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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

CA- puppy

October 5, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	CERTIFECT FOR DOGS
	EPA Reg. No. /File Symbol:	65331-T
	DP Barcode:	DP 377588
	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
Oct-5-2010
M Hashmi
T.L. Toxicology

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:

"Sub bean for companion animal for puppies…"

BACKGROUND:

The material received includes a companion animal safety study (in MRID 47914236) with beagle puppies (~8 weeks old at first treatment), 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 47914236. 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 day 1 treatment-related effects which included increased incidences of decreased activity, reduced food consumption, decreased heart rate, decreased mean respiratory rate, decreased body temperature, and some hematology and clinical chemistry parameters (increased mean serum glucose, elevated BUN), 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, the study in MRID 47914236 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study

(OPPTS 870.7200) in beagle puppies. This conclusion may change 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, FIPRONIL
[A COMBINATION OF ML-2,095,988 509T AND ML-3,948,906]

OPPTS 870.7200
STUDY TYPE: COMPANION ANIMAL SAFETY STUDY- PUPPIES
MRID 47914236 and 47914234

Prepared for

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

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Disclaimer

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

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EPA Secondary Reviewer: Byron T. Backus, Ph.D.
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Signature: Byron T. Backus
Date: Oct-5-2010

EPA Tertiary Reviewer: Kit Farwell, D.V.M.
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Signature: K Farwell
Date: OCT. 5 2010

Template version 02/06

DATA EVALUATION RECORD

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

PC CODES: 105402, 106201, 129121 **DP BARCODE:** 377588

TEST MATERIAL (PURITY): ML-2,095,988 509T [9.99% (w/v) ML-2,095,988 (fipronil); 8.97% (w/v) ML-3,335,716 ((S)-methoprene)] and ML 3,948,906 [20.0% (w/v) Amitraz]

SYNONYMS: CERTIFECT® for dogs

CITATIONS: Drag, M., R. Tessman, C. Courtney, et al. (2009) Safety of a combination of ML-2,095,988 509T and ML-3,948,906 when administered once topically at 1, 3 and 5X the target dose in eight week old puppies. Merial Missouri Research Center, Fulton, Missouri. Study Number PR&D 0176501, October 19, 2009. MRID 47914236. Unpublished.

Harriman, J. (2009) Safety summary for CERTIFECT® for dogs (Fipronil + (S)-Methoprene + Amitraz) topical spot-on. No study number provided, November 11, 2009. MRID 47914234. Unpublished.

SPONSOR: Merial Limited, 3239 Satellite Blvd., Duluth, Georgia.

EXECUTIVE SUMMARY: In an 28-day companion animal safety study (MRID 47914236), the combination of ML-2,095,988 509T [Frontline Plus, containing 9.99% (w/v) ML-2,095,988 (fipronil); 8.97% (w/v) ML-3,335,716 ((S)-methoprene)] and ML 3,948,906 [20.0% (w/v) Amitraz] was applied topically to the skin on the dorsal midline on two sites, one between the base of the skull and the shoulder blades, and the other at the front of the shoulder blades (each puppy in the non-control groups was treated with both Frontline Plus and the amitraz formulation). Groups of six male and six female approximately 8-week-old beagle puppies were treated at 1X (0.67 mL ML-2,095,988 509T; 0.40 mL ML-3,948,906; total 1.07 mL/puppy), 3X (2.01 mL-2,095,988 509T; 1.20 mL ML-3,948,906; total 3.21 mL/puppy), or 5X (3.35 mL ML 2,095,988 509T; 2.00 mL ML-3,948,906; total 5.35 mL/puppy) the total dosing volumes for the end use product (in puppies weighing less than 22 pounds; MRID 47914234). Physiologic saline was applied in identical manner to a control group of 6 male and 6 female animals at a dosing volume of 5.35 mL/puppy. To prevent run-off in the negative control, 3X, and 5X doses, the total volume was evenly divided into five, three, and five administrations, respectively, that were applied approximately 30 (± 15) minutes apart. Animals were treated on day 0 and sacrificed on day 28.

Animals in the 3X and 5X groups had treatment-related increased incidences of decreased activity, including sleepiness and observations that particular puppies were "less active." These signs were first seen during the day 0 physical examination (ten hours after the last treatment), and almost all of the affected puppies recovered by day 2. The exception was one 5X male, who remained affected on day 2 but recovered by day three. At necropsy, this animal was found to have a porto-systemic shunt, which decreased the functional mass of the liver. The same two groups also had increased incidences of vomiting on days 1-2 (3X, n.s.) or 0-2 (5X, $p<0.05$), which were also considered treatment-related.

One 3X female exhibited the following transient abnormal findings on day 2: saliva "hanging" from her mouth; walking in a disoriented manner ("bumping into walls"); and bilateral green ocular discharge. These signs resolved without intervention ("returned to normal in a very short period of time"), and the study author did not consider them to be treatment-related.

Both the control and 1X groups consumed 100% of the food offered from the p.m. feeding on day 0 through the p.m. feeding on day 3. For the 3X group food consumption was significantly decreased at both feedings on day 1, with three of five pens consuming less than 100% of the food offered during one or both feedings. For the 5X group mean food consumption per pen was significantly decreased beginning at the post-treatment p.m. feeding on day 0 and continuing through the p.m. feeding on day 3, and all five pens of puppies consumed less than 100% of the offered food during one or more of the feedings during this time frame. These differences are considered treatment-related.

The 5X group also had decreased mean percentages of food consumed during a.m. feedings on days 7-9 and the p. m. feeding on day 8 (65-85% vs. 95-100% for controls), with all five 5X pens consuming less than 100% of the offered food during two or more feedings between days 6 and 9. These differences are considered potentially treatment-related. On days 14-27, all pens of puppies in all groups consumed all of the food offered at both feedings.

On day 1 the mean heart rate was significantly decreased in all treated groups relative to their controls (1X: -15%; 3X: -35%; 5X: -33%), the mean respiratory rate was significantly decreased in the 3X and 5X groups, and the mean temperature (by sex) was decreased (although possibly not significantly) for both males and females in the 3X and 5X groups. Values had returned to normal by day 7, the next time these parameters were measured. No problems were seen in beagle puppies in a controlled laboratory environment, but a similar decrease in respiratory rate might be problematic in a brachycephalic puppy (which would include such breeds as Boxer, King Charles spaniel, Pekingese and Pug) in a warm, humid environment. Likewise, similar degrees of bradycardia and temperature decrease could increase a toy breed puppy's susceptibility to hypothermia when outdoors in the winter.

On day 1 the 5X group had slight (but statistically significant at $p < 0.01$) increases in mean hematocrit, hemoglobin and red blood cell counts, consistent with hemoconcentration due to dehydration or a fluid shift to the visceral organs. Similar effects were observed in the 3X group (statistically significant at $p < 0.05$ for hematocrit and red blood cell count).

Increased mean serum glucose (5X: $p<0.05$; 1X and 3X: $p<0.01$) in all treated groups on day 1 is considered treatment-related. One 1X puppy, seven 3X puppies, and one 5X puppy had serum

levels greater than or equal to 180 mg/dL on day 1; at levels higher than 180 mg/dL (the renal threshold), glucose spills into the urine and is lost. On day 1 BUN was elevated in all treatment groups (3X and 5X $p<0.01$) relative to controls.

Observations of coccidia-related soft, loose, and/or watery stools, with or without blood, were recorded for every single animal on the study. Because the puppies were group-housed throughout most of the study (except treatment and the ten hours immediately following the last application on day 0), certain observations, such as abnormal stools or vomiting, were mostly recorded by "pen" of puppies, i.e. as though every puppy in a particular pen had that particular clinical sign. The incidences of abnormal stools may have been overestimated quite a bit by recording these data in this manner, with initial onset varying from as early as day -14 during acclimation to as late as day 2. The puppies were treated with oral sulfadimethoxine for four days: replicates 1-5 were treated on days 1-4; replicates 6-10 were treated on days -6 to -3; and replicates 11-12 were treated on days -13 to -10. Following treatment with sulfadimethoxine there were continued observations of diarrhea. Two of the animals also exhibited signs of respiratory disease during the week immediately prior to treatment, and both of these animals were given an antibiotic (enrofloxacin) until one or two days prior to treatment. Guideline OPPTS 870.7200 states that the animals should be free of infectious diseases, which could complicate the interpretation of the study results. In addition to the physical signs related to these two infectious diseases, the antibiotic and/or the sulfadimethoxine could *themselves* have adverse effects or could interact with the test material to either worsen or mitigate its effects.

Because of treatment-related effects on food consumption, and day 1 effects (significant decreases in heart rate in all treated groups, significant decreases in mean respiratory rate in the 3X and 5X groups, decreases in mean temperature for both males and females in the 3X and 5X groups, increased mean serum glucose in all treated groups), the margin of safety for this combined formulation was not established at 5X or 3X the recommended dose; in addition, some effects (such as serum glucose levels) occurred at 1X. The Agency recommends that the registrant address these issues 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)..." The registrant should also address the issue of the health of the puppies, the treatments they received, and possible effects on the results of this study.

Based on the treatment-related findings and the health of the puppies, the study in MRIDs 47914236 and 47914234 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study (OPPTS 870.7200) in puppies. The study is classified as supplementary, but is potentially upgradeable if the registrant adequately addresses the toxicological concerns and health of puppies as 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 for the study report (MRID 47914238). Signed and dated GLP and Data Confidentiality statements were provided for the safety summary (MRID 47914234); the GLP Compliance Statement consisted of a declaration that this volume "is not subject to GLP certification" because no study was being submitted.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

a. ML-2,095,988 509T [Trade Name: Frontline® Plus]

Description:	Clear amber solution
Lot #:	D62705AR
Purity:	9.99% (w/v) ML-2,095,988 (fipronil); 8.97% (w/v) ML-3,335,716 [(S)-methoprene]; as analytically verified certificate of analysis
Compound Stability:	Expiration date: April 16, 2011
CAS #:	Fipronil Technical: 120068-37-3; Methoprene: 65733-16-6

b. ML-3,948,906

Description:	Not described
ID #:	ML 3,948,906 500A 001
Purity:	20.0% (w/v) Amitraz (ML-3,948,906)
Compound Stability:	Expiration Date: April 2009
CAS #:	Amitraz Technical: 33089-61-1

2. Vehicle and/or positive control: 0.9% Saline solution (Vedco, Inc.; Lot No. 703090F; Expiration Date: March 2010) was used as a control.

3. Test animals:

Species:	Dog	
Breed:	Beagle	
Age/weight at study initiation:	7.3 to 8.4 weeks old/ Males: 2.34-3.85 kg; Females: 2.19-3.72 kg	
Source:	Ridglan Farms, Mount Horeb, Wisconsin	
Housing:	Prior to weaning (day -20 or day -13): puppies were housed with dams in 1.66 m^2 cages; After weaning until allocation: littermates were co-housed in either 0.83 or 1.66 m^2 cages; After allocation: 2-3 puppies allocated to the same treatment group were co-housed in either 0.83 m^2 (2 puppies) or 1.66 m^2 (3 puppies) cages. Following treatment: puppies were separated for 10 hrs (± 30 min) Cages were stainless-steel with plastic-coated expanded aluminum floors.	
Diet:	Science Diet® Puppy Small Bites (Hills Pet Nutrition), 75 or 100 g/puppy twice daily. Forti Flora™ (Nestle Purina Pet Care Co.) was added to the diet of all puppies at unspecified times (perhaps throughout the study). Several puppies were supplemented with Science Diet® A/D and/or cottage cheese and milk replacer, as needed to stimulate appetite.	
Water:	*Ad libitum* water from an on site well	
Environmental conditions:	**Temperature:**	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	Not reported
Acclimation period:	At least fourteen days.	

B. STUDY DESIGN:

1. **In life dates:** Start: June 18, 2008; End: July 30, 2008

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups according to age (date of birth) and body weight, using a stratified randomized block design. The study was conducted in twelve replicates in such a manner that puppies were treated at approximately 8 weeks of age (7.3 to 8.4 weeks of age). One animal was allocated to the incorrect group due to the body weight being recorded incorrectly. All non-control puppies were dosed with both Frontline and the amitraz formulation.

TABLE 1. Study design [a]

Test Group	Dosing volume (mL/puppy)				Number assigned	
	ML-2,095,988 509T	ML-3,948,906	0.9% NaCl	Total	Males	Females
1. Control	0	0	5.35	5.35	6	6
2. 1X	0.67	0.40	0	1.07	6	6
3. 3X	2.01	1.20	0	3.21	6	6
4. 5X	3.35	2.00	0	5.35	6	6

[a] Data taken from p. 14 and 16, MRID 47914236.

3. **Dose selection rationale:** Dose selection rationale was not explicitly stated in the study report.

4. **Treatment:** The control or test material, as appropriate, was applied topically on day 0. For each application, the fur was parted on the midline of the neck, and the material was applied directly to the skin on two separate sites. One site was between the base of the skull and the shoulder blades, and the other site was at the front of the shoulder blades. To prevent run-off at the negative control, 3X, and 5X doses, the total volume was evenly divided into five, three, and five administrations, respectively, that were applied approximately 30 minutes (± 15 minutes) apart. The products were measured and applied using one-mL syringes with 1/100 mL graduations. The dosing volumes were calculated based on the body weight recorded on day -2.

5. **Statistics:** Continuous data, such as body weight, respiration rate, temperature, heart rate, and plasma chemistry and hematology values (except albumin/globulin ratio and percentage values for white blood cell differentials), were analyzed using repeated measures analysis of covariance (RMANCOVA) including treatment, sampling day, sex, and the interaction terms "treatment by sex," "treatment by sampling day," "sex by sampling day," and "treatment by sex by sampling day" as fixed effects. The animal was identified as the subject in the repeated statement. The covariate was the pretreatment baseline measurement or the arithmetic mean of the two pretreatment baseline measurements.

   The Akaike Information Criterion (AIC) was used to select a reasonably representative covariance structure for each repeated measures analysis. The matrix structure was selected

from among the following structures: compound symmetry, heterogeneous compound symmetry, spatial power, and unstructured.

Variables were first examined to determine if there was a significant "treatment by day by sex" interaction at α=0.05. If "treatment by sex by sampling day" interaction was significant, further analysis was not performed. If "treatment by sex by sampling day" interaction was not significant, the interactions of "treatment by sex" and "treatment by sampling day" were evaluated. If "treatment by sex" was significant at α=0.05, further analysis was not performed. If "treatment by sampling day" was significant at α=0.10, pair-wise comparisons of each treated group least squares mean was performed against the control group mean at each sampling day. The pair-wise comparisons used an unadjusted significance level of a=0.10. If the "treatment" main effect was significant at a=0.10, "treatment by sampling day" interaction was not significant, and neither the 3-way interaction nor the 2-way interaction with "sex" was significant, then treatment means from the main effect of "treatment" were compared.

Descriptive statistics (minimum, maximum, standard deviation, and mean) were calculated and reported as follows: at each time point for each treatment group with pooled sexes if "treatment by sampling day" was significant and both the 3-way and 2-way interactions with "sex" were not significant; for each treatment group by sex if either the 3-way or 2-way interactions with "sex" was significant; and overall for each treatment group if "treatment" main effect was significant and none of the other interactions were significant.

Food consumption was analyzed using repeated measures analysis of variance including treatment, sampling day, and "treatment by sampling day" interaction as fixed effects and "pen by treatment" as a random effect. The first-order autoregressive was used as the matrix structure for food consumption due to equally spaced time points. According to the study report, ordinal data were converted to continuous data as percent consumed. It is assumed that this statement meant that the mean percentage of food consumed per pen calculated for each measuring point was left alone rather than rounding it to the nearest quartile.

Profile plots were provided for all continuous variables that were collected multiple times during the study. These were based upon the individual observations for each animal and included baselines (arithmetic means of pre-study data) and all data collected from day 1 through the end of the study. A separate plot was given for each treatment group, with males and females indicated with different symbols or types of lines. The upper and lower limits of the reference range were included on the plots when appropriate.

Incidence data pertaining to gross or microscopic findings were analyzed using a Fisher's Exact Test to compare each treated group to the control group. A significance level of 0.10 was used.

Observations of diarrhea, vomiting, and decreased activity were summarized as counts and percentages and analyzed using a Pearson chi-square test at a significance level of α=0.10. Other "miscellaneous" health abnormalities were summarized using counts and percentages but were not analyzed.

Some inconsistent or inappropriate individual direct bilirubin values were attained by the Olympus AU 400e Chemistry Analyzer that was used in the study. In cases where a negative direct bilirubin value was reported, a value of 0.0 mg/dL was substituted and used for statistical analysis. In cases where the direct bilirubin value was higher than the total bilirubin value, the results were not included in the statistical analysis.

C. METHODS:

1. Observations:

a. General health observations: Beginning on day -14 (or earlier in some cases), the animals were observed cageside twice daily for mortality or health problems. Particular attention was directed to observations of central nervous system signs (seizures, tremors, salivation), vomiting, and diarrhea. The animals were also observed at approximately 15 minutes and then hourly (±45 minutes) for three hours following completion of application of the total volume on treatment days.

b. Clinical assessments: All animals received a physical and neurological examination some time between days -29 and -15 and on day -8, day -2 or -1, day 0 (approximately 10 hours ±2 hours post-treatment), and on days 1, 7, 14, 21, and 27. Some animals received additional examinations at other time points. The examination included assessment of general condition, the eyes (nystagmus, congestion, discharge, signs of visual impairment), mucous membranes, respiratory, circulatory, and autonomic and central nervous system, as well as somatomotor activity and behavior. Respiratory rate, heart rate, and temperature were recorded. After treatment, the application site was observed for changes to the skin and fur.

2. Body weight: The animals were weighed daily from arrival through day -1 and on days 1 through day 5 or day 6. Thereafter they were weighed during the physical examinations (days 7, 14, 21, and 27).

3. Food consumption: Beginning on day -1, the quantity of dry diet consumed per pen of two or three puppies was recorded twice daily. These data were reported qualitatively, as "all food consumed" or the approximate percentage of food that remained uneaten, reported in increments of 25%, i.e. "approximately 25% (or 50% or 75%) remaining."

4. Hematology & Clinical Chemistry: Each animal had blood collected for hematology and clinical chemistry evaluation twice prior to treatment and on days 1, 7, and 27. Most of the pre-treatment collections were done on days -8 and -2, but in some instances they were done on day -15, day -9, and/or day -1 instead. There was no mention of withholding food and/or

water prior to collection, and the venipuncture site was not reported. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count* (absolute and percentage)
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Morphology (if indicated)
	(Thromboplastin time)*	X	Heinz body formation
	(Clotting time)		
	(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*
	Magnesium	X	Urea nitrogen (BUN)*
X	Phosphorus*	X	Cholesterol
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)		Indirect bilirubin
	Creatine phosphokinase	X	Total protein (TP)*
	Lactic acid dehydrogenase (LDH)	X	Triglycerides
X	Alanine aminotransferase (ALT/also SGPT)*		Serum protein electrophoresis
X	Aspartate aminotransferase (AST/also SGOT)*	X	Albumin/globulin ratio
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

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

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Necropsy and Pathology:** On day 28, the surviving animals were euthanized via intravenous injection of pentobarbital sodium and subjected to exsanguination and gross necropsy. The indicated (X) organs or tissues were collected and preserved in 10% neutral buffered formalin or Davidsons solution (eyes). All collected samples from animals of the control and 5X groups and the skin application sites and any gross lesions from the animals of the 1X and 3X group were processed and stained and examined microscopically. Bone marrow smears were collected but were not examined because no abnormalities were noted in the day 27 hematology results.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta and carotid arteries	X	Brain
X	Salivary glands	X	Heart	X	Peripheral nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord (cervical, with bone)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		**GLANDULAR**
X	Ileum			X	Adrenal gland
X	Cecum		**UROGENITAL**	X	Parathyroid
X	Colon	X	Kidneys	X	Thyroid
	Rectum	X	Urinary bladder		
X	Liver	X	Testes		**OTHER**
X	Gall bladder	X	Epididymides	X	Bone (femur)
	Bile duct	X	Prostate	X	Skeletal muscle
X	Pancreas		Seminal vesicles	X	Skin (application site)
	RESPIRATORY	X	Ovaries	X	Gross lesions and masses (selected)
X	Trachea	X	Uterus		
X	Lung	X	Vagina		
	Nose				
	Pharynx				
	Larynx				

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Selected clinical signs data are given in Table 2. Because the puppies were group-housed throughout most of the study (except treatment and the ten hours immediately following the last application on day 0), certain observations, such as abnormal stools or vomiting, were mostly recorded by "pen" of puppies, i.e. as though every puppy in a particular pen had that particular clinical sign. The incidences of vomiting and abnormal stools may have been overestimated quite a bit by recording these data in this manner.

   The most common clinical signs were soft, loose, and/or watery stools, with or without blood, and these signs were grouped and considered together as "diarrhea." Every pen of puppies exhibited diarrhea on at least one day, although the day of onset varied by replicate. Fecal samples tested positive for coccidia, and all of the puppies were treated with oral sulfadimethoxine for four days: replicates 1-5 were treated on days 1-4; replicates 6-10 were treated on days -6 to -3; and replicates 11-12 were treated on days -13 to -10. Following treatment with sulfadimethoxine, there were continued observations of diarrhea. The cumulative incidence of "diarrhea" was significantly increased in the 1X group and significantly decreased in the 3X and 5X groups (pooled sexes).

   Animals in the 3x and 5x groups had treatment-related increased incidences of decreased activity, including sleepiness and observations that particular puppies were "less active." These signs were first seen during the day 0 physical examination (ten hours after the last

treatment), and almost all of the affected puppies recovered by day 2. The exception was one 5X male, who remained affected on day 2 but recovered by day three. At necropsy, this animal was found to have a porto-systemic shunt, which decreased the functional mass of the liver. The same two groups also had increased incidences of vomiting on days 1-2 (3X, n.s.) or 0-2 (5X, $p<0.05$), which were also considered treatment-related.

One 3X female exhibited the following transient abnormal findings on day 2: saliva "hanging" from her mouth; walking in a disoriented manner; and bilateral green ocular discharge. These signs resolved without intervention, and there is no indication within the report that the study author considered them to be treatment-related. It is reported (p. 26 of MRID 47914236) that: "...the episode was transient and the puppy was healthy for the reminder [sic] of the study. No abnormalities were noted for this animal at necropsy."

Non-treatment-related observations and physical examination findings included unilateral or bilateral prolapsed third eyelid(s), undescended testes, inguinal hernias, and clear epiphora.

TABLE 2: Clinical signs from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906 [a]

Observation	Dosage			
	Control	1X	3X	5X
Diarrhea [b]:				
Incidence [c]	115	150 **	43 **	69 **
# Affected pups	12	12	12	12
First / last days noted	0 / 28	0 / 28	0 / 28	0 / 28
Vomiting:				
Incidence [c]	6	0 *	10	19 *
# Affected	6	0	10	10
Days noted	2 and 21	Not applicable	1-2	0-2
Decreased activity [d]:				
Incidence [c]	0	0	14 **	23 **
# Affected	0	0	12	12
Days noted	Not applicable .	Not applicable	0-1	0-2

[a] Data taken from pp. 207 and 389-399, MRID 47914236. Data from days 0 through 28 of the study.
[b] Includes soft, loose and /or watery stools, with or without blood.
[c] Counts are relative to 29 days of observations per puppy, with a total count of 348 possible
[d] Includes findings recorded as 'sleepy' or 'less active'
*statistically different ($p<0.05$) from the control
** statistically different ($p< 0.01$) from the control

2. **<u>Quantitative physical signs parameters</u>:** The quantitative physical signs data are given in Table 3. Treatment-related effects included significantly decreased ($p<0.10$) mean heart rate in the 3X and 5X animals on day 0 (10 hours post-treatment), significantly decreased mean heart rate at all three treatment levels on day 1, significantly decreased mean respiratory rate in the 5X group on day 0 (10 hours post-treatment), and significantly decreased mean heart rate in the 3X and 5X groups on day 1. These data were analyzed and reported for the pooled sexes. On day 1, mean body temperatures of the 5X males and females were 1.7-1.8° F less than those of their respective controls, and mean body temperature of the 3X females was 2.4° F. less than controls.

TABLE 3: Physical examination parameters from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906[a]				
Study day	**Dosage**			
	Control	**1X**	**3X**	**5X**
Heart rate in beats/min for the pooled sexes (n~ 12 for all groups/timepoints)				
Baseline[b]	195.5	201.0	185.2	189.7
Day 0	197 ± 12	188 ± 18	149* ± 18 (-24)[c]	162* ± 14 (-18)
Day 1	200 ± 17	171* ± 19 (-15)	130* ± 30 (-35)	134* ± 24 (-33)
Day 7	199 ± 16	206 ± 20	214* ± 18 (+8)	197 ± 18
Day 27	195 ± 21	188 ±27	186 ± 14	200 ± 13
Respiratory rate in breaths/min for the pooled sexes (n~ 12 for all groups/timepoints)				
Baseline[b]	79.0	78.5	77.5	79.0
Day 0	78 ± 6	79 ± 11	74 ± 12	70* ± 19 (-10)
Day 1	66 ± 10	66 ± 6	55* ± 17 (-17)	49* ± 12 (-26)
Day 7	73 ± 8	73 ± 12	75 ± 12	70 ± 13
Day 27	72 ± 11	64* ± 11 (-11)	68 ± 14	68 ± 9
Temperature (°F) in males (n=6 for all groups)				
Baseline[b]	100.98	101.01	101.16	101.03
Day 0	101.3 ± 0.2	101.5 ± 0.6	101.0 ± 0.8	100.5 ± 0.9
Day 1	100.7 ± 0.4	100.9 ± 0.5	100.2 ± 0.9	99.0 ± 0.9
Day 7	101.2 ±± 0.3	101.1 ± 0.6	101.1 ± 0.3	100.7 ± 0.5
Day 27	101.5 ± 0.3	101.6 ± 0.5	101.4 ± 0.6	101.6 ± 0.3
Temperature (°F) in females (n=6 for all groups)				
Baseline[b]	101.03	100.89	100.92	100.96
Day 0	101.1 ± 0.3	101.3 ± 0.7	100.6 ± 0.8	100.0 ± 0.7
Day 1	100.6 ± 0.3	100.5 ± 0.9	98.2 ± 1.3	98.8 ± 1.0
Day 7	101.0 ±± 0.3	101.0 ± 0.4	101.2 ± 0.3	100.9 ± 0.4
Day 27	101.5 ± 0.4	101.5 ± 0.3	101.4 ± 0.2	101.8 ± 0.4

[a] Data taken from pp. 101-102 and 106-107, in MRID 47914236. Values are mean ± standard deviation with group sizes as indicated.

[b] Baseline mean calculated using values from day -8 and day-1 (or day -2)

[c] Numbers in parentheses equal percent different from control

*Statistically different ($p<0.10$) from the control

3. **Cosmetic effects:** According to the "Material and Methods" section of the study report, the application sites were to be observed for changes to the skin and fur as part of the physical examinations; however, no abnormal observations were reported, and there was no statement that all application sites appeared normal throughout the study.

4. **Mortality:** There were no deaths or moribund sacrifices.

B. BODY WEIGHT AND WEIGHT GAIN:

Selected body weight data are given in Table 4. Statistical analysis indicated that there was a significant "treatment by time" interaction, and, when analyzed with the sexes combined, the mean body weight of the 5X group was significantly less than controls ($p=0.07$) on day 2.

This difference reflected mean body weight losses for 5X animals of both sexes between days -1 and 2 (vs. mean weight gains in controls, 1X, and 3X animals of both sexes). These differences are considered treatment-related.

TABLE 4: Body weight data from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906 [a]

Parameter/ Study day or interval		Dosage			
		Control	1X	3X	5X
Males					
Body Weight (kg):	**Day -6**	2.73±0.418	2.81±0.447	2.78±0.231	2.69±0.494
	Day -1	3.01±0.593	3.19±0.551	3.10±0.306	2.98±0.624
	Day 1	3.07±0.615	3.18±0.570	3.18±0.336	2.92±0.618
	Day 2	3.13±0.641	3.24±0.652	3.18±0.325	2.81±0.579 (-10) [b]
	Day 7	3.34±0.698	3.58±0.689	3.45±0.297	3.12±0.676
	Day 14	3.70±0.849	3.95±0.837	3.77±0.365	3.47±0.894
	Day 21	3.99±0.966	4.36±0.881	4.12±0.423	3.86±1.003
	Day 27	4.32±0.947	4.64±0.963	4.56±0.510	4.28±0.971
BW gain (kg):	**Days -6 to -1**	0.28±0.184	0.38±0.181	0.32±0.172	0.30±0.210
	Days -1 to 2	0.12±0.076	0.06±0.157	0.09±0.156	-0.17±0.115
	Days 2 to 7	0.20±0.155	0.34±0.092	0.27±0.111	0.30±0.214
	Days 7 to 14	0.36±0.220	0.37±0.172	0.32±0.167	0.36±0.238
	Days 14 to 21	0.30±0.190	0.41±0.230	0.35±0.150	0.39±0.194
	Days 21 to 27	0.33±0.157	0.28±0.166	0.44±0.140	0.42±0.113
	Days -1 to 27	1.31±0.566	1.45±0.535	1.47±0.504	1.30±0.400
Females					
Body Weight (kg):	**Day -6**	2.62±0.517	2.77±0.507	2.50±0.308	2.42±0.335
	Day -1	2.99±0.563	3.12±0.528	2.73±0.360	2.69±0.384
	Day 1	3.05±0.591	3.19±0.526	2.76±0.524	2.66±0.382
	Day 2	3.16±0.592	3.27±0.498	2.74±0.419	2.64±0.514 (-17)
	Day 7	3.36±0.798	3.53±0.537	3.07±0.453	2.92±0.483
	Day 14	3.72±0.850	3.84±0.515	3.33±0.509	3.20±0.573
	Day 21	4.10±0.821	4.13±0.502	3.70±0.644	3.60±0.564
	Day 27	4.50±0.847	4.45±0.541	4.01±0.712	3.99±0.596
BW gain (kg):	**Days -6 to -1**	0.37±0.101	0.35±0.050	0.23±0.111	0.28±0.080
	Days -1 to 2	0.18±0.074	0.15±0.126	0.01±0.140	-0.06±0.178
	Days 2 to 7	0.20±0.232	0.26±0.114	0.33±0.158	0.28±0.091
	Days 7 to 14	0.36±0.137	0.31±0.136	0.26±0.080	0.28±0.162
	Days 14 to 21	0.38±0.065	0.26±0.150	0.37±0.185	0.41±0.153
	Days 21 to 27	0.40±0.219	0.32±0.231	0.31±0.121	0.39±0.125
	Days -1 to 27	1.52±0.465	1.33±0.452	1.29±0.378	1.30±0.266

[a] Data calculated by reviewer from individual data on pp. 365-368, MRID 47914236. Values are Mean ± Standard Deviation, with n=6 for all groups.

[b] Numbers in parentheses equal percent different from control.

C. FOOD CONSUMPTION:

Selected food consumption data are given in Table 5. The mean percentage of food consumed per pen was only reported for the combined sexes and for those measuring intervals where there was a statistically significant difference between a treated group and the control ($p<0.10$). At the 5X treatment level, mean food consumption per pen was significantly decreased beginning at the evening feeding, post-treatment on day 0 and

continuing through the evening feeding on day 3, and all five pens of puppies consumed less than 100% of the offered food during one or more of the feeding during this time frame. At the 3X treatment level, food consumption was decreased at both feedings on day 1, with three of five pens consuming less than 100% of the food offered during one or both feedings. These differences are considered treatment-related.

The 5X group also had decreased mean percentage of food consumed during the morning feeding on days 7-9 and the evening feeding on day 8 (65-85% vs. 95-100% for controls), with all five 5X pens consuming less than 100% of the offered food during two or more feedings between days 6 and 9. These differences are considered potentially treatment-related. On days 14-27, all pens of puppies in all groups consumed all of the food offered at both feedings.

TABLE 5: Selected food consumption data from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906 (Mean percentage of food consumed per pen with sexes combined)[a] showing decreased food consumption at 3X and 5X

Study day/time	Dosage			
	Control	1X	3X	5X
Day 0, p.m.	100%	100% [b]	95% [b]	80% *
Day 1, a.m.	100%	100% [b]	80% *	40% *
Day 1, p.m.	100%	100% [b]	75% *	45% *
Day 2, a.m.	100%	100% [b]	95% [b]	60% *
Day 2, p.m.	100%	100% [b]	100% [b]	85% *
Day 3, a.m.	100%	100% [b]	100% [b]	85% *
Day 3, p.m.	100%	100% [b]	100% [b]	90% *

[a] Data taken from p. 108 and 369-388, MRID 47914236.

[b] Calculated by reviewer.

* Statistically different ($p<0.10$) from the control.

D. BLOOD ANALYSES:

1. **Hematology:** Selected hematology data are given in Table 6. On day 1, the animals of the 5X group had slight statistically significant increases in mean hematocrit, hemoglobin, and red blood cell count values, and all of these fell just outside the provided reference ranges. This is consistent with hemoconcentration due to dehydration or fluid shift to the visceral organs. [See II.D.2, below.] Statistically significant differences or sex by time or treatment by time interactions were found for some white blood cell parameters. However, these data showed a lot of variability within groups, and examination of the individual values indicated that some animals had increases or decreases outside the reference range that started prior to treatment. The reviewer therefore considered it inappropriate to use these data to draw conclusions about treatment-related effects.

TABLE 6: Selected hematology parameters from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906 [a]				
Study day	**Dosage**			
	Control	**1X**	**3X**	**5X**
Hematocrit (%) for the pooled sexes [n=12 for all groups at most time points]				
Baseline [b]	30.58	29.79	30.48	30.54
Day 1	32.28±1.73	32.29±2.20	33.70±2.37 *	34.87±3.19 **
Day 7	34.06±2.60	32.60±1.86	33.44±1.66	32.93±1.65
Day 27	35.86±2.48	36.14±1.89	36.56±1.50	35.43±2.39
Hemoglobin (g/dL) for the pooled sexes [n=12 for all groups at most time points]				
Baseline [b]	10.13	9.77	10.07	10.03
Day 1	10.65±0.53	10.58±0.74	10.99±0.87	11.26±1.14 **
Day 7	11.32±0.79	10.76±0.65	11.10±0.65	10.86±0.68 *
Day 27	12.13±0.86	12.19±0.67	12.43±0.59	11.92±0.90
Red blood cell count (10^6/µL) for the pooled sexes [n=12 for all groups]				
Baseline [b]	4.50	4.34	4.48	4.53
Day 1	4.75±0.24	4.70±0.37	4.92±0.38 *	5.09±0.44 **
Day 7	5.05±0.41	4.81±0.30	5.01±0.30	4.96±0.27
Day 27	5.47±0.32	5.46±0.37	5.59±0.29	5.41±0.34

[a] Data taken from pp. 85-86 and 98-100, MRID 47914236. Values are Mean ± Standard Deviation, with group sizes as indicated.

[b] Baseline mean calculated using values from day -8 and day -1 (or day -2)

* Statistically different ($p<0.05$) from the control.

** Statistically different ($p<0.01$) from the control.

2. **Clinical Chemistry:** Increased mean serum glucose ($p<0.05$) in all treated groups on day 1 is considered significant and treatment-related even though there was not a clear dose response and the mean value for the 5X group was within the reference range at that time. Increased serum glucose with concurrent low insulin is a known effect of amitraz in dogs. At levels higher than 180 mg/dL (the renal threshold), glucose spills into the urine and is lost. One 1X animal, seven 3X animals, and one 5X animal had serum glucose levels greater than or equal to 180 mg/dL on day 1.

   Minimal, statistically significant treatment-related increases in mean sodium and blood urea nitrogen (BUN) levels were seen in the 3X- and 5X-treated animals on day 1, and recovery was evident by day 7. These changes may be associated with dehydration or fluid shift to the visceral organs. Statistically significant differences or sex by time or treatment by time interactions were found for some other parameters, but all differences were of small magnitude and were present without a dose-related trend and/or occurred with the mean and most individual values remaining within the reference range; thus none were considered biologically significant.

TABLE 7: Selected clinical chemistry parameters from puppies treated with a combination of ML-2,095,988 509T and ML-3,948,906 [a]				
Study day	Dosage			
	Control	1X	3X	5X
Glucose (mg/dL) for the pooled sexes [Reference Range=92-148 mg/dL]				
Baseline [b]	121.58	124.17	125.71	119.75
Day 1	118.92±7.95	150.08±30.16 **	171.00±50.68 **	140.00±40.05 *
Day 7	116.5±10.11	114.58±7.01	121.92±7.89	105.42±11.51
Day 27	116.5±9.66	115.58±10.52	126.33±8.72	108.25±13.39
Blood urea nitrogen (mg/dL) for the pooled sexes [Reference Range=6-18 mg/dL]				
Baseline [b]	10.83	12.13	12.21	11.50
Day 1	10.92±2.71	13.58±2.47	20.33±4.27 **	22.58±3.68 **
Day 7	13.33±2.96	11.58±3.18	10.58±2.97 *	10.17±1.85 **
Day 27	13.92±2.27	11.83±2.41 *	15.75±4.09	10.00±1.91 **
Sodium (mmol/L) for the pooled sexes [Reference Range=137-147]				
Baseline [b]	143.79	144.08	143.46	143.54
Day 1	144.50±1.83	145.83±2.04	147.58±2.61 **	146.67±4.36 *
Day 7	142.92±2.07	142.83±2.21	142.75±2.90	143.17±2.29
Day 27	145.58±2.39	146.50±1.24	145.50±1.62	147.00±1.35

[a] Data taken from pp. 82-84, 91-94, and 218, MRID 47914236. Values are Mean ± Standard Deviation, with group size n=12.

[b] Baseline mean calculated using values from day -8 and day -1 (or day -2).

* Statistically different ($p<0.05$) from the control.

** Statistically different ($p<0.01$) from the control.

E. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related gross lesions or changes of the skin at the application sites. Reported findings included a congenital liver malformation (almost complete absence of the left lateral lobe) in one 5X male, lung discoloration (red/brown, dull red, or red foci) in seven animals, and corroboration of physical examination findings such as prolapsed third eyelid(s), undescended testes, and inguinal hernias. None of the gross observations exhibited a dose response.

2. **Microscopic pathology:** There were no treatment-related microscopic lesions or changes. Observations from the skin of the cranial and caudal application sites included hyperkeratosis, Malassezia (a yeast organism), and acanthosis. The most common observations elsewhere included inflammatory foci of the liver, alveolitis of the lungs, and erythrophagocytosis in the mediastinal lymph node. Microscopic changes noted in the liver malformation of the 5X male were characteristic of congenital portosystemic shunt. None of the microscopic observations showed a dose-related increase in incidence or severity in the 5X group as compared to controls.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that topical application of a combination of ML-2,095,988 509T and ML 3,948,906 at 1X, 3X, and 5X the intended dosing volumes did not result in any toxicologically relevant effects in 7- to 8-week-old puppies. The applied doses amounted to up to 152 mg/kg ML-2,095,988, up to 137 mg/kg ML-3,335,716, and up to 182 mg/kg ML-3,948,906 at the 5X treatment level, and these doses were well tolerated.

B. REVIEWER COMMENTS: Guideline OPPTS 870.7200 states that the animals should be free of infectious diseases, which could complicate the interpretation of the study results. In this study, all of the animals were reported to have diarrhea, and at least some of the animals tested positive for coccidia; how many tested positive and when cannot be determined from the study report. Two of the animals exhibited signs of respiratory disease during the week immediately prior to treatment, and both of these animals were given an antibiotic until one or two days prior to treatment. In addition to the physical signs related to these two infectious diseases, the antibiotic and/or the sulfadimethoxine that all of the puppies were given to treat the coccidia could *themselves* have adverse effects or could interact with the test material to either worsen or mitigate its effects.

The guideline also states that animals should be vaccinated, dewormed, and acclimated for two weeks prior to the initiation of the study. The study report stated that the animals were *not* vaccinated, and there was no mention of deworming, so it is assumed that this was not done either. Given that coccidia infection poses a known challenge in the laboratory setting, it would have been prudent to wean the puppies slightly earlier (around five weeks of age) and give all of the puppies fecal examinations shortly thereafter. This would have left time to complete a sufficient course of treatment for any puppy requiring it (usually no shorter than five days) and give the puppies a two-week "wash-out" period during acclimation.

As another deficiency, the puppies were not fed *ad libitum* by offering them more food than they were likely to eat. This limited the usefulness of the food consumption data and decreased the likelihood that the body weight data would detect and accurately reflect an adverse effect.

In disagreement with the study author, this reviewer does consider the day 1 treatment-related decreases in body temperature, respiratory rate and heart rate, along with elevations in serum glucose and BUN, toxicologically relevant, in addition to the decreased activity that occurred in the 3X and 5X groups. It is also noted that there were no blood pressure measurements done. Alpha-2 adrenergic agents are known to cause transient increases and decreases in blood pressure in conjunction with bradycardia and diuresis, and either an increase or a decrease could cause damage to internal organs that could go undetected. Moreover, young animals are less able to maintain homeostasis. No problems were seen in beagle puppies in a controlled laboratory environment, but a similar decrease in respiratory rate might be problematic in a brachycephalic puppy (which would include such breeds as Boxer, King Charles spaniel, Pekingese and Pug) in a warm, humid environment. Likewise, similar degrees of bradycardia and temperature decrease could increase a toy breed puppy's

susceptibility to hypothermia when outdoors in the winter. Certainly a stressed out puppy could show an exacerbation of effects.

Based on the treatment-related findings and the health of the puppies, the study in MRIDs 47914236 and 47914234 does not satisfy the safety margin established in the guideline requirement for a companion animal safety study (OPPTS 870.7200) in puppies. The study is classified as supplementary, but is potentially upgradeable if the registrant adequately addresses the toxicological concerns and health of puppies as indicated above. Refer also to the additional study deficiencies (provided in Section C, below).

C. **STUDY DEFICIENCIES:** In addition to the major deficiencies mentioned above, the following deficiencies were identified:

- If the product label states that a treatment can be or is supposed to be repeated at an interval shorter than thirty days, this study should have included a repeat treatment. The proposed label 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, however, for puppies application would have to be at 30-day intervals.
- Because the puppies were group-housed, certain observations, such as abnormal stools or vomiting, were recorded by "pen" of puppies, i.e. as though every puppy in a particular pen had that particular clinical sign. The incidences of vomiting and abnormal stools may have been overestimated quite a bit by recording these data in this manner.
- The clinical signs data (individual clinical findings) were not summarized in a clear and concise manner.
- Means and standard deviations should have been calculated and reported separately by sex for all of the parameters, rather than only the ones with significant "sex" interactions.
- The results of the fecal evaluations were not provided, except for sporadic mentions that results were negative.
- The puppies were not weighed on day 0.
- The certificate of analysis for ML 3,948,906 did not give proper results. The percentage active ingredient was reported as "100.1% LC w/w," with no indication of what "LC" stood for. According to the "Material and Methods" section of the study report, ML 3,948,906 is supposed to contain 20.0% w/v amitraz.
- The statistical analyses using RMANCOVA were needlessly complex and at times made it difficult to accurately evaluate the results. All of the parameters should have been analyzed separately by sex, regardless of whether or not "sex" interactions were found. This is particularly important where sex differences are known to exist, such as hematology, clinical chemistry, and body weight. Statistical "power" gained by combining the sexes mainly served to find statistical significance in various aspects of the study that were not biologically or toxicologically relevant while increasing the likelihood of missing an indication that one sex is more sensitive to the test material than the other.

- Although not a guideline requirement, environmental housing conditions are typically provided in reports to ensure proper care of the dogs took place. They were not provided in the study report.

1. **DP BARCODE:** DP 377588
2. **PC CODES:** 129121 (Fipronil); 105402 (S-Methoprene); 106201 (Amitraz)
3. **CURRENT DATE:** October 5, 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/beagle puppies Merial Missouri Research Center, Fulton, Missouri Study No. PR&D 0176501 / Oct. 19 2009	47914236 47914234	Groups of 6M & 6F ~8 week old beagle puppies 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 5.35 mL/puppy saline solution. Test material or saline was administered on Day 0. No deaths occurred. Following dosage, puppies in the 3X and 5X groups had treatment-related increased incidences of decreased activity, with recovery by day 3. 3X & 5X groups also had increased incidences of vomiting on days 1-2 (3X) or 0-2 (5X). 3X group food consumption was depressed on day 1; 5X group consumed less from day 0 to 3. On Day 1 mean heart rate was significantly decreased in all treated groups, mean respiratory rate and mean temperature were decreased at 3X & 5X. Values had returned to normal on day 7. There was increased mean serum glucose in all treated groups on day 1; considered treatment-related. On day 1 BUN was elevated in all treatment groups relative to controls.	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