment Tablet Dosing Schedule

	Tablet Dosed (Control or Spinosad)[a]				
Treatment Group	**Study Day 0[b]**	**Study Day 1**	**Study Day 2**	**Study Day 3**	**Study Day 4**

A-Control	C	C	C	C	C
B-1.5X	S	C	C	C	C
D-4.4X	S	S	S	C	C
C-7.4X	S	S	S	S	S

[a] C=Control tablet dosed; S=Spinosad tablet dosed at 1.5X the maximum recommended therapeutic dose level.
[b] Dose interval 1 is shown. The same relationship schedule was followed in 28 day intervals for the second through the sixth dose interval.

Parameters Measured: Repeated measures: body weight, clinical chemistry, hematology, and coagulation. Single endpoints: urinalysis and organ weights.

Statistical Methods: Repeated measures data was analyzed with a repeated measures mixed-effects model with fixed effects treatment, sex, observation day (time), treatment*sex*time and all two-way interactions and random effects litter and litter by sex interaction. A baseline covariate was also included as a fixed effect. The minimum Akaike Information Criterion was used to select the covariance matrix. Single-dose endpoints were analyzed using a mixed-effect linear model with fixed effects for treatment, sex and treatment by sex interaction, and random effects for litter and litter by sex interaction.

Results: A female in the control group and a female in the 1.5X group were euthanized early due to poor condition. They were diagnosed with diabetes mellitus and malabsorption, respectively, unrelated to spinosad treatment. All other animals survived to the end of the study.

Vomiting was the most common observation. The incidence of vomiting one hour following spinosad treatment decreased over time, with substantial reduction after the second day of treatment. The incidence of vomiting fell sharply by the third dosing interval (14-week-old puppies) across all treatment groups.

From the third dosing interval onward, at least two of the three spinosad groups had significantly lower mean body weights than the control group (Table 16). Both treatment and control groups remained within the expected normal body weights for laboratory Beagle puppies.

Table 16: Mean Body Weight by Dosing Interval[a]

Dosing Interval	Treatment Group	Raw Mean BW (kg)	P-value
3	0X	6.17	-
	1.5X	5.87	0.42
	4.4X	**4.97**	**0.03**
	7.4X	**5.06**	**0.08**
4	0X	7.97	-
	1.5X	**7.35**	**0.08**
	4.4X	**6.65**	**0.02**
	7.4X	**6.66**	**0.02**

5	0X	8.95	-
	1.5X	**8.33**	**0.09**
	4.4X	7.87	0.11
	7.4X	**7.73**	**0.06**
6	0X	9.84	-
	1.5X	**9.09**	**0.05**
	4.4X	8.76	0.13
	7.4X	**8.34**	**0.01**
Study End	0X	10.26	-
	1.5X	**9.46**	**0.08**
	4.4X	9.06	0.12
	7.4X	**8.60**	**0.01**

[a] The **bolded** values are statistically significant. There were no statistically significant differences observed between baseline and dosing intervals 1 and 2.

No clinically relevant spinosad-related changes occurred in hematology, urinalysis or coagulation parameters during the study. Of the clinical pathology parameters, ALT was statistically significantly different from control as shown in Table 17.

ALT for the 7.4X spinosad group was statistically significantly increased from control at puppy day (PD) 140 (20 wks of age), PD168 (24 wks of age), and at PD196 (28 wks of age). These changes were not outside of the upper limits of the normal range for ALT; however they follow a rising trend and appear to be related to spinosad treatment. ALT for the control, 1.5, and 4.4X groups were not statistically significantly different.

Table 17: Statistically Significant Mean ALT Values[a]

Treatment Group	Puppy Day	ALT (U/L)	P-value
0X	140	31.5	-
	168	30.3	-
	196	32.7	-
7.4X	140	**42.3**	**0.07**
	168	**45.3**	**0.00**
	196	**51.4**	**0.00**

[a] The **bolded** values are statistically significant

There were no lesions associated with spinosad treatment found on gross necropsy. Vacuolation of lymphoid tissue was a treatment effect, affecting some dogs in the 4.4X group and all dogs in the 7.4X group. The tonsils were usually the most prominently affected. Vacuolation was comparable between sexes, although the 7.4X males had a slightly higher incidence of the observation in Peyer's patches of the ileum, cecum, and jejunum. While the incidences were the same, the severity of vacuolation in the tonsils was slightly greater in the 7.4X males than the 7.4X females. One dog in each of the

control, 1.5 and 7.4X groups had necrosis or depletion of bone marrow, but this could not be attributed to treatment with spinosad.

Conclusions: The administration of spinosad at doses up to 7.4X was clinically well tolerated in the puppies Treatment related effects included vomiting, primarily observed in the one-hour post-dose period; reduced growth rate in all spinosad-treated groups; and histological vacuolation (phospholipidosis) of the lymphoid tissue in the 4.4 and 7.4X groups. The vomiting, as well as the variation in body weights, did not have an impact on the overall health of the puppies. Vacuolation (phospholipidosis) of the lymphoid tissue had no apparent clinical impact on the dogs during the course of the study, and was not observed at the 1.5X dose. The long-term clinical relevance of phospholipidosis in dogs is unknown.

C. Study T9C420230

Title: A Domestic Animal Safety Study (One-Generation Reproduction) of Spinosad in the Female Beagle Dog

Type of Study: Target animal safety study conducted in accordance with GLPs for non-clinical laboratory studies

Purpose: To evaluate the safety of spinosad administered every 28 days at 0X and up to 1.3 and 4.4X the maximum recommended therapeutic dose, prior to mating, during gestation, and postpartum.

Study Director: Michael A. Schnell, DVM, MBA, White Eagle Laboratories, Inc., Doylestown, PA

Animals: 33 purpose bred female Beagles in 3 groups of 11 dogs per group

Dosage Form: Flavored spinosad tablet

Route of Administration: Oral

Dosage Groups: Treatment group 1 (0 mg/kg (0X) delivered in the chewable tablet formulation without the active ingredient) received one control tablet on days 0, 7, and 14. Treatment group 2 (1.3X) received one spinosad tablet on day 0, and one control tablet on days 7 and 14. Treatment group 3 (4.4X) received one spinosad tablet on days 0, 7, and 14. Doses ranged from approximately 49-82 mg/kg for the 1.3X treatment group and approximately 134-264 mg/kg for the 4.4X treatment group.

Study Duration: 482 days

Study Design: This was a parallel, controlled, one-generation study with the adult dam as the experimental unit. Based on start date and previous litter history, dams were randomized to one of three treatment groups. Dams were housed individually and administered a combination of tablets, as described above, on days 0, 7, and 14 of every 28-day interval prior to mating, during gestation, and during a six-week lactation period. Each treatment group consisted of eleven dams (total of 33) with the first ten coming into estrus mated.

Parameters Measured: Clinical observations, physical examinations, fertility, viability, mortality, body weight, gross pathology, and histopathology

Statistical Methods: Dam body weights were analyzed using a linear mixed model with fixed effects baseline covariate, treatment, time (i.e., study day), and treatment by time interaction, with individual female as the random effect. Separate analyses were computed on data from the pre-mating, gestation, and parturition phases.

For reproductive indices, a linear one-way analysis of variance (ANOVA) model was used to test for treatment differences. Body weights of pups were analyzed using a linear mixed model with litter as the experimental unit and fixed effects for baseline covariate, maternal treatment, time, and the treatment by time interaction, with litter as a random effect. Vomiting within one hour of dosing was summarized with frequency tables across the entire study by reproductive phase.

Results: All dams survived the study. All 30 dams that were bred in this study became pregnant. No effect was noted on any parameter for the successful litters, such as number of pups born and viability parameters. One 4.4X female aborted her litter on gestation day 49. No late term abortions were noted in the 1.3X or control group. Two females (1.3 and 4.4X), both of which had abnormally rapid progression through their estrus cycle, experienced early pregnancy loss. Subsequent necropsy findings confirmed that embryo resorption occurred.

Spinosad tablet administration was accompanied by vomiting within one hour of dosing in six dams in each of the 1.3 and 4.4X treatment groups. A greater frequency of vomiting occurred during the pre-mating phase when comparing both the 1.3 and 4.4X treatment groups to the control group. Vomiting within one hour of dosing was not noted for any control animal. Vomiting outside of this one-hour post-dose period occurred in all (0, 1.3, and 4.4X) groups without any clear relationship to dose.

No treatment-related effects were noted for mortality and body temperature for the dams and puppies or dam body weights. No spinosad-related findings were noted for necropsy or histopathology findings. Puppy loss from full term pregnancies occurred across all treatment groups, with no clear relation to dose group, nor were reasons for either death or euthanasia established. The puppies from the treated dams experienced more lethargy (4.4X group only), dehydration, weakness, and felt cold to the touch (4.4X group only). Puppies from dams treated at 1.3X the maximum recommended therapeutic dose had lower body weights than puppies from control dams. However, no difference was found between the 4.4X group and the control group.

Conclusions: With the exception of vomiting, spinosad administrations throughout the female Beagle reproductive cycle were generally clinically well-tolerated in the adult dogs. The relationship between spinosad to the abortion in the one dam in the 3X group and litter resorptions in the two dams from each treated group is unclear. However, spinosad administration appeared to contribute to a general increase in adverse clinical effects in puppies from treated dams versus control dams.

D. Study T9C750101

Title: Colostrum, Plasma and Milk Study of Spinosyns Following Oral Dosing of Spinosad to Beagle Dogs

Type of Study: Pilot Target Animal Safety

Purpose: To provide pilot information regarding the safety of spinosad at 1.5X the maximum recommended therapeutic dose in pregnant bitches and their offspring, to determine if spinosad partitions into colostrum/milk and if so, how these compare to reference plasma levels, and to calculate the approximate amount of spinosyns ingested by the average puppy through suckling over a 1-2 week period as it relates to the therapeutic adult dose.

Study Director: Michael A. Schnell, DVM, MBA, White Eagle Laboratories, Inc., Doylestown, PA

Animals: 3 pregnant female dogs

Dosage Form: Capsule containing spinosad technical powder (experimental formulation)

Route of Administration: Oral

Dosage Groups: There was one treatment group. All three dogs received spinosad.

Study Duration: 59 days

Study Design: Dogs were dosed twice at 90 mg/kg, once at day 28 of gestation and once 1 day prior to imminent parturition.

Parameters Measured: Clinical observations, physical examinations, and body weights for dams and puppies, maternal clinical pathology, milk/colostrum spinosyn concentrations, puppy histopathology and gross pathology

Results: All dams survived the study. No treatment-related effects were noted for clinical observations, physical examinations, body weights, or clinical pathology in the dams. The spinosad milk: reference plasma ratio ranged from 2.2-3.5. Mortality and morbidity varied across the three litters. Of the total 24 puppies born, 10 (41%) completed the study. Four puppies (16.7%) were immediately culled to reduce litter size, according to standard laboratory procedures. Causes of mortality for the other 10 puppies included stillborn (3 puppies) and death or euthanasia due to deteriorating health (7 puppies). Puppy morbidity included lethargy, dehydration, weakness, and thinness. Mortality and morbidity were greatest in puppies from the dam with the highest spinosyns level in milk (calculated dose of 2.2 mg/kg over a 9-day period). This dam also had the largest litter (10 puppies), which likely contributed to puppy mortality.

Conclusions: Spinosyns preferentially partition into milk over plasma, suggesting that in post-parturient bitches, the mammary gland serves to excrete spinosyns. This mammary excretion of spinosyns may negatively affect nursing puppies, as morbidity and mortality were highest in the puppies from the bitch that had the highest milk

spinosyn concentrations. However, it is difficult to interpret the relationship between spinosyn concentrations in milk and puppy health because of a lack of a control group, limited animal numbers, and confounding influence of the large litter size in one dam.

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 COMFORTIS chewable tablets:

Human Warnings are provided on the product label as follows: "Not for human use. Keep this and all drugs out of the reach of children."

The material safety data sheet (MSDS) contains more detailed occupational safety information. To report adverse reactions in users or to obtain a copy of the MSDS for this product call 1-888-545-5973.

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 COMFORTIS chewable tablets, when used according to the label, are safe and effective for killing fleas and for the treatment and prevention of flea infestations.

A. Marketing Status:

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is needed to monitor for and respond to adverse reactions

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

C. Patent Information:

COMFORTIS chewable tablets are under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
6, 664, 237 B1	August 12, 2019

VII. ATTACHMENTS:

Facsimile Labeling:

- Package Insert
- Client Information Sheet
- Dispensing Carton (140 mg, 270 mg, 560 mg, 810 mg, 1620 mg)
- Display Carton (140 mg, 270 mg, 560 mg, 810 mg, 1620 mg)
- Blister Pack (140 mg, 270 mg, 560 mg, 810 mg, 1620 mg)
- Stickers