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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

CA - dogs

September 22, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	CERTIFECT FOR DOGS
	EPA Reg. No. /File Symbol:	65331-T
	DP Barcode:	DP 372058
	Decision No.:	423378
	Action Code:	R320
	PC Codes:	129121 (Fipronil); 105402 (S-Methoprene); 106201 (Amitraz)

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

Byron T. Backus
SEPT-22-2010

M Hasler

To: Autumn Metzger/John Hebert RM 07
Insecticide-Rodenticide Branch
Registration Division (7505P)

Registrant: MERIAL LIMITED

FORMULATION FROM LABEL:

Side A

Active Ingredient(s):		By wt.
129121 Fipronil		9.8%
105402 (S)-Methoprene		8.8%
Other Ingredient(s):		81.4%
	TOTAL	100.00%

Side B

Active Ingredient(s):		By wt.
106201 Amitraz		22.1%
Other Ingredient(s):		77.9%
	TOTAL	100.0%

"The amount of active ingredients in the total volume is equivalent to 6.4% Fipronil, 5.8% (S)-Methoprene, and 7.6% Amitraz."

ACTION REQUESTED: The Risk Manager requests:

"...Please review the following data submission for the newly proposed spot-on for dogs. The formulation is made up of half a currently registered product and half a new product with a new ai, however, the two are separated within the container. The new data submitted does test the entire combined product. Please see the company's cover letter for more information..."

BACKGROUND:

The material received includes a companion animal safety study (in MRID 47914235) with adult (9-10 months old at first treatment) beagles, a proposed label (dated 11/13/09), a cover letter dated November 13, 2009, and a CSF dated Dec. 9, 2009.

COMMENTS AND RECOMMENDATIONS:

1. An Agency contractor, Oak Ridge National Laboratory, conducted the primary review of the companion animal safety study in MRID 47914235. TRB and HED conducted the secondary and tertiary reviews and made changes as necessary.

2. The study was conducted in a scientifically acceptable manner. However, the Agency's interpretation of respiratory rate, heart rate and body temperature changes was difficult based on the lack of key information. For example, statistics were not performed on separate gender group mean data. Interpretation of clinical chemistry data was difficult because statistics were reported as the number and percentage of individual dogs with values above reference ranges, rather than on actual measured clinical chemistry values. Given that females appeared more sensitive than males with regard to some clinical chemistry parameters, separate statistical analysis of males and females should have been conducted for both hematology parameters and clinical signs. These statistical concerns should be addressed.

3. Because of treatment-related effects on heart rate, body temperature, and some hematology and clinical chemistry parameters, the margin of safety for this combined formulation was not established at 5X or 3X the recommended dose; in addition, there were sporadic indications of effects at the 1X dose level. The Agency recommends that these issues be addressed according to the 870.7200 Guidelines which state: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..."

4. As noted in the attached DER, the proposed label states that the product is intended for once a month application for control of flea, ticks, and chewing lice. However, the proposed label also states that: "CERTIFECT® aids in the control of sarcoptic mange infestations. Multiple monthly treatments are recommended for the elimination of mites." It is not clear whether the term "Multiple monthly treatments" means more than once a month, or a number of once-a-month treatments. Clarification of the intended dosing schedule is important, especially in light of the fact that this study was conducted with repeated dosing at two-week intervals.

5. Based on the treatment-related findings and the lack of adequate statistically measured parameters, the study in MRID 47914235 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the dog. The study is potentially upgradeable if the registrant adequately addresses the statistical reporting and toxicological concerns indicated above. Refer also to the study deficiencies (provided in Section C of the attached DER).

6. Refer to the attached DER for additional comments regarding this study.

DATA EVALUATION RECORD

S-METHOPRENE, AMITRAZ, AND FIPRONIL
OPPTS 870.7200
COMPANION ANIMAL SAFETY STUDY- DOGS
MRID 47914235

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
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Prepared by

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Signature: Robert H. Ross
Date: JUL 27 2010

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: JUL 27 2010

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE_AC05_00OR22725.

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

Signature: Byron T. Backus
Date: 9/22/2010

EPA Tertiary Reviewer: Ayaad Assaad, DVM, Ph.D.
Toxicology and Epidemiology Branch, HED (7509P)

Signature: A Assaad
Date: 9/22/2010

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- dogs [OPPTS 870.7200]

PC CODES: 105402; 106201; 129121 **DP BARCODE:** 372058

TEST MATERIAL (PURITY): S-Methoprene (9%), Amitraz (20%), and Fipronil (10%). Purity is given as % by weight of each component in the formulation.

SYNONYMS: Frontline Plus; ML-2,095,988 509T (S-Methoprene : 8.97% + Fipronil : 9.99% ; this formulation is registered under EPA Reg. No. 65331-5). ML-3,489,906 (Amitraz: 20.0% w/v; 22.1% w/w; specific gravity = 0.906 g/mL).

CITATION: Gerhardy, Cecilia. (2009) Safety of a combination of ML-2,095,988 509T and ML-3,948,906 when topically administered at 1, 3, and 5 times the target dose in beagle dogs. MDS Pharma Services, 329 Impasse du Domaine Rozier, Les Oncins, 69210 Saint Germain sur L'Arbresle, France. Study Number PR&D 0164201 (Sponsor), AA73007 (MDS), October 23, 2009. MRID 47914235. Unpublished.

SPONSOR: Merial Limited, 3239 Satellite Blvd., Duluth, GA 30096-4640, USA.

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47914235), groups of six male and six female beagle dogs/group were topically administered either 1X, 3X, or 5X the recommended dose of the combination of FRONTLINE® Plus [Fipronil (10% a.i.) and (S)-methoprene (9% a.i.)] and amitraz (20% a.i.). The product, CERTIFECT® for Dogs, is proposed as a novel dual applicator. One chamber of the applicator contains the EPA-registered product Frontline® Plus for Dogs [9.8% w/v fipronil and 8.8% w/v (S)-methoprene], while the other chamber contains 22.1% w/v amitraz (these percentages of active ingredients are the same as those on the proposed label). The amounts for each active in the total volume are 6.4% Fipronil, 5.8% (S)-Methoprene and 7.6% Amitraz. Control groups of six males and six females were treated with saline at 5x the recommended volume of the proposed product. Treatments were twice monthly for approximately three months (on Days 0, 14, 28, 42, 56 and 70). At first treatment, the dogs were 9-10 months old, males weighed 6.1-9.7 kg and females weighed 4.9-7.8 kg. The 1X dosage rates for dogs weighing up to 10 kg were 0.67 mL ML-2,095,988 509T and 0.40 mL ML-3,948,906; for dogs weighing 10.1 to 20.0 kg dosage rates were 1.34 mL ML-2,095,988 509T and 0.80 mL ML-3,948,906. The dosage rates for ML-2,095,988 509T are consistent with those for EPA Reg. No. 65331-5 (FRONTLINE® Plus).

No deaths were observed. Body weight and food consumption were unaffected by treatment. Dogs in the 5x group had significantly ($p<0.10$) decreased respiratory rates after treatment in weeks 1, 5, 7, and 9 (statistics were performed for male and female dogs combined). The first observation performed the day after each treatment showed decreased mean heart rates for 3 of 6 treatments for 1X males, 4 of 6 treatments for 3X males, 3 of 6 treatments for 3X females, 6 of 6 treatments for 5X males, and 5 of 6 treatments for 5X females when compared to both pre-test values and values measured prior to each treatment. Dogs (males and females combined) in the 5X group had significantly ($p<0.10$) decreased heart rates for all treatment weeks. Compared with pre-treatment values, animals in group 4 had a decrease in mean body temperature after each treatment.

Decreased erythrocyte counts, packed cell volume, and hemoglobin concentration were observed at all dose levels the day after each treatment, compared to control and pre-test values. Changes in reticulocyte counts paralleled changes in erythrocyte counts, packed cell volume, and hemoglobin concentration. Increased ($p<0.10$) total white blood cell, monocyte, and neutrophil counts were noted in the 3X and 5X groups the day after treatment. Treatment-related clinical chemistry effects included increases in Blood Urea Nitrogen (BUN), glucose, calcium, phosphorus, potassium, and sodium values; females were affected more than males, especially with regard to BUN, calcium, phosphorus and potassium. The frequency of hematological and clinical chemistry effects increased with proximity to dosing, with recovery and/or the absence of statistically significant changes noted by 7 or 14 days post-dosing (Day 84 only).

At necropsy, there were no treatment-related organ weight changes, macroscopic effects or systemic microscopic effects. Perifollicular/periadnexal inflammation was noted in all groups and was characterized by rare neutrophils around sebaceous glands and hair follicles. This effect was slightly increased in severity and incidence at the shoulder application site of the 5x group dogs.

The study was conducted in a scientifically acceptable manner. However, the Agency's interpretation of respiratory rate, heart rate and body temperature changes was difficult based on the lack of key information. For example, statistics were not performed on separate gender group mean data. Interpretation of clinical chemistry data was difficult because statistics were reported as the number and percentage of individual dogs with values above reference ranges, rather than on actual measured clinical chemistry values. Given that females appeared more sensitive than males with regard to some clinical chemistry parameters, separate statistical analysis of males and females should have been conducted for both hematology parameters and clinical signs. These statistical concerns should be addressed.

Because of treatment-related effects on heart rate, body temperature, and some hematology and clinical chemistry parameters, the margin of safety for this combined formulation was not established at 5X or 3X the recommended dose; in addition, there were sporadic indications of effects at the 1X dose level. The Agency recommends that these issues be addressed according to the 870.7200 Guidelines which state: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..."

Based on these treatment-related findings and the lack of adequate statistically measured parameters, the study in MRID 47914235 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the dog. The study is potentially upgradeable if the registrant adequately addresses the statistical reporting and toxicological concerns indicated above. Refer also to the study deficiencies (provided in Section C).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:

	ML-2,095,988 509T (Frontline® Plus for dogs)	ML-3,948,906
Description:	Colorless liquid	Normally a pale yellow solution (the color was not determined by the dispensary unit in error)
Batch #:	D62705AR	500A 001
Purity:	10% w/v Fipronil; 9% w/v S-Methoprene	20% w/v Amitraz
Compound Stability:	Expiration date: April, 2011 (relevant stability data on file with Merial)	Expiration date: April, 2011 (relevant stability data on file with Merial)
CAS #:	Fipronil: 120068-37-3 S-Methoprene: 65733-16-6	Amitraz: 33089-61-1

2. Vehicle and/or positive control: Sterile physiological saline (0.9% NaCl) [Batch No. 5001373 (first three treatments) and 5001549 (last three treatments)] was used as a control.

3. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Males: 9 to 10 months/ 6.1-9.7 kg Females: 9 to 10 months/ 4.9-7.8 kg	
Source:	Marshall Bioresources, North Rose, NY, through Marshall BioResources Lyon, France	
Housing:	Individually in pens (except for a 2 hour per day socialization period in groups of three animals of the same sex)	
Diet:	Pelleted complete commercial diet (Diet 125 C3 Safe) at 300 g/dog/day	
Water:	Filtered water, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 3^0 C
	Humidity:	≥35%
	Air changes:	Minimum of 10/hour
	Photoperiod:	12 hr light/12 hr dark
Acclimation period:	20 days	

B. STUDY DESIGN:

1. **In life dates:** Start: May 15, 2008; End: August 7, 2008 (males) or August 8, 2008 (females)

2. **Animal assignment:** During the pretest period, the dogs were ranked by decreasing body weight within gender; six replicates of four males were formed. The four heaviest males formed replicate 1; the next four heaviest male dogs formed replicate 2, and so on. This was repeated with female dogs. Within replicates, one dog was randomly allocated to each of the four treatment groups. Each of the four treatment groups contained 6 males and 6 females.

TABLE 1. Study design[a]

Test Group	Body weight range	Dose Volume (mL/dog)				Dose level in relation to target dose	Number of males	Number of females
		NaCl	Frontline® Plus	ML-3,948,906	Total			
Group 1	Up to 10 kg	5.35	0	0	5.35	-	6	6
	10.1 to 20 kg	10.70	0	0	10.70			
Group 2	Up to 10 kg	0	0.67	0.40	1.07	1x	6	6
	10.1 to 20 kg	0	1.34	0.80	2.14			
Group 3	Up to 10 kg	0	2.01	1.20	3.21	3x	6	6
	10.1 to 20 kg	0	4.02	2.40	6.42			
Group 4	Up to 10 kg	0	3.35	2.00	5.35	5x	6	6
	10.1 to 20 kg	0	6.70	4.00	10.70			

[a] Data from p. 26 in MRID 47914235

3. **Dose selection rationale**: The doses used for group 2 are the dose levels of the end-use product. The dose levels used for groups 3 and 4 are 3X and 5X the dose levels of the end use product, respectively. According to the proposed label (dated 11/13/09) dogs weighing up to 22 lbs would receive 1.07 mL, those 23-44 lbs would receive 2.14 mL; 45-88 lbs would receive 4.28 mL, and 89-132 lbs would receive 6.42 mL.

4. **Preparation and treatment**: Dogs were weighed 24 hours prior to each treatment. Treatments were twice monthly for approximately three months. On Days 0, 14, 28, 42, 56, and 70 control or test material was applied via syringes filled just prior to administration. The hair was parted and equal volumes of formulations were applied directly onto the skin on two separate sites. One site was on the midline of the neck between the base of the skull and the shoulder blades, and the other site was between the shoulder blades. To avoid product run-off in treatment groups 3 and 4, the total volume was divided into multiple administrations applied approximately 30 minutes apart. For group 1 animals, the placebo did not diffuse through the haircoat; therefore, it was spread on the application site with a finger in order to avoid run-off.

Directions for application on the proposed label state: "Place applicator tip through dog's hair to the skin level. Squeeze applicator, applying entire contents in two separate spots on the neck between the base of the skull and the shoulder blades."

5. **Statistics**: Descriptive statistics (mean and standard deviation) for body weight, body weight gain, food consumption, rectal temperature, heart rate, respiratory rate, hematology, coagulation, serum chemistry, terminal body weight and organ weight were calculated for each group and sex. Terminal body weight and organ weights were assessed for normality of distribution using Kolmogorov's test. Variance of homogeneity across groups was assessed with Barlett's test. Normally distributed and homogeneous data were then tested by ANOVA followed by Dunnett's test. Non-normal or non-homogeneous data were assessed by the Kruskal-Wallis test followed by Dunn's test.

   Continuous plasma chemistry and hematology values (expect percentages for white blood cell differentials), respiration rate, temperature, heart rate, and body weight were analyzed by repeated measures analysis of covariance (RMANCOVA). Five variance-covariance structures were compared for each variable using the Akaike Information Criterion and the one with the lowest value (matrix structure) was selected.

   The interaction of "treatment by sex by sampling day" was evaluated at the significance level of alpha = 0.05. If significance was attained, pair-wise comparisons of dose within sex and sampling day would not have sufficient power to be useful. If "treatment by sex by sampling day" was not significant, the terms "treatment by sex" and "treatment by sampling day" were assessed. If "treatment by sex" was significant at alpha = 0.05, no further statistical testing was performed. If "treatment by sampling day" was significant at alpha = 0.10, pair-wise comparisons of each non-zero dose group were performed against the control group at each sampling day; pair-wise comparisons used a significance level of alpha = 0.10. If neither of the two-way interaction terms were significant, the main effect of treatment was evaluated at alpha = 0.10. If this term was significant, the hypothesis of no difference between treatment groups was rejected. Each non-zero dose group was compared with the saline control group; these comparisons were performed using linear contrasts at alpha = 0.10.

   Incidence of histopathological or gross evaluation findings were analyzed using a Fisher's Exact Test. Each treatment group was compared to the control at a significance level of alpha = 0.10.

C. METHODS:

1. Observations

1a. General health observations: Morbidity and mortality observations were conducted on each animal at least twice daily.

1b. Clinical assessments: Animals were also observed at the following times: Three times pretest [Day 15 (no heart rate measured)], Day -9, and Day -2; On treatment days prior to dosing and 10 minutes, at hourly intervals for four hours, and 8 hours after dosing; Twice per day (at least 4 hours apart) on other days except Days 2, 3, 16, 43, and 45 when the

minimum time between observations was 2 hours 55 minutes. The parameters evaluated included skin/fur/application site (alopecia, erythema, pruritus, skin lesions); eyes (nystagmus, papillary changes, photophobia, blepharospasm, conjunctivis, congestion, discharge); respiratory rate and abnormal respiration, mucous membrane reddening or pallor, heart rate and rhythm, ataxia, weakness, abnormal behavior, tremors, salivation, vomiting, diarrhea, and rectal temperature.

2. **Body weight**: Animals were weighed on Study Days -10, and -2, 24 hours prior to each treatment (Days -1, 13, 27, 41, 55, and 69), and the day prior to necropsy (Day 83 for males and 84 for females).

3. **Food consumption**: Food consumption was measured daily for each dog and reported as g/animal/day. Food consumption were recorded starting two weeks prior to initiation of treatment. (No food consumption data were available for one group 1 female from Days 21 to 22, two group 2 males (Days 76 to 77 and Days 2 to 3), one group 2 female (Days 48 to 49), and one group 4 female (Days 43 to 44) due to aberrant data.

4. **Hematology and clinical chemistry**: Blood was collected for hematology and clinical chemistry assessments on control and treated animals twice pretest (Days -10 and -6) and 24 hours after each treatment (Days 1, 15, 29, 43, 57, 71), and on Days 7, 35, 63, and 84. Blood was drawn from the jugular vein of manually restrained, unanesthetized animals. Animals had been fasted for at least 15 hours prior to sampling. 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
	Blood clotting measurements*	X	Red cell morphology
X	(Thromboplastin time)	X	Large unstained cells
X	(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*
	Sodium/Potassium ratio	X	Urea nitrogen*
X	Phosphorus *	X	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 protein*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase	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
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		
* Recommended for a companion animal safety evaluation based on OPPTS 870.7200			

5. **Necropsy and pathology**: Two weeks after the last dosing (Day 84 for males and Day 85 for females), all animals were euthanized and necropsied. Animals were fasted overnight, administered a pre-anesthetic (Tiletamine and Zolazepam injection), weighed, and euthanized by an intraveneous injection of sodium pentabarbitone followed by exsanguination. A gross necropsy was performed on all animals, and the following were examined: external surface, all orifices, cranial cavity, external surface of the brain and samples of the spinal cord, thoracic and abdominal cavities and organs, and the carcass.

   The CHECKED (X) tissues were collected and fixed in 10% neutral formalin, stained with hematoxylin-eosin (H&E) stain and examined. (Bone marrow smears were air dried and stained with May Grunwald Giemsa, Perl's blue and Masson's trichrome, and testes, epididymides, eyes and optic nerves were fixed in modified Davidson's fluid. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta, thoracic	XX	Brain (multiple sections)
X	Salivary glands	XX	Heart	X	Periph.nerve
X	Esophagus	X	Bone marrow	X	Spinal cord
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (retina, optic nerve)
X	Jejunum	XX	Thymus	X	GLANDULAR
X	Ileum			X	Adrenal gland
X	Cecum	X	UROGENITAL		Lacrimal gland
X	Colon	XX	Kidneys	XX	Parathyroids
	Rectum	X	Urinary bladder	XX	Thyroids
XX	Liver	XX	Testes	X	OTHER
X	Gall bladder	XX	Epididymides	X	Bone (sternum and/or femur)
	Bile duct	X	Prostate	X	Skeletal muscle
X	Pancreas		Seminal vesicle	X	Skin
X	RESPIRATORY	XX	Ovaries	X	All gross lesions and masses
X	Trachea	XX	Uterus		
X	Lung	X	Mammary gland		
	Nose	X	Vagina/Oviducts		
	Pharynx				
	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: No clinical signs summary data tables were provided; this is considered a reporting deficiency. (Individual animal data are presented in Appendix 1 of the study report, pages 381-1294). Clinical signs data were summarized as a narrative as follows. Slight to marked hair loss of the whole body was noted in all groups throughout the study. The incidence in control and 5X-treated males was similar; therefore, the effect is not considered treatment-related for males. However, the incidence was higher in 5X-treated females compared to controls up to the fifth treatment. The study author states that areas of alopecia were not observed during the treatment period, and therefore, this finding was not considered a toxicological effect.

   Vomiting was observed only in treated groups and was significantly ($p<0.05$) different from the control. The study author stated that this effect was considered incidental to treatment, because it was not specifically noted after treatment days and was also observed in Group 3 and 4 females during pretest.

   Slight to abundant liquid feces were observed throughout the study in all dose groups; significance ($p<0.05$) was attained only in the 3X group. The study author stated that this effect was considered incidental to treatment, because it was not specifically noted after treatment days and was also observed during pretest and in the control group.

Red buccal mucous and ocular membranes and colored skin were noted throughout the study in all treatment groups and controls, and were not considered treatment-related. Scabs, sores, and swollen mammary glands were also considered incidental to treatment, because they are "normal or occur spontaneously in this species."

2. **Cosmetic effects**: White deposits, greasy fur, and clumping of hair on the application area were noted throughout the study in all groups starting 10 minutes after application. On treatment days, these effects were noted in essentially all treated animals, and persisted for 6 to 7 days in the 1x group, 8 days in the 3X group, and 9 or 10 days in the 5X group. Greasy fur was significantly ($p<0.05$) increased in all treated groups compared to the control.

3. **Mortality**: No animals died in the control or treated groups.

4. **Respiratory rate**: Dogs in the 5X group had significantly ($p<0.10$) decreased respiratory rates after treatment in weeks 1, 5, 7, and 9 (statistics were performed for male and female dogs combined). Statistical significance was also attained for 1X and 3X dogs at 4 and 8 weeks; however, no effect was noted in the 5X animals at these time points. Statistical analyses were not performed on separate male and the female data. The author stated that although a treatment-related effect could not be excluded, the facts that individual animal values remained close to pretest levels and lowest control levels, and no accompanying clinical signs were noted suggested that this effect was not toxicologically significant. Selected respiratory rate data are summarized in Table 2.

TABLE 2: Mean respiratory rate (breaths/min) data in dogs treated with S-methoprene, amitraz, and fipronil[a]

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-2	29.7±4.3	27.8±8.0	29.2±5.2	25.8±5.2	29.0±3.5	29.0±5.6	25.0±5.8	26.7±5.2
0[b]	26.0±4.6	26.3±2.0	31.8±2.5	29.2±2.9	30.5±5.7	34.2±2.0	24.8±5.7	25.7±5.8
7[c]	21.7±2.9	25.3±6.7	26.0±4.6	23.3±1.0	26.0±4.0	25.0±4.3	27.7±6.6	23.7±1.6
14[b]	27.3±5.5	26.5±8.5	29.8±4.2	30.0±5.1	26.7±2.9	23.8±3.8	30.0±4.9	23.7±2.4
21[c]	23.3±3.1	24.5±7.8	28.0±4.6	23.3±6.1	27.0±5.6	25.3±5.6	27.7±3.5	25.0±4.4
28[b]	24.8±2.3	27.5±5.5	23.5±2.2	25.8±4.8	22.7±5.5	23.7±3.7	23.0±3.1	20.0±2.6
35[c]	22.3±2.3	26.0±5.4	23.3±4.3	26.7±3.9	21.0±4.1	24.0±4.7	23.0±6.0	25.7±3.2
42[b]	22.3±5.8	25.3±4.5	27.5±4.0	27.2±5.8	27.8±3.4	26.2±3.8	21.0±2.4	19.0±2.1
49[c]	25.5±4.0	21.7±3.7	24.3±4.6	29.0±10.9	25.0±4.1	27.2±5.9	29.0±4.3	24.3±3.6
56[b]	26.0±7.6	23.2±4.6	27.2±6.9	26.7±5.2	27.7±4.3	24.5±4.2	25.7±3.5	17.3±2.1
63[c]	24.7±3.1	26.2±6.9	27.3±2.9	31.8±7.0	22.3±3.5	28.2±5.3	22.8±3.1	26.2±4.7
70[b]	27.0±4.9	29.7±6.6	23.7±6.8	26.3±7.6	27.2±4.7	30.3±6.2	29.2±6.4	20.2±2.2
84[c]	26.2±5.0	22.7±6.3	40.8±18.7	30.0±6.5	29.0±4.1	28.2±4.3	28.0±5.6	22.5±1.9

[a]Extracted from pages 55-106, MRID 47914235.
[b]4 hours post-treatment
[c]First measurement of the day

5. **Heart rate**: The first observation performed the day after each treatment showed decreased mean heart rates for 3 of 6 treatments for 1X males, 4 of 6 treatments for 3X males, 3 of 6 treatments for 3X females, 6 of 6 treatments for 5X males, and 5 of 6 treatments for 5X females when compared to both pre-test values and values measured immediately prior to each treatment. Dogs (males and females combined) in the 5X group had significantly ($p<0.10$) decreased heart rates for all treatment weeks. Statistical analyses were not performed on separate male and the female data. The report states that "most of the time, the decrease was noted on treatment days, but the relevance of the decrease was difficult to

assess, since the heart rate was probably increased as a result of frequent handling on treatment days." The report also states that individual values were "rarely outside the control range and between two treatments, they were comparable to pretest values." The author concluded that the decreased heart rate was treatment- and dose-related, but without toxicological significance. Selected heart rate data are summarized in Table 3.

TABLE 3: Mean heart rate (beats/min) ± S.D. in dogs treated with S-methoprene, amitraz, and fipronil[a]

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-2	81.0±10.6	83.7±15.4	86.0±29.4	79.7±9.0	75.7±11.8	87.3±5.9	81.3±9.1	83.3±10.2
0[b]	94.3±9.5	89.0±7.2	89.3±14.1	89.7±12.6	84.7±18.1	93.3±13.4	84.7±13.1	82.0±11.6
0[c]	88.7±8.7	93.3±9.9	88.3±15.9	86.3±10.0	80.0±11.5	82.3±12.2	82.3±10.1	76.0±8.2
1[d]	97.7±14.4	97.7±20.0	76.7±18.8	80.0±13.3	72.7±8.8	93.7±12.8	78.7±9.0	**65.7±2.9**
7[d]	78.7±12.5	102.3±22.1	90.0±8.9	94.3±12.5	95.3±9.9	102.3±13.2	93.3±6.2	102.3±17.3
14[b]	88.7±11.8	98.0±15.5	92.3±7.3	89.3±8.0	87.3±6.2	91.0±11.4	91.3±6.0	79.0±5.5
15[d]	80.7±7.3	70.3±5.0	73.3±8.8	**69.7±4.1**	**69.0±5.8**	79.0±6.4	**66.2±2.6**	**69.3±6.3**
21[d]	89.7±13.5	88.7±18.0	82.3±14.7	91.7±6.4	96.0±8.4	93.7±7.5	100.3±11.3	89.3±9.9
28[b]	92.0±12.1	92.0±11.7	88.3±7.1	92.0±11.0	84.3±5.3	94.7±6.4	83.3±9.0	88.0±3.1
29[d]	81.7±8.1	91.0±7.8	**69.7±11.6**	89.7±23.0	79.7±10.5	85.0±8.6	71.7±9.9	79.0±10.3
35[d]	89.3±14.3	94.0±11.4	94.7±9.6	88.3±12.0	100.0±21.7	102.7±14.9	98.0±8.9	93.0±10.9
42[b]	86.7±8.5	96.3±12.7	87.7±5.6	93.0±8.1	95.7±15.9	82.3±10.4	86.3±11.9	83.0±9.0
43[d]	95.0±14.6	100.0±8.3	91.0±4.5	87.0±10.9	91.0±7.0	79.3±9.1	94.7±5.8	**60.7±7.0**
49[d]	93.3±16.9	96.0±15.4	97.0±13.7	98.0±15.0	104.4±16.6	104.7±13.5	94.0±7.6	90.7±11.7
56[b]	93.0±11.6	95.0±9.3	80.7±9.3	93.7±5.7	87.0±10.9	85.3±11.4	80.0±5.8	86.0±8.4
57[d]	85.3±10.7	103.0±19.6	80.3±7.9	88.0±6.8	74.3±18.0	84.7±10.5	70.0±4.2	71.3±10.3
63[d]	99.7±14.2	108.7±10.3	104.0±10.4	119.3±10.6	112.0±10.1	117.3±11.8	115.0±9.8	112.7±7.8
70[b]	78.3±15.6	85.0±17.9	86.3±12.0	94.3±9.8	87.3±14.3	90.3±14.3	80.3±7.3	85.3±7.7
71[d]	85.3±12.3	102.0±21.3	88.0±7.2	95.7±7.2	74.7±10.3	86.7±15.1	79.3±8.9	71.3±7.8
84[d]	80.0±7.7	99.3±13.1	94.7±19.1	102.3±9.8	94.0±15.0	94.3±8.5	102.3±11.6	99.3±7.9

[a] Extracted from pages 108-149, MRID 47914235.
[b] 4 hours post-treatment
[c] 8 hours post-treatment
[d] First measurement of the day

On Day 1 the mean heart rate for Group 4 (5X) females was 65.7 beats/minute, with values that were all within a narrow range (62-70 bpm);

6. **Body temperature**: Compared with pre-treatment values, animals in group 4 (5X) had a decrease in mean body temperature after each treatment; the lowest mean temperature was most often measured 8 hours after treatment. The maximum decrease, noted on day 43, when compared to values recorded before the fourth treatment, was 1.32°C for group 4 males (approx. 3.4% decrease) and 1.35°C (approx. 3.5% decrease) for group 4 females; mean variation for controls was -0.18°C (approx. 0.46% decrease) for males and +0.21°C (approx. 0.54% increase) for females. The report states that individual values were "only occasionally outside the control range and 48 hours after treatment, individual values were comparable with pretest values." The author concluded that the decreased body temperature was treatment-related, but without toxicological significance. Lesser decreases in mean body temperature were noted in lower dose groups; the author stated that a treatment-related effect is questionable for these dose levels. No statistical significance was attained. Selected body temperature data are summarized in Table 4. Group mean body temperatures of less than 38°C are in bold; these occurred primarily in Group 4 females, and, to a lesser extent, in Group 4 males and Group 3 females.

TABLE 4: Mean body temperature (°C) data in dogs treated with S-methoprene, amitraz, and fipronil[a]								
	Group 1		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
0[b]	39.15±0.52	39.15±0.45	39.22±0.33	38.97±0.44	39.05±0.35	39.03±0.35	39.22±0.33	38.65±0.39
0[c]	38.75±0.37	38.73±0.41	38.63±0.50	38.28±0.55	38.43±0.39	38.18±0.61	38.08±0.33	**37.55±0.21**
1[d]	39.27±0.61	39.10±0.27	38.97±0.34	38.40±0.49	38.68±0.35	38.35±0.44	38.75±0.19	38.02±0.29
14[b]	38.88±0.42	38.87±0.52	38.55±0.38	38.57±0.51	38.87±0.16	38.87±0.39	38.98±0.39	38.88±0.27
14[c]	38.43±0.27	38.35±0.29	38.33±0.18	38.30±0.23	38.15±0.34	38.03±0.33	**37.98±0.28**	**37.77±0.14**
15[d]	38.58±0.44	38.90±0.47	38.42±0.31	38.23±0.34	38.38±0.70	38.07±0.94	38.02±0.43	**37.52±0.51**
28[b]	38.78±0.51	38.87±0.48	39.05±0.60	38.93±0.55	38.67±0.56	38.77±0.34	39.13±0.52	38.55±0.41
28[c]	38.22±0.26	38.43±0.27	38.38±0.26	38.40±0.41	38.25±0.27	**37.97±0.21**	38.00±0.37	**37.67±0.18**
29[d]	38.73±0.27	38.92±0.25	38.67±0.27	38.48±0.26	38.57±0.48	38.00±0.29	38.17±0.37	**37.77±0.37**
42[b]	39.03±0.29	38.82±0.41	39.05±0.36	38.87±0.47	39.05±0.61	38.85±0.32	39.22±0.53	39.00±0.34
42[c]	38.33±0.44	38.35±0.24	38.05±0.25	38.25±0.29	38.50±0.52	**37.85±0.31**	**37.97±0.21**	**37.77±0.40**
43[d]	38.88±0.34	39.03±0.40	38.55±0.27	38.27±0.37	38.70±0.40	38.03±0.28	38.40±0.14	**37.65±0.57**
43[e]	38.85±0.36	38.72±0.34	38.45±0.16	38.30±0.35	38.45±0.43	38.00±0.63	**37.90±0.41**	**37.80±0.51**
56[b]	39.12±0.65	38.87±0.16	39.07±0.27	38.88±0.32	38.82±0.29	38.72±0.21	38.82±0.42	38.52±0.26
56c	38.42±0.33	38.20±0.21	38.03±0.16	38.13±0.26	38.58±0.40	38.02±0.12	**37.98±0.17**	**37.87±0.36**
57[d]	38.73±0.29	38.80±0.35	38.35±0.22	38.57±0.27	38.07±0.62	38.28±0.35	38.32±0.16	**37.90±0.27**
70[b]	38.42±0.62	38.70±0.19	38.68±0.42	38.25±0.59	38.45±0.24	38.10±0.42	38.72±0.56	**37.90±0.48**
70[c]	38.30±0.20	38.40±0.23	38.27±0.16	38.25±0.10	38.43±0.45	38.20±0.33	38.15±0.14	**37.85±0.34**
71[d]	38.45±0.23	38.57±0.08	38.20±0.40	38.12±0.35	38.25±0.29	**37.97±0.24**	38.00±0.38	**37.82±0.40**
84[d]	38.40±0.30	38.65±0.29	38.98±0.30	38.47±0.18	38.50±0.49	38.57±0.28	38.87±0.29	38.48±0.21

[a]Extracted from pages 150-188, MRID 47914235.
[b]Before treatment [c]8 hours post treatment [d]First measurement of day [e]Second measurement of day

Occurrences of increased incidences of individual body temperature values below 38°C are shown in Table 5. Incidences of greater than 20% are shown in bold. Again, females in Group 4 were most susceptible following treatment, but females in Group 3 and males in Group 4 were also affected.

TABLE 5: Incidence of individual body temperature values below 38°C data/total number of temperature measurements between and following treatments for dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Period	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	M	F	M	F	M	F	M	F
Day -15 to before treatment on Day 0	0/24	1/24	0/24	2/24	1/24	2/24	0/24	1/24
10 min. after treatment – Day 1	0/48	0/48	0/48	4/48	0/48	**13/48**	3/48	**14/48**
Day 2 to before treatment on Day 14	5/150	3/150	1/150	15/150	2/150	7/150	2/150	7/150
10 min. after treatment[b]– Day 15[c]	0/48	0/48	1/48	4/48	6/48	**11/48**	5/48	**23/48**
Day 16 to before treatment on Day 28	1/150	0/150	0/150	4/150	2/150	2/150	2/150	6/150
10 min. after treatment[b] to Day 29[c]	2/48	2/48	0/48	5/48	2/48	5/48	8/48	**24/48**
Day 30 to before treatment on Day 42	0/150	0/150	4/150	1/150	1/150	1/150	1/150	7/150
10 min. after treatment[b] to Day 43[c]	1/48	0/48	2/48	6/48	1/48	**11/48**	**12/48**	**21/48**
Day 44 to before treatment on Day 56	0/150	2/150	0/150	1/150	4/150	5/150	3/150	5/150
10 min. after treatment[b] to Day 57[c]	1/48	2/48	3/48	2/48	5/48	5/148	9/48	**25/48**
Day 58 to before treatment on Day 70	7/149	1/150	2/150	9/150	7/150	7/150	6/150	26/150
10 min. after treatment[b] to Day 71[c]	2/48	3/48	3/48	8/48	8/48	**14/48**	7/48	**30/48**
Day 72 to Day 85	3/162	0/162	0/162	13/162	3/162	2/162	2/162	19/162

[a]Extracted from pages 1796-1947, MRID 47914235.
[b]Temperatures were taken at 10 minutes and at 1, 2, 3, 4 and 8 hours after each treatment
[c]Includes both measurements taken on the day after dogs were treated.

B. BODY WEIGHT AND WEIGHT GAIN:

Body weight data are presented in Table 6. There was no statistically significant finding (males and females combined) for body weight at any dose level during this study. Statistical analyses were not performed on separate male and the female data.

TABLE 6: Mean body weight (kg) data in dogs treated with S-methoprene, amitraz, and fipronil[a]

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-1	7.82±0.98	5.93±0.74	7.75±0.98	6.00±1.03	7.90±1.16	6.03±0.84	7.65±1.04	6.03±0.50
27	7.82±0.83	6.23±0.54	8.00±1.12	6.33±0.72	8.18±1.21	6.22±0.91	8.07±1.19	6.00±0.70
55	7.85±0.68	6.33±0.66	8.30±1.10	6.37±0.81	8.30±1.34	6.28±1.03	8.23±1.05	5.98±0.74
83/84	8.13±0.68	6.35±0.69	8.53±1.19	6.65±0.78	8.68±1.41	6.40±1.16	8.53±1.17	6.10±0.71

[a]Extracted from pages 190-191, MRID 47914235.

C. FOOD CONSUMPTION:

Selected mean food consumption data are presented in Table 7. No treatment-related effects were observed. However, the study report notes that on treatment days food consumption was decreased for isolated animals in all groups. This finding was attributed to the high number of clinical examinations performed on treatment days. Summary data tables indicated that no statistical analyses were performed.

TABLE 7: Overall mean food consumption (g/dog) data in dogs treated with S-methoprene, amitraz, and fipronil[a]

Study Day/ Gender	Group 1 (Control)	Group 2 (1X)	Group 3 (3X)	Group 4 (5X)
-14 to 84 (male)	27313.6±2702.23	28808.3±479.02	26849.7±2300.46	27103.9±1886.34
-12 to 85 (female)	23191.1±2289.59	20768.7±2782.65	21446.5±2785.51	21371.3±1362.05

[a]Extracted from pages 199 & 209, MRID 47914235.

D. BLOOD ANALYSES:

1. **Hematology**: Treatment-related effects included changes in erythrocyte (RBC) counts, hemoglobin, packed cell volume and reticuloctyes, and increases in total white blood cell, monocyte, and neutrophil counts.

   Decreased erythrocyte counts, packed cell volume, and hemoglobin concentration were observed at all dose levels the day after each treatment, compared to control and pre-test values. The frequency of statistically significant decreases increased with proximity to dosing, and recovery and/or the absence of statistically significant decreases were noted by 7 or 14 days post-dosing (Day 84). Changes in reticulocyte counts paralleled changes in erythrocyte counts, packed cell volume, and hemoglobin concentration. Increased ($p<0.10$) total white blood cell, monocyte, and neutrophil counts were noted in the 3x and 5x groups the day after treatment.

   These effects were compatible with increased red blood cell attrition that affected mature and immature erythrocytes. Increases in neutrophils, monocytes, and white blood cells were interpreted as evidence of increased erythropoietic stimulation of bone marrow in response to red blood cell attrition. Selected statistically significant ($p<0.10$) hematology information is presented in Table 8. Table 9 presents the percentage of monocytes and neutrophil values above reference ranges, and Table 10 summarizes statistically significant ($p<0.05$) treatment by sex interactions for lymphocytes. However, values remained within the reference range.

TABLE 8: Summary of statistically significant ($p<0.10$) hematology data in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples)[a]

Parameter (units)	Reference range	Control group range	Least squares Means and (% Difference from Control group)		
			Dose		
			1X	3X	5X
Sampling times- One day after previous dosing					
Day 1					
Hemoglobin (g/dL)	13.3-18.6	14.0-16.9	-	13.96 (↓ 8%)	14.04 (↓ 7%)
PCV (%)	38.6-53.4	42.2-51.4	43.49 (↓ 4%)	41.73 (↓ 8%)	42.27 (↓ 7%)
RBC (10^6/µL)	5.81-7.99	6.19-7.08	6.30 (↓ 4%)	6.07 (↓ 7%)	6.06 (↓ 8%)
Reticulocyte (%)	0.0-1.1	0.3-2.1	0.44 (↓ 41%)	0.52 (↓ 31%)	0.50 (↓ 33%)
Day 15					
Hemoglobin (g/dL)	13.3-18.6	13.9-16.9	13.97 (↓ 8%)	14.01 (↓ 8%)	14.60 (↓ 4%)
PCV (%)	38.6-53.4	41.8-49.7	41.70 (↓ 8%)	41.83 (↓ 7%)	-
RBC (10^6/µL)	5.81-7.99	6.25-7.29	6.09 (↓ 8%)	6.11 (↓ 8%)	6.33 (↓ 4%)
Reticulocyte (%)	0.0-1.1	0.2-1.2	0.43 (↓ 23%)	-	-
Monocyte (10^3/µL)	0.09-0.81	0.37-0.87	-	0.76 (↑ 21%)	-
Neutrophil (10^3/µL)	2.23-10.05	4.57-9.33	-	8.13 (↑ 27%)	7.95 (↑ 24%)
WBC (10^3/µL)	4.92-14.46	7.20-13.39	-	12.09 (↑ 17%)	-
Day 29					
Hemoglobin (g/dL)	13.3-18.6	14.0-16.2	14.15 (↓ 5%)	14.15 (↓ 5%)	14.23 (↓ 5%)
PCV (%)	38.6-53.4	39.3-46.6	41.04 (↓ 4%)	-	-
RBC (10^6/µL)	5.81-7.99	6.17-7.03	6.09 (↓ 5%)	6.11 (↓ 5%)	6.09 (↓ 5%)
Monocyte (10^3/µL)	0.09-0.81	0.28-0.84	-	0.85 (↑ 52%)	0.89 (↑ 59%)
Neutrophil (10^3/µL)	2.23-10.05	3.35-7.88	-	9.77 (↑ 70%)	9.21 (↑ 60%)
WBC (10^3/µL)	4.92-14.46	5.87-11.10	-	13.43 (↑ 46%)	12.68 (↑ 38%)
Day 43					
Hemoglobin (g/dL)	13.3-18.6	12.7-16.4	14.41 (↓ 5%)	14.12 (↓ 7%)	14.43 (↓ 5%)
PCV (%)	38.6-53.4	37.0-47.5	42.06 (↓ 4%)	41.21 (↓ 6%)	42.26 (↓ 4%)

TABLE 8: Summary of statistically significant (p<0.10) hematology data in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples)[a]					
Parameter (units)	Reference range	Control group range	Least squares Means and (% Difference from Control group)		
			Dose		
			1X	3X	5X
RBC (10^6/µL)	5.81-7.99	5.71-7.24	6.25 (↓ 5%)	6.12 (↓ 7%)	6.22 (↓ 5%)
Monocyte (10^3/µL)	0.09-0.81	0.30-1.05	-	-	0.75 (↑ 44%)
Day 57					
Hemoglobin (g/dL)	13.3-18.6	14.1-17.0	-	14.67 (↓ 6%)	14.77 (↓ 6%)
PCV (%)	38.6-53.4	41.5-50.1	-	43.90 (↓ 5%)	44.20 (↓ 5%)
RBC (10^6/µL)	5.81-7.99	6.39-7.36	-	6.47 (↓ 7%)	6.46 (↓ 7%)
Day 71					
Hemoglobin (g/dL)	13.3-18.6	13.5-17.2	-	14.78 (↓ 4%)	14.59 (↓ 5%)
PCV (%)	38.6-53.4	39.8-51.1	-	-	43.17 (↓ 4%)
RBC (10^6/µL)	5.81-7.99	6.17-7.62	6.50 (↓ 5%)	6.51 (↓ 5%)	6.39 (↓ 6%)
Monocyte (10^3/µL)	0.09-0.81	0.27-0.74	-	-	0.66 (↑ 38%)
Sampling time- Seven days after previous dosing					
Day 7					
Reticulocyte (%)	0.0-1.1	0.2-0.9	-	0.67 (↑ 29%)	0.76 (↑ 46%)
Day 35					
Neutrophil (10^3/µL)	2.23-10.05	3.98-7.74	7.14 (↑ 17%)	-	-
Day 63					
Hemoglobin (g/dL)	13.3-18.6	14.1-17.0	16.33 (↑ 4%)	-	-
PCV (%)	38.6-53.4	42.6-49.7	47.96 (↑ 5%)	-	-
RBC (10^6/µL)	5.81-7.99	6.44-7.43	7.11 (↑ 4%)	-	-
Sampling time- Fourteen days after previous dosing					
Day 84					
Hemoglobin (g/dL)	13.3-18.6	14.1-16.7	16.22 (↑ 5%)	16.87 (↑ 9%)	-
PCV (%)	38.6-53.4	40.6.-49.0	47.27 (↑ 5%)	49.04 (↑ 9%)	-
RBC (10^6/µL)	5.81-7.99	6.28-7.34	-	7.33 (↑ 8%)	-
Reticulocyte (%)	0.0-1.1	0.3-2.1	-	0.62 (↑ 68%)	-

[a]Extracted from pages 2871-2874, MRID 47914235.

TABLE 9: Number (and percentage) of individual monocyte and neutrophil values above reference ranges one day after previous dosing sampling times in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples) (N= 72 individual values /dose for the one day after previous dosing sampling times combined)[a]					
Parameter (units)	Reference range	Dose group			
		0X	1X	3X	5X
Monocyte (10^3/µL)	0.09-0.81	7 (10%)	9 (13%)	20 (28%)	23 (32%)
Neutrophil (10^3/µL)	2.23-10.05	6 (8%)	4 (6%)	9 (13%)	13 (18%)

[a]Extracted from page 2875, MRID 47914235.

TABLE 10: Summary of statistically significant (p<0.05) interactions by sex for hematology parameters in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples) (n=60 measurements/sex/group)[a]					
Parameter (units)	Reference range	Control group range	Dose group		
			1X	3X	5X
Sampling days 1, 7, 15, 29, 35, 43, 57, 63, 71, and 84 combined					
Females					
Lymphocytes (10^3/µL)	1.44-3.90	1.53-4.02	2.89 (↑ 9%)	2.79 (↑ 5%)	2.84 (↑ 7%)
Males					
Lymphocytes (10^3/µL)	1.42-3.46	1.63-4.23	2.65 (0%)	2.50 (↓ 6%)	2.75 (↑ 4%)

[a]Extracted from page 2876, MRID 47914235.

2. **Clinical chemistry**: Treatment-related effects included increases in BUN, glucose, calcium, phosphorus, potassium, and sodium values. Interpretation of these data was made difficult because statistics were reported as the number and percentage of individual dogs with values above reference ranges, rather than on actual measured clinical chemistry values.

Dose-related increases in BUN were observed one day after dosing sampling times for all treated groups. Females were affected more than males. The increase extended to the 1x dose at sampling days 1, 15, 29, 43, 57, and 71 for females; whereas, males were affected at sampling days 15, 29, 43, 57, and 71. The cause of the increase is unknown, but is not clearly related to decreased kidney function because no creatinine effects or renal histopathology were noted. It is unclear why females were affected more than males.

TABLE 11: Mean Urea Values (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a] Means above the reference range upper limits (>6.80 for females and >7.69 for males) are in bold.

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	5.87±1.19	4.84±0.25	4.57±1.21	5.19±0.69	4.78±1.16	4.79±1.30	4.71±1.37	5.70±2.51
-6	5.47±0.52	4.89±0.49	4.81±1.51	4.95±0.74	4.49±0.76	4.86±1.22	4.48±0.81	4.48±1.09
1	5.72±1.20	4.94±0.79	5.49±1.48	**7.21±1.06**	6.05±0.75	6.42±1.87	6.91±1.37	**7.57±0.69**
7	6.04±1.22	5.51±1.22	5.19±1.30	5.33±0.92	5.64±0.79	6.13±1.72	5.47±0.79	5.40±1.38
15	5.95±0.75	5.31±0.17	6.20±1.46	6.22±0.42	6.68±1.23	**6.89±2.32**	6.85±1.53	**8.51±3.05**
29	5.46±0.86	4.81±0.49	6.15±1.44	5.90±0.89	6.38±0.89	**6.96±1.40**	6.45±0.53	**8.12±1.79**
35	5.55±0.66	5.25±0.72	4.54±1.41	5.15±0.46	4.46±0.77	5.27±1.68	5.20±0.58	5.75±1.82
43	5.64±0.95	4.94±0.68	6.27±1.67	6.05±0.83	6.40±0.80	**7.30±1.19**	6.66±0.67	**7.30±2.01**
57	5.89±0.96	4.69±0.71	6.00±1.82	6.09±0.79	6.03±1.10	**6.83±2.03**	6.34±1.17	**8.16±2.48**
63	5.85±0.78	4.74±0.52	5.08±1.39	5.51±0.98	4.56±0.33	5.79±1.63	5.59±0.45	5.56±0.84
71	5.64±1.24	5.51±0.91	6.31±2.07	6.03±0.92	6.66±1.44	6.75±1.31	6.91±0.61	**7.86±2.12**
84	5.88±0.86	4.83±0.57	5.30±2.04	5.82±0.72	4.90±0.76	5.43±1.88	5.75±1.10	4.86±1.28

[a]Extracted from pages 340-341 and 2641-2648, MRID 47914235.

The above values are in millimoles/L; urea is normally reported as mg/dL in the United States. According to the conversion table on p. 3167 of MRID 47914235 a value for serum urea reported in mM/L would have to be divided by 0.166 to obtain the value in mg/dL. Page 3169 of MRID gives the following reference ranges for urea: females: 14.46-40.96 mg/dL; males: 17.47-45.78 mg/dL. Multiplying these values by 0.166 would give 2.40-6.80 mM/L for females and 2.90-7.69 mM/L for males. Looking at the individual data gives the following incidences (Table 13) of males with urea values >7.69 mM/L and females with >6.80 mM/L. Occurrences of 50% or more are shown in bold.

TABLE 13: Incidences of males wth urea values > 7.69 mM/L and females with urea values > 6.80 mM/L in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1/6	0/6	0/6	0/6	0/6	1/6	0/6	1/6
-6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
1	1/6	0/6	0/6	**4/6**	0/6	2/6	1/6	**6/6**
7	1/6	1/6	0/6	1/6	0/6	2/6	0/6	1/6
15	0/6	0/6	1/6	1/6	1/6	**4/6**	1/6	**4/6**
29	0/6	0/6	1/6	1/6	1/6	**3/6**	0/6	**4/6**
35	0/6	0/6	0/6	0/6	0/6	1/6	0/6	2/6
43	0/6	0/6	1/6	1/6	0/6	**4/6**	0/6	**3/6**
57	0/6	0/6	1/6	1/6	0/6	**3/6**	1/6	**4/6**
63	0/6	0/6	0/6	1/6	0/6	2/6	0/6	1/6
71	1/6	1/6	1/6	2/6	1/6	**3/6**	1/6	**4/6**
84	0/6	0/6	1/6	0/6	0/6	1/6	0/6	0/6

[a]Extracted from pages 2641-2648, MRID 47914235.

Dose-related increases in the numbers of individual glucose values that exceeded the reference ranges were observed for female and male groups across the one day after previous dosing sampling times (Days 1, 15, 29, 43, 57, and 71 combined). Glucose values for individual dogs returned to levels below the upper boundaries of the reference ranges at 7 and 14 days after previous dosing sampling times; with the exceptions of one value for a 1X female at day 35, one value for a 3X female at day 7, and one value for a 5X female at the day 84 sampling time. The glucose values as reported are in mmol/L; glucose is reported as mg/dL in the United States. The reference ranges for glucose are given (p. 3168) as 78.18-109.82 mg/dL for females and 76.18 mg/dL for males. From the conversion factor given on p. 3167 [mg/dL = (mmol/L) ÷ 0.055] these values correspond to 4.30-6.04 mM/L for females and 4.19-5.97 mM/L for males. Means >6.04 mM/L for females and >5.97 mM/L for males are shown in bold; all occurred on days following treatment.

TABLE 14: Mean Glucose Values (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	5.35±0.36	4.92±0.51	4.72±0.57	4.87±0.35	5.00±0.67	5.12±0.47	5.12±0.68	4.98±0.62
-6	5.58±0.39	4.98±0.49	5.03±0.38	5.14±0.26	5.27±0.35	5.54±0.49	5.55±0.35	5.18±0.61
1	4.48±0.34	4.62±0.30	4.56±0.52	5.35±0.26	5.38±0.35	**6.20±0.59**	5.66±1.37	**6.52±0.55**
7	5.01±0.26	4.60±0.27	5.18±0.35	5.01±0.19	5.04±0.52	5.33±0.53	5.03±0.26	4.61±0.38
15	5.48±0.34	4.92±0.45	5.75±0.30	5.82±0.37	**7.08±1.39**	5.83±0.64	**6.30±1.06**	**7.43±0.48**
29	5.04±0.42	5.25±0.43	4.98±0.80	5.53±0.52	5.03±0.82	5.95±0.52	5.76±1.68	5.99 ±0.85
35	5.18±0.61	4.99±0.56	5.33±0.25	5.36±0.42	5.22±0.35	5.10±0.34	5.22±0.25	4.92±0.48
43	5.29±0.42	5.03±0.55	5.51±0.28	5.56±0.15	5.62±0.37	5.97±0.79	**6.70±1.05**	**7.45±1.11**
57	5.12±0.35	4.83±0.24	5.14±0.30	5.29±0.21	5.67±0.36	5.56±0.66	**6.00±0.81**	**7.03±1.26**
63	5.24±0.33	5.05±0.30	5.11±0.33	5.24±0.41	5.04±0.43	5.19±0.41	4.90±0.51	4.76±0.19
71	5.26±0.33	5.20±0.49	5.72±0.52	5.65±0.32	5.80±0.66	**6.08±0.72**	**6.00±1.34**	**6.66±1.02**
84	5.20±0.38	5.18±0.33	4.94±0.25	4.99±0.38	4.93±0.45	5.01±0.48	4.89±0.62	5.57±0.52

[a]Extracted from pages 2633-2640, MRID 47914235.

Examining the individual data gives the following incidences (Table 15) of males with glucose values >5.97 mM/L and females with >6.04 mM/L. Occurrences of 50% or more are shown in bold, with all occurring on days following treatment.

TABLE 15: Incidences of elevated Glucose Values(males >5.97 mM/L; females >6.04 mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
-6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
1	0/6	0/6	0/6	0/6	0/6	2/6	2/6	**5/6**
7	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
15	1/6	0/6	2/6	1/6	**5/6**	**3/6**	**4/6**	**6/6**
29	0/6	0/6	1/6	1/6	1/6	1/6	1/6	**3/6**
35	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6
43	0/6	0/6	0/6	0/6	1/6	2/6	**5/6**	**6/6**
57	0/6	0/6	0/6	0/6	1/6	1/6	**4/6**	**4/6**
63	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
71	0/6	0/6	2/6	0/6	**3/6**	**3/6**	2/6	**4/6**
84	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6

[a]Extracted from pages 2633-2640, MRID 47914235.

Dose-related increases in the numbers of individual calcium values that exceeded the reference ranges were observed for 3X and 5X female and male groups at the one day after previous dosing sampling times. The values below are in millimoles/L; serum calcium is normally reported in mg/dL in the U.S. From p. 3168 of MRID 47914235 the reference range for females is given as 9.84-10.96 mg/dL, and for males as 9.92-10.96 mg/dL. From information on p. 3167 the conversion is mg/dL = (mmol/L) ÷ 0.25. The reference ranges, expressed in mmol/L would then be 2.46-2.74 for females and 2.48-2.74 for males. Mean values above 2.74 in Table 16 below are shown in bold.

TABLE 16: Mean Calcium Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	2.53±0.09	2.48±0.06	2.49±0.07	2.49±0.06	2.49±0.07	2.53±0.05	2.50±0.08	2.51±0.06
-6	2.62±0.11	2.65±0.10	2.59±0.05	2.64±0.06	2.62±0.05	2.66±0.07	2.63±0.06	2.65±0.05
1	2.54±0.10	2.56±0.05	2.50±0.05	2.59±0.06	2.59±0.08	2.55±0.05	2.64±1.12	2.66±0.08
7	2.64±0.12	2.63±0.05	2.58±0.06	2.68±0.06	2.65±0.07	2.69±0.13	2.67±0.06	2.65±0.09
15	2.66±0.10	2.67±0.07	2.62±0.06	2.67±0.07	**2.75±0.09**	2.72±0.13	**2.82±0.15**	**2.86±0.09**
29	2.66±0.14	2.62±0.04	2.63±0.05	2.65±0.06	2.66±0.06	2.66±0.06	**2.75±0.13**	**2.79±0.11**
35	2.65±0.11	2.67±0.07	2.64±0.06	2.71±0.05	2.68±0.06	2.67±0.05	2.71±0.06	2.64±0.08
43	2.62±0.08	2.64±0.10	2.60±0.08	2.61±0.04	2.64±0.07	2.66±0.11	2.71±0.06	**2.76±0.07**
57	2.65±0.11	2.63±0.06	2.64±0.05	2.70±0.05	2.68±0.08	**2.76±0.15**	2.73±0.13	**2.78±0.10**
63	2.63±0.08	2.61±0.07	2.59±0.04	2.67±0.05	2.63±0.04	2.66±0.10	2.65±0.06	2.55±0.06
71	2.66±0.07	2.67±0.09	2.57±0.06	2.70±0.06	2.67±0.07	**2.78±0.14**	2.73±0.14	2.69±0.06
84	2.60±0.07	2.63±0.11	2.58±0.07	2.66±0.04	2.64±0.06	2.61±0.06	2.65±0.10	2.59±0.07

[a]Extracted from pages 334-335 and 2617-2624, MRID 47914235.

Examining the individual data gives the following incidences (Table 17) of males and females with calcium values >2.74. Occurrences of 50% or more are shown in bold, all occurring on days following treatment.

TABLE 17: Incidences of dogs with Calcium Values > 2.74 mM/L[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
-6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6
1	0/6	0/6	0/6	0/6	0/6	0/6	1/6	1/6
7	0/6	0/6	0/6	1/6	0/6	2/6	0/6	1/6
15	1/6	1/6	0/6	1/6	**4/6**	2/6	**5/6**	**5/6**
29	1/6	0/6	0/6	0/6	1/6	0/6	**3/6**	**4/6**
35	2/6	0/6	0/6	2/6	0/6	0/6	1/6	0/6
43	0/6	1/6	0/6	0/6	0/6	1/6	2/6	**4/6**
57	1/6	0/6	0/6	1/6	1/6	2/6	2/6	**5/6**
63	0/6	0/6	0/6	1/6	0/6	1/6	0/6	0/6
71	1/6	2/6	0/6	1/6	1/6	2/6	**3/6**	1/6
84	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6

[a]Extracted from pages 2617-2624, MRID 47914235.

Dose-related increases in the numbers of individual phosphorus values that exceeded the pretreatment range were observed for females and males across the one day after previous dosing sampling times (Days 1, 15, 29, 43, 57, and 71 combined). [Reference ranges are given on p. 3168 as 3.28-5.42 mg/dL for females and 3.37-5.26 mg/dL for males; the conversion factor, given on p. 3167, is mg/dL = (mmol/L) ÷ 0.323. This means the reference range would be 1.06-1.46 mmol/L for females and 1.09-1.70 mmol/L for males]. The reference ranges were not used to evaluate individual phosphorus values, because the mean baseline (pretreatment) values for phosphorus for individual dogs frequently (29%) exceeded the upper boundaries of the reference ranges Therefore, individual phosphorus values were compared to the respective pretreatment ranges. Increased phosphorus values of females and males in the 3x and 5x groups were considered treatment-related. Decreases in phosphorus values for individual dogs below the upper boundaries of the pretreatment ranges were noted at 7 (Days 7, 35, and 63) and 14 (Day 84) days after previous dosing sampling times.

TABLE 18: Mean Phosphorus Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1.58±0.13	1.70±0.19	1.70±0.15	1.68±0.25	1.75±0.18	1.73±0.18	1.66±0.21	1.71±0.17
-6	1.47±0.17	1.73±0.23	1.67±0.08	1.55±0.09	1.67±0.15	1.72±0.08	1.63±0.18	1.60±0.16
1	1.48±0.22	1.59±0.11	1.69±0.12	1.77±0.19	1.86±0.14	1.77±0.20	**2.05±0.28**	**1.94±0.13**
7	1.39±0.17	1.41±0.10	1.56±0.11	1.50±0.11	1.66±0.19	1.61±0.22	1.57±0.15	1.60±0.27
15	1.51±0.08	1.54±0.07	1.75±0.11	1.62±0.10	1.83±0.08	**1.98±0.23**	**2.04±0.26**	**1.94±0.17**
29	1.47±0.11	1.43±0.07	1.70±0.11	1.55±0.15	1.70±0.23	1.78±0.27	**1.85±0.14**	**1.94±0.12**
35	1.48±0.15	1.39±0.11	1.47±0.13	1.46±0.22	1.55±0.22	1.45±0.15	1.52±0.09	1.47±0.28
43	1.42±0.06	1.39±0.11	1.74±0.23	1.46±0.07	1.75±0.32	1.77±0.33	**1.83±0.15**	**1.87±0.26**
57	1.36±0.11	1.31±0.06	1.54±0.15	1.41±0.09	1.62±0.19	1.86±0.31	1.71±0.17	**1.88±0.20**
63	1.33±0.10	1.26±0.10	1.41±0.10	1.33±0.13	1.38±0.11	1.52±0.15	1.44±0.09	1.22±0.34
71	1.37±0.13	1.39±0.26	1.47±0.08	1.52±0.21	1.71±0.19	1.78±0.34	1.73±0.19	1.78±0.27
84	1.32±0.09	1.38±0.21	1.27±0.09	1.39±0.18	1.36±0.19	1.36±0.15	1.45±0.09	1.24±0.22

[a]Extracted from pages 336-337 and 2625-2632, MRID 47914235. Values in bold are ≥ 110% the mean for that sex and group for study days -10 and -6.

Individual potassium values that exceeded reference ranges [given on p. 3168 as 4.00-4.90 mEq/L for females and 4.00-5.00 mEq/L for males] were noted in all groups, including

TABLE 8: Summary of statistically significant (p<0.10) hematology data in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples)[a]					
Parameter (units)	Reference range	Control group range	Least squares Means and (% Difference from Control group)		
			Dose		
			1X	3X	5X
RBC (10^6/µL)	5.81-7.99	5.71-7.24	6.25 (↓ 5%)	6.12 (↓ 7%)	6.22 (↓ 5%)
Monocyte (10^3/µL)	0.09-0.81	0.30-1.05	-	-	0.75 (↑ 44%)
Day 57					
Hemoglobin (g/dL)	13.3-18.6	14.1-17.0	-	14.67 (↓ 6%)	14.77 (↓ 6%)
PCV (%)	38.6-53.4	41.5-50.1	-	43.90 (↓ 5%)	44.20 (↓ 5%)
RBC (10^6/µL)	5.81-7.99	6.39-7.36	-	6.47 (↓ 7%)	6.46 (↓ 7%)
Day 71					
Hemoglobin (g/dL)	13.3-18.6	13.5-17.2	-	14.78 (↓ 4%)	14.59 (↓ 5%)
PCV (%)	38.6-53.4	39.8-51.1	-	-	43.17 (↓ 4%)
RBC (10^6/µL)	5.81-7.99	6.17-7.62	6.50 (↓ 5%)	6.51 (↓ 5%)	6.39 (↓ 6%)
Monocyte (10^3/µL)	0.09-0.81	0.27-0.74	-	-	0.66 (↑ 38%)
Sampling time- Seven days after previous dosing					
Day 7					
Reticulocyte (%)	0.0-1.1	0.2-0.9	-	0.67 (↑ 29%)	0.76 (↑ 46%)
Day 35					
Neutrophil (10^3/µL)	2.23-10.05	3.98-7.74	7.14 (↑ 17%)	-	-
Day 63					
Hemoglobin (g/dL)	13.3-18.6	14.1-17.0	16.33 (↑ 4%)	-	-
PCV (%)	38.6-53.4	42.6-49.7	47.96 (↑ 5%)	-	-
RBC (10^6/µL)	5.81-7.99	6.44-7.43	7.11 (↑ 4%)	-	-
Sampling time- Fourteen days after previous dosing					
Day 84					
Hemoglobin (g/dL)	13.3-18.6	14.1-16.7	16.22 (↑ 5%)	16.87 (↑ 9%)	-
PCV (%)	38.6-53.4	40.6.-49.0	47.27 (↑ 5%)	49.04 (↑ 9%)	-
RBC (10^6/µL)	5.81-7.99	6.28-7.34	-	7.33 (↑ 8%)	-
Reticulocyte (%)	0.0-1.1	0.3-2.1	-	0.62 (↑ 68%)	-

[a]Extracted from pages 2871-2874, MRID 47914235.

TABLE 9: Number (and percentage) of individual monocyte and neutrophil values above reference ranges one day after previous dosing sampling times in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples) (N= 72 individual values /dose for the one day after previous dosing sampling times combined)[a]					
Parameter (units)	Reference range	Dose group			
		0X	1X	3X	5X
Monocyte (10^3/µL)	0.09-0.81	7 (10%)	9 (13%)	20 (28%)	23 (32%)
Neutrophil (10^3/µL)	2.23-10.05	6 (8%)	4 (6%)	9 (13%)	13 (18%)

[a]Extracted from page 2875, MRID 47914235.

TABLE 10: Summary of statistically significant (p<0.05) interactions by sex for hematology parameters in dogs treated with S-methoprene, amitraz, and fipronil (gender pooled samples) (n=60 measurements/sex/group)[a]					
Parameter (units)	Reference range	Control group range	Dose group		
			1X	3X	5X
Sampling days 1, 7, 15, 29, 35, 43, 57, 63, 71, and 84 combined					
Females					
Lymphocytes (10^3/µL)	1.44-3.90	1.53-4.02	2.89 (↑ 9%)	2.79 (↑ 5%)	2.84 (↑ 7%)
Males					
Lymphocytes (10^3/µL)	1.42-3.46	1.63-4.23	2.65 (0%)	2.50 (↓ 6%)	2.75 (↑ 4%)

[a]Extracted from page 2876, MRID 47914235.

2. **Clinical chemistry**: Treatment-related effects included increases in BUN, glucose, calcium, phosphorus, potassium, and sodium values. Interpretation of these data was made difficult because statistics were reported as the number and percentage of individual dogs with values above reference ranges, rather than on actual measured clinical chemistry values.

Dose-related increases in BUN were observed one day after dosing sampling times for all treated groups. Females were affected more than males. The increase extended to the 1x dose at sampling days 1, 15, 29, 43, 57, and 71 for females; whereas, males were affected at sampling days 15, 29, 43, 57, and 71. The cause of the increase is unknown, but is not clearly related to decreased kidney function because no creatinine effects or renal histopathology were noted. It is unclear why females were affected more than males.

TABLE 11: Mean Urea Values (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]
Means above the reference range upper limits (>6.80 for females and >7.69 for males) are in bold.

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	5.87±1.19	4.84±0.25	4.57±1.21	5.19±0.69	4.78±1.16	4.79±1.30	4.71±1.37	5.70±2.51
-6	5.47±0.52	4.89±0.49	4.81±1.51	4.95±0.74	4.49±0.76	4.86±1.22	4.48±0.81	4.48±1.09
1	5.72±1.20	4.94±0.79	5.49±1.48	**7.21±1.06**	6.05±0.75	6.42±1.87	6.91±1.37	**7.57±0.69**
7	6.04±1.22	5.51±1.22	5.19±1.30	5.33±0.92	5.64±0.79	6.13±1.72	5.47±0.79	5.40±1.38
15	5.95±0.75	5.31±0.17	6.20±1.46	6.22±0.42	6.68±1.23	**6.89±2.32**	6.85±1.53	**8.51±3.05**
29	5.46±0.86	4.81±0.49	6.15±1.44	5.90±0.89	6.38±0.89	**6.96±1.40**	6.45±0.53	**8.12±1.79**
35	5.55±0.66	5.25±0.72	4.54±1.41	5.15±0.46	4.46±0.77	5.27±1.68	5.20±0.58	5.75±1.82
43	5.64±0.95	4.94±0.68	6.27±1.67	6.05±0.83	6.40±0.80	**7.30±1.19**	6.66±0.67	**7.30±2.01**
57	5.89±0.96	4.69±0.71	6.00±1.82	6.09±0.79	6.03±1.10	**6.83±2.03**	6.34±1.17	**8.16±2.48**
63	5.85±0.78	4.74±0.52	5.08±1.39	5.51±0.98	4.56±0.33	5.79±1.63	5.59±0.45	5.56±0.84
71	5.64±1.24	5.51±0.91	6.31±2.07	6.03±0.92	6.66±1.44	6.75±1.31	6.91±0.61	**7.86±2.12**
84	5.88±0.86	4.83±0.57	5.30±2.04	5.82±0.72	4.90±0.76	5.43±1.88	5.75±1.10	4.86±1.28

[a]Extracted from pages 340-341 and 2641-2648, MRID 47914235.

The above values are in millimoles/L; urea is normally reported as mg/dL in the United States. According to the conversion table on p. 3167 of MRID 47914235 a value for serum urea reported in mM/L would have to be divided by 0.166 to obtain the value in mg/dL. Page 3169 of MRID gives the following reference ranges for urea: females: 14.46-40.96 mg/dL; males: 17.47-45.78 mg/dL. Multiplying these values by 0.166 would give 2.40-6.80 mM/L for females and 2.90-7.69 mM/L for males. Looking at the individual data gives the following incidences (Table 13) of males with urea values >7.69 mM/L and females with >6.80 mM/L. Occurrences of 50% or more are shown in bold.

TABLE 13: Incidences of males wth urea values > 7.69 mM/L and females with urea values > 6.80 mM/L in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1/6	0/6	0/6	0/6	0/6	1/6	0/6	1/6
-6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
1	1/6	0/6	0/6	**4/6**	0/6	2/6	1/6	**6/6**
7	1/6	1/6	0/6	1/6	0/6	2/6	0/6	1/6
15	0/6	0/6	1/6	1/6	1/6	**4/6**	1/6	**4/6**
29	0/6	0/6	1/6	1/6	1/6	**3/6**	0/6	**4/6**
35	0/6	0/6	0/6	0/6	0/6	1/6	0/6	2/6
43	0/6	0/6	1/6	1/6	0/6	**4/6**	0/6	**3/6**
57	0/6	0/6	1/6	1/6	0/6	**3/6**	1/6	**4/6**
63	0/6	0/6	0/6	1/6	0/6	2/6	0/6	1/6
71	1/6	1/6	1/6	2/6	1/6	**3/6**	1/6	**4/6**
84	0/6	0/6	1/6	0/6	0/6	1/6	0/6	0/6

[a]Extracted from pages 2641-2648, MRID 47914235.

Dose-related increases in the numbers of individual glucose values that exceeded the reference ranges were observed for female and male groups across the one day after previous dosing sampling times (Days 1, 15, 29, 43, 57, and 71 combined). Glucose values for individual dogs returned to levels below the upper boundaries of the reference ranges at 7 and 14 days after previous dosing sampling times; with the exceptions of one value for a 1X female at day 35, one value for a 3X female at day 7, and one value for a 5X female at the day 84 sampling time. The glucose values as reported are in mmol/L; glucose is reported as mg/dL in the United States. The reference ranges for glucose are given (p. 3168) as 78.18-109.82 mg/dL for females and 76.18 mg/dL for males. From the conversion factor given on p. 3167 [mg/dL = (mmol/L) ÷ 0.055] these values correspond to 4.30-6.04 mM/L for females and 4.19-5.97 mM/L for males. Means >6.04 mM/L for females and >5.97 mM/L for males are shown in bold; all occurred on days following treatment.

TABLE 14: Mean Glucose Values (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	5.35±0.36	4.92±0.51	4.72±0.57	4.87±0.35	5.00±0.67	5.12±0.47	5.12±0.68	4.98±0.62
-6	5.58±0.39	4.98±0.49	5.03±0.38	5.14±0.26	5.27±0.35	5.54±0.49	5.55±0.35	5.18±0.61
1	4.48±0.34	4.62±0.30	4.56±0.52	5.35±0.26	5.38±0.35	**6.20±0.59**	5.66±1.37	**6.52±0.55**
7	5.01±0.26	4.60±0.27	5.18±0.35	5.01±0.19	5.04±0.52	5.33±0.53	5.03±0.26	4.61±0.38
15	5.48±0.34	4.92±0.45	5.75±0.30	5.82±0.37	**7.08±1.39**	5.83±0.64	**6.30±1.06**	**7.43±0.48**
29	5.04±0.42	5.25±0.43	4.98±0.80	5.53±0.52	5.03±0.82	5.95±0.52	5.76±1.68	5.99 ±0.85
35	5.18±0.61	4.99±0.56	5.33±0.25	5.36±0.42	5.22±0.35	5.10±0.34	5.22±0.25	4.92±0.48
43	5.29±0.42	5.03±0.55	5.51±0.28	5.56±0.15	5.62±0.37	5.97±0.79	**6.70±1.05**	**7.45±1.11**
57	5.12±0.35	4.83±0.24	5.14±0.30	5.29±0.21	5.67±0.36	5.56±0.66	**6.00±0.81**	**7.03±1.26**
63	5.24±0.33	5.05±0.30	5.11±0.33	5.24±0.41	5.04±0.43	5.19±0.41	4.90±0.51	4.76±0.19
71	5.26±0.33	5.20±0.49	5.72±0.52	5.65±0.32	5.80±0.66	**6.08±0.72**	**6.00±1.34**	**6.66±1.02**
84	5.20±0.38	5.18±0.33	4.94±0.25	4.99±0.38	4.93±0.45	5.01±0.48	4.89±0.62	5.57±0.52

[a]Extracted from pages 2633-2640, MRID 47914235.

Examining the individual data gives the following incidences (Table 15) of males with glucose values >5.97 mM/L and females with >6.04 mM/L. Occurrences of 50% or more are shown in bold, with all occurring on days following treatment.

TABLE 15: Incidences of elevated Glucose Values(males >5.97 mM/L; females >6.04 mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
-6	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
1	0/6	0/6	0/6	0/6	0/6	2/6	2/6	**5/6**
7	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
15	1/6	0/6	2/6	1/6	**5/6**	**3/6**	**4/6**	**6/6**
29	0/6	0/6	1/6	1/6	1/6	1/6	1/6	**3/6**
35	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6
43	0/6	0/6	0/6	0/6	1/6	2/6	**5/6**	**6/6**
57	0/6	0/6	0/6	0/6	1/6	1/6	**4/6**	**4/6**
63	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
71	0/6	0/6	2/6	0/6	**3/6**	**3/6**	2/6	**4/6**
84	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6

[a]Extracted from pages 2633-2640, MRID 47914235.

Dose-related increases in the numbers of individual calcium values that exceeded the reference ranges were observed for 3X and 5X female and male groups at the one day after previous dosing sampling times. The values below are in millimoles/L; serum calcium is normally reported in mg/dL in the U.S. From p. 3168 of MRID 47914235 the reference range for females is given as 9.84-10.96 mg/dL, and for males as 9.92-10.96 mg/dL. From information on p. 3167 the conversion is mg/dL = (mmol/L) ÷ 0.25. The reference ranges, expressed in mmol/L would then be 2.46-2.74 for females and 2.48-2.74 for males. Mean values above 2.74 in Table 16 below are shown in bold.

TABLE 16: Mean Calcium Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	2.53±0.09	2.48±0.06	2.49±0.07	2.49±0.06	2.49±0.07	2.53±0.05	2.50±0.08	2.51±0.06
-6	2.62±0.11	2.65±0.10	2.59±0.05	2.64±0.06	2.62±0.05	2.66±0.07	2.63±0.06	2.65±0.05
1	2.54±0.10	2.56±0.05	2.50±0.05	2.59±0.06	2.59±0.08	2.55±0.05	2.64±1.12	2.66±0.08
7	2.64±0.12	2.63±0.05	2.58±0.06	2.68±0.06	2.65±0.07	2.69±0.13	2.67±0.06	2.65±0.09
15	2.66±0.10	2.67±0.07	2.62±0.06	2.67±0.07	**2.75±0.09**	2.72±0.13	**2.82±0.15**	**2.86±0.09**
29	2.66±0.14	2.62±0.04	2.63±0.05	2.65±0.06	2.66±0.06	2.66±0.06	**2.75±0.13**	**2.79±0.11**
35	2.65±0.11	2.67±0.07	2.64±0.06	2.71±0.05	2.68±0.06	2.67±0.05	2.71±0.06	2.64±0.08
43	2.62±0.08	2.64±0.10	2.60±0.08	2.61±0.04	2.64±0.07	2.66±0.11	2.71±0.06	**2.76±0.07**
57	2.65±0.11	2.63±0.06	2.64±0.05	2.70±0.05	2.68±0.08	**2.76±0.15**	2.73±0.13	**2.78±0.10**
63	2.63±0.08	2.61±0.07	2.59±0.04	2.67±0.05	2.63±0.04	2.66±0.10	2.65±0.06	2.55±0.06
71	2.66±0.07	2.67±0.09	2.57±0.06	2.70±0.06	2.67±0.07	**2.78±0.14**	2.73±0.14	2.69±0.06
84	2.60±0.07	2.63±0.11	2.58±0.07	2.66±0.04	2.64±0.06	2.61±0.06	2.65±0.10	2.59±0.07

[a]Extracted from pages 334-335 and 2617-2624, MRID 47914235.

Examining the individual data gives the following incidences (Table 17) of males and females with calcium values >2.74. Occurrences of 50% or more are shown in bold, all occurring on days following treatment.

TABLE 17: Incidences of dogs with Calcium Values > 2.74 mM/L[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
-6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6
1	0/6	0/6	0/6	0/6	0/6	0/6	1/6	1/6
7	0/6	0/6	0/6	1/6	0/6	2/6	0/6	1/6
15	1/6	1/6	0/6	1/6	**4/6**	2/6	**5/6**	**5/6**
29	1/6	0/6	0/6	0/6	1/6	0/6	**3/6**	**4/6**
35	2/6	0/6	0/6	2/6	0/6	0/6	1/6	0/6
43	0/6	1/6	0/6	0/6	0/6	1/6	2/6	**4/6**
57	1/6	0/6	0/6	1/6	1/6	2/6	2/6	**5/6**
63	0/6	0/6	0/6	1/6	0/6	1/6	0/6	0/6
71	1/6	2/6	0/6	1/6	1/6	2/6	**3/6**	1/6
84	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6

[a]Extracted from pages 2617-2624, MRID 47914235.

Dose-related increases in the numbers of individual phosphorus values that exceeded the pretreatment range were observed for females and males across the one day after previous dosing sampling times (Days 1, 15, 29, 43, 57, and 71 combined). [Reference ranges are given on p. 3168 as 3.28-5.42 mg/dL for females and 3.37-5.26 mg/dL for males; the conversion factor, given on p. 3167, is mg/dL = (mmol/L) ÷ 0.323. This means the reference range would be 1.06-1.46 mmol/L for females and 1.09-1.70 mmol/L for males]. The reference ranges were not used to evaluate individual phosphorus values, because the mean baseline (pretreatment) values for phosphorus for individual dogs frequently (29%) exceeded the upper boundaries of the reference ranges Therefore, individual phosphorus values were compared to the respective pretreatment ranges. Increased phosphorus values of females and males in the 3x and 5x groups were considered treatment-related. Decreases in phosphorus values for individual dogs below the upper boundaries of the pretreatment ranges were noted at 7 (Days 7, 35, and 63) and 14 (Day 84) days after previous dosing sampling times.

TABLE 18: Mean Phosphorus Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1.58±0.13	1.70±0.19	1.70±0.15	1.68±0.25	1.75±0.18	1.73±0.18	1.66±0.21	1.71±0.17
-6	1.47±0.17	1.73±0.23	1.67±0.08	1.55±0.09	1.67±0.15	1.72±0.08	1.63±0.18	1.60±0.16
1	1.48±0.22	1.59±0.11	1.69±0.12	1.77±0.19	1.86±0.14	1.77±0.20	**2.05±0.28**	**1.94±0.13**
7	1.39±0.17	1.41±0.10	1.56±0.11	1.50±0.11	1.66±0.19	1.61±0.22	1.57±0.15	1.60±0.27
15	1.51±0.08	1.54±0.07	1.75±0.11	1.62±0.10	1.83±0.08	**1.98±0.23**	**2.04±0.26**	**1.94±0.17**
29	1.47±0.11	1.43±0.07	1.70±0.11	1.55±0.15	1.70±0.23	1.78±0.27	**1.85±0.14**	**1.94±0.12**
35	1.48±0.15	1.39±0.11	1.47±0.13	1.46±0.22	1.55±0.22	1.45±0.15	1.52±0.09	1.47±0.28
43	1.42±0.06	1.39±0.11	1.74±0.23	1.46±0.07	1.75±0.32	1.77±0.33	**1.83±0.15**	**1.87±0.26**
57	1.36±0.11	1.31±0.06	1.54±0.15	1.41±0.09	1.62±0.19	1.86±0.31	1.71±0.17	**1.88±0.20**
63	1.33±0.10	1.26±0.10	1.41±0.10	1.33±0.13	1.38±0.11	1.52±0.15	1.44±0.09	1.22±0.34
71	1.37±0.13	1.39±0.26	1.47±0.08	1.52±0.21	1.71±0.19	1.78±0.34	1.73±0.19	1.78±0.27
84	1.32±0.09	1.38±0.21	1.27±0.09	1.39±0.18	1.36±0.19	1.36±0.15	1.45±0.09	1.24±0.22

[a]Extracted from pages 336-337 and 2625-2632, MRID 47914235. Values in bold are ≥ 110% the mean for that sex and group for study days -10 and -6.

Individual potassium values that exceeded reference ranges [given on p. 3168 as 4.00-4.90 mEq/L for females and 4.00-5.00 mEq/L for males] were noted in all groups, including

controls; increases were most frequently observed in the 3x and 5x groups at one day after dosing sampling times. Females appear to be more affected than males. Levels resolved to values below the upper boundaries of the reference ranges at the 7 and 14 days after previous dosing sampling times. When individual values below the reference range occurred, they were limited to the 7 and 14 days after previous dosing sampling times.

TABLE 19: Mean Potassium Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	4.77±0.33	4.65±0.19	4.60±0.20	4.55±0.21	4.67±0.26	4.73±0.16	4.68±0.19	4.75±0.30
-6	4.67±0.31	4.80±0.26	4.58±0.25	4.58±0.34	4.65±0.18	4.70±0.22	4.78±0.12	4.55±0.27
1	4.70±0.24	4.55±0.21	4.77±0.18	4.60±0.27	4.83±0.15	4.82±0.27	**5.03±0.36**	**5.13±0.34**
7	4.55±0.14	4.53±0.16	4.35±0.14	4.33±0.22	4.47±0.10	4.78±0.26	4.65±0.20	4.48±0.53
15	4.78±0.08	4.75±0.19	4.83±0.18	4.62±0.25	4.80±0.24	4.82±0.25	4.87±0.23	4.67±0.24
29	4.75±0.14	4.38±0.22	4.68±0.19	4.47±0.27	4.80±0.23	4.82±0.27	4.88±0.17	4.90±0.24
35	4.72±0.25	4.58±0.33	4.47±0.37	4.33±0.29	4.57±0.32	4.45±0.10	4.52±0.17	4.45±0.43
43	4.65±0.19	4.52±0.19	4.70±0.25	4.65±0.16	4.93±0.18	4.90±0.13	**5.07±0.27**	4.65±0.40
57	4.62±0.18	4.42±0.15	4.68±0.18	4.52±0.23	4.87±0.15	4.73±0.14	4.83±0.24	4.87±0.23
63	4.65±0.30	4.52±0.15	4.23±0.18	4.40±0.21	4.57±0.23	4.70±0.32	4.63±0.16	4.40±0.25
71	4.58±0.15	4.37±0.26	4.53±0.27	4.47±0.15	4.75±0.19	4.67±0.30	4.73±0.21	4.85±0.30
84	4.60±0.24	4.47±0.24	4.32±0.28	4.40±0.27	4.65±0.29	4.37±0.37	4.58±0.15	4.08±0.23

[a]Extracted from pages 330-331 and 2601-2608, MRID 47914235. Values in bold are means exceeding the reference ranges (females: >4.90 mM/L; males: >5.00 mM/L).

TABLE 19: Mean Potassium Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]; Incidences of animals with values exceeding upper limits (females: >4.90 mM/L; males: >5.00 mM/L).

Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1/6	1/6	0/6	0/6	0/6	1/6	0/6	1/6
-6	1/6	2/6	0/6	0/6	0/6	0/6	0/6	0/6
1	0/6	0/6	0/6	0/6	0/6	1/6	2/6	**5/6**
7	0/6	0/6	0/6	0/6	0/6	2/6	0/6	1/6
15	0/6	1/6	0/6	0/6	1/6	2/6	1/6	1/6
29	0/6	0/6	0/6	0/6	0/6	1/6	1/6	**3/6**
35	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6
43	0/6	0/6	0/6	0/6	2/6	**3/6**	2/6	1/6
57	0/6	0/6	0/6	0/6	0/6	0/6	0/6	**3/6**
63	1/6	0/6	0/6	0/6	0/6	2/6	0/6	0/6
71	0/6	0/6	0/6	0/6	0/6	1/6	0/6	2/6
84	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6

[a]Extracted from pages 2601-2608, MRID 47914235.

Treatment-related increases in the numbers of individual sodium values that exceeded the reference ranges were observed for 5X female and male groups at the one day after previous dosing sampling times. [Reference ranges are given on p. 3168 as 143.0-150.0 mEq/L for both sexes]. Females were affected more than males. No values below the lower boundary of the reference ranges were observed.

TABLE 20: Mean Sodium (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	148.0±2.0	146.2±2.1	147.5±0.5	146.5±1.4	146.8±1.0	146.3±1.0	146.7±0.8	146.7±0.8
-6	146.3±4.1	147.2±1.0	147.7±0.5	147.7±0.8	146.3±1.9	146.0±0.9	146.7±0.5	146.5±1.0
1	147.2±1.5	147.5±2.0	148.0±0.9	146.7±1.6	147.7±1.4	147.2±0.8	149.2±1.6	147.5±1.6
7	144.2±1.7	144.3±1.2	145.2±1.2	144.7±1.4	144.8±1.2	144.0±0.6	145.0±1.4	144.8±1.3
15	146.0±0.9	146.0±0.9	147.0±1.7	146.0±1.9	148.3±2.4	147.2±1.8	147.8±2.3	**151.0±2.1**
29	145.8±1.7	146.0±1.3	147.0±0.6	145.8±1.0	146.7±1.0	146.8±1.2	147.8±1.3	149.8±2.5
35	146.7±1.6	146.8±1.5	146.5±1.2	147.3±1.9	146.2±1.0	147.3±0.5	147.2±1.6	147.0±1.7
43	146.3±0.8	145.3±0.5	146.3±0.8	145.7±1.0	146.3±1.4	147.2±1.9	147.8±1.7	149.3±2.1
57	146.2±1.5	145.7±1.0	146.8±1.7	146.5±1.4	146.7±1.4	147.8±2.2	147.2±2.3	149.0±1.7
63	146.5±1.0	145.3±0.5	145.8±1.0	146.3±1.2	145.7±0.5	145.3±0.8	145.5±0.8	146.7±2.2
71	146.0±1.3	145.3±1.8	146.0±1.3	145.7±1.2	147.7±2.2	147.3±1.2	147.7±1.9	147.0±2.1
84	145.8±1.5	144.8±1.7	145.8±1.2	146.2±1.2	145.3±1.5	145.3±0.5	145.7±1.0	146.0±1.3

[a]Extracted from pages 328-329 and xxxx-xxxx, MRID 47914235. Values in bold are means exceeding the reference ranges (females: >4.90 mM/L; males: >5.00 mM/L).

TABLE 19: Mean Sodium Levels (mM/L) in dogs treated with S-methoprene, amitraz, and fipronil[a]; Incidences of animals with values exceeding upper limit (both sexes: >150.0 mM/L). Values in bold indicate an incidence of 50% or more.								
Study Day	Group 1 (Control)		Group 2 (1X)		Group 3 (3X)		Group 4 (5X)	
	Males	Females	Males	Females	Males	Females	Males	Females
-10	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
-6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
15	0/6	0/6	0/6	0/6	1/6	0/6	1/6	**5/6**
29	0/6	0/6	0/6	0/6	0/6	0/6	0/6	**3/6**
35	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
43	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6
57	0/6	0/6	0/6	0/6	0/6	1/6	1/6	2/6
63	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6
71	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6
84	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

[a]Extracted from pages 2593-2600, MRID 47914235.

3. Necropsy

At necropsy, there were no treatment-related organ weight changes, macroscopic effects or systemic microscopic effects. Microscopic analysis of skin application sites revealed the following: hyperkeratosis, granulomas, and mononuclear cell infiltrates in the epidermis and/or dermis. These effects were described as "mild" and no dose-response relationship was noted. Perifollicular/periadnexal inflammation was noted in all groups and was characterized by rare neutrophils around sebaceous glands and hair follicles. This effect was slightly increased in severity and incidence at the shoulder application site of group 4 dogs. (Incidence: Males: 4/6- control group, 4/6- 1X group, 2/6- 3X group, 6/6- 5X group. Females: 2/6- control group, 2/6- 1X group, 6/6- 3X group, 6/6- 5X group).

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author provided the following overall conclusions:

The test article was clinically well-tolerated at both the general and local level from a safety standpoint.

Local findings (clumping of hair, white deposits, greasy fur at application sites) are considered cosmetic, not clinical effects and are attributed to the nature of the test material and route of administration.

Decreases in heart and respiratory rates and body temperature were transient and of no clinical significance.

Transient decreases in hemoglobin, packed cell volume, red blood cells and reticulocytes, and increases in monocytes, neutrophils, and white blood cells were noted.

Transient increases in serum glucose were noted.

Transient increases in blood urea nitrogen and electrolytes (calcium, potassium, phosphorus, and sodium) were noted.

Effects on hematological and clinical chemical analysis were noted in all test-article treated groups. Recovery was noted for all of these effects. Coagulation was not affected.

No treatment-related effects on gross observations, organ weights, or microscopic observations were noted at necropsy.

Treatment did not induce any adverse effects.

B. REVIEWER COMMENTS:

Application of S-Methoprene, Amitraz, and Fipronil to dogs at 1X, 3X and 5X the recommended dose twice monthly for three months produced no deaths. Treatment-related clinical signs included decreases in heart and respiratory rates and body temperature. No treatment-related changes in body weight or food consumption were observed. Treatment-related hematological effects included decreases in erythrocyte (RBC) counts, hemoglobin, packed cell volume and reticuloctyes, and increases in total white blood cell, monocyte, and neutrophil counts. Treatment-related clinical chemistry effects included increased glucose, BUN, calcium, sodium, potassium, and phosphorus; females were affected more than males with regard to BUN, potassium, and sodium. At necropsy, there were no treatment-related organ weight changes, macroscopic effects or systemic microscopic effects. Microscopic analysis of skin application sites revealed hyperkeratosis, granulomas, and mononuclear cell infiltrates in the epidermis and/or dermis. These effects were described as "mild" and no

dose-response relationship was noted. Perifollicular/periadnexal inflammation was noted in all groups.

According to one reference (Gupta, R.C. 2007. Veterinary Toxicology: Basic and Clinical Principles) "Amitraz stimulates the α_2-adrenoreceptor, resulting in impairment of consciousness, respiratory depression, convulsions, bradycardia, hypotension, hypothermia, and hyperglycemia... In addition to [being an] α_2-adrenergic agonist, amitraz is a potent inhibitor of monoamine oxidase (MAO) enzyme... Animals exposed to amitraz may show signs of CNS depression or CNS stimulation, depending on the dose level and to some extent the species involved. Generally, high doses have a CNS depressive effect with reduced spontaneous activity, bradycardia, respiratory depression, and hypothermia. Death results from respiratory failure. At low doses CNS stimulation may occur, as manifested by hyperactivity to external stimuli such as handling, and considerably increased food consumption." The effects seen in this study included bradycardia, hypothermia and hyperglycemia.

Because of treatment-related effects on heart rate, body temperature, and some hematology and clinical chemistry parameters, the margin of safety for this formulation combination was not established at 5X or 3X the recommended dose; in addition, there were sporadic indications of effects at the 1X dose level. The Agency recommends that these issues be addressed according to the 870.7200 Guidelines which state: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..."

Based on these treatment-related findings and the lack of adequate statistically measured parameters, the study in MRID 47914235 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the dog. The study is potentially upgradeable if the registrant adequately addresses the statistical reporting and toxicological concerns indicated above. Refer also to the study deficiencies (provided in Section C).

C. STUDY DEFICIENCIES:

According to the "Safety Summary for CERTIFECT® for dogs (Fipronil + (S)-Methoprene + Amitraz) Topical Spot-On" (MRID 47914234), the product is proposed as a novel dual applicator. One chamber of the applicator contains the EPA-registered product Frontline® Plus for Dogs [9.8% w/v fipronil and 8.8% w/w (S)-methoprene], while the other chamber contains 22.1% w/w amitraz. (The solutions are separated in the applicator to enhance stability.) In this study, the percentage of active ingredients was not exactly the same as in the proposed product. The percentages used were 10% w/v fipronil, 9% w/v (S)-methoprene and 20% w/v amitraz.

The proposed label states that the product is intended for once a month application for control of flea, ticks, and chewing lice. However, the proposed label also states that: "CERTIFECT® aids in the control of sarcoptic mange infestations. Multiple monthly treatments are recommended for the elimination of mites." It is not clear whether the term

"Multiple monthly treatments" means more than once a month, or a number of once-a-month treatments. Clarification of the intended dosing schedule is important, especially in light of the fact that this study was conducted with repeated dosing at two-week intervals.

No clinical signs summary data tables were provided; this is considered a reporting deficiency.

Interpretation of respiratory rate, heart rate, and body temperature data was difficult due to the way the study was presented. No statistics were performed on separate gender group mean data.

Interpretation of clinical chemistry data was made difficult because statistics were reported as the number and percentage of individual dogs with values above reference ranges, rather than on actual measured clinical chemistry values.

Given the fact that females appeared more sensitive than males with regard to some clinical chemistry parameters, separate statistical analysis of males and females should have been conducted for both hematology parameters and clinical signs.

1. **DP BARCODE:** DP 372058
2. **PC CODES:** 129121 (Fipronil); 105402 (S-Methoprene); 106201 (Amitraz)
3. **CURRENT DATE:** September 21, 2010
4. **TEST MATERIAL:** CERTIFECT® for Dogs: 62.6% by weight ML-2,095,988 509T [a clear, colorless liquid, specific gravity = 1.019 g/mL, assaying 9.99% (w/v) Fipronil and 8.97% (w/v) S-Methoprene] and 37.4% by weight ML-3,948,906 [a pale yellow liquid, specific gravity = 0.9044 g/mL, assaying 22.12-22.67% Amitraz].

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety/adult dog MDS Pharma Services, France Lab Study No. AA73007; Sponsor Study No. PR&D 0164201 / Oct. 23 2009	47914235	Groups of 6M & 6F 9-10 month old beagle dogs were topically administered 1X, 3X or 5X the recommended dose of CERTIFECT® for Dogs [0.67 mL 9.99% w/v Fipronil, 8.97% w/v S-Methoprene and 0.40 mL 22.1% Amitraz for dogs weighing less than 10 kg]. A control group received an application of 5X saline solution. Test material or saline was administered on Days 0, 14, 28, 42, 56 and 70. No deaths occurred. Body weight and food consumption were unaffected. Dogs in the 5X group had significantly decreased respiratory rates after treatment on days 1, 5, 7 & 9. 5X dogs had significantly decreased heart rates for all treatment weeks. Compared with pretreatment values, dogs at 5X had a decrease in mean body temperature after each treatment. Treatment-related clinical chemistry effects included increases in BUN, glucose, calcium, phosphorus, potassium and calcium levels with females affected more than males. Interpretation of effects was difficult due to the way data were presented; margin of safety was not established at 5X or even 3X and there were sporadic indications of effects at 1X. Registrant should address the toxicological significance of these effects.	N/A	Does not satisfy safety margin established in OPPTS 870.7200 (potentially upgradeable)

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