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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

August 17, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	L899 INSECTICIDE SPINETORAM
	EPA Reg. No. /File Symbol:	72642-O
	DP Barcode:	DP 379867
	Decision No.:	422561
	Action Code:	R270
	PC Code:	110009 (Spinetoram: 39.6%)

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

Byron T. Backus
AUG-17-2010

To: Samantha Hulkower/Mark Suarez RM 13
Insecticide Branch
Registration Division (7505P)

Registrant: ELANCO ANIMAL HEALTH

FORMULATION FROM LABEL:

Active Ingredient(s):		By wt.
110009 Spinetoram		39.60%
Other Ingredient(s):		60.40%
	TOTAL	100.00%

ACTION REQUESTED: The Risk Manager requests:

"Please review Elanco's response to the companion animal safety study…the Agency deemed unacceptable but upgradeable."

BACKGROUND:

The material received includes a response (MRIDs 48161300 and 48161301) to a previous Agency review (dated June 3, 2010) of a companion animal study in 8-week old kittens (MRIDs 47899911 and 4789912), as well as a revised label.

COMMENTS AND RECOMMENDATIONS:

1. In the original review, it was noted that the 1X dosages in the companion animal safety studies were 0.7 mL, while the proposed label for this product indicates the individual applicators contain 0.55 mL (0.019 fl oz) of the formulation. From 48161301: "At the time the Companion Animal Safety (CAS) studies were conducted, the final dose for the product had not been chosen by the Registrant. The 1X dose in the CAS studies was a 0.7 mL application, whereas the final dose based on product performance testing was determined to be 0.5 mL (note the applicator is filled to 0.57 mL to ensure a 0.5 mL dose is delivered to the cat as a small amount of residual stays in the applicator tube…). Therefore, these studies represented approximate doses of 1.4X, 4.2X and 7X. Also note the initial label that was submitted stated the volume as 0.55 mL. The registrant reconfirmed [redid?] the calculation and recognized there was an error. The volume is 0.57 mL which still correlates to 0.019 fl oz. The correction has been made on the label that was resubmitted with this response." This response to the Agency adequately addresses dosage level issues in the cat and kitten companion animal safety studies.

2. One 5X-treated male (#124) is reported (p. 458 of MRID 47899912) to have exhibited ataxia on day 2. This incident is not reported in the summary of detailed clinical observations (see pages 134-136 of MRID 47899912) or in the short summary on page 23. An examination of the hematology and clinical chemistry data from this animal on that date indicates no abnormalities. From MRID 48161301 ataxia was observed in #124 on day 2 at 7:02 AM, but at 7:23 AM #124 was normal; the observations were by two different technicians. Except for the report of ataxia on Day 2 #124 was normal from Day 1 to 44. Ataxia was observed one or more times during the morning of day 2 in 7 other kittens, all in the 5X-vehicle control group. There were no other possible treatment-related clinical signs in the main study animals, and there were no treatment-related effects on mortality, body weight, food consumption, hematology, coagulation, or clinical chemistry. A 5X-treated female (#147) had impaired function and swelling of the left hind limb on days 8 through 10, and was treated with Clavamox (an antibiotic) and Meloxicam (to relieve pain). From MRID 48161301 this animal was not removed from the study because the problem with the hind limb was considered to be a minor ailment that did not impact the animal's survivability and was easily treatable with routine medication without compromising the data that was obtained from it. The statement is made in MRID 48161301 that: "The treatments used were not considered to have masked or obscured any abnormalities as except for soft feces (observed on Day 2, resolved on Day 3, prior to treatment with the medications), there were no other clinical observations to obscure." This statement is not understood by this reviewer, as, according to information on p. 480 of MRID 47899912, #147 had soft feces at the AM observation on days 15 and 37, and (from p. 484) at the PM observations on days -7, 7, 14, and 36, with no report of soft feces for day 2. Treatment with Clavamox can cause soft feces or diarrhea in cats, and the occurrences of soft feces at the PM observation on day 14 and the AM observation on day 15 may have been associated with this antibiotic (presumably this kitten was treated with Clavamox through day 16). It is also stated in MRID 48161301 that: "In addition,

the clinical pathology endpoints following dosing on Days 1 and 30 (before and after medication) were comparable with other animals in that treatment group. Clavamox and Meloxicam are routine therapies used in veterinary medicine and are likely to be typical of concomitant medications cats could receive along with L899 insecticide."

3. The material received in MRID 48161301, despite the question of whether or not soft feces observed for #147 on days 14 and 15 may have been caused by the Clavamox, adequately addresses the concerns in the TRB kitten study review of June 3, 2010. This companion animal safety study in kittens is reclassified as **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in kittens. It supports the use of this product (packaged in applicators containing 0.57 mL and delivering 0.5 mL of formulation) in 8-week-old kittens with 30 days (or one month) between doses.

4. Refer to the attached DER for additional comments regarding the kitten study.

5. The material in MRID 48161301 also includes the following regarding the death of a 5X vehicle control adult cat in the previously reviewed (June 16, 2010) study in MRID 47899910: "Elanco does not agree with EPA's assessment that the death of cat #125 was associated with the treatment with vehicle control. EPA cited the primary reason for death to be hypocalcemia and decreased bicarbonate as measured in the day 31 blood sampling. These two blood values loosely correlated to values observed in the serum chemistries from the kittens in the 5X vehicle control in Study 130-163. What does not correlate, however, are other diagnostic tests (CBC, Coagulation, and Serum Chemistry), clinical observations, and necropsy. None of these pathological processes are consistent with exposure to the solvent. The observed changes in calcium and bicarbonate, in and of themselves, do not suggest a connection between the solvent administered to animal #125 and those adverse events that occurred in Study 130-163 especially given the trauma concomitantly experienced by the animal shortly before death."

EPA did not state that hypocalcemia and decreased bicarbonate were the reasons for death. The statement made in the review of June 16, 2010 was the following: "However, this animal had the lowest bicarbonate and calcium levels (from p. 651 of MRID 47899910 10 mEq/L and 6.7 mg/dL, respectively) of all adult cats on the study on Day 31, values consistent with those observed (bicarbonate: range of 7-13 mEq/L; calcium: 4.9-8.3 mg/dL) for Day 2 in the 5X vehicle control (same material used in the adult study) kittens sacrificed *in extremis* (study in MRID 47899912). It is concluded that this death in the adult female was a result of 5X exposure to the vehicle on Day 30." The Agency interprets the low bicarbonate and calcium levels as effects of significant exposure to the vehicle, not as the cause(s) of death. As #125 was found dead on Day 33 (more than 24 hours after blood was taken on Day 31), additional hematology and clinical chemistry parameters (including potassium, sodium and chloride levels) may have been affected by the time of death. For necropsy findings, in the kitten study (MRID 47899912) it is stated (p. 676) with respect to the pathology interpretation from the euthanized kittens that: "A cause of moribundity could not be determined based on macroscopic findings." This reviewer considers it highly unlikely that cat #125 simply died from trauma to a paw 3 days after treatment, and the conclusion that death was a result of 5X exposure to the vehicle remains unchanged.

EPA Reviewer: Byron T. Backus, Ph.D. **Signature:** Byron T. Backus
Technical Review Branch, Registration Division (7505P) **Date:** 8/17/2010

EPA Secondary Reviewer: Ayaad Assaad, DVM, Ph.D. **Signature:** Ayaad Assaad
Toxicology and Epidemiology Branch, HED (7509P) **Date:** 8/17/2010

Template version 02/06

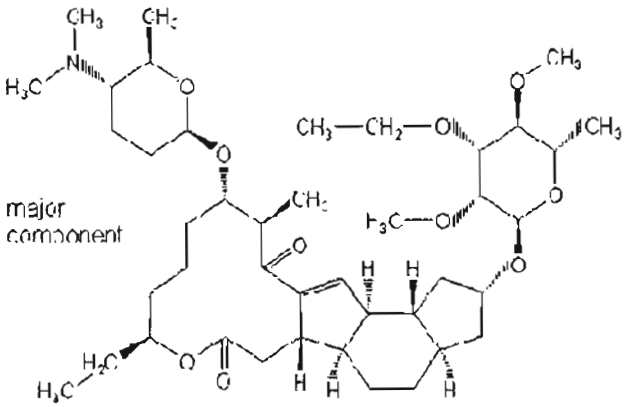
DATA EVALUATION RECORD

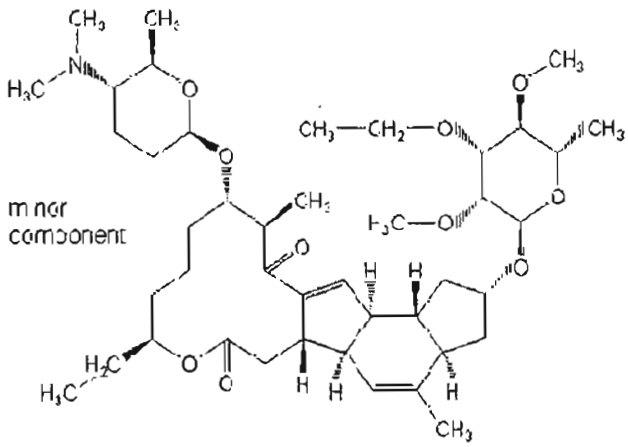
STUDY TYPE: Companion Animal Safety Study - Kittens; OPPTS 870.7200

PC CODE: 110009 **DP BARCODE:** 372448

TEST MATERIAL (PURITY): L899 Insecticide [39.58% (w/w) Spinetoram; Lot No. PP-09-192-L899-09-03-22]

STRUCTURE OF ACTIVE:





SYNONYMS: None provided.

CITATIONS: Lloyd, Z. (2009) Safety evaluation study of topically applied L899 Insecticide on eight-week-old kittens. MPI Research Inc., Mattawan, Michigan. Study Number 130-163, September 28, 2009. MRID 47899912.

Weatherston, I. (2009) Adverse incident in relation to safety evaluation study of topically applied L899 Insecticide on eight-week-old kittens. Technology Sciences Group Inc., Goodyear, Arizona [Submitter]. No study number provided, October 26, 2009. MRID 47899911. Unpublished.

Weatherston, I. (2010) Elanco Response to EPA's Data Evaluation Records on L899 Insecticide. Technology Sciences Group Inc., Goodyear, Arizona. No study number provided, July 16, 2010 (date of cover letter). MRID 48161301. Unpublished.

SPONSOR: Elanco Animal Health, A Division of Eli Lilly & Company, 2001 W. Main Street, Greenfield, Indiana.

EXECUTIVE SUMMARY: In a 45-day companion animal safety study (MRID 47899912), L899 Insecticide [39.58% (w/w) Spinetoram; Lot No. PP-09-192-L899-09-03-22] was applied topically to groups of six male and six female 8-week-old (54-58 days old on Day 0) kittens at 1X (0.7 mL), 3X (2.1 mL), or 5X (3.5 mL) dosing volumes (nominally 294.2, 882.3, or 1470.7 mg Spinetoram per kitten; according to the proposed label and information from MRID 48161301 individual applicators will contain 0.57 mL and will dispense 0.5 mL of the formulation, so the 1X, 3X and 5X study values represent 1.4X, 4.2X and 7X of the actual dosage rates, respectively). "L899 Insecticide Placebo" (Lot No. 09-01-81) was applied in identical manner to control groups of six male and six female animals at dosing volumes of 2.0 mL (group 1; the same amount of inert ingredients as applied for the 5X formulation dose) or 0.4 mL (group 6; inert ingredients at the same levels as for the 1X formulation dose). On Day 0 male kittens weighed from 0.59 to 1.04 kg and female kittens from 0.56 to 0.99 kg; the kittens were obtained from Liberty Research, Waverly, NY.

The test material or vehicle was applied to the dorsal midline of the animal at one discrete site between the shoulder blades and extending cranially and caudally as needed to prevent runoff. Animals were treated twice, at a 29-day interval (on days 1 and 30), except group 1 (5X vehicle control) was only treated once (on day 1). Surviving animals were euthanized on day 45. Group 6 was added to the study 31 days after the other groups due to a very high mortality rate in the original 5X vehicle control group; this group was treated, observed, and tested in identical manner to the other groups, but on a staggered schedule.

Initial (5X) vehicle controls: Three males and five females were sacrificed *in extremis* on day 2 following such unremitting, worsening treatment-related clinical signs as decreased activity, prostration, skin cold to touch, tremors, vocalization, tonic convulsions, piloerection, ataxia, hypersensitivity to touch, splayed limbs, abnormal head movements (nystagmus), and aggressive behavior. Treatment-related effects on clinical chemistry in these animals included moderate to marked decreases in sodium, potassium, chloride, and bicarbonate, consistent with metabolic acidosis with an increased anion gap. The kittens that were sacrificed also had mild to moderate increases in alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities, moderate increases in creatine kinase and lactate dehydrogenase activities, moderate

decreases in calcium and phosphorus concentration, and mild increases in triglyceride and glucose concentration.

Parameter	Pre-test Range (Mean)	Day 2 Range (Mean) for the 4 Surviving Kittens	Day 2 Range(Mean) for the 8 Kittens Which Were Sacrificed *in extremis*
Sodium	146-152 (148.7) mEq/L	148-150 (148.5) mEq/L	133-147 (140.0) mEq/L
Potassium	4.8-6.7 (5.83) mEq/L	5.1-6.1(5.5) mEq/L	3.8-5.9 (4.54) mEq/L
Chloride	113-122 (116.5) mEq/L	113-118 (115.5) mEq/L	91-107 (99.4) mEq/L
Bicarbonate	17-27 (22.5) mEq/L	18-23 (20.3) mEq/L	7-13 (9.5) mEq/L
Calcium	9.8-10.8 (10.29) mg/dL	9.7-10.3 (9.98) mg/dL	4.9-8.3 (6.23) mg/dL
Phosphorus	7.4-10.1 (8.27) mg/dL	7.2-10.1 (8.53) mg/dL	4.8-9.4 (7.13) mg/dL
Alkaline Phosphatase	54-100 (80.9) U/L	67-93 (77.8) U/L	55-511 (163.5) U/L
AST	12-26 (19.6) U/L	14-41 (22.3) U/L	46-352 (221.3) U/L
ALT	24-53 (32.9) U/L	28-96 (46.5) U/L	39-681 (296.5) U/L
Creatine Kinase	107-1042 (341.2) U/L	137-484 (258.0) U/L	2309-6941 (4265.1) U/L
LDH	156-523 (289.2) U/L	159-538 (345.0) U/L	622-3584 (2060.3) U/L
Triglycerides	21-59 (34.0) mg/dL	19-37 (25.5) mg/dL	56-140 (92.1) mg/dL
Glucose	92-125 (103.6) mg/dL	101-116 (107.5) mg/dL	49-260 (192.6) mg/dL

*Data calculated from data provided on p. 794-801 and 810-817, MRID 47899912.

The surviving animals of this group did not exhibit any treatment-related clinical signs and had normal body weights, body weight gain, and food consumption for kittens of this age living under laboratory conditions; however, they did have minimal decreases in erythrocyte count, hematocrit, and hemoglobin concentration on days 2 and 8 (males) or day 8, only (female), with recovery by day 31.

Main study: One 5X-treated male (#124) is reported (p. 458 of MRID 47899912) to have exhibited ataxia on day 2. This incident is not reported in the summary of detailed clinical observations (see pages 134-136 of MRID 47899912) or in the short summary on page 23. An examination of the hematology and clinical chemistry data from this animal on that date indicates no abnormalities. From MRID 48161301 ataxia was observed in #124 on day 2 at 7:02 AM, but at 7:23 AM #124 was normal; the observations were by two different technicians. Except for the report of ataxia on Day 2 #124 was normal from Day 1 to 44. Ataxia was observed one or more times during the morning of day 2 in 7 other kittens, all in the 5X-vehicle control group. There were no other possible treatment-related clinical signs in the main study animals, and there were no treatment-related effects on mortality, body weight, food consumption, hematology, coagulation, or clinical chemistry. A 5X-treated female (#147) had impaired function and swelling of the left hind limb on days 8 through 10, and was treated with Clavamox (an antibiotic) and Meloxicam (to relieve pain). From MRID 48161301 this animal was not removed from the study because the problem with the hind limb was considered to be a minor ailment that did not impact the animal's survivability and was easily treatable with routine medication without compromising the data that was obtained from it. The statement is made in MRID 48161301 that: "The treatments used were not considered to have masked or obscured any abnormalities as except for soft feces (observed on Day 2, resolved on Day 3, prior to treatment with the medications), there were no other clinical observations to obscure." This statement is not understood by this reviewer, as, according to information on p. 480 of MRID 47899912, #147 had soft feces at the AM observation on days 15 and 37, and (from p. 484) at the

PM observations on days -7, 7, 14, and 36, with no report of soft feces for day 2. Treatment with Clavamox can cause soft feces or diarrhea in cats, and the occurrences of soft feces at the PM observation on day 14 and the AM observation on day 15 may have been associated with this antibiotic (presumably this kitten was treated with Clavamox through day 16). It is also stated in MRID 48161301 that: "In addition, the clinical pathology endpoints following dosing on Days 1 and 30 (before and after medication) were comparable with other animals in that treatment group. Clavamox and Meloxicam are routine therapies used in veterinary medicine and are likely to be typical of concomitant medications cats could receive along with L899 insecticide."

The material received in MRID 48161301, despite the question of whether or not soft feces observed for #147 on days 14 and 15 may have been caused by the Clavamox, adequately addresses the concerns in the TRB kitten study review of June 3, 2010. This companion animal safety study in kittens is reclassified as **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in kittens. It supports the use of this product (packaged in applicators containing 0.57 mL and delivering 0.5 mL of formulation) in 8-week-old kittens with 30 days (or one month) between doses.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** L899 Insecticide

Description:	Clear or slightly hazy, amber liquid
Lot #:	PP-09-192-L899-09-03-22
Purity:	39.58% (w/w) Spinetoram
Compound Stability:	Retest date: August 5, 2009 (post-study); analytically confirmed to be stable for the duration of the study
CAS #:	Not provided

2. **Vehicle and/or positive control:** "L899 Insecticide Placebo" (Lot No. 09-01-81) was used as a control. The clear, colorless liquid was comprised of the same solvents and inerts in the same relative proportions as in L899 Insecticide [information as to composition is Confidential Business Information].

3. **Test animals:**

	Species:	Cat	
	Breed:	Domestic Shorthair	
	Age/weight at study initiation:	54-58 days old/ Males: 0.59-1.04 kg; Females: 0.56-0.99 kg	
	Source:	Liberty Research, Waverly, New York	
	Housing:	Individually in mobile stainless-steel cages with mesh flooring, a resting board, litter box, and enrichment toys.	
	Diet:	One can of Hill's Prescription (a/d) Diet per day and Lab Diet® Feline Diet #5003, PMI Nutrition International, Inc., *ad libitum*, except while the animals were fasting	
	Water:	*Ad libitum* tap water	
	Environmental conditions:	Temperature: Humidity: Air changes: Photoperiod:	64-84° F. 30-70% Not provided Approximately 12 hrs light/12 hrs dark, with occasional interruption of the dark portion of the cycle due to study-related activities
	Acclimation period:	Two weeks.	

B. STUDY DESIGN:

1. **In life dates:** Start: June 3, 2009; End: August 14, 2009

2. **Animal assignment:** Study design is given in Table 1. The original animals were assigned to groups 1 through 4 according to body weight using a standard randomization procedure. Group 6 was added 31 days after the study was in progress, due to the *in extremis* sacrifices of three males and five females from the initial 5X vehicle control group. For the purpose of blinding, the animals were identified by a common group number (Group 5) for all purposes other than dosing.

TABLE 1. Study design[a]

Test Group	Dosing volume (mL/animal)	mg Spinetoram per animal	Spinetoram dose (mg/kg)[b]		Number assigned	
			Day 1	Day 30	Males	Females
1. Vehicle control (5X)	2.0	0	0	0	6	6
2. 1X	0.7	294.2	306.5 - 439.1	181.5 - 255.8	6	6
3. 3X	2.1	882.3	848.4 - 1225.4	534.7 - 741.4	6	6
4. 5X	3.5	1470.7	1414.1 - 2334.4	774.1 - 1516.2	6	6
6. Vehicle control (1X)	0.4	0	0	0	6	6

[a]Data taken from p. 15 and 866, MRID 47899912.
[b]Calculated by reviewer using individual body weights on day 1 and day 29.

3. **Dose selection rationale:** The initial doses were selected based on OPPTS 870.7200 guidelines and based on a proposed clinical dose of 0.7 mL "per kitten." When the additional vehicle control group of six males and six females was added to the study, the animals were treated with the vehicle at 1X the proposed label dosage in order to avoid the toxicity that occurred at the higher 5X dose. The study author stated that the 0.7 mL "per kitten" dose applied to all kittens within a certain weight range, but that weight range was not provided in the study report. According to the proposed label, an applicator (which would presumably be used for both kittens and adult cats) contains 0.019 fl oz (0.55 mL) of product.

4. **Treatment:** The control or test material, as appropriate, was applied topically using a disposable syringe on days 1 and 30 (for groups 2, 3, 4, and 6) or on day 1 only (for group 1). For each application, the appropriate volume was applied by dragging the syringe along the dorsal midline of the animal so that the contents were applied at one discrete site between the shoulder blades and extending cranially and caudally as needed to prevent runoff. The study author stated that the application sites were not shaved. There was no mention of whether the fur was parted in order to apply the product directly to the skin (the product label states to apply the contents of the applicator tube to a single spot on the skin of the cat).

5. **Statistics:** The experimental unit was the individual animal. Mean and standard deviation and/or incidence counts (for categorical variables) were calculated for each endpoint. Data from the animals in groups 2, 3, and 4 were compared statistically to data from the animals in group 6 (1X vehicle controls). Data from the surviving animals of the initial 5X vehicle control group were not included in the statistical analyses.

   Body weights and clinical pathology parameters (hematology, coagulation, and clinical chemistry) were analyzed using a Repeated Measures Analysis of Covariance (RMANCOVA) mixed-effects model. The fixed effects included Treatment, Sex, and Time, and interactions between and among the three factors. The pretest clinical pathology value or Day -2 body weight was used as the covariate.

   The covariance structures used were "AR(l)," "ARH(1)," and "UN" for data collected on equal time intervals, or "CS," "CSH," and "UN" for data collected on unequal time intervals. The covariance structure that minimized the Akaike's Information Criterion (AIC) was used.

   Food consumption was analyzed using a Repeated Measures Analysis of Variance (RMANOVA) mixed-effects model. The fixed effects in the model included Treatment, Sex, and Time, and interactions between and among the three factors. The covariance structures used were "AR(l)," "ARH(1)," and "UN" for data collected on equal time intervals. The covariance structure that minimized the Akaike's Information Criterion (AIC) was used.

   According to the study author for both types of analyses, if the Treatment × Sex × Time interaction was significant ($p<0.05$), then the Treatment × Time interaction was examined for each Sex at $\alpha = 0.10$. If the three way interaction was not significant, then the Treatment × Sex interaction was evaluated at $\alpha = 0.05$, and the Treatment × Time interaction was evaluated at $\alpha = 0.10$. If the Treatment × Sex interaction was significant, then treatment effect was evaluated separately for each sex at $\alpha = 0.10$. Regardless of whether Treatment × Sex interaction was significant, if the Treatment × Time interaction was significant, pair-wise contrasts of each non-zero dosing (1X, 3X, 5X) mean against the vehicle control mean at each time using the "time by dose group" LS means were evaluated at $\alpha = 0.10$. If neither two-way interaction was significant, then the main effects were checked, i.e., evaluated the main effect of treatment. If this term was significant at $\alpha = 0.10$, then pair-wise comparisons between non-zero dosing vs. the vehicle control were performed and evaluated at $\alpha = 0.10$.

For both the RMANCOVA and RMANOVA analyses, Sex × Time was included in the model for completeness but was not evaluated, and, although a randomized block design was employed, block was not included as a random effect in the model.

The study report also included profile plots for all endpoints that had multiple measurement times. These were were presented in two ways: 1) all animals' observed values for a single treatment were plotted on the Y-axis against time (study days) on the X-axis, and 2) group means of all treatment groups were plotted on the Y-axis against time (study days) on the X-axis.

C. METHODS:

1. **Observations:** Throughout acclimation and the study interval, the animals were observed cageside twice daily, at least six hours apart for mortality, moribundity, injury, and availability of food and water. Beginning on day -7, more detailed observations were made twice daily (at least six hours apart) and pre-dosing and 15 minutes, and 1, 2, 3 and 4 hours post-dosing on treatment days (days 1 and 30). The observations included, but were not limited to, evaluation of the skin and hair, eyes, and mucous membranes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory system, circulatory system, autonomic and central nervous system, somatomotor system and behavior patterns. A staff veterinarian gave all animals complete physical examinations on day -6, and, throughout the study, a veterinarian was consulted as needed, with all diagnostic testing, treatments, and observations recorded.

2. **Body weight:** The animals were weighed three times per week during acclimation and the study interval.

3. **Food consumption:** Individual food consumption was measured daily beginning on day 1, and these values were used to calculate weekly food consumption.

4. **Hematology & Clinical Chemistry:** Pretest (day -6 or -7) and on days 2 and 31, following a 4- to 6-hour fast (drinking water not withheld), blood was collected from the jugular vein for hematology, clinical chemistry, and coagulation evaluation. If an animal had altered values on day 2 (as compared to pretest), additional sampling for hematology and/or clinical chemistry evaluation was done on day 8. 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	X	Reticulocyte count (aggregate and punctuate, absolute)
	Blood clotting measurements	X	Morphology (blood smear)
X	(Thromboplastin time)*		
	(Clotting time)		
X	(Prothrombin time)*		

* Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. **Clinical Chemistry:**

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
X	Magnesium	X	Urea nitrogen (BUN)*
X	Phosphorus*	X	Cholesterol
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)		Indirect bilirubin
X	Creatine phosphokinase	X	Total protein (TP)*
X	Lactic acid dehydrogenase (LDH)	X	Triglycerides
X	Alanine aminotransferase (ALT/also SGPT)*		Serum protein electrophoresis
X	Aspartate aminotransferase (AST/also SGOT)*	X	Albumin/globulin ratio
	Sorbitol dehydrogenase	X	Bicarbonate
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Amylase		

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200.

5. **Sacrifice and Pathology:** On day 45, the surviving animals were euthanized via administration of sodium pentobarbital and discarded without further evaluation.

   Animals that died or were sacrificed moribund during the study were subjected to complete necropsy, and the indicated (X) organs or tissues were collected and preserved in neutral buffered formalin or a modified Davidson's fixative (eyes and testes) for potential future histopathological evaluation. Moribund sacrifices were done by intraperitoneal administration of sodium pentobarbital solution.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Peripheral nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		GLANDULAR
X	Ileum			X	Adrenal gland
X	Cecum	X	UROGENITAL	X	Parathyroid
X	Colon	X	Kidneys	X	Thyroid
X	Rectum	X	Urinary bladder		
X	Liver	X	Ureters	X	OTHER
X	Gall bladder	X	Testes	X	Bone
X	Pancreas	X	Epididymides	X	Joint
X	RESPIRATORY	X	Prostate	X	Skin
X	Trachea		Seminal vesicles	X	Skeletal muscle
X	Lung	X	Ovaries	X	Mammary gland (females)
	Nose	X	Oviduct	X	Peyer's patch
	Pharynx	X	Uterus with cervix	X	All gross lesions and masses
X	Larynx	X	Vagina		

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity:

a. Main study: One 5X-treated male exhibited ataxia during an unscheduled detailed clinical observation on day 2, and this is considered to be the only potentially treatment-related clinical sign seen in the main study animals of the treated groups 2 though 4 and group 6 (1X vehicle control). This finding was omitted from the summary table and the discussion of clinical signs in the text. One 5X female had impaired function and swelling of the left hind limb on days 8-10 and was treated with oral Clavamox (8 days) and subcutaneous Meloxicam (6 days). The most common clinical signs included vomiting, soft or watery feces, lacrimation, sparse hair, abrasions or scabbed areas, and coughing. No dose response patterns were seen; therefore none were considered treatment-related.

b. Initial 5X vehicle control group: Three males and five females exhibited treatment-related abnormal clinical signs on day 2 (during the morning observation and unscheduled observations). These included the following: decreased activity, prostration, skin cold to touch, tremors, vocalization, tonic convulsions, piloerection, ataxia, hypersensitivity to touch, splayed limbs, abnormal head movements (up and down), and aggressive behavior. The remaining animals did not exhibit any treatment-related clinical signs.

2. Cosmetic effects and migration or runoff of the test or control article: There was no evaluation of cosmetic effects or the migration or runoff of the test or control articles.

3. **Mortality:** There were no deaths or moribund sacrifices of animals in groups 2 through 4 or group 6. Three males and five females of the initial 5X vehicle control group were sacrificed *in extremis* on day 2; these were the animals that had treatment-related abnormal clinical signs on day 2, as described above in section II.A.1.b.

B. BODY WEIGHT AND WEIGHT GAIN:

a. **Main study:** Selected body weight data are given in Table 2. There were no treatment-related effects on body weight. Statistical analysis indicated that there were significant main effects and sex by time and treatment by time interactions, but a consistent dose response was not seen in either sex.

b. **Initial 5X vehicle control group:** The surviving animals of group 1 gained weight over the course of the remainder of the study, and their body weights and body weight gain were normal for kittens of this age living under laboratory conditions.

TABLE 2: Body weight and food consumption data[a]

Parameter/Study day or interval		Dose			
		Vehicle control (1X)	1X	3X	5X
Males					
Body Weight (kg):	Day -2	0.903 ± 0.132	0.778 ± 0.108	0.733 ± 0.122	0.755 ± 0.130
	Day 1	0.950 ± 0.121	0.825 ± 0.101	0.840 ± 0.115	0.830 ± 0.146
	Day 8	1.11 ± 0.117	0.917 ± 0.094	0.985 ± 0.120	0.937 ± 0.153
	Day 15	1.28 ± 0.141	1.08 ± 0.125	1.15 ± 0.121	1.11 ± 0.218
	Day 29	1.66 ± 0.163	1.47 ± 0.095	1.45 ± 0.142	1.51 ± 0.240
	Day 43	2.07 ± 0.194	1.82 ± 0.093	1.82 ± 0.157	1.89 ± 0.262
Days -2 to 43 BW gain in kg[b]		1.67	1.042	1.087	1.135
Days -2 to 43 BW gain as % BW on day -2		129%	134%	135%	150%
Overall food consumption in g/animal/day		39.84 ± 5.001	38.25 ± 12.91	34.92 ± 15.21	32.69 ± 12.02
Females					
Body Weight (kg):	Day -2	0.772 ± 0.098	0.758 ± 0.090	0.768 ± 0.124	0.733 ± 0.107
	Day 1	0.822 ± 0.096	0.812 ± 0.097	0.862 ± 0.118	0.777 ± 0.106
	Day 8	0.968 ± 0.103	0.883 ± 0.105	0.948 ± 0.129	0.843 ± 0.103
	Day 15	1.10 ± 0.130	1.01 ± 0.165	1.13 ± 0.15	0.98 ± 0.135
	Day 29	1.40 ± 0.161	1.33 ± 0.111	1.39 ± 0.172	1.23 ± 0.176
	Day 43	1.70 ± 0.176	1.61 ± 0.138	1.68 ± 0.185	1.42 ± 0.272
Days -2 to 43 BW gain in kg[b]		0.928	0.852	0.912	0.687
Days -2 to 43 BW gain as % BW on day -2		120%	112%	119%	94%
Overall food consumption in g/animal/day		27.73 ± 10.10	25.96 ± 6.39	29.40 ± 9.98	31.98 ± 27.02

[a]Data from pp. 24, 195-204, 216, and 218, MRID 47899912. Values are Mean ± Standard Deviation (where available), with n=6 for all groups.

[b]Calculated by reviewer using group mean body weight values; not analyzed statistically.

C. FOOD CONSUMPTION:

1. **Main study:** Mean overall food consumption is given in Table 2. There was a statistically significant treatment by sex by time interaction, but there were no biologically or statistically significant differences in the weekly mean food consumption values of the treated males and females relative to their respective controls.

2. **Initial 5X vehicle control group:** Food consumption of the surviving animals of this group was considered normal for kittens of this age living under laboratory conditions.

D. BLOOD ANALYSES:

1. Main study:

a. **Hematology and coagulation:** Statistically significant treatment effects, treatment by time interactions, and/or treatment by sex interactions were found for a number of parameters, but none were considered biologically significant. When mean values for the 1X, 3X, and 5X males and females were compared to their respective controls at each separate time point, the differences from control lacked a dose response, the mean values fell within two standard deviations of the control mean, and/or the direction of change (increase or decrease) was not toxicologically relevant. One female from each of the 3X and 5X groups had increased activated partial thromboplastin times (APTT) at all measuring intervals, including pre-study, with the highest values (52.0 and 39.3 seconds) observed on Day 2. These differences were not considered treatment-related and the prothrombin times for these animals were normal.

b. **Clinical Chemistry:** Although statistically significant treatment effects, treatment by time interactions, and/or treatment by sex interactions were found for a number of parameters, none were considered biologically significant. When data for the individual sexes at each separate time point were examined, the differences from control lacked a dose response, the mean values fell within two standard deviations of the control mean, and/or the direction of change (increase or decrease) was not toxicologically relevant. GGT activity could not be evaluated because most of the results of the analyses done on days 2 and 31 were outside the linear range of the assay.

2. Initial 5X vehicle control group:

a. **Hematology and coagulation:** On day 2, most of the animals that were symptomatic and killed *in extremis* (both sexes) exhibited a stress leukogram, i.e. neutrophilia and concurrent lymphopenia, with or without a mildly elevated leukocyte count. The surviving animals had minimal decreases in erythrocyte count, hematocrit, and hemoglobin concentration: in males, the decreases were first seen on day 2, persisted through day 8, and resolved by day 31; and the female first showed a decrease on day 8 and recovered by day 31. It is possible that similar changes in the erythron also occurred in the symptomatic animals but were masked by concurrent dehydration. [See below.]

TABLE 3: Comparison of pre-test (all kittens) and day 2 selected hematology parameters for the surviving kittens of the original 5X control group and those that were sacrificed *in extremis*.			
Parameter	Pre-test Range (Mean)	Day 2 Range (Mean) for the 4 Surviving Kittens	Day 2 Range(Mean) for the 8 Kittens Which Were Sacrificed *in extremis*
Neutrophils	3.28-10.89 (6.25) $10^3/\mu L$	1.96-5.20 (3.54) $10^3/\mu L$	4.62-26.46 (15.99) $10^3/\mu L$
Lymphocytes	2.38-11.45 (6.52) $10^3/\mu L$	0.60-7.32 (3.17) $10^3/\mu L$	0.40-5.50 (1.88) $10^3/\mu L$
Leukocytes	6.7-22.1 (14.4) $10^3/\mu L$	4.7-12.4 (7.1) $10^3/\mu L$	5.2-28.6 (18.6) $10^3/\mu L$
RBC	17-27 (22.5) mEq/L	18-23 (20.3) mEq/L	7-13 (9.5) mEq/L
Hematocrit	9.8-10.8 (10.29) mg/dL	9.7-10.3 (9.98) mg/dL	4.9-8.3 (6.23) mg/dL
Hemoglobin	7.4-10.1 (8.27) mg/dL	7.2-10.1 (8.53) mg/dL	4.8-9.4 (7.13) mg/dL

*Data calculated from data provided on p. 733, 741, 760, 768, MRID 47899912.

b. **Clinical Chemistry**: On day 2, the symptomatic animals had moderate to marked decreases in sodium, potassium, chloride, and bicarbonate, consistent with metabolic acidosis with an increased anion gap. These animals also had mild to moderate increases in alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities, moderate increases in creatine kinase and lactate dehydrogenase activities, moderate decreases in calcium and phosphorus concentration, and mild increases in triglyceride and glucose concentration. The surviving/asymptomatic animals did not exhibit these same changes on day 2 or the later measuring intervals. A comparison of these parameters is given in Table 4, below:

TABLE 4: Comparison of pre-test (all kittens) and day 2 selected clinical chemistry parameters between the surviving kittens of the original 5X control group and those that were sacrificed *in extremis*.			
Parameter	Pre-test Range (Mean)	Day 2 Range (Mean) for the 4 Surviving Kittens	Day 2 Range(Mean) for the 8 Kittens Which Were Sacrificed *in extremis*
Sodium	146-152 (148.7) mEq/L	148-150 (148.5) mEq/L	133-147 (140.0) mEq/L
Potassium	4.8-6.7 (5.83) mEq/L	5.1-6.1(5.5) mEq/L	3.8-5.9 (4.54) mEq/L
Chloride	113-122 (116.5) mEq/L	113-118 (115.5) mEq/L	91-107 (99.4) mEq/L
Bicarbonate	17-27 (22.5) mEq/L	18-23 (20.3) mEq/L	7-13 (9.5) mEq/L
Calcium	9.8-10.8 (10.29) mg/dL	9.7-10.3 (9.98) mg/dL	4.9-8.3 (6.23) mg/dL
Phosphorus	7.4-10.1 (8.27) mg/dL	7.2-10.1 (8.53) mg/dL	4.8-9.4 (7.13) mg/dL
Alkaline Phosphatase	54-100 (80.9) U/L	67-93 (77.8) U/L	55-511 (163.5) U/L
AST	12-26 (19.6) U/L	14-41 (22.3) U/L	46-352 (221.3) U/L
ALT	24-53 (32.9) U/L	28-96 (46.5) U/L	39-681 (296.5) U/L
Creatine Kinase	107-1042 (341.2) U/L	137-484 (258.0) U/L	2309-6941 (4265.1) U/L
LDH	156-523 (289.2) U/L	159-538 (345.0) U/L	622-3584 (2060.3) U/L
Triglycerides	21-59 (34.0) mg/dL	19-37 (25.5) mg/dL	56-140 (92.1) mg/dL
Glucose	92-125 (103.6) mg/dL	101-116 (107.5) mg/dL	49-260 (192.6) mg/dL

*Data calculated from data provided on p. 794-801 and 810-817, MRID 47899912.

E. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Gross findings in the animals from the initial 5X vehicle control group that were sacrificed *in extremis* were limited to dermal edema and/or subcutis in two (of three) males and two (of four) females. In two animals the edema was noted on the dorsal thoracic and lumbar regions and was characterized as mild. In one animal, the edema was noted on treated skin and characterized as mild and "more pronounced on the right side." In one animal, the edema was noted on the left lateral abdomen and characterized as moderate.

2. **Microscopic pathology:** None of the samples collected at necropsy were examined microscopically.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that two topical applications of the test material to 8-week-old kittens at dose volumes of 0.7, 2.1, or 3.5 mL per kitten (or 294.2, 882.3, or 1470.7 mg Spinetoram per kitten) 29 days apart for a total of two treatments did not result in effects on clinical signs, body weight, food consumption, hematology, coagulation, or clinical chemistry. The study author stated that the proposed clinical regimen of 0.7 mL per kitten (equivalent to 294.2 mg Spinetoram per kitten) applied topically every thirty days would be well tolerated.

The study author stated that investigation confirmed that the initial 5X vehicle control animals were dosed with the correct material, at the correct volume. The study author also stated that the results of independent GC/MS analysis indicated that the L899 Insecticide Placebo and the L899 Insecticide both contained the same proportional amounts of solvents and/or inerts and that the analysis did not reveal significant contamination of the L899 Insecticide Placebo. The study author concluded that the clinical signs and altered clinical pathology parameters of the animals treated with 2.0 mL (5X) of the vehicle control were dose- and treatment-related.

Additional comments from Iain Weatherston, regulatory consultant for the submitter and Byron Backus (EPA, OPP Companion Animal Team) were provided in MRID 47899911. These included suggestions that the reduced viscosity of the vehicle compared to the end-use product may have resulted in greater skin penetration, or that the absence of the active ingredient made the formulation less unpalatable, so that the kittens were grooming themselves more readily, thus ingesting a greater quantity of the excipients.

B. REVIEWER COMMENTS:

The study deviated from OPPTS 870.7200 guidelines by using a non-concurrent vehicle control group and by treating that group at a 1X, rather than a 5X level. This was done after application of the excipients at the maximum levels that would appear in a 5X dosage of the end-use product resulted in excessive toxicity. This study attained the 3X and 5X exaggerated doses through modified use of the actual end-use product, rather than using

specifically prepared formulations that contained higher concentrations of the active ingredient. This means that the animals in the 5X-treated group were exposed to 5X levels of all the excipients without exhibiting the same degree of toxicity seen in the animals of the initial 5X vehicle control group.

Three males and five females were sacrificed *in extremis* on day 2 following such unremitting, worsening treatment-related clinical signs as decreased activity, prostration, skin cold to touch, tremors, vocalization, tonic convulsions, piloerection, ataxia, hypersensitivity to touch, splayed limbs, abnormal head movements (nystagmus), and aggressive behavior. Treatment-related effects on clinical chemistry in these animals included moderate to marked decreases in sodium, potassium, chloride, and bicarbonate, consistent with metabolic acidosis with an increased anion gap. The kittens that were sacrificed also had mild to moderate increases in alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities, moderate increases in creatine kinase and lactate dehydrogenase activities, moderate decreases in calcium and phosphorus concentration, and mild increases in triglyceride and glucose concentration.

[In the adult cat study (MRID 47899910) 5X vehicle control animals were dosed with 2.0 mL of material with the same batch number (09-01-81) as that received by the controls of the kitten study, and there were no mortalities or symptoms following the first dosage. Following the second dosage on Day 30 one female was found dead on Day 33, with approximately an inch and a half of its front left paw caught in the floor grate near the middle of the cage. The death was reported as accidental. However, this animal had the lowest bicarbonate and calcium levels (10 mEq/L and 6.7 mg/dL, respectively) of all adult cats on the study on Day 31, values consistent with those observed (bicarbonate: range of 7-13 mEq/L; calcium: 4.9-8.3 mg/dL) for Day 2 in the 5X vehicle control (same material used in the adult study) kittens sacrificed *in extremis* (study in MRID 47899912)].

The reviewer agrees that the application of the end-use product at dose volumes of 0.7 or 2.1 mL (or a 0.4 mL volume of the control) did not result in significant adverse effects. However, one 5X (3.5 mL) male was reported to have ataxia on day 2 [see p. 458 of MRID 47899912, although according to information on p. 134 all 6 males in the 5X group were normal at the AM and PM observations on Day 2]. The Agency has previously (review dated June 3, 2010) stated the need for a clarification as to the occurrence and severity of ataxia in 5X male #124 on day 2. From MRID 48161301: "The unscheduled observation of ataxia was recorded…on June 4, 2009…at 7:02 AM. Animal #124 was also observed as normal on June 4, 2009 (Day 2) at 7:23 AM by [a second technician]. Animal #124 was observed at least twice daily from Day 1 through Day 44 and was considered to be normal at every one of these observations except for this one instance on Day 2. Ataxia was not noted by veterinary staff for animal #124 on Day 2…" With regard to the severity: "As a standard, ataxia is not graded by severity within the glossary of the Provantis™ data collection software. The severity of ataxia was not recorded on this study by technical staff… Ataxia was described by veterinarians during consultation as severe in seven of eight 5X vehicle control animals that ultimately were euthanized in extremis. The remaining kitten which was not classified as severely ataxic was already classified as laterally recumbent at the 8:00 AM consultation (the same observation time when the other 7 kittens were noted as severely

ataxic). Animal #124 was not mentioned at any time point by the veterinary staff as ataxic or severely ataxic."

The Agency also previously (review of June 3, 2010) asked for additional information on the veterinary observations for 5X female #147, as well as a justification as to why this animal was left on the study while she had a health problem and was receiving medications that could alter lab findings and/or obscure treatment-related clinical signs. From MRID 48161301: "Animal #147 was observed on June 10, 2009 (Day 8) at 8:32 AM with impaired function of the left hind limb. The veterinary staff was consulted and a radiograph of the limb was taken on June 10... The radiograph confirmed that the limb was not fractured. At the recommendation of the veterinarian, consultation with the Sponsor, and approval of the Study Director, this animal was treated orally with...Clavamox® (62.5 mg) BID to treat a possible infection and once daily via subcutaneous injection with...Meloxicam (2 mg) to relieve pain through June 18, 2009 (Day 16) [this reviewer interprets this sentence as indicating the animal was treated with both Clavamox and Meloxicam through June 18]. The limb function was described...as slightly impaired by veterinary staff through June 14, 2009... The animal was not removed from the study on the basis that impairment of hind limb function was considered to be a minor ailment that did not impact the animal's survivability and was easily treatable with routine medication without compromising...the data that could be obtained from animal #147. The statement is made in MRID 48161301 that: "The treatments used were not considered to have masked or obscured any abnormalities as except for soft feces (observed on Day 2, resolved on Day 3, prior to treatment with the medications), there were no other clinical observations to obscure." This statement is not understood by this reviewer, as, according to information on p. 480 of MRID 47899912, #147 had soft feces at the AM observation on days 15 and 37, and (from p. 484) at the PM observations on days -7, 7, 14, and 36, with no report of soft feces for day 2. Treatment with Clavamox can cause soft feces or diarrhea in cats, and the occurrences of soft feces at the PM observation on day 14 and the AM observation on day 15 may have been associated with this antibiotic (presuming this kitten was treated with Clavamox through day 16). It is also stated in MRID 48161301 that: "In addition, the clinical pathology endpoints following dosing on Days 1 and 30 (before and after medication) were comparable with other animals in that treatment group. Clavamox and Meloxicam are routine therapies used in veterinary medicine and are likely to be typical of concomitant medications cats could receive along with L899 insecticide."

The material received in MRID 48161301, despite the question of whether or not soft feces observed for #147 on days 14 and 15 may have been caused by the Clavamox, adequately addresses the concerns in the TRB kitten study review of June 3, 2010. This companion animal safety study in kittens is reclassified as **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in kittens. It supports the use of this product (packaged in applicators containing 0.57 mL and delivering 0.5 mL of formulation) in 8-week-old kittens with 30 days (or one month) between doses.

1. **DP BARCODE:** 372448,
2. **PC CODE:** 110009 (Spinetoram)
3. **CURRENT DATE:** July 30, 2010
4. **TEST MATERIALS:** Original controls (Group 1): 5X vehicle (L899 Insecticide Placebo, Lot No. 09-01-81, containing the same solvents and inerts in the same relative proportions as L899 Insecticide); Group 6: 1X vehicle (L899 Insecticide Placebo); Groups 2, 3, 4: 1X, 3X and 5X: L899 Insecticide [39.58% (w/w) Spinetoram; Lot No. PP-09-192-L899-09-03-22]

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Study/8 week-old Kittens MPI Research Inc., Mattawan, Michigan Elanco Animal Health, A Division of Eli Lilly & Company, Greenfield, Indiana	47899912 47899911 48161301	Five groups (each 6M & 6F) of 8 week old kittens were treated on Day 0. Group 1 (5X controls) was treated with 2.0 mL vehicle, Group 2 with 1X (0.7 mL) L899 Insecticide; Group 3 with 3X (2.1 mL) L899 Insecticide; Group 4 with 5X (3.5 mL) L899 Insecticide, and Group 6 (non-concurrent with other groups) with 1X (0.4 mL) vehicle. All groups except 1 were treated again at the same doses on Day 29. 3 males and 5 females from Group 1 were sacrificed *in extremis* on Day 2. There was no mortality in any of the other groups. One 5X male is reported to have exhibited ataxia on Day 2. A 5X female had impaired function and swelling of the left hind limb on Days 8-10 was treated with Clavamox and Meloxicam. No other effects noted.	N/A	A

Core Grade Key: A = Acceptable, S = Supplementary, U = Unacceptable, W = Waived