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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

February 10, 2011

MEMORANDUM

Subject: Companion Animal Safety, Response to Rebuttal from Merial
Name of Pesticide Product: CERTIFECT FOR DOGS
EPA Reg. No. /File Symbol: 65331-T
DP Barcode: DP 384668
Decision No.: 423378
Action Code: R320
PC Codes: 129121 (Fipronil: 9.8%)
105402 (S-Methoprene: 8.8%)
106201 (Amitraz: 22.1%)

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

Byron T. Backus
Feb-10-2011

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

Team Leader, Toxicology

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	100.0%

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 CA safety rebuttal paper from the registrant. Please also send to HED (Kit Farwell) for a secondary review to determine if a 5x safety margin can be determined. Please let RD definitely know if this product should or should not be registered based on your findings."

BACKGROUND:

The material received for review includes a 76-page document from the registrant (dated December 7, 2010) titled: "Merial Response to EPA's Comments on the Review of the Companion Animal Safety Studies for CERTIFECT™ for Dogs (MRIDs 47914235, 47914236, and 47914237)."

In addition, in a previous TRB review dated January 4, 2011 it was stated that the registrant should provide information regarding the Treatment Group 2 dog which died (refer to p. 33 of MRID 48321001) in the field conditions study. The material received for review includes the following (from an Email dated 01/20/2011 from Timothy Dotson at Merial to Autumn Metzger of RD):

Case 3022 was a 14-year old spayed, female Labrador with a history of epileptic-type signs prior to enrollment in the study. This dog was first treated with ML-3,481,564 on 09May09. 6 days after treatment, the dog had a severe epileptic fit in the night and exhibited disorientation and slight ataxia the next day. The dog was hospitalized that same day and received intravenous fluid therapy with diazepam and corticosteroids. The dog recovered and was returned to its Owner with daily administration of Phenobarbital and corticosteroids. The dog was still under treatment with Phenobarbital and corticosteroids when the 2nd treatment with ML-3,481,564 was administered on 07Jun09. On 08Jun09, the dog exhibited paresis of the hind limbs in the hours following the treatment (i.e. difficulty to get up) but did not have signs of epilepsy, and the dog had a good appetite. The dog was in a stationary posture the day after. The Study Monitor and Investigator agreed on 10Jun09 to remove the dog from the study. On 18Jun09 (8 days after removal from the study and 11 days after treatment), in the early morning the dog had another epileptic seizure and died one hour later without regaining consciousness. The Owner was not overly concerned because the dog was old and the Owner was not willing to do blood work, tissue sampling or necropsy.

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For completeness and transparency we are providing the following two cases where the dogs were removed from the study due to extenuating circumstances that involved 1) Case 2833 – a dog euthanized per the request of the Owner due to the age of the animal and the fact that the Owner was leaving the country for an indefinite period of time, and 2) Case 3310 – one dog dying by accidental poisoning. Neither case was utilized in the statistical calculations for this study. Case 3310 listed below is from Site 13 and is being provided although the Investigator's involvement was limited throughout the study and data did not generally satisfy the

documentation requirements for recording. Data from this site were therefore not combined with those from the 14 other sites for data summarization purposes.

Case 2833 was a 14-year old Rough Collie female spay which according to the owner had a "fever" the day after the 1st treatment of ML -3,481,564, and a Vet on duty prescribed amoxicillin/clavulanic acid for 5 days. 3 days after the 3rd treatment the dog was observed to have a loss of appetite, was sleeping continually and drinking a lot of water. 4 days after the 3rd treatment the dog still had a "fever" (39.5°C), was prostrate with a loss of equilibrium and showed increased capillary recoloration time. These signs were considered likely to be related to the age of the dog from the Investigator's viewpoint. The Owner requested the dog be euthanized considering the age of the dog and the fact that she (the Owner) was at that time leaving the country for an indefinite period of time. The animal was euthanized the same day.

Case 3310 was a 4 year-old male German Shepherd that started vomiting 3 days after the 2nd treatment in Treatment Group 2 and was treated with aluminum phosphate by the Owner. The animal showed quick deterioration of its general condition with bloody vomiting, then diarrhea and loss of consciousness and died soon after, 24 hours after the observation of the first clinical signs. The dog was not seen by the Investigator and the Owner did not consent to a necropsy or blood/tissue samplings. From the nurse's description of the clinical signs and the fact that there was a cat from the same neighborhood that died 24 hours later with similar signs, the Investigator determined that both animals were poisoned with an anticoagulant.

COMMENTS AND RECOMMENDATIONS:

1. The registrant has addressed (attachment No. 1 to the letter dated December 7, 2010) the EPA comments regarding the adult dog study in MRID 47914235. On the basis of the registrant's responses, TRB has come to the following conclusion:

   Although there were treatment-related effects on heart rate, body temperature, and some hematology and clinical chemistry parameters, especially at 5X and 3X the recommended dose, with sporadic indications of effects at the 1X dose level, the effects were transient and non-life-threatening. The 870.7200 Guidelines 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..." It is concluded that this product can be registered. If this product is registered, the decrease in heart rate and the increase in glucose levels should be addressed with labeling that states this product should not be used in dogs that have heart problems, are diabetic or overweight. The label should also indicate that temporary lethargy and application site pruritus in dogs may occur as a result of treatment with this product. In addition, the statement (in reference to sarcoptic mange infestations) "Multiple monthly treatments" should be revised to indicate it refers to a number of once-a-month treatments.

   The study in MRID 47914235, when combined with additional information in MRID 48321001 and attachment No. 1 to the registrant's letter dated December 7, 2010, is classified as acceptable. Refer to the revised executive summary (attached) for this study.

2. The effects observed in the puppy study (MRID 47914236) were similar to those seen in the adult dog study. These included a decrease in heart rates, primarily in the 3X and 5X groups (mean values in bpm: 3X females: Day -1: 188; Day 0 at 10 hrs: 150; Day 1: 120; day 7: 198; 3X males: Day -1: 192; Day 0 at 10 hrs: 148; Day 1: 140; Day 7: 192; 5X females: Day -1: 196; Day 0 at 10 hrs: 160; Day 1: 146; Day 7: 200; 5X males: Day -1: 200; Day 0 at 10 hours: 164; Day 1: 122; Day 7: 194 (values calculated from pp. 356 of MRID 47914236). The effect seemed to be more pronounced in 3X females than their 5X counterparts, as three 3X females had heart rates lower on than 100 bpm on day 1 as compared to none of the 5X females. There may have been slight effects at 1X (one male had a heart rate of 192 bpm on day -1 and 132 bpm on day 1, recovering to 180 bpm on day 7).

   The background provided by the registrant regarding the health of the puppies, including the fact that they arrived at the testing facility at the ages of 4-5 weeks with their dams, and their subsequent sulfadimethoxine treatment for coccidiosis, adequately addresses the health issue raised in the initial review of MRID 47914236. It is concluded that the puppy study in MRID 47914236, when combined with additional information in attachment No. 2 to the registrant's letter dated December 7, 2010, is reclassified as acceptable.

3. The registrant Agency concerns in the material (Attachment No. 3) dated December 7, 2010. For puppies born between June 2007 and May 2008 there were two cases (0.22%) of hydrocephalus and 3 cases (0.33%) of abnormal head. For June 2009 to June 2010 there were three cases (0.37%) of hydrocephalus, and none of abnormal head. The marked head

deformity of a stillborn pup in the 0X placebo-treated control group and the minimal hydrocephalus of one pup in the 3X treatment group were the only two cases of head deformity born at the laboratory in the period 06/01/08 and 06/12/09. A review of the ancestry of all hydrocephalus cases in the Liberty Research beagle colony in the past three years found that the 3X pup with hydrocephalus had common ancestors with all control cases of hydrocephalus detected in this colony. The incidence of congenital diaphragmatic hernia (1 case in 2750 pups or 0.04 percent) at Liberty Research is low, particularly in comparison with the 0.68 to 0.89 percent of beagle pups produced at another facility (Marshall BioResources) over a period of five years. It is concluded that the incidences seen in the CERTIFECT™ study were consistent with historical control data and not related to treatment.

4. The circumstances related to the 3 dog deaths in the field study do not indicate that the test material was responsible. Two of these dogs were fourteen years old (because of age considerations they probably should not have been in this study to begin with); both had health issues before treatment, and the rough collie was running a slight fever (exposure to amitraz causes a drop in body temperature) before she was euthanized. The product label would include (from PR 96-6) a statement to consult a veterinarian before using this product on debilitated or aged animals. We can accept that the death (with bloody vomiting) of the third dog, a 4-year-old male German shepherd was due to poisoning from an anticoagulant.

5. TRB is not stating that a 5X margin of safety exists for this product. The effects noted (reduced heart rate or bradycardia, lower body temperature, lethargy) are clearly adverse, and would be considered as such for the purposes of human risk assessment. However, the 870.7200 Guidelines 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)..."

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

Signature: Byron T. Backus
Date: Feb - 16 - 2011

EPA Secondary Reviewer: Kit Farwell, DVM
Risk Assessment Branch VII, HED (7509P)

Signature: Kit Farwell
Date: 2/10/2011

Template version 02/06

DATA EVALUATION RECORD [PREFACE AND REVISED EXECUTIVE SUMMARY ONLY]

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.

Additional comments in: Dotson, T. (2010) Merial Response to EPA's Comments on the Review of the Companion Animal Safety Studies for CERTIFECT™ for Dogs (MRIDs 47914235, 47914236 and 47914237). Attachment No. 1. December 7, 2010.

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 occurred. 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. Similar results were reported in the puppy study (MRID 47914236). It has been reported that IV administration of amitraz to dogs at 1 mg/kg resulted in a dramatic reduction of heart rate (from 108 ± 8 bpm to 47 ± 5 bpm at 55 minutes; W. H. Hsu and S. V. McNeel. Amitraz-Induced Prolongation of Gastrointestinal Transit and Bradycardia in Dogs and their Antagonism by Yohimbine: Preliminary Study. Drug and Chemical Toxicology, 8(4), 239-253. (1985)). This would be consistent with such observations as a heart rate of 50 bpm in group 4 female 415 on day 43. Also, from an additional source (http://www.dog-health-guide.org/heartarrhythmiacanine.html):

"A common form of heart arrhythmia canine is called Bradycardia or a slower than normal heartbeat. A slower heartbeat means there is a slower blood flow that reduces the amount of oxygen in your dogs body. With less oxygen your dog will act tired. The weakness will increase as the condition worsens. Less oxygen also affects the brain causing your dog to act dizzy or sometimes confused. The reduced levels of oxygen could also lead to fainting and collapse.

"This condition is diagnosed if a large dog's heartbeat at rest is below 60 beats per minute and in a small dog the heartbeat is less than 80 beats per minute. This disease is sometimes attributed and misdiagnosed to a dog getting older instead of an issue with their heartbeat."

Whether or not the lethargy observed in some dogs in the CAS studies (and at 5.11% in the field study) is solely due to a reduced heart rate is debatable. However, one concern that this reviewer has is that dogs with underlying heart disease may be at increased risk as a result of exposure to this formulation. For this reason, this reviewer recommends that the label of this product includes a statement that it is not to be used on dogs known to have heart problems.

Compared with pre-treatment values, animals in group 4 had a decrease in mean body temperature after each treatment; the lowest measurement (in female 414 of the 5X group) was 36.7° on study day 43 [this was the only recorded value below 37.0°]. The lowest measurement in a male was 37.3° (dog 410 of the 5X group on day 15). The Merck Veterinary Manual lists a normal temperature range for the dog of 38.4-39.4°C (mean of 38.9°C). From the material received December 10, 2010 mild hypothermia (shivering and/or lethargy) in the dog is described as 35.5-37.2°C, and moderate (loss of ability to shiver, clumsiness, slowing of pulse

and breathing rates, possible loss of consciousness) is 32.2-35.5°C. Although the temporary decrease in body temperature is an adverse effect, it is transient and non-life-threatening

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 appeared to be affected more than males, especially with regard to BUN, calcium, phosphorus and potassium. However, the material in Attachment No. 1 to the letter dated December 7, 2010 includes a statistical analysis of the clinical pathology and clinical observations data by sex, and demonstrates that there were no substantive differences in the response of male and female dogs to dermal administration of the test material. 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. Although these effects can be considered to be adverse, they would usually be temporary and non-life-threatening, although the increase in glucose levels could impact more heavily on diabetic dogs. The label of the proposed product should include a statement that it is not to be used on diabetic or grossly overweight dogs.

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.

Although there were treatment-related effects on heart rate, body temperature, and some hematology and clinical chemistry parameters, especially at 5X and 3X the recommended dose, with sporadic indications of effects at the 1X dose level, the effects were transient and non-life-threatening. Based on 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...," it is concluded that this product can be registered. However, the decrease in heart rate and the increase in glucose levels should be addressed with labeling that states this product should not be used in dogs that have heart problems, or are diabetic or overweight. The label should also indicate that temporary (not more than 24 hours) lethargy in dogs may occur as a result of treatment with this product. In addition, the statement (in reference to sarcoptic mange infestations) should make it clear that the term "Multiple monthly treatments" means a number of once-a-month treatments.

The study in MRID 47914235, when combined with additional information in MRID 48321001 and attachment No. 1 to the registrant's letter dated December 7, 2010, is classified as acceptable.

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

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

Signature: Byron T. Backus
Date: Feb. 10 2011

Signature: K Farwell
Date: 2/10/2011

Template version 02/06

DATA EVALUATION RECORD
[PREFACE AND REVISED EXECUTIVE SUMMARY ONLY]

STUDY TYPE: Companion animal safety study- breeding dogs – Non-Guideline

PC CODES: 105402, 106201, 129121 **DP BARCODE:** DP 377589

TEST MATERIALS (PURITY): 1) ML-2,095, 988 509T [FRONTLINE® Plus which contains Fipronil (10% a.i.) and (S)-methoprene (9% a.i.)] and 2) ML-3,948, 906 [amitraz (20% a.i.)]

TRADE NAME: CERTIFECT® for Dogs

CITATION: Reagan, E., C. Berthelin-Baker, S. Yoon, *et al* (2009) A study to determine the safety of a topical treatment with a combination of ML-2,095,988 509T and ML-3,948,906 in breeding female dogs at one and three times the recommended dose. Liberty Research, Inc., Waverly, NY. Study Nos. PR&D 0169001, LRI 08.2791.020, October 21, 2009. MRID 47914237. Unpublished.

Additional comments in: Dotson, T. (2010) Merial Response to EPA's Comments on the Review of the Companion Animal Safety Studies for CERTIFECT™ for Dogs (MRIDs 47914235, 47914236 and 47914237). Attachment No. 3. December 7, 2010.

SPONSOR: Merial Limited, Duluth, GA

EXECUTIVE SUMMARY: In a non-guideline companion animal safety study (MRID 47914237), twelve proven breeding female beagle dogs/group were topically administered either 1X or 3X the recommended combined dose of FRONTLINE® Plus [Fipronil (10% a.i.) and (S)-methoprene (9% a.i.)] and an amitraz (20% a.i.) formulation. A 1X dose consisted of 1.07 mL (0.67 mL Frontline Plus and 0.40 mL of the 20% amitraz formulation) for dogs weighing up to 10 kg; and 2.14 mL (1.34 mL Frontline Plus and 0.80 mL of the 20% amitraz formulation) for dogs weighing 10.1 to 20 kg. A 3X dose consisted of 3.21 mL (2.01 mL Frontline Plus and 1.20 mL of the 20% amitraz formulation) for dogs weighing up to 10 kg; and 6.42 mL (4.02 mL Frontline Plus and 2.40 mL of the 20% amitraz formulation) for dogs weighing 10.1-20 kg. 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/w (S)-methoprene], while the other chamber contains 22.1% w/v amitraz. A control group of 12 females was treated with 0.9% saline at 3X the recommended volume of the proposed product. To avoid run-off in the groups treated with 3X of the combination product or

0.9% saline, the total volume was divided into 3 administrations that were applied 15-30 minutes apart. A group of proven males served as studs; these dogs were not treated. When estrus was observed in the females, they were co-housed with the males and allowed to mate until at least 3 matings had been witnessed, or until the female no longer showed signs of estrus. The topical treatment applications were administered simultaneously on the midline of the neck every 28 days beginning at various times prior to estrus and ending when the pups were weaned at Day 42 post-parturition. On the fourth day after placement with a male, the females were treated with the proposed product, regardless of the time span between treatments.

The following parameters were measured in females: clinical signs of toxicity, including hourly evaluations on treatment days with special attention to signs of amitraz toxicity; body weight; and reproduction assessments (mating and gestational indices). The number of pups and their health status, including body weight, was recorded on Days 7, 14, 28 and 42 after parturition. A weaning index was calculated for each litter. Pups were examined for congenital abnormalities. Vital signs (temperature, heart rate and respiration rate) were monitored in pups on Days 14, 28 and 42.

One female in the 1X group was euthanized 7 days after parturition due to a severe bacterial infection of the uterus (pyometra) that was unrelated to treatment. All other females survived until the end of the study. There were no clinical signs of toxicity in treated females or their pups at 1X or 3X the recommended dose during the observations or at any time after each treatment administration. Body weight was statistically significantly decreased compared to the control group at Day 42 post-partum for females in the 1X dose group. This is not considered treatment-related since no effects were observed in the 3X dose group. Body weight gain over the course of the study was decreased in both the 1X and 3X dose groups compared to the control group but there was no dose-response relationship.

There were no treatment-related effects on the reproduction parameters. There were no statistically significant changes in the litter and weaning parameters. The number of stillborn pups/litter was higher in the 3X group (2.6% vs. 0.3% in the control group) when expressed as retransformation of arcsine square root of (number of stillborn pups/numbers of pups born). However, the actual number of stillborn pups in the 3X group was 2 (in two litters) out of a total of 64 births, as compared to 2 (in one litter) out of a total of 62 births in the 0X group. Body weight in pups was statistically significantly decreased in the 1X group compared to the control group on Day 7 post-partum. This is not considered treatment-related since no change was observed at 3X. Body weight gain from Day 7 to weaning on Day 42 was not affected by treatment. Vital signs in pups on Days 14, 28 and 42 were comparable between control and treated groups. No congenital abnormalities were detected in the 1X group pups, giving a significantly ($p<0.01$) lower proportion of pups with abnormalities per litter in this group compared to the control. In the 3X group, one female pup was euthanized; on necropsy, she had minimal hydrocephalus, peritonitis and septicemia. This pup had the only occurrence of hydrocephalus in the breeding colony during the entire year of the study. In the previous year, two cases of hydrocephalus were reported in control animals. A stillborn pup in the 3X group had a congenital diaphragmatic hernia; no pups in the breeding colony had been observed with this finding in the previous year. One stillborn pup in the control group had brachiocephalic muzzle and large cleft palate. Other congenital abnormalities in the control and 3X groups were relatively minor (overbite, tip of tail bent, umbilical hernia, double rear dewclaws). The

registrant has addressed Agency concerns in the material (Attachment No. 3) dated December 7, 2010. For puppies born between June 2007 and May 2008 there were two cases (0.22%) of hydrocephalus and 3 cases (0.33%) of abnormal head. For June 2009 to June 2010 there were three cases (0.37%) of hydrocephalus, and none of abnormal head. The marked head deformity of a stillborn pup in the 0X placebo-treated control group and the minimal hydrocephalus of one pup in the 3X treatment group were the only two cases of head deformity born at the laboratory in the period 06/01/08 and 06/12/09. A review of the ancestry of all hydrocephalus cases in the Liberty Research beagle colony in the past three years found that the 3X pup with hydrocephalus had common ancestors with all control cases of hydrocephalus detected in this colony. The incidence of congenital diaphragmatic hernia (1 case in 2750 pups or 0.04 percent) at Liberty Research is low, particularly in comparison with the 0.68 to 0.89 percent of beagle pups produced at another facility (Marshall BioResources) over a period of five years. It is concluded that the incidences seen in the CERTIFECT™ study were consistent with historical control data and not related to treatment.

The companion animal safety study (MRID 47914237) in female breeding beagle dogs is reclassified as Acceptable/Non-Guideline.

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

1. **DP BARCODE:** DP 384668
2. **PC CODES:** 129121 (Fipronil); 105402 (S-Methoprene); 106201 (Amitraz)
3. **CURRENT DATE:** February 8, 2011
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 Additional material submitted December 7, 2010.	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. All of these effects were transient and non-life-threatening.	N/A	Acceptable

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

1. **DP BARCODE:** DP 384668
2. **PC CODES:** 129121 (Fipronil); 105402 (S-Methoprene); 106201 (Amitraz)
3. **CURRENT DATE:** February 8, 2011
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 breeding safety/adult dog Liberty Research, Inc. Waverly, NY LRI Study No. 08.2791.020; Sponsor Study No. PR&D 0169001 / Oct. 21 2009	47914237	12 proven breeding female beagle dogs/group were topically administered either 1X or 3X the recommended dose of the combination of FRONTLINE® Plus [Fipronil (10% a.i.) and (S)-methoprene (9% a.i.)] and amitraz (20% a.i.). A third group was dosed with saline at 3X the volume of the recommended dose. Dogs were dosed every 28 days beginning at various times prior to estrus and ending when the pups were weaned at Day 42 post-parturition. On the fourth day after placement with a male, the females were treated with the proposed product, regardless of the time span between treatments. Registrant has adequately addressed the one occurrence of minimal hydrocephalus and the one occurrence of congenital diaphragmatic hernia in 3X puppies; it is concluded that these were not related to treatment.	N/A	Acceptable

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