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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

OFFICE OF PESTICIDE PROGRAMS
REGISTRATION DIVISION (7505P)

July 26, 2011

MEMORANDUM

Subject:	Name of Pesticide Product:	SERGEANT'S FIPRONIL + ETOFENPROX + METHOPRENE SPOT-ON FOR CATS
	EPA Reg. No. /File Symbol:	2517-RUL
	DP Barcode:	DP 391870
	Decision No.:	442446
	Action Code:	R310
	PC Codes:	129121 (Fipronil: 9.8%) 128965 (Etofenprox: 15%) 105402 (S-Methoprene: 11.8%)

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

Byron T. Backus
July-26-2011

To: Bonaventure Akinlosotu/Richard Gebken, RM 10
Insecticide Branch
Registration Division (7505P)

Registrant: SERGEANT'S PET CARE PRODUCTS, INC.

FORMULATION FROM LABEL:

Active Ingredient(s):	by wt.
129121 Fipronil	9.80%
128965 Etofenprox	15.00%
105402 S-Methoprene	11.80%
Other Ingredient(s):	63.40%
TOTAL	100.00%

ACTION REQUESTED: The Risk Manager requests:

"For your review: Response to EPA's DER dated May 16, 2011 for CAS study (MRID 48302108) to upgrade initial review from supplemental to acceptable data, in support of the registration for 4 products (2517-RUL, RUA, RUT and RUI).

BACKGROUND:

The material received includes a document (MRID 48513201) titled: Support Data to Upgrade the Study "Companion Animal Safety Evaluation of a Spot-On Containing Two Toxicants and Two Insect Growth Regulators on Kittens and Cats" From Supplemental to Acceptable EPA Reg. No. 2517-RUT, 2517-RUA, 2517-RUL, 2517-RUI MRID 48302108.

COMMENTS AND RECOMMENDATIONS:

1. With the additional information in MRID 48513201, TRB has revised the DER for the companion animal safety study (MRID 48302108) and its accompanying data analysis report (MRID 48302109).

2. There were dermal effects. Two adult cats in group B1 (1X) scratched the application site and lost hair after application, and the report states that these lesions were considered to be a result of exposure to the test material. One of these cats (963 FE5) had preexisting alopecia (measuring 3 mm x 3 mm on the dorsal neck, between the ears) on days -7 and -1, and then had a scratch mark (20 mm x 10 mm) on days +1 and +7 at the application site, "moving anterior." The second (966 2C7) had no preexisting condition, but then had a scratch mark at the application site on study days +1, +7, and +14. In the discussion on p. 38 of MRID 48513201 it is stated that: "The *de novo* hair loss and local sensory irritation (scratch marks) recorded for cat 966 2C7 must be assumed to be a consequence of the application of Sergeant's test substance. However, its immediate onset (within 24 hours) suggests that this was not an irritant but rather an allergic reaction to a component, or components, of the test substance to which this cat may have been previously exposed. Although the history of this cat in this test facility is not available, it is known that...this facility was active in the testing of fipronil-containing me-too products, including spot-ons (that may have also contained the same or similar inerts). ...there is a substantial possibility that cat 966 2C7 had been previously used in such testing and recycled into Sergeant's protocol." None of the adult cats in the 5X group had any dermal effects.

   Because the dermal effects in the two 1X adult cats were relatively minor, did not require treatment, and because similar effects were not observed in the 5X group, TRB concludes that these effects should not impact on either the registration or labeling of the supported products.

   One kitten (CC0 F92) in the 1X (A1) group had scabs on the back of the head from day 7 to 14 and scabs between the shoulder blades on days 7 and 8; this kitten was an albino. One kitten (female CC2 AB1) in the 5X group (also an albino) had acute wet eczema on the neck and shoulders from day 3 to 14, and this required treatment with Dermavet Cream. It is stated (p. 36) that this lesion: "...was considered to be related to the application of the test substance."

   From page 37 of MRID 48513201 both of the albino kittens were littermates, and there were no other kittens (albino or otherwise) from this litter in this study. As noted on p. 37 of MRID 48513201: "It is unfortunate that albino kittens, a comparative rarity in the cat population, were included in a companion animal safety study. The literature on defects or susceptibilities associated with albinism in cats describes a propensity for deafness, but no references to dermatologic effects. However, one must suspect that the unique skin reactions in the area of test substance application to these two albino kittens may not properly be extrapolated to what will occur in the US cat population when this product may be released onto the market."

3. TRB has concluded that the most probable explanation for the observed effects in the albino kittens is that the toxicity was due to a fipronil photodegradate (MB46513, also known as fipronil-desulfinyl) which is more toxic than the parent compound. It has been established that fipronil accumulates in sebaceous glands. The dose-related response observed in the albino kittens (which lack melanin, which would normally block light from reaching fipronil) is consistent with formation of this photodegradate (particularly as effects appeared on day 3 in the 5X kitten and on day 7 in the 1X kitten). The possibility also exists that the presence of one or more of the other ingredients in this formulation may have enhanced the formation of this photodegradate in the albino kittens.

4. The registrant should provide the Agency with any additional information available from this study relevant to the formation of the fipronil degradate, including (if possible) the fur coloration of the animals at the application site and the level and type of lighting that the cats and kittens were exposed. This information should be received within 6 months of the issuance of a registration.

5. This companion animal safety study in adult cats and kittens is upgraded to Acceptable and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in cats and/or kittens with the stipulation that labeling indicates that this product is not to be used on albino cats or kittens.

6. Refer to the attached DER for additional comments.

EPA Primary Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: July 26, 2011

EPA Tertiary Reviewer: Kit Farwell, D.V.M.
Risk Assessment Branch VII, HED (7509P)

Signature: KFarwell
Date: 7/26/2011

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- cats/kittens – OPPTS 870.7200

PC CODES: 129121: Fipronil; 105401: S-Methoprene; 128965: Etofenprox; 129032: Pyriproxyfen

DP BARCODE: 385149

TEST MATERIAL (PURITY): Cat Spot on Soup, containing Fipronil (9.83%), S-Methoprene (11.76% w/w), Etofenprox (15.78%) and Pyriproxifen (2.34%)

TRADE NAME: Not provided

CITATIONS: Delport, P. (2010) Companion Animal Safety Evaluation of a Spot-On Containing Two Toxicants and Two Insect Growth Regulators On Kittens and Cats (Sergeant's Fipronil + Etofenprox + Methoprene Spot-On for Cats). Project Number: CV/09/660, SER/1009/1. July 13, 2010. Unpublished study prepared by Clin Vet International. 118 p. MRID 48302108.

Miller, T. (2010) Companion Animal Safety Evaluation of a Spot-On Containing Two Toxicants and Two Insect Growth Regulators on Kittens and Cats (Sergeant's Fipronil + Etofenprox + Methoprene Spot-On for Cats). Project Number: CV/09/660. April 13, 2010. Unpublished study prepared by Thomas A. Miller. 13 p. MRID 48302109.

Hoskins, K. (2011) Support Data to Upgrade the Study "Companion Animal Safety Evaluation of a Spot-On Containing Two Toxicants and Two Insect Growth Regulators on Kittens and Cats" From Supplemental to Acceptable. Project Number: CV/09/660/SUPPLEMENT. June 16, 2011. Unpublished study prepared by Sergeant's Pet Care Products, Inc. 41 p. MRID 48513201.

SPONSOR: Sergeant's Pet Care Products, Plano, Texas

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 48302108), three groups, each containing 3 male and 3 female adult (≥ 1 year of age) cats (body weights: males: 2.98-4.92 kg; females: 1.92-3.06 kg; source: ClinVet cattery) and three groups, each containing 3 male and 3 female kittens (12-16 weeks of age; body weights: males: 1.08-1.84 kg; females: 0.82-1.68 kg) were treated with either 0.5 mL (1X) or 2.5 mL (5X 0.5 mL) of Cat Spot on Soup (containing Fipronil: 9.83%; S-Methoprene: 11.76%; Etofenprox: 15.78%; and Pyriproxyfen: 2.34%) or with 1.45 mL (5X 0.29 mL) of the control substance or placebo. The groups and the amounts of test material or placebo administered to each cat or kitten are given below:

Kittens	Adult Cats
Group A1 (n=6) Kittens (12-16 weeks old);single (0.5 mL) application of Cat Spot on Soup	Group B1 (n=6) Adult cats (> 1 year old) Single (0.5 mL) application of Cat Spot on Soup
Group A2 (n=6) Kittens (12-16 weeks old); five 0.5 mL applications of Cat Spot on Soup administered at one hour intervals	Group B2 (n=6) Adult cats (> 1 year old); five 0.5 mL applications of Cat Spot on Soup administered at one hour intervals
Group C1 (n=6) Kittens (12-16 weeks old); five 0.29 mL applications of Placebo administered at one hour intervals	Group C2 (n=6) Adults (> 1 year old); five 0.29 mL applications of Placebo administered at one hour intervals

The test and control substances were applied by syringe along the back of each animal, starting on the neck behind the ears and then extending towards the base of the tail. According to the report the objective was to limit application of the test material to areas where it would be difficult for the animal to "lick off" (and presumably ingest) the substances, although any material at the base of the tail (or even along part of the back) would be accessible for grooming purposes. Cats and kittens receiving 0.5 mL Cat Spot on Soup received a single application; cats and kittens receiving 2.5 mL received five 0.5 mL applications, with approximately one hour between applications. Cats and kittens receiving 1.45 mL control substance received five 0.29 mL applications, with approximately one hour between applications.

The animals were observed daily for general health. Clinical examinations (which included pulse rate, respiratory rate, rectal temperature, and examination of mucous membranes, eyes, motility, lymph nodes, abdominal palpation, thoracic auscultation and evaluation of skin condition) were performed on days -14, -7, -1, +1, +7, and +14, and the animals were also weighed on these days. Blood samples were collected for clinical chemistry and hematology on days -6, +1, +7 and +14. All animals were sedated (Rompun 2%, 0.12 mL/kg IM) to facilitate blood specimen collection.

All animals survived to the end of the study, and there were no indications of any systemic toxicity. No effects were observed in the hematology or blood chemistry results or body weight values. Most cats and kittens showed no dose-related changes in food consumption, although one Group A2 (5X) female kitten scored 2 for meat consumption on Day +2 (kittens received pellets and meat, with separate scoring for their consumption of these two items). Of 522 observations for the consumption of meat, there were 518 "4's" (>75 to 100% consumption); 3 "3's" (>50-75% consumption), and one "2" (>25-50% consumption). Remarkably, the score of "2" involved the 5X albino kitten (CC2 AB1) observed to have a "hotspot on side of neck behind ear" starting on Day +3 which necessitated treatment. On every other pre- and post-treatment day of the study this kitten scored "4" for meat consumption.

There were dermal effects. Two adult cats in group B1 (1X) scratched the application site and lost hair after application, and the report states that these lesions were considered to be a result of exposure to the test material. One of these cats (963 FE5) had preexisting alopecia (measuring 3 mm x 3 mm on the dorsal neck, between the ears) on days -7 and -1, and then had a scratch mark (20 mm x 10 mm) on days +1 and +7 at the application site, "moving anterior." The second (966 2C7) had no preexisting condition, but then had a scratch mark at the application site on study days +1, +7, and +14. In the discussion on p. 38 of MRID 48513201 it is stated that: "The *de novo* hair loss and local sensory irritation (scratch marks) recorded for cat 966 2C7 must be assumed to be a consequence of the application of Sergeant's test substance. However, its immediate onset (within 24 hours) suggests that this was not an irritant but rather an allergic reaction to a component, or components, of the test substance to which this cat may have been previously exposed. Although the history of this cat in this test facility is not available, it is known that...this facility was active in the testing of fipronil-

containing me-too products, including spot-ons (that may have also contained the same or similar inerts). ...there is a substantial possibility that cat 966 2C7 had been previously used in such testing and recycled into Sergeant's protocol." None of the adult cats in the 5X group had any dermal effects. One kitten (CC0 F92) in the 1X (A1) group had scabs on the back of the head from day 7 to 14 and scabs between the shoulder blades on days 7 and 8; this kitten was an albino. One kitten (female CC2 AB1) in the 5X group (also an albino) had acute wet eczema on the neck and shoulders from day 3 to 14, and this required treatment with Dermavet Cream. It is stated (p. 36) that this lesion: "...was considered to be related to the application of the test substance."

From page 37 of MRID 48513201 both of the albino kittens were littermates, and there were no other kittens (albino or otherwise) from this litter in this study. As noted on p. 37 of MRID 48513201: "It is unfortunate that albino kittens, a comparative rarity in the cat population, were included in a companion animal safety study. The literature on defects or susceptibilities associated with albinism in cats describes a propensity for deafness, but no references to dermatologic effects. However, one must suspect that the unique skin reactions in the area of test substance application to these two albino kittens may not properly be extrapolated to what will occur in the US cat population when this product may be released onto the market."

TRB has concluded that the most probable explanation for the observed effects in the albino kittens is that the toxicity was due to a fipronil photodegradate (MB46513, also known as fipronil-desulfinyl) which is more toxic than the parent compound. It has been established that fipronil accumulates in sebaceous glands. The dose-related response observed in the albino kittens (which lack melanin, which would normally block light from reaching fipronil) is consistent with formation of this photodegradate (particularly as effects appeared on day 3 in the 5X kitten and on day 7 in the 1X kitten). The possibility also exists that the presence of one or more of the other ingredients in this formulation may have enhanced the formation of this photodegradate in the albino kittens.

Hourly observations for adult cats and kittens for the four hours after last application are reported in MRID 48513201. For adults (from p. 11 of MRID 48513201) one B2 female (8B1 C65) showed restless rolling with a duration of ~10 minutes after the third application (this observation was unique, as it was made before the series of 5 applications of test material had been completed), but had calmed down one hour later, then (from p. 9 of MRID 48513201) was restless at the first hourly observation post-application, and was showing signs of estrus at the next observation, but showed no signs at the next two observations. Two B2 males showed crystals (presumably from evaporation at the application site), one at the 2 hour observation, and both at the 3 and 4-hour observations. The crystals were still present on one male on Days 1 and 2.

For kittens, no signs were observed in the four hours following the last application, and the only signs that were observed were dermal effects in 5X albino kitten CC2 AB1 (first noted on Day 3) and scabs behind head and between shoulder blades in 1X albino kitten CCO F92 (first observed on Day 7).

From the confidential attachment to MRID 48513201, it has been confirmed that the solvent(s) and/or inerts present in the test material are the same as those in the products which will be supported by this study.

This companion animal safety study in adult cats and kittens is upgraded to Acceptable and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in cats

and/or kittens with the stipulation that labeling indicates that this product is not to be used on albino cats or kittens.

COMPLIANCE: Signed and dated GLP, Quality Assurance and [No] Data Confidentiality statements were provided for ClinVet International. According to the GLP statement, Pathcare Veterinary Laboratory (Bloemfontein), which conducted the hematology and clinical chemistry analyses is an ISO 15189 accredited facility and their clinical veterinary department is GLP accredited.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:

Cat Spot on Soup

Description:	Not provided
Batch #:	FPI0 12/09
Purity:	Fipronil 9.83%; S-Methoprene 11.76%; Etofenprox 15.78%; Pyriproxyfen 2.34%
Storage:	At room temperature
Compound Stability:	Expiration date: September 30, 2010
CAS #:	Not provided

Control substance

Description:	Not provided
Batch #:	012004-09
Purity:	Transcutol CG 99.92% [identified on the Internet as Diethylene glyeol monoethyl ether]
Storage:	At room temperature
Compound Stability:	Expiration date: February 2011
CAS #:	Not provided

2. Vehicle control: See control substance described above.

3. Test animals:

Species:	Domestic Cat (adults ≥ 1 year of age; kittens: 12-16 weeks of age)
Strain:	Not specified (short to medium haired)
Age/weight	Adult cats ≥ 1 year of age: males: 2.98-4.60 kg; females: 1.92-3.06 kg Kittens 12-16 weeks old: males: 1.08-1.84 kg; females: 0.82-1.68 kg
Source:	ClinVet cattery
Housing:	Individually housed in cages: [From p. 18 of MRID 48302108: adult cat cages: 0.74 m x 0.67 m x 0.6 m; kitten cages: 1.5 m x 1.5 m x 1 m (kittens were in larger eages than adults?)].
Diet:	Adults (>1 year) were fed IAMS Multi cat 1+ cat food (Eukanuba) Kittens (12-16 weeks) were fed kitten and junior 0-1 food and Purina Friskies
Water:	"...supplied in stainless steel bowls."
Environmental conditions:	
Temperature:	$20 \pm 4^{\circ}$ C
Humidity:	Not provided
Air changes:	12-15 air changes/hr
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Fourteen days

B. STUDY DESIGN:

1. **In life dates:** Adult: Acclimatization start (day -14): October 27, 2009; Experimental start (day 0): November 10, 2009; Experimental termination date: November 24, 2009.

   Kitten: Acclimatization start (day -14): December 15, 2009; Experimental start (day 0): December 29, 2009; Experimental termination date: January 12, 2010.

2. **Animal assignment:** Thirty-six animals were assigned to the study, with an additional four adults and four kittens enrolled on day -14 as possible replacements.

   For the adult cats, the genders were separated and the cats allocated to study groups within the age requirements using randomization through minimization with body weight as the only criterion. Within each gender, animals were blocked into three blocks of three cats each. The three heaviest cats formed block 1; the next three heaviest cats formed block 2 and the lightest cats formed block 3. Within blocks, cats were allocated to one of three groups, 1, 2 or 3. The first animal of block 1 was assigned to group 1, the second to group 2 and the third to group 3. The first animal of block 2 was assigned to group 3, the second to group 2 and the third to group 1. The first animal of block 3 was assigned to group 1, the second to group 2 and the third to group 3. Groups 1, 2 and 3 were then randomly assigned to the actual groups B1, B2 and C2 using Excel randbetween. Kittens were similarly assigned to groups A1, A2 and C1.

Table 1. Dosage Groups

Kittens	Adult Cats
Group A1 (n=6) Kittens (12-16 weeks old);single (0.5 mL) application of Cat Spot on Soup	Group B1 (n=6) Adult cats (> 1 year old) Single (0.5 mL) application of Cat Spot on Soup
Group A2 (n=6) Kittens (12-16 weeks old); five 0.5 mL applications of Cat Spot on Soup administered at one hour intervals	Group B2 (n=6) Adult cats (> 1 year old); five 0.5 mL applications of Cat Spot on Soup administered at one hour intervals
Group C1 (n=6) Kittens (12-16 weeks old); five 0.29 mL applications of Placebo administered at one hour intervals	Group C2 (n=6) Adults (> 1 year old); five 0.29 mL applieations of Placebo administered at one hour intervals

3. **Dose selection rationale**: The 1X dosage rate (0.017 fluid oz. or 0.5 mL) is consistent with the proposed labeling for the products that this study would be used to support.

4. **Preparation and treatment**: On Day 0 the cats were held by hand and the test and control substances were applied as measured doses from syringes (without needles) to a furrow in the

hair on the back, starting high on the neck behind the ears and extending caudally towards the base of the tail.

Table 2: Doses		
Group	**Material applied**	**Total dose administered**
A1 (kittens)	Cat Spot on Soup	0.5 mL (1X)
A2 (kittens)	Cat Spot on Soup	2.5 mL (5X 0.5 mL)
B1 (adults)	Cat Spot on Soup	0.5 mL (1X)
B2 (adults)	Cat Spot on Soup	2.5 mL (5X 0.5 mL)
C1 (kittens)	Placebo	1.45 mL (5X 0.29 mL)
C2 (adults)	Placebo	1.45 mL (5X 0.29 mL)

Cat Spot on Soup contains Fipronil (9.83%), S-Methoprene (11.76% w/w), Etofenprox (15.78%) and Pyriproxifen (2.34%)
Placebo is identified (p. 12 of MRID 48302108) as Transcutol CG 99.92%.

5. **Statistics**: From p. 40 of MRID 48302108: "All analyses and calculations were performed using SAS Version 8.2. Statistical significance was declared at a two-side p-value of 0.05. The data from the adult cats and kittens were analysed separately and are presented as such..."

   The emphasis of the statistical analyses was the change from baseline of each of the hematology and clinical chemistry parameters. Individual hematology and clinical chemistry values on test days -6, 1, 7 and 14 were tabulated separately for each variable and each group, together with the following descriptive statistics: mean, standard deviation (SD), coefficient of variation (CV %), geometric mean, geometric SD, median, minimum, maximum and the number of observations in the group. The individual changes and percentage changes from baseline (test day -6) for each of the post-treatment days (1, 7 and 14) were calculated and tabulated for each variable and each group. The number of post-treatment values that fell outside the reference range was calculated.

   The post-treatment values were compared to the baseline values in an intra-treatment comparison by means of an analysis of variance (ANOVA) with an animal and observation time as effects. An inter-treatment comparison with respect to the changes from baseline was also performed. The post-treatment values of each the two treatment groups for adult cats (B1 and B2) were compared to their control group (C2). The post-treatment values of each of the two test treatment groups (A1 and A2) were compared to the control group (C1) for kittens. An analysis of covariance (CO-ANOVA) with a treatment effect and the baseline values as covariate was used for the analyses. In addition, the change from baseline values of each of the two treatment groups were compared to those of the corresponding control group (C2) for dogs and the two test groups (A1 and A2) were compared to those of the control group for adult cats and kittens by means of an ANOVA with a treatment effect.

C. METHODS:

1. Observations:

a. **General health observations**: The animals were observed daily for general health. Specific health observations were observed hourly on the day of application until four hours after application of the test material and twice daily, thereafter, for the duration of the study. Observations included, but were not limited to, changes in skin and fur, eyes and mucous membranes, nervous signs and behavior pattern as well as vomiting and diarrhea.

b. **Clinical examinations**: Physical examinations were conducted by a veterinarian on Days -14, -7, -1, +1, +7 and +14. The examinations included but were not limited to pulse rate, respiratory rate, rectal temperature, mucous membranes and skin, joints, eyes, ears, mouth and genital organs, and auscultation [using a stethoscope to listen to the sounds] of the heart and lungs.

2. **Body weight:** Animals were weighed on Days -14, -7, -1, +1, +7 and +14.

3. **Food consumption:** From p. 26 of MRID 48302108: The amount of food offered was measured and the food consumption was recorded daily and scored using the following scoring system.

   Fc 1 - 0- 25% Fc 3 - >50-75%
   Fc 2 - >25- 50% Fc 4 - >75-100%

4. **Hematology and clinical chemistry**: Blood was collected for hematology and clinical chemistry assessments on non-fasted animals on the following days: - 6, +1, +7 and +14. The tests were conducted by Pathcare, Bloemfontein, South Africa, because ClinVet International did not have the expertise and apparatus to perform the analyses. 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*[1]		Reticulocyte count
	Blood clotting measurements*		
X	(Activated Partial Thromboplastin time)		
X	(Prothrombin time)		

*Recommended for companion animals safety evaluation based on OPPTS 870.7200

[1]From p. 27 of MRID 48302108: "In some cases the platelets were microscopically clumped and in those eases the platelet count returned by the Celldyne 3500 was not correct, the actual eount should have been higher and the pathologist had to eomment on the number of platelets on a blood smear. The platelet count was then indieated as "reduced", "adequate" or "increased". For the sake of consistency, a value was linked to these classes. However the variable was handled as a discrete variable and not as a continuous variable.

The following criteria were used by the pathologist:

The historical control value for cats was 300-600 x 10^9/L
Platelets appeared reduced: approximately 250 x 10^9/L
Platelets appeared adequate: approximately 350 x 10^9/L
Platelets appeared increased: approximately 650 x 10^9/L

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium* (Ca)	X	Albumin*
X	Chloride* (Cl)	X	Creatinine*
	Magnesium (Mg)	X	Urea nitrogen*
X	Phosphorus * (P)		Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (Na)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total serum protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine kinase	X	Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
	Gamma glutamyl transferase (GGT)		TCO_2 Bicarbonate
	Amylase		
	Sorbitol dehydrogenase		

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

5. **Urinalysis:** Urinalysis was not conducted.

6. **Sacrifice and pathology**: The study did not have a scheduled necropsy.

II. RESULTS

A. **ACTUAL DOSES ADMINISTERED**: The mg/kg doses of active ingredients were not calculated. From information in Table 1 of MRID 48302109, the mg/kg doses to kittens were significantly higher than those to adults (refer to Tables 3 and 4).

Table 3. Dosages of Fipronil and Etofenprox for adult cats

GROUP	SEX	ANIMAL NUMBER	AGE (YEARS)	WEIGHT (KG)	DOSAGE OF FIPRONIL (mg/kg)	DOSAGE OF ETOFENPROX (mg/kg)
B1	Male	9B3 D3A	≥ 1	4.60	10.7	17.2
B1	Male	9B4 70C	≥ 1	4.26	11.5	18.5
B1	Male	963 FE5	≥ 1	3.26	15.1	24.2
B1	Female	966 2C7	≥ 1	2.88	17.1	27.4
B1	Female	CC5 325	≥ 1	2.34	21.0	33.7
B1	Female	CD5 170	≥ 1	2.00	24.6	39.5
Mean values for Group B1			≥ 1	3.22	16.7	26.8
B2	Male	965 009	≥ 1	4.44	55.3	88.9
B2	Male	B8D B1D	≥ 1	4.44	55.3	88.9
B2	Male	6DC 0CC	≥ 1	2.98	82.5	132.4
B2	Female	CD4 302	≥ 1	2.62	93.8	150.6
B2	Female	8B1 C65	≥ 1	2.46	99.9	160.4
B2	Female	9B4 08D	≥ 1	1.92	128.9	205.5
Mean values for Group B2			≥ 1	3.14	86.0	137.8
C2	Male	6D6 BEB	≥ 1	4.92	0	0
C2	Male	700 A83	≥ 1	3.96	0	0
C2	Male	70D F55	≥ 1	3.40	0	0
C2	Female	CD3 D77	≥ 1	3.06	0	0
C2	Female	6BD 8B8	≥ 1	2.28	0	0
C2	Female	CD3 E61	≥ 1	2.20	0	0
Mean values for Group C2			≥ 1	3.30	0	0

Table 4. Dosages of Fipronil and Etofenprox for kittens

GROUP	SEX	ANIMAL NUMBER	AGE (DAYS)	WEIGHT (KG)	DOSAGE OF FIPRONIL (mg/kg)	DOSAGE OF ETOFENPROX (mg/kg)
A1	Male	E46 53D	112	1.76	27.9	44.8
A1	Male	CC1 D85	92	1.34	36.7	58.9
A1	Male	CC0 F92	92	1.22	40.3	64.7
A1	Female	CC0 4A8	92	1.48	33.2	53.3
A1	Female	CC1 26E	84	1.34	36.7	58.9
A1	Female	CC1 C0A	84	1.00	49.2	78.9
Mean values for Group A1			92.7	1.36	37.3	59.9
A2	Male	E9F FD4	112	1.84	133.6	214.4
A2	Male	CC0 B97	100	1.32	186.2	298.9
A2	Male	CC2 D13	84	1.22	201.4	323.4
A2	Female	CC1 EED	113	1.68	146.3	234.8
A2	Female	CC0 38A	92	1.30	189.0	303.5
A2	Female	CC2 AB1	92	1.12	219.4	352.2
Mean values for Group A2			98.8	1.41	179.3	287.9
C1	Male	CC0 6CC	84	1.52	0.0	0.0
C1	Male	CC0 D09	100	1.48	0.0	0.0
C1	Male	CC3 AE0	84	1.08	0.0	0.0
C1	Female	EA1 63C	113	1.42	0.0	0.0
C1	Female	CC0 F9D	92	1.38	0.0	0.0
C1	Female	CC1 7F8	92	0.82	0.0	0.0
Mean values for Group C1			94.2	1.28	0.0	0.0

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B. OBSERVATIONS:

1. **Clinical signs and application site effects**: For the adult cats, two animals in group B1 (1X) scratched the application site and lost hair after application. One of these cats (966 2C7) had preexisting alopecia (days -7 and -1) measuring 3 mm x 3 mm on dorsal neck (between ears) at the time of application. For group B2 (5X) there were no abnormal signs, although one cat (CD4 302) had hair loss (5 mm x 5 mm) on the right ear and neck on days -7 and -1. One cat came into estrus on day +1. For the controls (C2), one cat had tongue ulcers on days +7 and +14 (improving on day +14), confirmed as a case of mycoplasmosis. For the kittens, one group A1 (1X) kitten (CC0 F92, an albino) developed scabs behind the head and between the shoulder blades on day +7, which were still present on day +14. For group A2 (5X) one kitten (CC2 AB1, an albino) had acute wet eczema on the neck and shoulders on day 3, and this was still present on day +14. This required treatment with Dermavet Cream. It is stated (p. 10 of MRID 48302108) that: "The test substance caused scabs and eczema in two albino kittens." One control kitten (CC0 F9D) had loose feces on day +9.

Table 5a - Signs in Group B1 (adults – 1X)

Animal ID	Sex	Study Days	Signs
9B3 D3A	M		No abnormal signs
9B4 70C	M		No abnormal signs (p. 30, 32) [but, from p. 29, treated on days +2 through +6 with Lincomycin HCl 50 mg by injection for mueopurulent nasal discharge (left)]
963 FE5	M	+1, +7, +14 +1 to +14	Scratch mark (20 mm x 10 mm) on application site, moving anterior (p. 30) Local hair loss on application site (p. 33)
966 2C7	F	-7, -1 +1, +7 +1 to +13 +6 to +12	Alopecia 3 mm x 3mm on dorsal neck (between ears) Scratch mark (20 mm x 10 mm) on application site, moving anterior Local hair loss on application site (from p. 33) Scratch mark (from p. 33)
CC5 325	F	-1	Scratch mark anterior to right ear (p. 30)
CD5 170	F		No abnormal signs

Table 5b - Signs in Group B2 (adults – 5X)

Animal ID	Sex	Study Days	Signs
965 009	M		No abnormal signs
B8D B1D	M		No abnormal signs
6DC 0CC	M		No abnormal signs
CD4 302	F	-7, -1	Hair loss right ear & neck 5 mm x 5mm (p. 31)
8B1 C65	F	-14, -7 +1	Alopecia left lateral neck (p. 31) Estrus (p. 31)
9B4 08D	F		No abnormal signs

Table 5c - Signs in Group C2 (adults – 0X)

Animal ID	Sex	Study Days	Signs
6D6 BEB	M		No abnormal signs
700 A83	M	+7, +14	Tongue ulcers (improving on day +14) (p. 31)
70D F55	M	-7	Thinning of hair – inner thighs (p. 31)
CD3 D77	F	-7, -1, +1, +7	Wound left lateral neck; estrus day +7 (p. 31)
6BD 8B8	F		No abnormal signs
CD3 E61	F		No abnormal signs

Table 6a - Signs in Group A1 (kittens – 1X)			
Animal ID	**Sex**	**Study Days**	**Signs**
E46 53D	M		No abnormal signs
CC1 D85	M		No abnormal signs
CC0 F92	M	+7, +14 +7 to +14 +7 to +8	Scabs behind head and between shoulder blades (p. 32) Scabs behind the head (p. 34) Scabs between shoulder blades (p. 34)
CC0 4A8	F		No abnormal signs
CC1 26E	F		No abnormal signs
CC1 C0A	F		No abnormal signs

Table 6b - Signs in Group A2 (kittens – 5X)			
Animal ID	**Sex**	**Study Days**	**Signs**
E9F FD4	M		No abnormal signs
CC0 B97	M	+1	Lacrimation in the right eye (p. 31)
CC2 D13	M		No abnormal signs
CC1 EED	F		No abnormal signs
CC0 38A	F		No abnormal signs
CC2 AB1	F	+3 to +14	Acute wet eczema (p. 32, 34)

Table 6c - Signs in Group C1 (kittens – 0X)			
Animal ID	**Sex**	**Study Days**	**Signs**
CC0 6CC	M		No abnormal signs
CC0 D09	M		No abnormal signs
CC3 AE0	M		No abnormal signs
EA1 63C	F		No abnormal signs
CC0 F9D	F	+9	Loose feces
CC1 7F8	F		No abnormal signs

From p. 33 of MRID 48302108 (for adult cats): "Cats 963 FE5 and 966 2C7 (group B1) lost hair on the application site and the lesions were visible from day +1 till the end of the study… A scratch mark was visible in this lesion of cat 966 2C7 from day +6 to +12. These lesions were considered to be a result of the application of the test substance… In group B2, two cats (965 009 and B8D B1D) developed crystals on the hair tips after application. On cat 965 009 it was visible on day 0 at the +2 hour and +3 hour observations, while the crystals were visible from +1 hour till day +2 on B8D B1D. These were considered to be cosmetic changes. 8B1 C65 was restless and came into oestrus on day 0… No abnormal signs were recorded amongst the cats in group C2."

From p. 34 of MRID 48302108 (for kittens): "CC0 F92 (group A1) developed scabs behind the head [that were detected?] on day +7 that lasted to the end of the study and scabs between the shoulder blades that disappeared by the end of day +8… CC2 AB1 (albino kitten in group A2) developed wet eczema on day +3, it became infected on day +7 and was bleeding on day +8. After therapy [from p. 29: Dermavet topical once or twice a day on days +8 to +10] the lesions started to heal towards the end of the study…" From p. 36: "CC2 AB1 (albino kitten in group A2) developed wet eczema behind the ear on day +3. On day +5 it had spread to the shoulder blades, the sides of the neck and below the chin. This lesion was improving by day +14 and was considered to be related to the application of the test substance."

2. **Mortality**: All adult cats and all kittens survived to the end of the study.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight data for adults are presented in Table 7, and for kittens in Table 8. There are no indications of any significant differences between the different adult dosage groups, or the different kitten dosage groups.

Table 7: Mean body weight and body weight changes for adult cats[a]

Group	N	Body weight (kg)				Body weight changes (kg)		
		Day -1	Day +1	Day +7	Day +14	Day -1 to +1	Day -1 to +7	Day -1 to +14
B1 (adult males) 1X	3	4.04	4.01	3.99	4.01	-0.03	-0.05	-0.03
B1 (adult females) 1X	3	2.41	2.41	2.47	2.51	0.00	+0.06	+0.10
B1 (combined) 1X	6	3.22	3.21	3.23	3.26	-0.01	+0.01	+0.04
B2 (adult males) 5X	3	3.95	3.88	3.92	4.02	-0.07	-0.03	+0.07
B2 (adult females) 5X	3	2.33	2.35	2.28	2.27	+0.02	-0.05	-0.06
B2 (combined) 5X	6	3.14	3.12	3.10	3.15	-0.02	-0.04	+0.01
C2 (adult males) 0X	3	4.09	4.08	3.98	3.99	-0.01	-0.11	-0.10
C2 (adult females) 0X	3	2.51	2.52	2.53	2.55	+0.01	+0.02	+0.04
C2 (combined) 0X	6	3.30	3.30	3.25	3.27	0.00	-0.05	-0.03

[a] Calculated by the reviewer from data on page 37, MRID 48302108

Table 8: Mean body weight and body weight changes for kittens[a]

Group	N	Body weight (kg)				Body weight changes (kg)		
		Day -1	Day +1	Day +7	Day +14	Day -1 to +1	Day -1 to +7	Day -1 to +14
A1 (male kittens) 1X	3	1.44	1.43	1.51	1.72	-0.01	+0.07	+0.28
A1 (female kittens) 1X	3	1.27	1.27	1.37	1.55	0.00	+0.10	+0.28
A1 (combined) 1X	6	1.36	1.35	1.44	1.64	-0.01	+0.08	+0.28
A2 (male kittens) 5X	3	1.46	1.45	1.53	1.68	-0.01	+0.07	+0.22
A2 (female kittens) 5X	3	1.37	1.37	1.47	1.61	0.00	+0.10	+0.24
A2 (combined) 5X	6	1.41	1.41	1.50	1.65	0.00	+0.09	+0.24
C1 (male kittens) 0X	3	1.36	1.33	1.43	1.59	-0.03	+0.07	+0.23
C1 (female kittens) 0X	3	1.21	1.21	1.25	1.39	0.00	+0.04	+0.18
C1 (combined) 0X	6	1.28	1.27	1.34	1.49	-0.01	+0.06	+0.21

[a] Calculated by the reviewer from data on pages 37-38, MRID 48302108

C. FOOD CONSUMPTION: No treatment-related effects were reported. Most cats and kittens showed no dose-related changes in food consumption, although one Group A2 (5X) female kitten scored 2 for meat consumption on Day +2 (kittens received pellets and meat, with separate scoring for their consumption of these two items). Of 522 observations for the consumption of meat, there were 518 "4's" (>75-100% consumption); 3 "3's" (>50%-75% consumption) and one "2" (>25-50% consumption. Remarkably, the score of "2" involved the 5X albino kitten (CC2 AB1) observed to have a "hotspot on side of neck behind ear" starting on Day +3 which necessitated treatment. On every other pre- and post-treatment day of the study, this kitten scored "4" for meat consumption.

The following Tables are from p. 39 of MRID 48302108:

Table 9. Mean daily and weekly food consumption values for the adult groups			
Study Day	**Group B1 (1X)**	**Group B2 (5X)**	**Group C2 (0X)**
-13	3.67	3.33	3.33
-12	3.17	3.67	3.17
-11	3.67	3.67	3.33
-10	3.83	3.83	3.33
-9	3.83	3.83	3.50
-8	3.83	3.50	3.33
-7	3.67	3.50	3.50
Mean days -13 to -7 (week 1)	**3.67**	**3.62**	**3.36**
-6	3.83	3.33	3.17
-5	3.00	2.67	2.83
-4	3.50	3.17	4.00
-3	3.67	3.33	3.67
-2	3.83	3.33	3.83
-1	3.83	3.67	3.67
0	3.67	3.50	3.50
Mean days -6 to 0 (week 2)	**3.62**	**3.29**	**3.52**
+1	4.00	3.33	3.50
+2	3.33	2.67	2.83
+3	3.83	3.17	3.83
+4	3.83	3.33	4.00
+5	3.33	3.50	3.17
+6	4.00	3.67	3.17
+7	3.33	3.50	3.17
Mean days +1 to +7 (week 3)	**3.67**	**3.31**	**3.38**
+8	3.33	3.00	2.83
+9	3.67	3.33	3.17
+10	3.83	3.50	3.83
+11	3.50	3.33	3.67
+12	4.00	3.67	3.17
+13	3.67	3.33	3.33
+14	3.83	3.17	3.50
Mean days +8 to +14 (week 4)	**3.69**	**3.33**	**3.36**

Food consumption was scored daily using the following scoring system.

1 = 0- 25%
2 = >25- 50%
3 = >50-75%
4 = >75-100%

Individual daily food consumption scores for adult cats from day -14 to +14 are reported on p. 6 of MRID 48513201.

Table 10. Mean daily and weekly food consumption values for the kitten groups						
Study Day	**Group A1 (1X)**		**Group A2 (5X)**		**Group C1 (0X)**	
	Pellets	Meat	Pellets	Meat	Pellets	Meat
-13	3.42	4.00	3.50	4.00	3.42	4.00
-12	3.92	4.00	4.00	4.00	3.83	3.83
-11	4.00	4.00	3.92	4.00	3.83	3.83
-10	3.92	3.83	3.92	4.00	3.92	4.00
-9	4.00	4.00	3.92	4.00	3.92	4.00
-8	3.83	4.00	3.92	4.00	3.75	4.00
-7	4.00	4.00	4.00	4.00	3.83	4.00
Mean days -13 to -7 (week 1)	**3.87**	**3.98**	**3.88**	**4.00**	**3.79**	**3.95**
-6	3.92	4.00	4.00	4.00	3.75	4.00
-5	3.83	4.00	3.92	4.00	3.75	4.00
-4	3.92	4.00	4.00	4.00	3.92	4.00
-3	4.00	4.00	4.00	4.00	3.58	4.00
-2	3.83	4.00	4.00	4.00	3.67	4.00
-1	3.83	4.00	4.00	4.00	3.92	4.00
0	3.83	4.00	4.00	4.00	4.00	4.00
Mean days -6 to 0 (week 2)	**3.88**	**4.00**	**3.99**	**4.00**	**3.80**	**4.00**
+1	3.92	4.00	4.00	4.00	3.75	4.00
+2	3.83	4.00	3.58	3.67	3.67	4.00
+3	4.00	4.00	3.83	4.00	4.00	4.00
+4	3.58	4.00	3.67	4.00	3.50	4.00
+5	3.92	4.00	4.00	4.00	3.83	4.00
+6	3.92	4.00	3.75	4.00	3.75	4.00
+7	4.00	4.00	3.92	4.00	3.42	4.00
Mean days +1 to +7 (week 3)	**3.88**	**4.00**	**3.82**	**3.95**	**3.70**	**4.00**
+8	4.00	4.00	3.92	4.00	3.67	4.00
+9	3.83	4.00	4.00	4.00	3.50	4.00
+10	3.58	4.00	4.00	4.00	3.67	4.00
+11	3.83	4.00	3.58	4.00	3.75	4.00
+12	4.00	4.00	3.83	4.00	4.00	4.00
+13	4.00	4.00	4.00	4.00	4.00	4.00
+14	3.83	4.00	3.83	4.00	3.67	4.00
Mean days +8 to +14 (week 4)	**3.87**	**4.00**	**3.88**	**4.00**	**3.75**	**4.00**

Food consumption was scored daily using the following scoring system.

1 = 0- 25%
2 = >25- 50%
3 = >50-75%
4 = >75-100%

Individual daily food consumption scores for kittens are reported on p. 20-21 of MRID 48513201.

D. CLINICAL PATHOLOGY ANALYSES:

1. **Statistical considerations:** From p. 43 of MRID 48302108: "The statistical analysis was designed to screen the results in order to point out differences between each of the 2 test treatment groups (B1 and B2 in the case of adult cats and A1 and A2 for kittens) and the corresponding control groups (C2 for adult cats and C1 for kittens) that should be considered more closely from a clinical and safety point of view. The following strategy was followed to point out these differences for further review:

a. If there were any values on either test days 1, 7 or 14 in at least one animal in a particular test group that were outside the reference range, and

b. If the difference in the mean values for a particular parameter between a specific test group and the control group was statistically significant from the between-group comparison (from either the ANOVA or CO-ANOVA analysis) and

c. If the within-group comparison for the particular test group from test day -6 (baseline) to the post-treatment day was statistical[ly] significant."

2. **Hematology**: From p. 44 of MRID 48302108 no treatment-related changes were observed in adult cats. For kittens: "The mean prothrombin time [for group A1, the 1X kittens] decreased from 8.52 to 7.03...from baseline (day -6) to day +7, with 5 of the 6 kittens (CC0 4A8, CC0 F92, CC1 26E, CC1 C0A and CC D85) having values 6.8, 6.8, 6.1, 7.2 and 7.5 respectively, all of which were below the reference range of 7.8 to 8.9." The group A2 kittens also had significantly reduced prothrombin times for days 7 and 14. For group A2 (5X kittens): "The mean aPTT [activated partial thromboplastic time] decreased from 15.33 to 13.25...from baseline (test day -6) to test day -1, with 1 of the 6 kittens (CC0 B97) having a value of 11.0, which was below the reference range of 12.7 to 17.9." However, pathology for PT and aPTT generally involves increased values.

Table 11. Statistically significant changes from baseline in hematology parameters for kitten groups

Hematology Parameter	Kitten Group	Day -6 Mean	Day +1 Mean	p-value	Day +7 Mean	p-value	Day +14 Mean	p-value
Prothrombin Time (sec)	A1(1X)	8.52	8.22	0.0913	7.03	0.0048	7.22	0.0006
Prothrombin Time (sec)	A2(5X)	8.43	8.58	0.6560	7.85	0.0127	7.57	0.0048
Partial thrombo-plastin time (sec)	A2(5X)	15.33	13.25	0.0375	13.97	0.1629	13.48	0.1259

2. **Clinical Chemistry**: For adult cats, from p. 44 of MRID 48302108: "The mean albumin value [of group B1] decreased from 30.0 to 29.0...from baseline (test day -6) to test day 7, with 1 of the adult cats (963 FE5) having a value of 24, which was below the reference range of 25 to 34."

For group B1: "The mean total bilirubin value increased from 7.17 to 8.0...from baseline (test day -6) to test day 14, with 1 of the 6 adult cats (CC5 325) having a value of 12, which was above the reference range of 4 to 11... At no other days in any of the two groups were all three of the conditions set out...[see statistical considerations, above] met for any other clinical chemistry parameters in the adult cats."

For kittens, from p. 45 of MRID 48302108 for group A1 (1X): "The mean total chloride value increased from 121.2 to 125...from baseline (test day -6) to test day 7, with 1 of the 6 kittens (CC1 C0A) having a value of 126, which was above the reference range of 117 to 125..."

Also for group A1: "The mean total protein value increased from 59.3 to 62.0...from baseline (test day -6) to test day 14, with 1 of the 6 kittens (CC1 C0A) having a value of 68, which was above the reference range of 56 to 67..."

These findings appear to be sporadic, and unrelated to exposure to the test material.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded [p. 116 of MRID 48302108] that two adult cats developed minor lesions after application of a single dose of the test substance, but no signs were recorded in the group that received five times the dose.

In group B2 (5X) two cats developed crystals on the hair tips after application. On one cat these were visible on day 0 at the 2- and 3-hour observations, while they were visible from 1 hour until day 2 on the other. These were considered to be cosmetic changes.

One kitten developed scabs after application of a single dose of the test substance and one kitten developed wet eczema after receiving five times the dose (both were albino kittens).

According to the discussion on p. 7 of MRID 48302109: "It is not apparent if the delayed appearance of eczema and scabs was coincidentally unrelated, or a direct consequence of the application of the test substance. An earlier CASS study report (MRID 46161309) in which cats and kittens were also treated at 1X and 5X but with larger until dose volumes (0.7 and 1.4 mL, instead of 0.5 mL) and with an etofenprox-Nylar test substance containing a much higher level of etofenprox (55% vs. 15%) did not describe any dermatopathology... However, the present CASS test substance also contained fipronil, a different class of active that has been very widely used now for years, apparently without gathering a reputation for inducing significant dermatopathology."

From p. 37 of MRID 48513201: "Kitten CC2 AB1 (group A2, 5x dose) had "hot spots" on the side of its neck and between its shoulder blades, first observed on the third day after treatment and persisting through day 14, reported as starting to resolve on day 9... Kitten CCO F92 (group A1, 1x dose) had scabs on the back of its head recorded from days 7 to 14 and between its shoulder blades on days 7 and 8 (resolved on day 9)... Both of these kittens were littermate albinos and there were no other kittens (albino or otherwise) from this litter enrolled in the study. There were no other dermatologic reaction reports for any of the other kittens in any group in the study. It is unfortunate that albino kittens, a comparative rarity in the cat population, were included in a companion animal safety study. ...However, one must suspect that the unique skin reactions in the area of test substance application to these two albino kittens may not properly be extrapolated to what will occur in the US cat population when this product may be released onto the market."

B. REVIEWER'S COMMENTS AND CONCLUSIONS: From the confidential attachment to MRID 48513201, it has been confirmed that the solvent(s) and/or inerts present in the test material are the same as those in the products which will be supported by this study.

All animals survived to the end of the study, and there were no indications of any systemic toxicity. There were no effects on body weights. Most cats and kittens showed no dose-related changes in food consumption, although one Group A2 (5X) female kitten scored 2 for meat consumption on Day +2 (kittens received pellets and meat, with separate scoring for their consumption of these two items). Of 522 observations for the consumption of meat, there were

518 "4's" (>75 to 100% consumption); 3 "3's" (>50-75% consumption), and one "2" (>25-50% consumption). Remarkably, the score of "2" involved the 5X albino kitten (CC2 AB1) observed to have a "hotspot on side of neck behind ear" starting on Day +3 which necessitated treatment. On every other pre- and post-treatment day of the study this kitten scored "4" for meat consumption.

Hourly observations for adult cats and kittens for the four hours after last application are reported in MRID 48513201. For adults (from p. 11 of MRID 48513201) one B2 female (8B1 C65) showed restless rolling with a duration of ~10 minutes after the third application (this observation was unique, as it was made before the series of 5 applications of test material had been completed), but had calmed down one hour later, then (from p. 9 of MRID 48513201) was restless at the first hourly observation post-application, and was showing signs of estrus at the next observation, but showed no signs at the next two observations. Two B2 males showed crystals (presumably from evaporation at the application site), one at the 2 hour observation, and both at the 3 and 4-hour observations. The crystals were still present on one male on Days 1 and 2.

For kittens, no signs were observed in the four hours following the last application, and the only signs that were observed were dermal effects in 5X albino kitten CC2 AB1 (first noted on Day 3) and scabs behind head and between shoulder blades in 1X albino kitten CCO F92 (first observed on Day 7).

There were dermal effects. Two adult cats in group B1 (1X) scratched the application site and lost hair after application, and the report states that these lesions were considered to be a result of exposure to the test material. One of these cats had preexisting alopecia (measuring 3 mm x 3 mm on the dorsal neck, between the ears) on days -7 and -1, and then had a scratch mark (20 mm x 10 mm) on days +1 and +7 at the application site, "moving anterior." The second had no preexisting condition, but then had a scratch mark at the application site on study days +1, +7, and +14. None of the adult cats in the 5X group showed any dermal signs. One kitten in the 1X group had scabs on the back of the head from day 7 to 14 and scabs between the shoulder blades on days 7 and 8; this kitten was an albino. One kitten in the 5X group (also an albino) had acute wet eczema on the neck and shoulders from day 3 to 14, and this required treatment with Dermavet Cream. It is stated (p. 36) that this lesion: "...was considered to be related to the application of the test substance."

From page 37 of MRID 48513201 both of the albino kittens were littermates, and there were no other kittens (albino or otherwise) from this litter in this study. As noted on p. 37 of MRID 48513201: "It is unfortunate that albino kittens, a comparative rarity in the cat population, were included in a companion animal safety study. The literature on defects or susceptibilities associated with albinism in cats describes a propensity for deafness, but no references to dermatologic effects. However, one must suspect that the unique skin reactions in the area of test substance application to these two albino kittens may not properly be extrapolated to what will occur in the US cat population when this product may be released onto the market."

TRB has concluded that the most probable explanation for the observed effects in the albino kittens is that the toxicity was due to a fipronil photodegradate (MB46513, also known as fipronil-desulfinyl) which is more toxic than the parent compound. It has been established that fipronil accumulates in sebaceous glands. The dose-related response observed in the albino kittens (which lack melanin, which would normally block light from reaching fipronil) is consistent with

formation of this photodegradate (particularly as effects appeared on day 3 in the 5X kitten and on day 7 in the 1X kitten). The possibility also exists that the presence of one or more of the other ingredients in this formulation may have enhanced the formation of this photodegradate in the albino kittens.

The registrant should provide the Agency with any additional information available from this study relevant to the formation of the fipronil degradate, including (if possible) the fur coloration of the animals at the application site and the level and type of lighting that the cats and kittens were exposed. This information should be received within 6 months of the issuance of a registration.

This companion animal safety study in adult cats and kittens is upgraded to Acceptable and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in cats and/or kittens with the stipulation that labeling indicates that this product is not to be used on albino cats or kittens.

1. **DP BARCODE:** 385149
2. **PC CODES:** 129121 (Fipronil); 105401: (S-Methoprene); 128965: (Etofenprox); 129032 (Pyriproxyfen)
3. **CURRENT DATE:** July 26, 2011
4. **TEST MATERIAL:** Cat Spot on Soup, containing Fipronil (9.83%), S-Methoprene (11.76% w/w), Etofenprox (15.78%) and Pyriproxifen (2.34%)

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Study/AdultCats & Kittens ClinVet International ; Bloemfontein, South Africa Study CV 09/660 / July 13, 2010.	48302108 48302109 48513201	Three groups, each containing 3M & 3F adult cats, and 3 groups, each containing 3M & 3F kittens (12-16 weeks of age) were treated (along the back of each animal) with either 0.5 mL (1X) or 2.5 mL (5X) of test material or with 1.45 mL (5 x 0.29 mL) of the control material. Dermal effects occurred in two 1X adults (but none of the 5X adults) and in one 1X and one 5X kitten. Both affected kittens were albino. No indications of any effect involving hematological, clinical chemistry, or body weights. Food (meat) consumption data indicates a possible effect on the 5X albino kitten on Day 2, shortly before the appearance (Day 3) of dermal effects. TRB has concluded that the most probable explanation for the observed effects in the albino kittens is that the toxicity was due to a photodegradate of fipronil. Labeling should stipulate that this product is not to be used on albino cats or kittens.	N/A	A

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