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

OFFICE OF
PREVENTION,

PESTICIDES

AND
TOXIC

SUBSTANCES

Date: June 1, 2004

MEMORANDUM

Subject: EPA File Symbol: 69332-G SPI #8208-55D
DP Barcode: D303497
Decision No.: 337588
PC Codes: 128965 (Etofenprox); 129032 (Nylar)

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

To: Kevin Sweeney/RM Team 13
Insecticide Branch
Registration Division (7505C)

Registrant: PET LOGIC, L.L.C.

ACTION REQUESTED: "Please review the attached animal safety study for the new formulation of the pending cat spot-on."

BACKGROUND: The proposed product, SPI #8208-55D ETOFENPROX SPOT-ON has the following label ingredient declaration:

Active Ingredients:
Etofenprox.....55.0%
Pyriproxyfen.....2.2%
Inert Ingredients.....42.8%

As proposed [at least for cats] the formulation would be packaged in 0.7 mL applicator tubes, and 0.6 mL of these contents have be applied as a spot or stripe on the cat's back between the shoulder blades for cats weighing between 2.2 and 5 lbs, and 1.2 mL

(from two 0.7 vials) would be applied for cats weighing over 5 lbs. The product claims are for effectiveness of at least 28 days ["Kills and repels adult fleas for up to 28 days (4 weeks)(1 month) per application."]

The following are the claims and use directions which pertain to cats [the proposed product labeling received by TRB also includes claims for use on dogs, with statements such as: "Formulated especially for toy breed dogs [Dogs weighing less than 15 lbs]."

FOR USE ON CATS:
Weighing 2.2 pounds to less than 5 pounds - Apply contents of one (0.7 mL) tube *[vial]* of Etofenprox Spot-On as a spot high on the back of the cat's neck behind its head.

Weighing 5 pounds or more - Apply contents of two (0.7 mL) tubews [vials] of Etofenprox Spot-On solution as a spot or stripe, starting high on the back of the cat's neck to in front of the shoulder blades.
Or apply one (1.4 mL) tube [vial] of Etofenprox Spot-On solution as a spot or stripe, starting high on the back of the cat's neck to in front of the shoulder blades.

HOW TO APPLY:
Remove product tube *[vial]* from the package. Holding tube *[vial]* with notched end pointing up and away from the face and body, cut or tear off the narrow end at the notches. While holding the animal with one hand, use the other hand to apply the solution. Invert the tube *[vial]* and use the narrow end to part the animal's hair while gently squeezing to apply to the animal's skin. Hold the animal for a few seconds to give the solution time to be absorbed into the animal's coat. When finished, wrap the tube *[vial]* in paper and put into the trash.

Labeling also includes a statement that households with more than one cat should not allow cats to groom each other until the solution has dried.

COMMENTS AND RECOMMENDATIONS:

1. This study is classified as **Acceptable** for a companion animal safety study (OPPTS 870.7200) in kittens and adult cats. While a decrease in food consumption was noted in the 1X kittens and adult cats following application of the test material, and the same finding was also observed at 5X (and, to some extent, in the vehicle control animals), what occurred at 5X did not impact on the survival of the cats and kittens and was reasonably transient. These effects may also have been, in part, due to the overnight fasting and blood collection on Day 1. The proposed dosage application rate (0.6 mL of a formulation containing 56.03% Etofenprox and 2.26% Nylar for kittens and cats weighing less than 5 lbs and 1.2 mL for cats weighing more than 5 lbs) at 30-day intervals is adequately supported by this study.

2. According to the proposed labeling, the product can be reapplied once every four weeks. The guideline states that repeat treatments are not required for products with re-treatment intervals of 14 to 30 days which have no observed toxicity following exposure to a 5X dose level; however, in this case toxicity - albeit minimal - was observed. From the criteria of OPPTS 870.7200 then the product label should have

a minimum retreatment interval of 30 days (rather than 28 days and/or 4 weeks, as suggested by some of the proposed label statements).

3. The material received for review by TRB includes (MRID 46161308) a study confirming that the amount of formulation delivered from a 0.7 mL vial is essentially 0.6 mL (0.59 mL with a standard deviation of 0.03 mL). It is noted that the proposed labeling indicates the product may be packaged in 1.4 mL tubes for cats weighing >5 lbs; we should have confirmation (which has not been received by TRB) that the amount of formulation delivered from a 1.4 mL vial is 1.2 mL.

DATA EVALUATION RECORD

Reviewer: Byron T. Backus, Ph.D. **Date:** May 28, 2004
Risk Manager (EPA): 03

STUDY TYPE: Companion Animal Safety - 12 week-old kittens and adult cats. OPPTS 870.7200

TEST MATERIAL (% a.i.): Etofenprox-IGR Spot-On for Kittens (Etofenprox 56.03%; Nylar 2.26%). Etofenprox Lot 8815. A colorless liquid packaged (received) in unidose tubes designed to deliver 0.6 mL.

SYNONYMS: PC Codes 128965 (Etofenprox), 129032 (Nylar)

CITATION: Kuhn, J.O. (2003) Companion Animal Safety Study for an Etofenprox-IGR Spot-On on Kittens and Cats. OPPTS No. 870.1100 [Note by reviewer: should be OPPTS 870.7200]. STILLMEADOW, Inc., 12852 Park One Drive, Sugar Land, TX 77478. Lab Study No. 7652-03. Study Completion Date: 18 November 2003. MRID 46161309. Unpublished.

SPONSOR: Sergeant's Pet Care Products, Inc., 2637 South 158th Plaza, Suite 100, Omaha, NE 68130-1703.

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46161309), Etofenprox-IGR (Etofenprox 56.03% and Nylar® 2.26%) Spot-On for Kittens was topically applied to groups of three male (1.1 to 1.5 kg) and three female (1.1 to 1.6 kg) 84 to 90-day old (12-week) kittens and three male (3.6 to 5.3 kg) and three female (2.3 - 4.2 kg) adult (at least one year old) cats. For Group A (1X) doses were: 0.6 mL for kittens (as each weighed less than 5 lbs) and 1.2 mL for adult cats (as each weighed more than 5 lbs); for Group B (5X): a cumulative dose of 3.0 mL for kittens and 6.0 mL for adult cats, and for Group C (5X inert vehicle): a cumulative dose of 1.284 mL vehicle (=5 x 0.6 mL x 0.428) for kittens and 2.568 mL (=5 x 2 x 0.6 mL x 0.428) for adult cats. Animals in Groups B and C each received 5 equal treatments at 1-hour intervals. Application was to a furrow in the skin starting high on the back of the neck to in front of the shoulder blades.

On the day of dosage, observations for mortality and signs of toxicity were made for Group A animals at 1, 2, 3, 4 and 6 hours following treatment. Observations of Group B and C animals were made just prior to each subsequent dosing following the first (this would be at about 1, 2, 3 and 4 hrs following the first dose), as well as 1, 2, 3, 4 and 6 hours after the final dosing. All groups were then observed twice daily through study termination (Day 14). Individual daily food consumption values were measured from day -7 through day 14. Individual weights were determined on Days -7, 0 (prior to dosing), 7 and 14. Blood samples were collected on study Days -7 and 1 by jugular venipuncture; on both occasions there was an overnight fast prior to blood collection.

One Group C (vehicle control) female was found dead on Day 3 with no abnormal external findings. The right horn of the uterus was very swollen, and the left horn was slightly swollen. Approximately 100 mL of reddish brown fluid was collected from the right horn. The probable cause of death was closed-cervix pyometra [a collection of pus in the uterine cavity]. This death was not considered to be related to exposure to the test material vehicle.

Cosmetic effects (wet, greasy and/or spiked fur and white deposits) were observed in all animals in all groups beginning at the one-hour observation. These effects were gone by Day 1 in Group A, by Day 3 in Group B and by Day 2 in Group C. On Day 1, one Group B kitten showed trembling and salivation, and another Group B kitten showed activity decrease and rapid respiration. One Group B adult cat had decreased appetite on Day 1, consuming only 0.7 g of food on Day 1 (consumption on Day 0 had been 105.6 g and on Day 2 82.8 g). Decreases in food consumption were also evident for a female adult cat in Group A, which consumed only 8.0 g on Day 1 (compared with 49.1 g on Day 0 and 35.1 g on Day 2) and a second Group A female adult consuming only 13.4 g on Day 1 and 12.2 g on Day 2 (compared with 18.7 g on Day 0 and 39.9 g on Day 2). #1692 and #1700 (both female kittens in Group B; 1692 indicated above as having activity decrease and rapid respiration) also had noticeably reduced food consumption values (19.4 g and 21.5 g, respectively) on Day 1.

There was a definite drop in food consumption in all groups on Day 1 compared with pretest means, and that this was most marked in Groups A (kittens: -24.3%; adults: -51.7%) and B (kittens: -44.2%; adults:-51.0%), but was also present in Group C (kittens: -24.6%; adults: -38.9%). However, kittens of all groups had definitely recovered by Day 4, and adults by Day 8. The kittens in groups A and B and adult cats in all 3 groups showed highly significant reduced food consumption in the period from day 0 to day 7 as compared to their pre-exposure means, and the mean food consumption values for kittens in groups A and B were lower and significantly different from the mean food consumption for kittens in group C during this period.

Group A and B female kittens showed mean weight losses in the period from Day 0 to 7 (although Group A female kittens showed a greater mean loss than those in Group B, but the Group A female kittens also had shown a considerable mean weight gain between Day -7 and Day 0), as did Group B male kittens. However, mean body weights for kittens in groups A, B and C were very similar on Day 14, so there was no indication of a permanent effect on this parameter. While one Group A adult female (#1560) showed a weight loss of 0.3 kg between Day 0 to Day 14, 5/6 Group B adult cats showed weight gains of 0.2 kg in the same period, and the remaining Group B adult showed a weight loss of only 0.1 kg. There was no indication then of an adverse dose-related effect on adult cat body weights.

There were no treatment related effects on hematology (including coagulation) or clinical chemistry parameters. There were occasional statistical differences between groups, but the mean values were usually within the provided reference ranges, with a few minor exceptions (the mean MCHC values for all groups and all individuals were below the stated reference range of 32 - 36 g/dL, as group means ranged from 30.0 - 30.6, and individual values ranged from 28.9 to 31.8 at both collection times). There

was no indication of any change in the measured hematology parameters as a result of exposure to the test material, as the only statistically significant differences between pretreatment and post-treatment hematology means occurred in Group C (the placebo controls) and involved WBC count and prothrombin time.

According to the package labeling, the product can be reapplied once every four weeks. The guideline states that repeat treatments are not required for products with re-treatment intervals of 14 to 30 days which have no observed toxicity following exposure to a 5X dose level; however, in this case toxicity was observed. From the criteria of OPPTS 870.7200 then the product label should have a minimum retreatment interval of 30 days (rather than 28 days and/or 4 weeks, as suggested by some of the proposed label statements).

Overall, this study is classified as **Acceptable** for a companion animal safety study (OPPTS 870.7200) in kittens and adult cats. While a decrease in food consumption was noted in the 1X kittens and adult cats following application of the test material, and the same finding was also observed at 5X (and, to some extent, in the vehicle control animals), what occurred at 5X did not impact on the survival of the cats and kittens and was reasonably transient. These effects may also have been, in part, due to the overnight fasting and blood collection on Day 1. The proposed dosage application rates of 0.6 mL of a formulation containing 56.03% Etofenprox and 2.26% Nylar for kittens and cats weighing less than 5 lbs and 1.2 mL for cats weighing more than 5 lbs at 30-day intervals are adequately supported by this study.

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

I. MATERIALS

A. MATERIALS

1. Test material: Etofenprox-IGR Spot-on for Kittens, with active ingredients Etofenprox (56.03%) and Nylar® (2.26%). Packaged in unit dose containers designed to deliver 0.6 mL. From p. 11 of MRID 46161309: "The 0.7-mL unidose tubes used in this study were expected [to] deliver 0.6 mL test substance.
   Description: A colorless liquid; from information in Appendix F the density was 1.06.
   Lot No.: SP 8815
   Storage: At room temperature.

2. Administration: Topical (spot-on)

3. Vehicle control: From p. 8 of MRID 46161309: "Formulation inert ingredients without actives (Lot # VE01C01)."

4. Test animals
   Species: Cat
   Breed: Domestic short-hair

Ages and weights at study initiation: Kittens - 12 weeks old; M 1.1 - 1.5 kg; F. 1.1 - 1.6 kg. Adult cats - at least one year old; M 3.6 - 5.3 kg; F 2.3 - 4.2 kg.
Source: Harlan Sprague Dawley, Madison, WI & STILLMEADOW, Inc.
Housing: Individually in stainless steel cages
Diet: PMI Feline Lab Diet #5003, fed once per day, "appropriate amount to meet nutritional requirements." From information in Appendix A (pp. 30-32 of MRID 46161309) kittens generally received a maximum of 90 g a day and adult received a maximum of 130 g/day (although, if the food consumption data on p. 32 can be believed kitten #1706 in Group C consumed 93.2 g on Day 9).
Water: Potable water, *ad libitum*
Environmental conditions:
Temperature: 22°C ± 3°C
Humidity: 30 - 70%
Air changes: 10 - 12 air changes per hour
Photoperiod: 12-hr light/dark cycle
Acclimation period: 7 days

II. STUDY DESIGN

A. IN LIFE DATES

Start: (from p. 8 of MRID 46161309): September 29, 2003 (animals treated); end: October 13, 2003 (laboratory portion of the study was terminated).

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

There were 3 male and 3 female and 3 male and 3 female adults per treatment group. From p. 10 of MRID 46161309: "On Day -4, using a computer-generated randomization procedure, the kittens and adult cats were randomly assigned to three groups (Groups A, B and C) to equalize the mean body weight and sex." Treatments "were administered by application to the skin in a furrow in the hair coat in a strip, starting high on the back of the cats' necks and extending backward to in front of the shoulder blades. The test substance tubes were held inverted for 15 seconds to ensure proper drainage of the contents. The test substance was applied to Group A animals at the single unit dose rate of 0.6 mL for kittens under 5 pounds, and 2 unit doses for older cats (=/>5 pounds). The test substance was applied to Group B animals at 5X the single dose rate by treating at the single dose rate five times at hourly intervals in the same manner as Group A. Group C was dosed with the inert vehicle in syringes at the 1X dose rate five times at hourly intervals. This dose rate was adjusted for the 57.2% of actives in the test substance (i.e., at 42.8% of the single dose volume...: 0.6 mL x 42.8% or 2 X 0.6 mL x 42.8%)."

TABLE 1. Study design

Group	Number of animals		Number of applicatio

Cumulative Dose

					(mL)		
	Kittens		Adult Cats				
	Male	Female	Male	Female	Kittens	Adult Cats	ns
A. 1X	3	3	3	3	0.6	1.2	1
B. 5X	3	3	3	3	3.0	6.0	5[b]
C. Vehicle control[a]	3	3	3	3	1.284[a]	2.568[a]	5[b]

Data taken from text (p. 9 & 10) of MRID 46161309.
[a] Vehicle only, with individual doses of 0.2568 mL; no exposure to active ingredients.
[b] Five individual treatments were applied at approximately one hour intervals.

C. DOSE SELECTION RATIONALE

According to the package labeling for this product, it is supplied in pre-measured 0.7 (delivering 0.6 mL) mL dose volumes, with 0.6 mL being the dosage for a cat or kitten weighing between 2.2 and 5 lbs, and 1.2 mL (either from a single 1.4 mL applicator or two 0.7 mL applicators) for cats weighing more than 5 lbs. (The material received includes a document titled: "Tube Net Contents Delivery Confirmation" in MRID 46161308 which demonstrates a mean of 0.59 mL with a standard deviation of 0.03 mL was delivered from 0.7 mL unit dose tubes; however, no data were provided for unit dose tubes containing 1.4 mL product).

From the proposed labeling, the product is to be reapplied on a monthly basis. Application for cats or kittens weighing 5 lbs or less should be as a spot-on (one application from a 0.7 mL applicator), "high on the back of the cat's neck behind its head." Application to cats weighing more than 5 lbs should either be as a spot or stripe (two applications from two 0.7 mL applicators), "starting high on the back of the cat's neck to in front of the shoulder blades."

D. EXPERIMENTAL DESIGN

The kittens and adult cats were observed daily from study day -7 (beginning of the acclimation period) through day 14. Individual daily food consumption values were measured from day -7 through day 14. Individual weights were determined on Days -7, 0 (prior to dosing), 7 and 14.

E. PATHOLOGICAL PARAMETERS

Blood samples were collected on study Days -7 and 1 by jugular venipuncture; on both occasions there was an overnight fast prior to blood collection. All samples were sent to IDEXX Veterinary Services (West Sacramento, CA) for analysis. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
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)*
		X	Total and direct bilirubin (T Bil & D
	ENZYMES	X	Bil)*
X	Alkaline phosphatase(ALPor ALK)*		Total serum protein (TP)*
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase	X	Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)		Albumin/Globulin (A/G) ratio
X	Serum alanine aminotransferase (ALT or		
X	SGPT)*		
	Serum aspartate aminotransferase(AST or SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

Data for weight, weight gain, food consumption, clinical chemistry, and hematology were segregated and tabulated by group (A, B or C), by age (kitten or adult cat). The hematology and blood chemistry data were also segregated by sex. From p. 70 of MRID 46161309: "The data were statistically analyzed by Student's "t" test, assuming equal variances, using the statistical program in Microsoft Excel, version 97-SR-1..."

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], Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

The dose per 0.6 mL application (based on a product specific gravity of 1.06 g/mL) 0.636 g. Since the product contained 56.03% Etofenprox and 2.26% Nylar each 0.6 mL dose then contained 0.356 g (=356 mg) Etofenprox and 0.0144 g (=14.4 mg) Nylar. The mean Day 0 weights of the kittens were, Group A (1X): 1.45 kg; Group B (5X): 1.35 kg and Group C, (5X vehicle):1.3 kg. Corresponding mean cumulative dosages of actives were: Group A: 245.8 mg/kg Etofenprox and 9.91 mg/kg Nylar; Group B: 1319.8 mg/kg Etofenprox and 53.24 mg/kg Nylar. The mean Day 0 weights of the adult cats were: Group A (1X): 3.73 kg; Group B (5X) 3.88 kg; Group C (5X vehicle): 3.73 kg. Corresponding mean cumulative dosages of actives for adults were: Group A: 190.9 mg/kg Etofenprox and 7.7 mg/kg Nylar; Group B: 917.6 mg/kg Etofenprox and 37.0 mg/kg Nylar. These values are summarized in the table below:

TABLE 2. Dosages

Group	Body weight		Cumulative Dose/Animal			Mean Dose mg/kg		
	Mean (kg)	S.D.	Product (mg)	Etofen-prox (mg)	Nylar (mg)	Product	Etofen-prox	Nylar
A. (Kitten) 1X	1.45	0.217	636	356.4	14.4	438.6	245.8	9.9

B. (Kitten) 5X	1.35	0.138	3180	1781.8	71.9	2355.6	1319.9	53.3
C. (Kitten) 5X vehicle	1.30	0.210	1284[a]	0	0	987.7[a]	0	0
A. (Adult Cat) 1X	3.73	1.174	1272	712.7	28.7	341.0	191.1	7.7
B. (Adult Cat) 5X	3.88	0.997	6360	3563.5	143.7	1639.2	918.4	37.0
C. (Adult) 5X vehicle	3.73	0.958	2568[a]	0	0	688.5[a]	0	0

Values calculated from information on p. 10 and data on p. 14 & 15 of MRID 46161309.

B. MORTALITY

One Group C (vehicle control) female was found dead on Day 3. There were no abnormal external findings. "Internally, the right horn of the uterus was swollen, measuring approximately 7 inches long by 5 inches in diameter, and the left horn was slightly swollen. Approximately 100 mL of reddish brown fluid was collected from the right horn. The probable cause of death was closed-cervix pyometra [a collection of pus in the uterine cavity]." This death was not considered to be related to exposure to the test material vehicle.

C. CLINICAL SIGNS

From p. 12 of MRID 46161309: "There were no pharmacologic or toxicologic effects seen during observations immediately post-dosing. Cosmetic effects (wet, greasy and/or spiked fur and white deposits) were observed in all animals regardless of treatment group beginning at the one-hour observation. These effects were seen in all animals on Day 0, but were no longer seen by Day 1 in Group A, by Day 3 in Group B and by Day 2 in Group C. On Day 1, trembling and salivation were noted in one Group B kitten (1705) [from p. 20 of MRID 46161309: both findings slight at the AM observation; very slight trembling at the PM observation] and activity decrease and rapid respiration were noted in another kitten (1692) in the same group [from p. 21 of MRID 46161309: both findings were moderate at the AM observation and both were slight at the PM observation]. One Group B adult cat (1370) had decreased appetite on Day 1 [from p. 21 of MRID 46161309: present at the PM observation only]." From information in Appendix A (p. 31 of MRID 46161309) this animal consumed only 0.7 g of food on Day 1 (consumption for #1370 on Day 0 had been 105.6 g and on Day 2 was 82.8 g). However decreases in food consumption were also evident for #1560 (a female adult cat) in Group A, which consumed only 8.0 g on Day 1 (compared with 49.1 g on Day 0 and 35.1 g on Day 2) and #1601 (also a female adult in Group A) consuming only 13.4 g on Day 1 and 12.2 g on Day 2

(compared with 18.7 g on Day 0 and 39.9 g on Day 3). #1692 and #1700 (both female kittens in Group B; 1692 indicated above as having activity decrease and rapid respiration) also had noticeably reduced food consumption values (19.4 g and 21.5 g, respectively) on Day 1.

Selected possible treatment-related clinical (non-cosmetic) observations are summarized in Table 3. It is noted that blood samples were taken from the animals (presumably in the AM) on Day 1 "following an overnight fast." It is not certain whether the reduced food consumption values and other symptoms observed on Day 1 could have been at least partially from stress on the animals from the overnight fasting and/or blood collecting.

TABLE 3. Possible treatment-related observations of kittens and/or cats treated with Etofenprox/Pyriproxyfen Spot-on Formulation

Clinical observations	Group	Number affected[a]	Duration
Decreased appetite	A. 1X (female adults: #1560, #1601)	2/6	#1560 - reduced food consumption on Day 1; #1601 - reduced food consumption on Days 1 & 2.
trembling with salivation	B. 5X (male kitten: #1705)	1/6	Study day 1, slight trembling and slight salivation at the AM observation; very slight trembling at the PM observation.
activity decrease, rapid respiration and decreased appetite	B. 5X (female kitten: #1692)	1/6	Study day 1, moderate activity decrease and moderate rapid respiration at the AM observation; slight activity decrease and slight rapid respiration at the PM observation; decreased food consumption on Days 1 and 2.
Decreased appetite	B. 5X (female kitten: #1700)	1/6	Decreased food consumption on Day 1.
Decreased appetite	B. 5X (male adult cat: #1370)	1/6	Study day 1, decreased appetite present at PM observation.

[a]Denominator is total number of kittens or cats in the Group.

Data taken and/or calculated from text p. 12 and Table 2 (Clinical Observations) pp. 20-21and Appendix A (p. 31) of MRID 46161309. Sexes from information in Appendix Table 1.1 (pp. 53-54 of MRID 46161309).

Although there were a few statistically significant differences between groups at several timepoints, none of these were biologically relevant. There were no significant differences between pretreatment (Day -7) and posttreatment (Day 1) means for Groups A and B with respect to hematology parameters. There were a few significant differences with respect to some clinical chemistry parameters (such as total and/or direct bilirubin, BUN, and, for Group B only, serum chloride); however, observed values were generally normal within normal ranges.

Reference ranges (presumably for adult cats) are given on p. 45 of MRID 46161309. As expected, the Alkaline Phosphatase (ALP) values for the kittens during this study (141-358 IU/L) are all well above the reference range of 0-62 IU/L. It is concluded that there were no treatment related effects on hematology and coagulation parameters, or on clinical chemistry values.

D. BODY WEIGHT AND WEIGHT GAIN

From p. 12 of MRID 46161309: "There were two significant differences in bodweight data for the treatment groups kittens between Day -7 and Day 14 and these differences are directly attributable to the rapid growth that occurs in 3-month-old adequately nourished kittens. There were no significant differences in the group weight data of the adult cats. The average weight gains from treatment through study termination for Groups A, B and C were 0.1, 0.2 and 0.1 kilograms, respectively. There were substantial numbers of significant group body weight changes in the kittens' data. The group daily weight change data for both groups of treated kittens showed significantly reduced daily growth rates over the seven days following treatment. The group daily growth rates of these kittens showed a compensating rebound during the following week. There were few significant differences between group daily weight gains or losses in the adult cats, and these differences were not treatment-related."

Table 4 shows the mean body weights for kittens by group and sex, as well as the mean weight gains from Day 0 to Day 14. Group A and B female kittens showed mean weight losses in the period from Day 0 to 7 (although Group A female kittens showed a greater mean loss than those in Group B, but the Group A female kittens also had shown a considerable mean weight gain between Day -7 and Day 0), as did Group B male kittens. However, mean body weights for groups A, B and C were very similar on Day 14, so there was no indication of a permanent effect on this parameter.

TABLE 4. Mean Body Weights for Kittens by Group and Sex

Group & Sex	kg ± S.D.				Wt gain Day 0-14
	Day -7	Day 0	Day 7	Day 14	kg ± S.D.
A(1X) Males	1.267 ± 0.115	1.300 ± 0.100	1.367 ± 0.208	1.767 ± 0.115	0.467 ± 0.058
A(1X) Females	1.267 ± 0.208	1.600 ± 0.200	1.267 ± 0.208	1.567 ± 0.252	-0.033 ± 0.379
B(5X) Males	1.300 ± 0.173	1.400 ± 0.173	1.333 ± 0.115	1.700 ± 0.100	0.300 ± 0.200
B(5X) Females	1.267 ± 0.153	1.300 ± 0.100	1.267 ± 0.208	1.533 ± 0.321	0.233 ± 0.252

C(5X vehicle) Males[a]	1.333 ± 0.208	1.333 ± 0.208	1.400 ± 0.100	1.700 ± 0.265	0.367 ± 0.058
C(5X vehicle) Females[a]	1.333 ± 0.252	1.267 ± 0.252	1.467 ± 0.252	1.533 ± 0.252	0.267 ± 0.058

[a]Assumed (from information given on p. 53 of MRID 46161309) that #1704, #1706 and #1709 were males and #1691, #1693 and #1696 were females.

Values calculated from data on pp. 14-15 of MRID 46161309.

E. FOOD CONSUMPTION

From p. 52 of MRID 46161309 for the kittens' daily food consumption data: "...There were several significant differences in values within each treatment group between the time periods... The mean daily food intake values for all groups of kittens (treated and controls) were significantly reduced during the week after treatment (days 0 to 7), compared with the following week (days 8 to 14) when food consumption rebounded. Although group daily mean food consumption values for the period immediately after treatment were reduced for all groups, compared with the respective pre-treatment values, these differences were not statistically significant. Although not statistically significant, reductions in food consumption values were greater in the group of kittens treated at five times label dose rate (13% depression) than in kittens treated at the single rate (9%) and in the placebo controls (4%). The differences in the daily food consumption data between these three groups over the immediate post-treatment period of days 0 to 7 were also statistically significant."

"There were reductions in the food consumption values for all three groups of adult cats (principals and controls) during the week immediately after treatment... that were similar to those recorded in the kittens' data. Although observed in the data of all three groups of adult cats, the reductions in daily food consumption were statistically significant for only the two groups of principal cats that were treated with the active test substance."

TABLE 5. Group Mean Food Consumption

Group	gram/day ± S.D.									
	Pretest Ave.	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 8**	**Day 11**	**Day 14**
A. (Kitten) 1X	59.35 ±5.63	50.02 ±10.76	44.90 ±6.31	48.67 ±9.57	50.08 ±6.64	57.43 ±10.41	61.20 ±14.56	56.50 ±15.20	62.55 ±12.33	76.03 ±13.35
B. (Kitten) 5X	58.85 ±8.85	58.58 ±16.47	33.05 ±11.03	47.43 ±23.68	46.57 ±12.91	61.67 ±12.55	49.15 ±20.47	70.22 ±19.35	67.93 ±16.75	77.00 ±17.09

C. (Kitten) 5X vehicle	65.30 ±8.38	56.50 ±16.36	49.23 ±8.35	68.37 ±13.03	61.67 ±11.35	65.16 ±13.61	62.83 ±10.35	80.23 ±15.27	74.40 ±14.38	75.37 ±13.22
A. (Adult Cat) 1X	77.40 ±9.44	63.22 ±37.29	37.42 ±21.62	48.88 ±28.18	57.32 ±13.79	56.28 ±12.06	55.65 ±19.45	66.67 ±16.02	61.60 ±16.38	62.97 ±11.89
B. (Adult Cat) 5X	85.43 ±18.12	84.05 ±34.95	41.87 ±22.50	60.02 ±21.41	70.72 ±31.07	69.85 ±35.51	63.45 ±22.52	79.43 ±29.76	82.68 ±25.13	98.25 ±30.45
C. (Adult) 5X vehicle	69.55 ±11.81	55.47 ±8.22	42.50 ±6.19	50.08 ±12.96	46.70 ±16.83	52.20 ±8.94	49.56 ±7.46	51.36 ±15.73	49.12 ±13.18	68.86 ±24.54

Values calculated from data in Appendix A (pp. 30-32) of MRID 46161309.

Kittens appeared to recover pre-exposure food consumption values faster than the adult cats. Refer to Table 6 (below).

TABLE 6. Daily Group Mean Food Consumption (g/day) and Percentage Change From Pretest Average										
	Pretest Ave.	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 7**	**Day 8**	**Day 11**	**Day 14**
A. (Kitten) 1X	59.35	44.90 - **24.3 %**	48.67 - **18.0 %**	50.08 - **15.6 %**	57.43 **-3.2%**	61.20 **+3.1 %**	49.18 - **17.1 %**	56.50 **-4.8%**	62.55 **+5.4 %**	76.03 **+28.1 %**
B. (Kitten) 5X	58.85	33.05 - **44.2 %**	47.43 - **19.4 %**	46.57 - **20.9 %**	61.67 **+4.8 %**	49.15 - **16.5 %**	56.65 **-3.7%**	70.22 **+19.3 %**	67.93 **+15.4 %**	77.00 **+30.8 %**
C. (Kitten) 5X vehicle	65.30	49.23 - **24.6 %**	68.37 **+4.7 %**	61.67 **-5.6%**	65.16 **-0.2%**	62.83 **-3.8%**	73.40 **+12.4 %**	80.23 **+22.9 %**	74.40 **+13.9 %**	75.37 **+15.4 %**
A. (Adult Cat) 1X	77.40	37.42 - **51.7 %**	48.88 - **36.8 %**	57.32 - **25.9 %**	56.28 - **27.3 %**	55.65 - **28.1 %**	58.02 - **25.0 %**	66.67 - **13.9 %**	61.60 - **20.4 %**	62.97 - **18.6 %**
B. (Adult Cat) 5X	85.43	41.87 - **51.0 %**	60.02 - **29.7 %**	70.72 - **17.2 %**	69.85 - **18.2 %**	63.45 - **25.7 %**	72.16 - **15.5 %**	79.43 **-7.0%**	82.68 **-3.2%**	98.25 **+15.0 %**
C. (Adult) 5X vehicle	69.55	42.50 - **38.9 %**	50.08 - **28.0 %**	46.70 - **32.9 %**	52.20 - **24.9 %**	49.56 - **28.7 %**	48.90 - **29.7 %**	51.36 - **26.2 %**	49.12 - **29.4 %**	68.86 **-1.0%**

Values calculated from data in Appendix A (pp. 30-32) of MRID 46161309.

It is concluded that there was a definite drop in food consumption in all groups on Day 1, and that this was most marked in Groups A and B. However, kittens of all groups had definitely recovered by Day 4, and adults had probably recovered by Day 8. The kittens in groups A and B and adult cats in all 3 groups (refer to p. 61-62 of MRID 46161309) showed highly significant reduced food consumption in the period from day 0 to day 7 as compared to their pre-exposure means, and the mean food consumption values for kittens in groups A and B were lower and significantly different from the mean food consumption for kittens in group C during this period.

F. HEMATOLOGY

There were no treatment related effects on hematology and coagulation parameters. There were occasional statistical differences between groups, but the mean values were usually within the provided reference ranges, with a few minor exceptions, particularly the mean MCHC values for all groups (and all individuals), which were below the stated reference range (p. 45: 32 - 36 g/dL; group means ranged from 30.0 - 30.6, and individual values ranged from 28.9 to 31.8) at both collection times. There was no indication of any change in the measured hematology parameters as a result of exposure to the test material, as the only statistically significant differences between pretreatment and post-treatment hematology means occurred in Group C (the placebo controls) and involved WBC count and prothrombin time.

G. CLINICAL CHEMISTRY

There were no indications of any treatment related effects on clinical chemistry parameters. Sporadic statistically significant differences (such as pretreatment vs post-treatment means for total and/or direct bilirubin and for BUN) occurred in Group C as well as Groups B and/or A, and were not biologically relevant, indicative of any pathology, and were usually within normal reference ranges.

H. NECROPSY FINDINGS

The one adult cat (#1331) in the vehicle control group (Group C) found dead on Day 3 showed no abnormal external findings. "Internally, the right horn of the uterus was swollen, measuring approximately 7 inches long by 5 inches in diameter, and the left horn was slightly swollen. Approximately 100 mL of reddish brown fluid was collected from the right horn. The probable cause of death was closed-cervix pyometra."

IV. DISCUSSION

The major effect related to exposure to the test material in both kittens and adult cats was a reduction in food consumption (or loss of appetite) at both the 1X and 5X dose levels for several days following application. This was presumably associated with a slight weight loss in kittens (particularly noticeable in females) during the period from Day 0 to Day 7. Controls (which received a 5X vehicle exposure) also showed a reduction in food consumption during this period (although not as much as in the cats exposed to the formulation with actives). Some of these effects may have been partially due to overnight fasting followed by blood collection on Day 1. Other effects observed in Group B (5X formulation) on Study Day 1 (the formulation was applied on Day 0) were trembling with salivation (seen in one male kitten), and activity decrease with rapid respiration in one female kitten. These effects were transient

There were no indications of any dose-related effects on clinical chemistry or hematology parameters.

Although there were effects at the 1X dose level, these were minor. Effects observed at the 5X dose level were also reasonably minor and transient. Mean body weights for kittens in groups A (1X), B (5X) and C (5X vehicle) were very similar on Day 14, so there was no indication of a permanent effect on this parameter. It is concluded then that there is an adequate margin of safety (at least 5X) between the exposure associated with the proposed use levels for this formulation in kittens and cats and the dose at which significant adverse toxicological effects would occur.

STUDY DEFICIENCIES: There are no major study deficiencies which would impact on its acceptability. There are a few minor problems (it appears that the maximum daily amount of food given to kittens was 90 grams, but the food consumption for kitten #1706 in Group C for Day 8 is reported as 93.2 g, which is

inconsistent with other data). A great deal of the statistical reporting combines data from kittens and adult cats, which is inappropriate, particularly for clinical chemistry parameters such as ALP - Alkaline Phosphatase - and Phos, or serum phosphate.

According to the package labeling, the product can be reapplied once every four weeks. The guideline states that repeat treatments are not required for products with re-treatment intervals of 14 to 30 days which have no observed toxicity following exposure to a 5X dose level; however, in this case toxicity - albeit minimal - was observed. From the criteria of OPPTS 870.7200 then the product label should have a minimum retreatment interval of 30 days (rather than 28 days and/or 4 weeks, as suggested by some of the proposed label statements).

Overall, this study is classified as **Acceptable** for a companion animal safety study (OPPTS 870.7200) in kittens and adult cats. While a decrease in food consumption was noted in the 1X kittens and adult cats following application of the test material, and the same finding was also observed at 5X (and, to some extent, in the vehicle control animals), what occurred at 5X did not impact on the survival of the cats and kittens and was reasonably transient. These effects may also have been, in part, due to the overnight fasting and blood collection on Day 1. The proposed dosage application rate (0.6 mL of a formulation containing 56.03% Etofenprox and 2.26% Nylar for kittens and cats weighing less than 5 lbs and 1.2 mL for cats weighing more than 5 lbs) at 30-day intervals is adequately supported by this study.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D303497
2. **PC CODES:** 128965 Etofenprox; 129032 Pyriproxyfen
3. **CURRENT DATE:** June 1, 2004
4. **TEST MATERIAL:** EPA File Symbol: 69332-G, Product Name: SPI#8208-55D; containing Etofenprox (55.0%) and Pyriproxyfen (2.2%) as active ingredients.

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety/kitten & cat/ STILLMEADOW Inc. Sugar Land, TX /Lab Study No. 7652-03/ 18-NOV-2003	46161309	Formulation applied to back of neck to groups of 3M & 3F 84-90 day old (12 week old) kittens and 3M & 3F adult (at least one year old) cats. Groups A, B and C were respectively treated at 1X (kittens which all weighed less than 5 lbs received 0.6 mL product; adults which all weighed more than 5 lbs were treated with 1.2 mL product); 5X (kittens all were <5 lbs and each received 3.0 mL applied as 5 0.6 mL treatments at 1-hr intervals; adults all were >5 lbs and each received 6.0 mL applied as 5 1.2 mL treatments at 1-hr intervals; 5X vehicle control (kittens received 5 0.2568 mL vehicle treatments at 1-hr intervals; adults received 5 0.5136 mL treatments at 1-hr intervals). One Group C female was found dead on Day 3 from a closed-cervix pyometra (not related to exposure to test material vehicle). Cosmetic effects (wet, greasy and/or spiked fur and white deposits) were seen in all kittens, gone by Day 1 in Group A, by Day 3 in Group B and by Day 2 in C. On Day 1 one Group B kitten showed trembling & salivation, and another Group B kitten showed activity decrease & rapid respiration. Drop in food consumption was seen in all groups on Day 1 (Group A: kittens: -24.3%; adults: -51.7%; Group B: kittens: -44.2%; adults: -51.0%; Group C: kittens: -24.6%; adults: -38.9%). However, kittens of all groups had definitely recovered by Day 4 and adults by Day 8. No indication of any effect on body weights on Day 14. No effects on hematology or clinical chemistry parameters. Study acceptable in supporting dose of 0.6 mL for 12-week-old+ kittens and cats weighing 2.2-5 lbs and 1.2 mL for cats weighing >5 lbs.	N.A.	Acceptable

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**