



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: November 24, 2004

MEMORANDUM

Subject: EPA File Symbol: 2517-IN SERGEANT'S CYPHENOTHHRIN + IGR
SQUEEZE-ON FOR DOGS
DP Barcode: D305948
Decision No.: 338118
PC Codes: 129013 Cyphenothrin (CAS #39515-40-7), 129032
Pyriproxyfen (CAS #95737-68-1)

From: Byron T. Backus, Ph.D.
Technical Review Branch
Registration Division (7505C)

Byron T. Backus
11-24-2004
✓CR

To: Linda DeLuise/George LaRocca RM 13
Insecticide Branch
Registration Division (7505C)

Applicant: SERGEANT'S PET CARE PRODUCTS, INC.

FORMULATION DECLARATION FROM LABEL:

<u>Active Ingredient(s):</u>	% by wt
Cyphenothrin (CAS #39515-40-7).....	40.0%
Nylar (CAS #95737-68-1).....	2.0%
<u>Inert Ingredients:</u>	58.0%
Total:	100.00%

ACTION REQUESTED:

The Risk Manager requests:

Tox waiver request for acute inhalation; review of 5 other acute studies and a companion animal study.

BACKGROUND:

This package includes an acute oral LD₅₀ study (rat, up-and-down procedure, defaulting to an acute toxic class procedure, MRID 46166103); acute dermal LD₅₀ study (rat; MRID 46166104); primary eye irritation study (rabbit; MRID 46166105); primary dermal irritation study (rabbit; 46166106) and a dermal sensitization study (guinea pig; 46166107), as well as a companion animal safety study in dogs (MRID 46166108). The five acute toxicity studies were conducted at Product Safety Labs, New Jersey. The companion animal safety study was conducted at Stillmeadow, Inc. In addition, there is a waiver request for an inhalation study. All studies were conducted on Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% Nylar.

RECOMMENDATIONS:

1. The companion animal (dog) safety study in MRID 46166108 has been reviewed and has been classified as acceptable for puppies (12 weeks and older) and adult dogs. It is concluded that there is an adequate margin of safety (at least 5X) between the exposure associated with the proposed use level for this formulation in dogs, and that at which significant adverse systemic effects (not seen in this study, but which might include ear twitching, muscle tremors, drooling) may occur. For dermal effects an effect was observed in one puppy in Group III (treated at essentially a 7.5X dose level), but in none of the other dogs (including the puppies in Group II, dosed at 1.5X) indicating a reasonably low potential for this effect in dogs treated at the proposed use level.
2. It is noted that the test material was supplied in (and applied from) unidose 1.5 mL ampules. However, the report states that the mean volume delivered from a single 1.5 mL ampule was 1.17 mL, and the registrant is proposing packaging this product in 3.0 and 4.5 (as well as 1.0 and 1.5) mL tubes. This is acceptable only if the 3.0 mL tubes deliver no more than 2.34 (2 x 1.17) mL and the 4.5 mL tubes deliver no more than 3.51 (3 x 1.17) mL.
3. The five acute toxicity studies have been reviewed and classified as acceptable. In addition, TRB has no objection to the registrant's waiver request for an acute inhalation study, based on the product form (a yellow liquid), its proposed packaging

(1.0, 1.5, 3.0 or 4.5 mL tubes or ampules), the method of application (as a spot-on or stripe-on to the dog's back), and the relatively low inhalation toxicity of technical Cyphenothrin (one report from the open literature gives a rat LC50 of 1.85 mg/L, or EPA toxicity category III; extrapolating from this the inhalation LC50 value for a 40% Cyphenothrin-60% inert product would then be greater than 4 mg/L, or EPA toxicity category IV by this exposure route).

4. Based on the results of the acute toxicity studies, the following is the acute toxicity profile for EPA File Symbol: 2517-IN SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS. The signal word of the product would be CAUTION, as proposed by the registrant:

<u>Study Type</u>	<u>Tox. Cat.</u>	<u>Classification & MRID #</u>
Acute Oral LD ₅₀ (rat)	III	Acceptable (#46166103)
Acute Dermal LD ₅₀ (rat)	III	Acceptable (#46166104)
Acute Inhalation LC ₅₀	IV	Waived
Primary Eye Irritation (rabbit)	III	Acceptable (#46166105)
Primary Dermal Irritation (rabbit)	IV	Acceptable (#46166106)
Dermal Sensitization (guinea pig)	Negative	Acceptable (#46166107)

5. Based on the acute toxicity profile and proposed uses, the following is the precautionary labeling for this product, as obtained from the Label Review System:

PRODUCT ID #: 002517-00080

PRODUCT NAME: SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS

SIGNAL WORD: CAUTION

PRECAUTIONARY STATEMENTS

Hazards to Humans and Domestic Animals:

Harmful if swallowed or absorbed through skin.. Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

First Aid:

If on skin:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a poison control center or doctor for treatment advice.

If swallowed:

- Call a poison control center or doctor immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to by a poison control center or doctor.
- Do not give anything to an unconscious person.

[Cyphenothrin 40%; Pyriproxyfen 2%]

EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHHRIN + IGR SQUEEZE-ON FOR DOGS

Reviewer: Byron T. Backus, Ph.D.
Risk Manager (EPA): 13

Date: November 19, 2004

STUDY TYPE: Acute Oral Toxicity - Rat; OPPTS 870.1100; OECD 425

TEST MATERIAL (% a.i.): Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared. From the certificate of analysis (p. 15 of MRID 4666103) this contained 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% Nylar. From information on p. 11 of MRID 46166104 the specific gravity of the test material was 1.061 g/mL. The test material is described as a clear, light yellow liquid.

SYNONYMS: The test material description is consistent with the proposed product 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs (although this product does not contain Methoprene) with a label declaration of: Cyphenothrin 40.0% and Pyriproxyfen (Nylar) 2.0%.

CITATION: Moore, G. (2003) Acute Oral Toxicity Up and Down Procedure in Rats: Cyphenothrin-IGR Squeeze-On for Dogs. Project Number: 13320, P320/UDP. Unpublished study prepared by Product Safety Labs, Food Product Laboratory and Silliker Laboratories of New Jersey, Inc. 16 p. Study Completion Date: May 20, 2003. MRID 46166103.

SPONSOR: MCLAUGHLIN GORMLEY KING COMPANY, 8810 Tenth Avenue North, Minneapolis, MN 55427

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 46166103), conducted using the up-and-down procedure but defaulting to the acute toxic class method, Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Gokilaht (Cyphenothrin), 3.0% Methoprene and 2.00% Nylar was administered by oral gavage at 2000 mg/kg to a single Sprague-Dawley derived 9-week-old albino fasted (overnight) female rat. When this rat survived, four additional fasted (overnight) female rats of the same strain, age, body weight range (164-182 g) and source (Ace Animals, Inc., Boyertown, PA) were also dosed at 2000 mg/kg.

On the day of dosage rats were observed for several hours for mortality and signs of gross toxicity for several hours post-dosing. They were then observed at least once a day for the remainder of the 14-day observation period.

On the day of dosage rats were observed at least 3 times within the first 4 hours after dosing for clinical signs of toxicity and mortality and then at least once daily for the remainder of the 14-day observation period. Individual body weights were recorded just prior to dosing (Day 0) and on days 7 and 14. Individual body weights were recorded predosing and on days 7 and 14.

Two rats died within 24 hours of dosage with no clinical signs observed prior to death. Two rats which survived showed reduced fecal volume, ventral staining and hypoactivity, with recovery by Day 4. All survivors gained weight in the period from Day 0 (predose) to Day 7 and again from Day 7 to 14.

[Cyphenothrin 40%; Pyriproxyfen 2%]
EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS

Postmortem necropsy findings in the rats which died showed discoloration of the lungs and intestines and fluid filled stomachs. Gross necropsy findings in rats surviving to terminal sacrifice were unremarkable.

Estimated Oral LD₅₀ in female rats > 2000 mg/kg.

EPA File Symbol 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 40% Cyphenothrin and 2% Pyriproxyfen (Nylar) is in EPA toxicity category III in terms of oral exposure based on the observed LD₅₀ (>2000 mg/kg) in female rats.

This acute oral study is classified as acceptable. It does satisfy the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 16), and [No] Data Confidentiality (p. 2) statements were provided.

RESULTS and DISCUSSION:

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: Friday, October 15, 2004, 4:37:11 PM

Data file name: Cyphenothrin-IGR.dat

Last modified: 10/15/2004 4:37:11 PM

Test/Substance: Cyphenothrin-IGR

Test type: Limit Test

Limit dose (mg/kg): 2000

Assumed LD50 (mg/kg): Default

Assumed sigma (mg/kg): 0.5

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
-----------	-----------	--------------	-------------------	------------------

1	8040	2000	O	O
2	8198	2000	O	O
3	8239	2000	O	O
4	8372	2000	X	X
5	9407	2000	X	X

(X = Died, O = Survived)

Dose Recommendation: The limit test is complete.

[Cyphenothrin 40%; Pyriproxyfen 2%]
EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHHRIN + IGR SQUEEZE-ON FOR DOGS

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
2000	3	2	5
All Doses	3	2	5

Statistical Estimates:

The LD50 is greater than 2000 mg/kg.

Dose (mg/kg bw)	Mortality/Number Tested		
	Males	Females	Combined
2000	-	2/5	-

Statistics - Not necessary to compute the oral LD₅₀.

A. Mortality - As noted in the table above.

B. Clinical observations - Two rats died within 24 hours of dosage with no clinical signs observed prior to death. Two rats which survived showed reduced fecal volume, ventral staining and hypoactivity, with recovery by Day 4. All survivors gained weight in the period from Day 0 (predose) to Day 7 and again from Day 7 to 14.

C. Gross Necropsy - Postmortem necropsy findings in the rats which died showed discoloration of the lungs and intestines and fluid filled stomachs. Gross necropsy findings in rats surviving to terminal sacrifice were unremarkable.

D. Reviewer's Conclusions: The study is acceptable. EPA File Symbol 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 40% Cyphenothrin and 2% Pyriproxyfen (Nylar) is in EPA toxicity category III in terms of oral toxicity based on the observed LD₅₀ (>2000 mg/kg) in female rats.

E. Deficiencies - None

Reviewer: Byron T. Backus, Ph.D.
Risk Manager (EPA): 13

Date: November 22, 2004

STUDY TYPE: Acute Dermal Toxicity - Wistar rats - OPPTS 870.1200; OECD 402

TEST MATERIAL (% a.i.): Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared. From the certificate of analysis (p. 15 of MRID 4666103) this contained 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% Nylar. From information on p. 11 of MRID 46166104 the specific gravity of the test material was 1.061 g/mL. The test material is described as a clear, light yellow liquid.

SYNONYMS: The test material description is consistent with the proposed product 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs (although this product does not contain Methoprene) with a label declaration of: Cyphenothrin 40.0% and Pyriproxyfen (Nylar) 2.0%.

CITATION: Moore, G. (2003) Acute Dermal Toxicity Study in Rats - Limit Test: Cyphenothrin-IGR Squeeze-On for Dogs. Project Number: 13321, P322. Unpublished study prepared by Product Safety Labs, Food Product Laboratory and Silliker Laboratories of New Jersey, Inc. 16 p. Study Completion Date: May 20, 2003. MRID 46166104.

SPONSOR: MCLAUGHLIN GORMLEY KING COMPANY, 8810 Tenth Avenue North, Minneapolis, MN 55427

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID #46166104), a group (5M & 5F) of Sprague-Dawley derived albino rats (source: Ace Animals, Inc., Boyertown, PA; Males: 300-318 g; Females: 178-204 g; young adult [indicated by body weight data]) were dermally exposed (approximately 10% of body surface) for 24 hrs to 2000 mg/kg of undiluted Cyphenothrin-IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen). The test material was held in contact by a gauze pad and Durapore tape.

Rats were observed several times after application on day 0 and once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing (day 0) and on days 7 and 14.

There was no mortality and there were no signs of systemic toxicity. Three males showed some dermal irritation (erythema and/or edema) with clearing by day 2. All rats gained weight from day 0 to 7 and from day 7 to 14.

No gross abnormalities were observed at post-sacrifice necropsy.

Dermal LD₅₀ Males > 2000 mg/kg (0/5 died)
Females > 2000 mg/kg (0/5 died)
Combined > 2000 mg/kg (0/10 died)

Based on the rat LD₅₀ > 2000 mg/kg, Cyphenothrin-IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar is in EPA toxicity category III in terms of dermal toxicity.

[Cyphenothrin 40%; Pyriproxyfen 2%]
EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS

This acute dermal study is classified as acceptable. It does satisfy the guideline requirement for an acute dermal study (OPPTS 870.1200; OECD 402) in the rat.

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 16), and [No] Data Confidentiality (p. 2) statements were provided.

RESULTS and DISCUSSION:

Dose (mg/kg bw)	Mortality/Number Tested		
	Males	Females	Combined
2000	0/5	0/5	0/10

Statistics - Not necessary to compute the dermal LD₅₀.

A. Mortality - None, as noted in the table above.

B. Clinical observations - There were no signs of systemic toxicity. Three males showed some dermal irritation (erythema and/or edema) with clearing by day 2. All rats gained weight from day 0 to 7 and from day 7 to 14.

C. Gross Necropsy - No gross abnormalities were observed at post-sacrifice necropsy.

D. Reviewer's Conclusions: The study is acceptable. Based on the rat LD₅₀ > 2000 mg/kg, Cyphenothrin-IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen) is in EPA toxicity category III in terms of dermal toxicity.

E. Deficiencies - None

Reviewer: Byron T. Backus, Ph.D.
Risk Manager (EPA): 13

Date: November 23, 2004

STUDY TYPE: Primary Eye Irritation - NZW Rabbit; OPPTS 870.2400; OECD 405

TEST MATERIAL (% a.i.): Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared. From the certificate of analysis (p. 15 of MRID 4666103) this contained 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% Nylar. From information on p. 11 of MRID 46166104 the specific gravity of the test material was 1.061 g/mL. The test material is described as a clear, light yellow liquid.

SYNONYMS: The test material description is consistent with the proposed product 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs (although this product does not contain Methoprene) with a label declaration of Cyphenothrin 40.0% and Pyriproxyfen (Nylar) 2.0%.

CITATION: Moore, G. (2003) Primary Eye Irritation Study in Rabbits: Cyphenothrin-IGR Squeeze-On for Dogs. Project Number: 13322, P324. Unpublished study prepared by Product Safety Labs, Food Products Laboratory and Silliker Laboratories of New Jersey, Inc. 17 p. Study Completion Date: May 20, 2003. MRID 46166105.

SPONSOR: MCLAUGHLIN GORMLEY KING COMPANY, 8810 Tenth Avenue North, Minneapolis, MN 55427

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 46166105), 0.1 mL of undiluted Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen), was instilled into the conjunctival sac of one eye of each of 3 adult New Zealand White Rabbits (weights: not reported; ages: young adult; source: Davidson's Mill Farm, South Brunswick, NJ), with observations and scoring at 1, 24, 48 and 72 hours after instillation.

No corneal opacity was observed (with 2% ophthalmic fluorescein sodium used at 24 hours to verify the absence of corneal opacity at that reading). 3/3 eyes were positive for conjunctival redness (score of 2) at 1 and 24 hours. All eyes were completely clear (all scores zero) at 72 hours.

In this study, Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen) is in EPA toxicity category III based on the presence of grade 2 conjunctival redness in 3/3 eyes at 24 hrs which subsequently cleared by 72 hrs.

This study is classified as acceptable. It does satisfy the guideline requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in the rabbit.

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 17), and [No] Data Confidentiality (p. 2) statements were provided.

RESULTS AND DISCUSSION:

Observations	Number "positive"/number tested			
	1 hr	24 hrs ²	48 hrs	72 hrs
Corneal Opacity	0/3	0/3	0/3	0/3
Iritis	0/3	0/3	0/3	0/3
Conjunctivae:				
Redness ¹	3/3	3/3	0/3	0/3
Chemosis ¹	0/3	0/3	0/3	0/3
Discharge ¹	3/3	0/3	0/3	0/3

¹Score of 2 or more considered positive

²Fluorescein staining was used to verify the absence of corneal opacity.

A. Observations - No systemic effects were observed. 3/3 eyes were positive for conjunctival redness (score of 2) at 1 and 24 hours. All eyes were completely clear (all scores zero) at 72 hours.

B. Reviewer's Conclusions: The study adequately defines a Toxicity Category III hazard potential in terms of eye exposure potential for Cyphenothrin-IGR Squeeze-On for Dogs, a clear, light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nyilar (Pyriproxyfen).

C. Deficiencies - None

[Cyphenothrin 40%; Pyriproxyfen 2%]

EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS

Reviewer: Byron T. Backus, Ph.D.
Risk Manager (EPA): 13

Date: November 23, 2004

STUDY TYPE: Primary Dermal Irritation - NZW Rabbit; OPPTS 870.2500; OECD 404

TEST MATERIAL (% a.i.): Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared. From the certificate of analysis (p. 15 of MRID 4666103) this contained 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% Nylar. From information on p. 11 of MRID 46166104 the specific gravity of the test material was 1.061 g/mL. The test material is described as a clear, light yellow liquid.

SYNONYMS: The test material description is consistent with the proposed product 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs (although this product does not contain Methoprene) with a label declaration of Cyphenothrin 40.0% and Pyriproxyfen (Nylar) 2.0%.

CITATION: Moore, G. (2003) Primary Skin Irritation Study in Rabbits: Cyphenothrin-IGR Squeeze-On for Dogs. Project Number: 13323, P326. Unpublished study prepared by Product Safety Labs, Food Products Laboratory and Silliker Laboratories of New Jersey, Inc. 17 p. Study Completion Date: May 20, 2003. MRID 46166106.

SPONSOR: MCLAUGHLIN GORMLEY KING COMPANY, 8810 Tenth Avenue North, Minneapolis, MN 55427

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 46166106), 0.5 mL aliquots of undiluted Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen), were applied to dermal sites on each of 3 (2M & 1F) young adult New Zealand White albino rabbits (source: Davidson's Mill Farm, South Brunswick, NJ) with 4-hour semioccluded exposure.

After 4 hours, the gauze patch and holding tape were removed. The test sites were scored (Draize) at 1, 24, 48 and 72 hrs and at 7 and 10 days.

No edema was observed (all scores for edema were zero). All sites scored one for erythema at 1 hour and 2 at 24, 48 and 72 hours. One site scored 2 for erythema on day 7 while the other two scored 1. All scores were zero on day 10. The PII (average of scores at 1, 24, 48 & 72 hrs) = 1.75.

In this study, Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen) is in EPA Toxicity Category IV for dermal irritation effects, based on the PII of 1.75 and relatively low score (grade 2, characterized as well-defined) for erythema at 72 hrs [EPA Toxicity Category III would be characterized by moderate or grade 3 erythema at 72 hrs] following 4-hr semi-occluded exposure.

This study is classified as acceptable. It does satisfy the guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.

[Cyphenothrin 40%; Pyriproxyfen 2%]

EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHHRIN + IGR SQUEEZE-ON FOR DOGS

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 17), and [No] Data Confidentiality (p. 2) statements were provided.

RESULTS and DISCUSSION:

A. Observations - No edema was observed (all scores for edema were zero). All sites scored one for erythema at 1 hour and 2 at 24, 48 and 72 hours. One site scored 2 for erythema on day 7 while the other two scored 1. All scores were zero on day 10. The PII (average of scores at 1, 24, 48 & 72 hrs) = 1.75.

B. Results - The PII (average of 1, 24, 48 and 72-hour scores) = 1.75. The mean irritation score on day 3 was 2.0 (erythema: 2.0; edema: 0.0).

C. Reviewer's Conclusions - The study adequately demonstrates a Toxicity Category IV hazard potential in terms of dermal irritation for Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen).

D. Deficiencies - None

[Cyphenothrin 40%; Pyriproxyfen 2%]

EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHRIN + IGR SQUEEZE-ON FOR DOGS

Reviewer: Byron T. Backus, Ph.D.
Product Manager (EPA): 13

Date: November 23, 2004

STUDY TYPE: Dermal Sensitization - albino Guinea Pig; OPPTS 870.2600; OECD 406, 429

TEST MATERIAL (% a.i.): Cyphenothrin-IGR Squeeze-On for Dogs (2824) MGK GLP Project #1683A - Lab Prepared. From the certificate of analysis (p. 15 of MRID 4666103) this contained 39.87% Gokilaht (Cyphenothrin), 3.00% Methoprene and 2.00% NyLar. From information on p. 11 of MRID 46166104 the specific gravity of the test material was 1.061 g/mL. The test material is described as a clear, light yellow liquid.

SYNONYMS: The test material description is consistent with the proposed product 2517-IN Sergeant's Cyphenothrin + IGR Squeeze-On for Dogs (although this product does not contain Methoprene) with a label declaration of Cyphenothrin 40.0% and Pyriproxyfen (NyLar) 2.0%.

CITATION: Moore, G. (2003) Dermal Sensitization Study in Guinea Pigs: Buehler Method: Cyphenothrin-IGR Squeeze-On for Dogs. Project Number: 13324, P328. Unpublished study prepared by Product Safety Labs, Food Products Laboratory and Silliker Laboratories of New Jersey, Inc. 25 p. Study Completion Date: May 20, 2003. MRID 46166107.

SPONSOR: MCLAUGHLIN GORMLEY KING COMPANY, 8810 Tenth Avenue North, Minneapolis, MN 55427

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 46166107) with Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% NyLar (Pyriproxyfen), a group of 20M Hartley albino guinea pigs (373-428 g; young adult; source: Elm Hill Breeding Labs, Chelmsford, MA) were each dermally exposed (6 hours) to a 0.4 mL aliquot of test material on a once-a-week basis for 3 consecutive weeks. After a two week rest period they were then dermally challenged with 0.4 mL of a 75% w/w mixture of the test material in mineral oil at a previously unexposed site. An additional 10 previously unexposed male guinea pigs received were similarly treated. Challenge sites on all 30 guinea pigs were evaluated and scored for erythema at 24 and 48 hours after the application.

Following challenge, 7/20 previously exposed guinea pigs showed very slight (score of 0.5) erythema at 24 hours; all scored zero at 48 hours. 4/10 controls showed very slight (score of 0.5) erythema at 24 hours; all scored zero at 48 hours.

The report includes results from a positive control study (PSL Study #12371) which was conducted with technical (85%) alpha-Hexylcinnamaldehyde and completed on August 15, 2002. The results (3/10 previously exposed, 0/5 naive control guinea pigs showing a positive response at challenge) were appropriate. The study dates for the testing with Cyphenothrin-IGR Squeeze-On for Dogs were from March 13 to April 11, 2003. While slightly outside the 6-month period indicated in the Guidelines, it is concluded the overall study findings are acceptable.

[Cyphenothrin 40%; Pyriproxyfen 2%]

EPA File Symbol 2517-IN: SERGEANT'S CYPHENOTHHRIN + IGR SQUEEZE-ON FOR DOGS

In this study there were no indications that Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen) is a dermal sensitizer.

This study is classified as acceptable. It does satisfy the guideline requirement for a dermal sensitization study (OPPTS 870.2600; OECD 406, 429) in the Guinea pig.

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 25), and [No] Data Confidentiality (p. 2) statements were provided.

I. PROCEDURE

A. Induction - Each of 20 male Hartley albino guinea pigs was treated once a week for 3 consecutive weeks to a 6-hour exposure to 0.4 mL undiluted Cyphenothrin-IGR Squeeze-On for Dogs.

B. Challenge - Twenty-seven days after the first induction exposure 0.4 mL of a 75% w/w mixture of the test material in mineral oil was applied to a naive site on the right side of each guinea pig at a previously unexposed site. These sites were evaluated and scored for erythema at 24 and 48 hours after the challenge application.

C. Naive Controls - At the time the 20 previously induced guinea pigs were challenged, 10 previously unexposed (negative control) guinea pigs were similarly challenged.

II. RESULTS and DISCUSSION:

A. Reactions and duration - Following challenge, 7/20 previously exposed guinea pigs showed very slight (score of 0.5) erythema at 24 hours; all scored zero at 48 hours. 4/10 controls showed very slight (score of 0.5) erythema at 24 hours; all scored zero at 48 hours.

B. Positive control - The report includes results from a positive control study (PSL Study #12371) which was conducted with technical (85%) alpha-Hexylcinnamaldehyde and completed on August 15, 2002. The results (3/10 previously exposed, 0/5 naive control guinea pigs showing a positive response at challenge) were appropriate. The study dates for the testing with Cyphenothrin-IGR Squeeze-On for Dogs were from March 13 to April 11, 2003. While slightly outside the 6-month period indicated in the Guidelines, it is concluded the overall study findings are acceptable.

C. Reviewer's Conclusions: Based on the results of this study Cyphenothrin-IGR Squeeze-On for Dogs, a clear light yellow liquid with a specific gravity of 1.061 g/mL containing 39.87% Cyphenothrin, 3.00% Methoprene and 2.00% Nylar (Pyriproxyfen) is not a dermal sensitizer.

D. Deficiencies - The final date for the cited positive control study is approximately 7 months before the initiation of this study. However, TRB can accept the results of this study.

EPA Primary Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505C)
EPA Secondary Reviewer: William Dykstra, Ph.D.
Health Effects Division (7509C)

Signature: Byron T. Backus
Date: 11/15/2004
Signature: William Dykstra
Date: 11/12/04

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Dogs OPPTS 870.7200

PC CODES: 129013 (Cyphenothrin), 129032
RISK MANAGER: (EPA): 13

DP BARCODE: D305948
DECISION NO.: 338118

PRODUCT AND TEST MATERIAL: Cyphenothrin-IGR Spot-on for Dogs. [EPA File Symbol 2517-IN]; a liquid labeled "Gokilaht Spot-On w/IGR's (2824) 40.00% RS-Gokilaht; 3.00% S-Methoprene; 2.00% Nylar." According to a certificate of analysis (p. 47 of MRID 46166108) the formulation contained 39.87% Gokilaht, 3.00% S-Methoprene and 2.00% Nylar. Packaged in unidose ampules containing 1.5 mL product.

CITATION: Kuhn, J. (2003) Companion Animal Safety Study in Dogs: Cyphenothrin-IGR Spot-on for Dogs. Final Report. Project Number 7650/03. Unpublished study prepared by Stillmeadow, Inc. and Miller, Thomas A. 53 p. Study Completion Date: 20 October 2003. MRID 46166108.

SPONSOR: Sergeant's Pet Care Products, Inc. Omaha, NE 68130-1703.

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46166108), groups of 12 dogs (from 5 to 9 males in each group) with each group including three 12-week old puppies weighing 4.1-6.1 kg, 2 or 3 dogs weighing 6.8-15 kg, 3 or 4 dogs weighing 15.1-29.5 kg, and 3 weighing >29.5 kg) were dosed with: 1) the amount of vehicle contained in a single dose (Group I, controls); 2) at 1X the label use directions (except for puppies, which were dosed at 1.5X) in Group II, and 3) at 5X the label use directions (except for puppies, which were dosed at 7.5X) in Group III. Group III dogs were treated five times with one hour between each treatment.

The test material was supplied in unidose 1.5 mL ampules. However, the report states that the mean volume delivered from one of these ampules was 1.17 mL. One dose for puppies less than 15 lbs [= 6.8 kg] are to be treated with 1.0 mL, for dogs weighing 15-33 lbs (6.8-15 kg) it was 1.5 mL, for dogs weighing 15.1-29.5 kg it was the contents of two 1.5 mL ampules (the proposed label says two 1.5 mL or one 3.0 mL ampule), and for dogs weighing >29.5 kg it was three 1.5 mL ampules (the proposed label says three 1.5 mL or one 4.5 mL ampules). The control material was supplied in bulk and placebo controls were treated with this formulation (without actives) at the rate of 55.13% of the active product dose volumes.

Administration was according to the proposed label directions and involved application of the test substance (or control vehicle) to the skin in a line along the spine starting at the back of the neck. Label directions specify application of the product as a spot-on or stripe treatment between the shoulder blades to dogs weighing up to 15 kgs. For dogs weighing between 15 and 29.5 kg application would be as a spot-on or stripe treatment at two sites on the back, one

16

15

between the shoulder blade and one directly in front of the base of the tail. For >29.5 kg the contents of one 1.5 mL ampule would be applied to the back as a spot-on or stripe between the shoulder, and the contents of the other two 1.5 mL ampules would be applied as a stripe on the back in front of the base of the tail.

Each dog in Groups I and II was observed at 1, 2, 3 and 4 hours following treatment on Day 0. Group III dogs were also observed "between the hourly dosings" (1, 2, 3, 4, 5, 6, 7 and 8 hours after the first treatment). All dogs were then observed twice (a.m. and p.m.) on Days 1-15.

Individual body weights were determined on Days -7, -3, 7 and 14. Individual food consumption was determined on a daily basis from Day -7 through Day 15 by measuring the amount of food given to each dog in the morning and subtracting the amount left at the end of the day. Blood samples were taken on Days -7 and 1 following overnight fasts.

Possible systemic effects related to exposure to the test material included ocular discharge and salivation. In the immediate period following treatment, ocular discharge was observed in one Group II dog (a puppy) at 4 hours post-dose, and in 4 Group III dogs (including all three puppies). Salivation (mostly very slight, but in some cases moderate) was observed in five Group III dogs (including 1/3 puppies), and was observed (very slight) in one adult 32.2 kg dog at 1 hour postdose (so at this time this dog had presumably been treated with only 1 or 2 applications of test material). Salivation was seen in another Group III adult starting at 3 hours, and in two additional Group III adults starting at 4 hours. During the subsequent 15-day observation period, ocular discharge was frequently observed (including continuously from day 10 to 15) in one control puppy, in none of the Group II (1X) dogs, and in one Group III puppy (days 1-2) and one Group III adult (days 1-6, then again on Days 13-15); both of these Group III animals had also shown ocular discharge in the period immediately following the first treatment. Salivation was observed in one Group III adult male at the AM observation on Day 1 (this dog had also showed salivation during the 8-hour period following the first treatment). One Group III male puppy showed a lesion (or lesions) on both sides of the shoulder (presumably at or near the application site) from Day 5 through 15, and was observed to scratch this area frequently.

Group III adults showed a mean weight loss between days -3 and +7. The incidences of adult dogs showing weight losses between days -3 and +7 were: Group I: 3/9; Group II: 4/9; Group III: 6/9. It is concluded then that for adult dogs exposure to a 5X dosage of test material was associated with a slight mean weight decrease in the period from Day -3 to Day 7.

While Group I puppies showed a greater mean weight gain in the period from Day -3 to +7 than Groups II and III, their mean weight gain/day in the subsequent period from Day 7 to 14 (0.053 kg/day) was comparable to the mean weight gains from Day -3 to +7 for Groups II (0.033 kg/day) and III (0.047 kg/day). Group III puppies also showed a greater mean weight gain in the period from Day -3 to 7 than did Group II puppies. There is no indication then that exposure to the test material affected body weight gain in puppies.

Puppies in Groups II and III showed lower mean food consumption values on Days 0 and 1 relative to their controls. This has to be considered as treatment-related. A similar effect in the adults was not evident.

No effects were noted on hematological or clinical chemistry parameters.

While the Guidelines for this type of study state that the targeted adequate margin of safety is 5X, it is also stated that: "Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)." The test material was not tested at 3X and effects were noted at 5X. However, the

effects noted at 5X, including ocular discharge (also noted in one puppy dosed at what was essentially 1.5X) and salivation were minimal (most occurrences of both ocular discharge and salivation were described as "very slight.") and reasonably transient. It is noteworthy that no systemic neurological signs (such as tremors or ataxia) were observed, and the salivation may have been due to ingestion of small amounts of test material from licking or biting the application area. In addition, the performing laboratory has demonstrated in the past extremely meticulous reporting of observational data in companion animal safety studies, and it is quite likely that these observations would not have been reported from some of the other laboratories which conduct this type of study.

For local dermal effects, one puppy treated at what was essentially a 7.5X dose level showed subsequent shoulder lesions and was noted to scratch this area frequently. Dermal exposure to pyrethroids can cause a burning and/or itching sensation at the application site, and this has to be considered an effect (unless the registrant can provide additional information demonstrating otherwise). However, this was an isolated case, and the three adult dogs treated with 3 unit doses/application with a total of 5 applications (for a total of fifteen 1.5-mL ampules in all) did not show a similar response.

However, one area of concern is that the 1.5 mL ampules (the only size tested in this study) delivered only an average of only 1.17 mL of test material, and the registrant is proposing packaging this product in 3.0 and 4.5 mL (as well as 1.0 and 1.5 mL) tubes. This is acceptable only if the 3.0 mL tubes deliver no more than 2.34 (2 x 1.17) mL and the 4.5 mL tubes deliver no more than 3.51 (3 x 1.17) mL.

This study is classified as **Acceptable** as a companion animal safety study (OPPTS 870.7200) for puppies (12 weeks and older) and adult dogs. **It is concluded that there is an adequate margin of safety (at least 5X) between the exposure associated with the proposed use level for this formulation in dogs and that at which significant adverse systemic effects (not seen in this study, but which might include ear twitching, muscle tremors, drooling) may occur.** For dermal effects an effect was observed in one puppy in Group III (treated at essentially a 7.5X dose level), but in none of the other dogs (including the puppies in Group II), indicating a reasonably low potential for this effect in dogs treated at the proposed use level.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements were present.

I. MATERIALS

A. MATERIALS

1. **Test material:** Cyphenothrin-IGR Spot-on for Dogs, with a label declaration for active ingredients of RS-Gokilaht [=Cyphenothrin] (40.00%), S-Methoprene (3.00%), and Nylar (2.00%). According to a certificate of analysis on p. 47 of MRID 46166108 the respective analytical values were 39.87%, 3.00% and 2.00%. Packaged in unit dose ampules containing 1.5 mL.

Description: A liquid;
Lot No.: #1683B
Storage: Room Temperature

2. **Administration:** Topical (spot-on)
3. **Vehicle control:** X-5699-03 (Placebo Control); From Study 0310: Lot #03390A0100. liquid which was stored at room temperature.

4. Test animals

Species: Dog

Breed: From p. 9 of MRID 46166108: "Beagles and other breeds..."

Ages and weights at study initiation: "Animals were at least 3 months old at dosing.

There were 3 animals from each group in each of the following weight ranges: <15, 15-33, 34-65 and >65 pounds (<6.8, 6.8-15, 15.1-29.5 and >29.5 kg). All dogs less than 15 pounds were pups that were 12 weeks old at dosing." [Note by reviewer: The alkaline phosphatase measurements from Dog 2854F (controls), 3085M (Group II) and 2156M (Group III) were relatively high - refer to pp. 32-34 - suggesting these were fairly young dogs too].

Sources: Butler Farms (Clyde, NY), Martin Creek Kennels (Wiliford, AR), Ridgland Farms (Mt. Horeb, WI) and STILLMEADOW, Inc.

Housing: Individually in kennels measuring 3' x 5.5'.

Diet: PMI Canine High Density Diet 5L18.

Water: Tap water, *ad libitum*

Environmental conditions:

Temperature: $22^{\circ} \pm 3^{\circ}\text{C}$

Humidity: 30 - 70%

Air changes: 10 - 12/hr

Photoperiod: 12 hr dark/12 hr light

Acclimation period: 2 weeks

II. STUDY DESIGN

A. IN LIFE DATES

From the report cover: study initiation date: 13 August 2003; study completion date: 20 October 2003.

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

There were a total of 12 dogs per dosage group. Group 1 (1X vehicle; note: observation schedule on p. 16 of MRID 46166108 is the same for Groups 1 and 2, consistent with a single application of placebo on dogs in Group 1) consisted of 9 males and 3 females; Group II (1X) consisted of 5 males and 7 females, and Group III (5X) consisted of 8 males and 4 females. Assignment was on the basis of weight. From p. 10 of MRID 46166108: "Animals selected for testing were randomly assigned to three groups... Since there were three dose sizes (based on animal body weight) to be used in each treatment group, three dogs of each weight range were included in each group. The weight ranges were <15, 15-33, 34-65 and >65 pounds (<6.6, 6.8-15, 15.1-29.5 and >29.5 kg). The dogs <15 pounds were 3-month old puppies."

From p. 10 of MRID 46166108: "The test substance and placebo were applied to the skin in a line along the spine starting at the back of the neck. The test substance was supplied in unit dose plastic tubes, each containing 1.5 mL and were administered to each animal according to its body weight. The placebo control substance was provided in bulk. On Day 0, a label dose (1X) of the test substance was administered to each Group II animal according to body weight. Group III animals received the test substance at five times the label dose (5X) administered as single doses once every hour for 5 hours. The single dose volumes were: dogs <15 pounds, 1.5 mL (one unit dose); dogs 13 [15?]-33 pounds, 1.5 mL (one unit dose); dogs 34-65 pounds, 3 mL (two unit doses); and dogs >65 pounds, 4.5 mL (three unit doses). Group I was treated with the placebo control material in a volume equivalent to that of the normal label dose of the test substance less the volume of that dose that was occupied by the active ingredients [or approximately

55% of a normal 1X dose volume]..."

TABLE 1. Study design							
Group & Weight Range (kg)		Number of dogs or puppies		Cumulative Dose/dog			Number of applications
		Male	Female	Total/Dog	Mean mL/kg	Mean Dosage Cyphenothrin (mg/kg) ^d	
I (control)	<6.6 ^a	3	0	0.83 mL ^b	0.16 ^b	0	1
	6.8-15	1	1	0.83 mL ^b	0.08 ^b	0	
	15.1-29.5	2	2	1.65 mL ^b	0.09 ^b	0	
	>29.5	3	0	2.48 mL ^b	0.07 ^b	0	
II (1X)	<6.6 ^a	1	2	1.17 mL ^c	0.28 ^c	112 ^d [121] ^a	1
	6.8-15	1	2	1.17 mL ^c	0.09 ^c	36 ^d [39] ^a	
	15.1-29.5	1	2	2.34 mL ^c	0.12 ^c	48 ^d [52] ^a	
	>29.5	2	1	3.51 mL ^c	0.11 ^c	42 ^d [45] ^a	
III (5X)	<6.6 ^a	1	2	5.85 mL ^c	1.26 ^c	503 ^d [543] ^a	5
	6.8-15	0	2	5.85 mL ^c	0.51 ^c	207 ^d [224] ^a	
	15.1-29.5	3	1	11.7 mL ^c	0.64 ^c	255 ^d [275] ^a	
	>29.5	3	0	17.55 mL ^c	0.50 ^c	199 ^d [215] ^a	

Data calculated from information on p. 14-15 in MRID 46166108.

^a Puppies

^b Placebo

^c Test material (with actives); amount delivered based on 1.17 mL/application.

^d Based on a specific gravity for the test material of 1.00 g/mL (consistent with the calculations for dosage as reported on pp. 14-15 of MRID 46166108) and based on 1.17 mL delivered/tube.

^e Based on a specific gravity of 1.08 g/mL

Note: According to the CSF the specific gravity of the proposed product is about 1.08; assuming the product contains 40% Cyphenothrin then 1.5 mL would be 1.62 g and would contain 648 mg of Cyphenothrin. The calculations of dosage of Cyphenothrin in the report (see p. 14-15 of MRID 46166108) appear to be based on a specific gravity for the proposed product of about 1.00. Example: Dog 3067 F weighing 4.2 kg received one 1.5 mL dose of the product and this is reported as a dosage of 142 mg/kg Cyphenothrin. 142 mg/kg x 4.2 kg = 596.4 mg; dividing this by 0.3987 (the analytical percentage) gives 1496 mg (= 1.496 g) total product applied. However, in Appendix G (see p. 52 of MRID 46166108) it is stated that the unit dose containers did not deliver the entire target dose of 1.5 mL, as there was a mean delivered volume of 1.17 mL. A statement in Appendix G ("...5X dose rates ranged from 494 to 630 mg/kg for the smallest subjects.") is consistent with delivery of 1.17 mL/dose and a specific gravity of about 1.08 g/mL.

C. DOSE SELECTION RATIONALE

According to the proposed label this product will be packaged in unidose 1.0, 1.5, 3.0 and 4.5 mL applicator tubes. These correspond to single treatments for dogs weighing 15 lbs and under, 15-33 lbs, 33-66 lbs and >66 lbs. However, in this study, Group 2 (1X) dogs (puppies) weighing less than 15 lbs received 1.5 mL (instead of 1.0 mL), while Group 3 (5X) dogs (puppies) weighing less than 15 lbs received 5 x 1.5 mL = 7.5 mL (instead of 5 x 1.0 mL = 5.0 mL).

D. EXPERIMENTAL DESIGN

From p. 10 of MRID 46166108: "Each animal was observed at 1, 2, 3 and 4 hours following dosing on Day 0 and then twice daily [AM and PM] for the duration of the study. Group III animals were also observed between the hourly dosings. Each animal was examined for signs of any pharmacologic and/or toxicologic effects. Only abnormalities were recorded."

Individual dogs were weighed on Days -7, -3, 7 and 14.

Individual food consumption was measured daily by measuring the amount of food given to each dog in the morning and subtracting the amount of food left at the end of the day.

Baseline blood samples were collected from each dog on Day -7 by jugular venipuncture following an overnight fast. Blood samples were also similarly collected on Day 1.

E. PATHOLOGICAL PARAMETERS

Blood samples were collected on Study Days -7, and 1 by jugular venipuncture following an overnight fast. The CHECKED (X) parameters were examined:

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time [PT])*		
X	(Activated partial thromboplastin time [APTT])*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Blood creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
	ENZYMES	X	Total and direct bilirubin (T Bil & D Bil)*
X	Alkaline phosphatase(ALP or ALK)*	X	Total serum protein (TP)*
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)	X	Albumin/Globulin (A/G) ratio
X	Serum alanine aminotransferase (ALT or SGPT)*		
X	Serum aspartate aminotransferase(AST or SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

The statistical report is found in Appendix G. It consists of a 6-page document (pages 48-53 of MRID 46166108). From p. 51 of MRID 46166108: "The data generated by the test facility...were statistically analyzed by Student's "t" test, assuming equal variances, using the statistical program in Microsoft Excel, version 97-SR-1... Since cyphenothrin is potentially the most toxic component of the product (pyriproxyfen and methoprene are known to be virtually mammalian-inert) cyphenothrin dosage was the focus. To ensure that some of the test subjects were treated at or above the target maximum dose rate of 100 mg of cyphenothrin per kg body weight, the 12-week-old Beagle pups were treated with the next higher unit dose volume. Although weighing between 9 and 11 lb at treatment, for which the proposed label dose rate is one dose of 1 mL, these pups received one 1.5 mL unit dose. To validate the dosage delivered to the principals, the expelled contents of six 1.5 mL unit dose containers were each weighed..." [Note: the report text then refers to Table 1.1, which is not present in the report]. From p. 52 of the report: "The dose validation data (Table 1.1) indicated that the unit dose containers, if filled at the target dose volume of 1.5 mL, were not capable of delivering the entire target volume (mean volume delivered was 1.17 mL).

G. DISPOSITION OF ANIMALS

Not stated. According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

H. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

The dose per 1.17 mL application (based on a product specific gravity of 1.08 g/mL) is 1.264 g. Since the test material contained (by analysis) 39.87% Cyphenothrin, 3.00% S-Methoprene and 2.00% Nylar, each 1.17 mL dose then contained 0.504 g (=504 mg) Cyphenothrin, 0.038 g (=38 mg) S-Methoprene and 0.025 g (=25 mg) Nylar. For Group II (1X) mean cumulative Cyphenothrin dosages were: puppies (<6.6 kg): 121 mg/kg; dogs 6.8-15 kg: 39 mg/kg; dogs 15.1-29.5 kg: 52 mg/kg; and dogs >29.5 kg: 45 mg/kg. For Group III (5X) dosages were: puppies (<6.6 kg): 543 mg/kg; dogs 6.8-15 kg: 224 mg/kg; dogs 15.1-29.5 kg: 255 mg/kg; and dogs >29.5 kg: 215 mg/kg. Refer to Table 1 of this DER. B. MORTALITY

There was no mortality, with all dogs surviving the 14-day observation period.

C. CLINICAL SIGNS

In the observation period immediately following treatment, no clinical signs of systemic toxicity were observed in Group I (controls). In Group II (1X) animal 3074 (a male pup weighing 4.1 kg) showed very slight ocular discharge from both eyes at 4 hours post-dose. In Group III (5X) four dogs (including all 3 puppies) showed very slight to moderate ocular discharge from one or both eyes in the period from one hour to 8 hours post-dosing. In addition, five dogs (including one puppy) showed very slight to moderate salivation during this period (in two dogs it was classified as very slight). Very slight salivation occurred in one animal at 1 hour (i.e., presumably following a single dosage of test material), and in two others it was first noted at 3 hours (after 3 application treatments)

Slight to moderate green ocular discharge (both eyes) was observed in one control puppy in the period from Day 1 to Day 6, and then again in this puppy from Day 10 to Day 15. No ocular discharge was observed in Group II in the period from Day 1 to Day 15. One Group III puppy showed clear ocular discharge from the left eye on Days 1 and 2, while an adult dog showed clear ocular discharge from the left eye from Day 1 through Day 6, then again from Day 13 through Day 15.

TABLE 2. Adverse Effects Observed in Dogs Treated with Cyphenothrin-IGR Spot-on In the Period Immediately Following Treatment ^a			
Parameter	Group I (Control)	Group II (1X)	Group III (5X)
Ocular discharge - one or both eyes in the immediate post-dosing period	0[0]	1[1/48]	4[12/96]
Salivation	0[0]	0[0]	5[18/96]
Soft stool	0[0]	0	2[2/96]
Spiked greasy hair at application site in the immediate post-dosing period	0[0]	2[2/48]	0[0]

^aData taken from Table 2 (p. 15) of MRID 46166108.

From p. 11 of MRID 46166108: "Five of the Group II [1X] animals exhibited greasy spiked fur and/or white deposits at the dose site through Day 3. The only other observation noted in this group was moderate white foamy vomit in one dog on Day 8. In Group III, greasy and/or spiked fur and/or white deposits were seen through Day 1 in two dogs, through Day 3 in three dogs, through Day 6 in three dogs and [from Day 5] through Day 15 in one dog. Other observations included slight clear ocular discharge through Day 2 and shoulder lesions through Day 15 in one animal (the dog was observed to scratch the irritated area frequently), and slight to moderate diarrhea on Days 3 and 4 in another animal. Another dog had very slight to moderate clear ocular discharge through study termination with slight to moderate redness around the eye on Days 3-6. One dog exhibited slight salivation on Day 1 and moderate diarrhea on Day 14, and another had a lesion on the back on Days 7-15."

The Group III dog with the lesions on the shoulder (from p. 18: "both sides") from Day 5 through 15 was 3070M (a male puppy), treated with five 1.5 mL applications, while the Group III dog with the lesion on the back (Days 7-15) was 2853F (a female adult) also treated with five 1.5 mL applications.

D. BODY WEIGHT AND WEIGHT GAIN

From p. 12 of MRID 46166108: "The average weight gain[s] for Groups I, II and III were 1.1, 1.0 and 0.6 kilograms, respectively. There were no significant differences among groups, and no dose related responses."

The values in Table 3 are calculated from individual body weight data (p. 14-15 of MRID 46166108):

TABLE 3. Mean Body Weights for Dogs by Group						
Group	kg $\pm$ S.D.				Mean Wt change Day -3 to 7	Mean Wt change Day -3 to 14
	Day -7	Day -3	Day 7	Day 14	kg $\pm$ S.D.	kg $\pm$ S.D.
I (Controls) puppies	4.63 $\pm$ 0.50	5.07 $\pm$ 0.93	6.13 $\pm$ 1.01	6.50 $\pm$ 1.10	1.07 $\pm$ 0.15	1.43 $\pm$ 0.31
I (Controls) adults	21.87 $\pm$ 9.93	22.34 $\pm$ 10.26	22.86 $\pm$ 10.90	23.31 $\pm$ 11.15	0.51 $\pm$ 0.87	0.97 $\pm$ 1.00
II (1X) puppies	4.80 $\pm$ 1.14	4.17 $\pm$ 0.06	4.50 $\pm$ 0.10	4.97 $\pm$ 0.31	0.33 $\pm$ 0.15	0.80 $\pm$ 0.36
II (1X) adults	21.64 $\pm$ 9.61	21.90 $\pm$ 9.16	22.53 $\pm$ 10.29	22.91 $\pm$ 10.29	0.41 $\pm$ 1.36	1.01 $\pm$ 1.61
III (5X) puppies	4.67 $\pm$ 0.71	4.63 $\pm$ 0.57	5.10 $\pm$ 0.70	5.63 $\pm$ 0.50	0.47 $\pm$ 0.15	1.00 $\pm$ 0.10
III (5X) adults	22.18 $\pm$ 10.00	22.38 $\pm$ 10.35	22.18 $\pm$ 10.40	22.86 $\pm$ 10.68	-0.20 $\pm$ 0.32	0.48 $\pm$ 0.58

Values calculated from data on p. 14 and 15 of MRID 46166108.

The possibility exists that there was a switch of puppies (or their bodyweights) as pup 3073M (assigned to controls) weighed 4.1 kg on day -7 but 6.1 kg on day -3, while pup 3074M (assigned to Group II or 1X) weighed 6.1 kg on day -7 but 4.1 kg on day -3. However, because this switch would have occurred before the dogs were treated, there would have been no impact on the study results.

The only group in which adults showed a mean weight loss between days -3 and +7 was Group III. The incidences of adult dogs showing weight losses between days -3 and +7 were the following: Group I: 3/9; Group II: 4/9; Group III: 6/9. It is concluded then that for adult dogs exposure to a 5X dosage of test material was associated with a slight mean weight decrease in the period from Day -3 to Day 7.

While Group I puppies showed a greater mean weight gain in the period from Day -3 to +7 than Groups II and III, their mean weight gain/day in the subsequent period from Day 7 to 14 (0.053 kg/day) was comparable to the mean weight gains from Day -3 to +7 for Groups II (0.033 kg/day) and III (0.047 kg/day). Group III puppies also showed a greater mean weight gain in the period from Day -3 to 7 than did Group II puppies. There is no indication then that exposure to the test material affected body weight gain in puppies.

TABLE 4. Mean Body Weight Gains for Puppies				
	Day -3 to +7	Day -3	Mean Pup Wt. Gain kg/Day Day -3 to +7	Mean Pup Wt. Gain kg/Day Day 7 to 14
I (Controls) puppies	1.07 ± 0.15	0.37 ± 0.15	0.107	0.053
II (1X) puppies	0.33 ± 0.15	0.47 ± 0.21	0.033	0.067
III (5X) puppies	0.47 ± 0.15	0.53 ± 0.25	0.047	0.076

Values calculated from data on p. 14 and 15 of MRID 46166108.

E. FOOD CONSUMPTION

Puppies in Groups II and III showed lower mean food consumption values on Days 0 and 1 relative to their controls. This has to be considered as treatment-related. A similar effect in the adults was not evident.

TABLE 5. Mean Diet ± S.D. (g) Consumed by Dog/Group by Day							
Group							
	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
I (Controls) puppies	227 ± 40	267 ± 14	263 ± 17	279 ± 27	315 ± 27	252 ± 41	275 ± 0
I (Controls) adults	431 ± 129	475 ± 131	525 ± 110	496 ± 138	504 ± 166	313 ± 173	496 ± 146
II (1X) puppies	193 ± 7	122 ± 3	89 ± 17	239 ± 45	200 ± 81	165 ± 11	209 ± 15
II (1X) adults	384 ± 218	387 ± 202	339 ± 248	410 ± 207	435 ± 216	310 ± 177	418 ± 192
III (5X) puppies	234 ± 27	85 ± 0	98 ± 82	201 ± 69	178 ± 51	167 ± 98	191 ± 0
III (5X) adults	445 ± 141	497 ± 122	379 ± 207	452 ± 175	435 ± 159	322 ± 236	437 ± 122

Values calculated from data on p. 26-29 of MRID 46166108.

F. HEMATOLOGY

From p. 12 of MRID 46166108: "The hematology values were within normal limits except for platelet counts, prothrombin time and/or activated partial thromboplastin time. These values were significantly elevated in all groups, including the placebo group, and therefore were not dose related."

There were no indications of any treatment related effects on hematology parameters. Alkaline phosphatase activity was elevated for puppies in all groups (and was usually above the reference range of 10-150 IU/L), but this is normal for puppies.

G. CLINICAL CHEMISTRY

There were no indications of any treatment related effects on clinical chemistry parameters. As indicated on p. 12 of MRID 46166108 clinical chemistry results "were within normal limits in males and females and the few significant differences among male or female means in any group or between group means did not appear to be related to treatment with the test substance."

H. NECROPSY FINDINGS

As there were no mortalities, there were no necropsy findings.

IV. DISCUSSION

Possible effects related to exposure to the test material included ocular discharge (seen in both eyes of one puppy in Group II at 4-hours post-dosing; classified as very slight; seen in 3 puppies and one adult in Group III in the period from one hour to eight hours following the first dose. In all 3 puppies ocular discharge, when it occurred during this period, was described as very slight. In the adult there was progression to a red, irritated, watery left eye at 8 hours following the first dosage. In addition, very slight to moderate salivation was noted in five dogs (including two puppies) of Group III in the one to eight hours following treatment. Salivation was seen in one adult (#3080, a 32.2-kg male receiving three 1.5-mL doses at each application) at the one hour observation (i.e., presumably one hour after the first treatment), and very slight salivation was seen in this one dog at 3 and 4 hours [following the first dosage], and then moderate salivation was seen at 8 hours. However, no effects ["No Observable Abnormalities"] were then seen in this dog for the remainder of the 14-day observation period.

Adult dogs dosed at the 5X level tended to show a slight mean weight loss in the week following treatment, although there was no indication of an effect on food consumption.

There was no indication of an effect on body weight in puppies at the 1X and 5X dose levels [actually 1.5X and 7.5X dose levels], although their mean food consumption levels for days 0 and 1 were noticeably lower than concurrent values of their controls as well as their own pre-exposure food consumption.

While the Guidelines for this type of study state that the targeted adequate margin of safety is 5X, it is also stated that: "Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)." The test material was not tested at 3X and effects were noted at 5X. However, the effects noted at 5X, including ocular discharge (also noted in one puppy dosed at what was essentially 1.5X) and salivation were minimal (most occurrences of both ocular discharge and salivation were described as "very slight.") and reasonably transient. It is noteworthy that no systemic neurological signs (such as tremors or ataxia) were observed, and the salivation may have been due to ingestion of small amounts of test material from licking or biting the application area. In addition, the performing laboratory has demonstrated in the past extremely meticulous reporting of observational data in companion animal safety studies, and it is quite likely that these observations would not have been reported from some of the other laboratories which conduct this type of study.

For local dermal effects, one Group III puppy (treated at what was essentially a 7.5X dose level) showed subsequent shoulder lesions (from day 5 through 15) and was noted to scratch this area frequently. Dermal exposure to pyrethroids can cause a burning and/or itching sensation at the application site, and this has to be considered an effect (unless the registrant can provide additional information demonstrating otherwise). However, this was an isolated case, and the three adult dogs treated with 3 unit doses/application with a total of 5 applications (for a total of fifteen 1.5-mL ampules in all) did not show a similar response.

However, one area of concern is that the 1.5 mL ampules (the only size tested in this study) delivered only an average of only 1.17 mL of test material, and the registrant is

proposing packaging this product in 3.0 and 4.5 mL (as well as 1.0 and 1.5 mL) tubes. This is acceptable only if the 3.0 mL tubes deliver no more than 2.34 (2×1.17) mL and the 4.5 mL tubes deliver no more than 3.51 (3×1.17) mL.

This study is classified as **Acceptable** as a companion animal safety study (OPPTS 870.7200) for puppies (12 weeks and older) and adult dogs. It is concluded then that **there is an adequate margin of safety (at least 5X) between the exposure associated with the proposed use level for this formulation in dogs and the dose at which significant adverse systemic toxicological effects (not seen in this study, but which might include ear twitching, muscle tremors, drooling) may occur.** For dermal effects an effect was observed in one puppy in Group III (treated at essentially a 7.5X dose level), but in none of the other dogs in this study (including the puppies in Group II), indicating a reasonably low potential for this effect in dogs treated at the proposed use level.

STUDY DEFICIENCIES: The test material was not tested at 3X and effects were noted at 5X. However, the effects noted at 5X, including ocular discharge (also noted in one puppy dosed at what was essentially 1.5X) and salivation were minimal (most occurrences of both ocular discharge and salivation were described as "very slight."). In addition, the performing laboratory has demonstrated in the past extremely good reporting of observational data, and it is quite possible that what was reported in this study would not have been reported from some of the other laboratories which conduct this type of study.