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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

CA - breeding dog

October 5, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	CERTIFECT FOR DOGS
	EPA Reg. No. /File Symbol:	65331-T
	DP Barcode:	DP 377589
	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 Heasley 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: 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 non-guideline companion animal safety study (in MRID 47914237) titled: "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."

COMMENTS AND RECOMMENDATIONS:

1. An Agency contractor, Oak Ridge National Laboratory, conducted the primary review of the non-guideline companion animal safety study in MRID 47914237. TRB and HED conducted the secondary review and made changes as necessary.

2. Because of Agency concerns regarding the occurrence of the major congenital abnormalities observed in two puppies in the 3X group, this companion animal safety study in female breeding beagle dogs is currently classified as **Supplementary/Non-Guideline**. The registrant should address the issue of these abnormalities. If possible, any historical control data (including incidences) from the laboratory, as well as information from the literature for these types of abnormalities in beagles should be provided to the Agency. If this issue is adequately addressed, the study can be upgraded to acceptable.

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

DATA EVALUATION RECORD

FIPRONIL, (S)-METHOPRENE, AMITRAZ
COMPANION ANIMAL SAFETY STUDY- BREEDING DOGS – NON-GUIDELINE
MRID 47914237

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Primary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H.	Signature: Date:	Robert H. Ross for Virginia Dobozy JUL 27 2010
Secondary Reviewers: Dana F. Glass, D.V.M.	Signature: Date:	Dana F. Glass JUL 27 2010
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross JUL 27 2010
Quality Assurance: Lee Ann Wilson, M.S.	Signature: Date:	L. A. Wilson 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: Oct-5-2010

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

Signature: Kit Farwell
Date: Oct 5-2010

Template version 02/06

DATA EVALUATION RECORD

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.

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 Agency concern is that the major congenital findings in the 3X group may have been treatment-related. The inclusion of a 5X treatment group in the study might have confirmed or refuted that the findings at 3X were treatment-related; however, the Agency does not have a specific protocol for reproductive safety studies in companion animals. This study used a protocol based on FDA Guideline 33 (which has been superseded by VICH GL43).

Because of Agency concerns regarding the occurrence of the major congenital abnormalities observed in two puppies in the 3X group, this companion animal safety study in female breeding beagle dogs is currently classified as Supplementary/Non-Guideline. The registrant should address the issue of these abnormalities. If possible, any historical control data (including incidences) from the laboratory, as well as information from the literature for these types of abnormalities in beagles should be provided to the Agency. If this issue is adequately addressed, the study can be upgraded to acceptable.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

1a. ML-2,095,988 509T (Frontline Plus®)

Description:	Clean, clear amber solution
Lot #:	D62705AR
Purity:	10% w/v ML-2,095,988 (fipronil) and 9% w/v ML-3,335,716 [(S)-Methoprene]
Storage:	Room temperature
Compound Stability:	Expiration date: April 16, 2011
CAS #:	120068-37-3 (fipronil) and 65733-16-6 [(S)-Methoprene]

1b. ML-3,948,906 (Amitraz)

Description:	Clear, pale yellow liquid
ID #:	ML-3,948,906 500A 001
Purity:	20% w/v amitraz
Storage:	Room temperature
Compound Stability:	Expiration date: "end of October 2009"
CAS #:	33089-61-1

2. Placebo control: 0.9% Saline Solution

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight	Males: 2.3 – 9.4 years; weight unknown Females: 2.0 to 9.4 years/6.46 - 18.53 kg before Day 0
Source:	Liberty Research, Inc. (Antibody Defined Colony)

Housing:	Females were housed in pens in groups of three by treatment group, except when moved to be co-housed with a male for mating. The males stayed in their own pens throughout the study. Approximately two weeks prior to the earliest estimated date of parturition and until pups were 42 days old, females were housed in individual maternity pens.
Diet:	Adult dogs (until two weeks prior to the calculated date of parturition of the females) were fed once daily and *ad libitum* for two hours with Laboratory Canine Diet 5006 if less than three years old or with Laboratory Canine diet-Dentaguard when three years and older. From two weeks prior to the calculated date of parturition, females were fed *ad libitum* with High Density Canine Diet 5L18. From three weeks of age, this diet was softened with Esbilac and offered to pups once daily *ad libitum*.
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	Not provided
Humidity:	Not provided
Air changes:	Not provided
Photoperiod:	Not provided
Acclimation period:	At least six days prior to the first treatment for the females and more than seven days prior to their first mating for the males.

B. STUDY DESIGN:

1. **In life dates:** Start: June 9, 2008; End: June 12, 2009

2. **Animal assignment:** Fifty animals were assigned to the study. All animals were proven breeders. Females and males had at least 2 litters with 3 normal healthy pups (i.e., litters of at least 3 pups with no congenital malformations). The study was a negative controlled, blinded safety study, which used a randomized block design with blocks based on the ratio of pups weaned to pups born (dead or alive) in the last two litters and parity. A total of 36 females were selected in two strata of 18. A random list for the 12 replicates of 3 similar females was generated using the PROC PLAN procedure. Each stratum was selected from a pool of 20 females before the first dosing. The first and second strata females, each with six replicates of three similar females were allocated to Replicates 1-6 and 7-12, respectively. Within each stratum selection, females were ranked by decreasing weaning ratio over the last two litters (ratio of pups weaned to the total number of pups born, dead or alive in the last two litters combined). Females with identical weaning ratios were ranked by lowest to highest parity (number of litters). The three females with the highest weaning ratio in the first stratum were assigned to replicate 1. The next three females were assigned to replicate 2, and so on, until the first six replicates were formed for stratum 1. The same procedure was followed for stratum 2. Within each replicate, females were allocated to one of the three treatment groups using the randomization list generated by the Sponsor. Each replicate and stratum had a common Day 0 (day of first treatment). Ten male breeding beagle dogs were not treated and were assigned to a random breeding order by lottery.

   A minimum sample size of 12 females per group was treated with either 0 (negative control), 1X the recommended dose of the proposed product or 3X the recommended dose (Table 1).

TABLE 1: Study design[a]					
Treatment group	**Drug**	**Total volume (mL)[b]**	**Route**	**Treatment days[c]**	**Total # of animals**
1	0.9% Saline Solution	3.21, 6.42 or 12.84	Topical	0, 28, etc.	12
2	ML-2,095,988 509T[d] + ML-3,948,906[e]	1.07 or 2.14	Topical	0, 28, etc.	12
3	ML-2,095,988 509T[d] + ML-3,948,906[e]	3.21 or 6.42	Topical	0, 28, etc.	12

[a] Data from p. 15 in MRID 47914237

[b] Based on body weight of dogs, i.e., 1.07 mL (Group 2) or 3.21 mL (Groups 1 and 3) if ≤10 kg, 2.14 mL (Group 2) or 6.42 mL (Groups 1 and 3) if ≥10.1 kg (one female in Group 1).

[c] Females were treated throughout breeding, pregnancy and lactation according to the schedule of operations.

[d] Frontline Plus®

[e] Amitraz

3. **Dose selection rationale**: ML-2,095,988 509 (Frontline Plus®) is an EPA-registered product (EPA Reg. No. 65331-5). The rationale for the dose of amitraz was not provided, but presumably involved dosing of the combination product at 1X and 3X.

4. **Mating:** Female dogs were examined daily for signs of estrus, such as vulvar swelling and vaginal discharge, until mating. Each female was co-housed with her selected male on the day after signs of estrus were observed. The pair was allowed to mate until at least three matings had been witnessed, or until the female no longer showed signs of estrus. Four females removed from their male's pen, on a day when they still showed signs of estrus and before three matings were observed, did not become pregnant. The date, time and duration of observed matings were recorded for each female. The estimated range of parturition dates was calculated by adding 63 days to the first and last day of pairing with the male. Whenever possible, males were given a five-day rest period between mating with different females.

5. **Preparation and treatment**: Saline solution (0.9%) or the combination of Frontline Plus with the amitraz formulation was applied to the females by parting the hair and applying directly onto the skin at two separate spots of approximately equal volume, both on the midline of the neck, one between the base of the skull and the shoulder blades, and the other just in front of the shoulder blades. To avoid product run-off in the control and 3X groups, the total volume was divided into three administrations applied to dogs 15 to 30 minutes apart. The products were administered with 1 mL plastic syringes graduated to1/100 mL. Dose calculations were based on the weight of the female recorded between Day -4 and Day -1 for the first treatment and within 5 days of each subsequent treatment. Day 0 (first treatment day) was not the same for females within the first and second stratum, but was the same for all females within each stratum. Subsequent treatments were administered every 28 days until the 4th day after placement with a male. Each female received between 1 and 8 treatments prior to being placed with a male. Once the mating was initiated, all females were treated at 5-7 days after placement with the male, i.e., at the estimated time of conception, regardless of the date of the last treatment. This "conception treatment" was followed by treatments every 28 days until the females completed the study, except for one control female that had one treatment postponed from Day 1 to Day 5 after parturition to avoid disrupting the litter during the neonatal period. Her next treatment was administered 56 days after her

last pre-partum treatment. Treatments continued until 42 days after parturition or at 30 days after the last estimated date of parturition for the five females that failed to deliver.

6. **Statistics**: The following were analyzed using analysis of variance for a randomized block design with the female as the experimental unit: number of pups born per litter; number of live pups born per litter; proportion of dead pups born per litter; proportion of pups with abnormalities per litter; average weight of pups at 7 days post-partum per litter; weaning index (number of pups weaned/number of pups born alive); sex ratio (number of male pups/total number of pups born); average weight gain of live pups from Day 7 to weaning (Day 42); weight of females before first treatment; weight of females at 7 days post-partum; and weight of females at 42 days post-partum. The arc-sine transformation was applied to proportion of dead pups born per litter, proportion of pups with abnormalities per litter, weaning index and sex ratio prior to analysis.

   Delivery rate (conception rate) and failure to deliver (incidence of abortion) were analyzed using Fisher's exact test to compare each treated group to the control. In all analyses, each treated group was compared to the control. A two-sided significance level of 0.10 was used for all tests.

   The following reproduction indices were summarized by treatment group: mating index (number of females with at least one mating observed/number paired); and gestation indices (number of females with live pups born/number of females pregnant, calculated in two fashions, first assuming that females placed with a male that did not whelp were not pregnant and secondly, assuming that females had been pregnant).

C. METHODS:

1. Observations:

a. **General health observations**: Morning and afternoon mortality checks were made on all study animals. The animals were observed daily for general health. Specific health observations on the day of application were observed hourly (± 30 minutes) for four hours after application of the test material. Post-treatment observations included, but were not limited to: assessment for lethargy, ataxia, recumbency, paralysis, coma, pruritis, hyperactivity, tremors, convulsions, abnormal mydriasis, abnormal miosis, dyspnea, tachypnea, coughing, abnormal appetite, abnormal salivation, vomiting, abnormal feces and abnormal urine. Staff was trained to look for any expected clinical signs of amitraz toxicity, including sedation, loss of reflexes, hypothermia, bradycardia, hypotension, bradypnea and mydriasis, third eyelid prolapsed, increased diuresis or vomiting. No application site examinations were reported.

b. **Veterinary examinations**: Physical examinations were conducted on females prior to allocation and at the end of the study. Each stud had a pre-allocation physical examination. The examinations were conducted by a veterinarian or a qualified designee and included evaluations of the following areas or systems: body weight; body temperature; heart and respiration rate; auscultation of the chest; head, eyes, ears, skin; abdominal palpation; genitalia; musculoskeletal and general neurological assessment.

c. **Body weight:** Females were weighed before each treatment for dose calculation and body weight monitoring and at 7 and 42 days after parturition.

2. **Food consumption:** Food consumption was not reported.

3. **Clinical pathology:** No clinical pathology examinations were conducted.

4. **Reproduction data:** The following reproductive indices were calculated by treated group:

   - Mating index: number of females with live pups born/number paired.
   - Gestational index: number of females with live pups born/number of females pregnant, calculated in two fashions, first assuming that females placed with a male that did not whelp were not pregnant and secondly, assuming that these females had been pregnant.

5. **Litter and weaning data:** The number and health status of the pups, including body weight, were recorded on Days 7, 14, 28 and 42 after parturition. A weaning index (number of pups weaned/number of pups born alive) was calculated for each litter. Pups were considered weaned at 42 days of age. Pups were examined for congenital abnormalities, which were defined as malformations detected at birth or during the early weeks of life and which appeared to be permanent. Vital signs (temperature, heart rate and respiration rate) were monitored in pups on Days 14, 28 and 42.

6. **Sacrifice and pathology**: The study did not have a scheduled necropsy. All animals that died during the study were weighed (except for two stillborn pups) and necropsy was performed. A cause of death was established, if possible.

II. RESULTS

A. **ACTUAL DOSES ADMINISTERED**: The doses of active ingredients to females over the course of the study ranged as high as to 39.70 mg/kg ML-2,095,988 (fipronil), 35.80 mg/kg ML-3,335,716 [(S)-methoprene] and 47.50 mg/kg ML-3,948,906 (amitraz). The timing of the conception treatment produced a shorter dosing interval during breeding for most females. At the 3X dose, the interval between doses administered around conception could be as short as 2-7 days. The volume of each formulation administered per weight group is presented in Table 2.

TABLE 2: Actual volumes of products administered[a]				
Dog wt. (kg)	0.9% Saline (mL)	Frontline Plus® (mL)	Amitraz (mL)	Total volume (mL)
Group 1 (control)				
up to 10	1.07 adm. 3 times	-	-	3.21
10.1- 20	2.14 adm. 3 times	-	-	6.42
20.1 – 40	4.28 adm. 3 times	-	-	12.84
Group 2 (1X)				
up to 10	-	0.67*	0.40*	1.07*
10.1- 20	-	1.34	0.80	2.14
Group 3 (3X)				
up to 10	-	0.67 adm. 3 times	0.40 adm. 3 times	3.21 (1.07 adm. 3 times)
10.1- 20	-	1.34 adm. 3 times	0.80 adm. 3 times	6.42 (2.14 adm. 3 times)

[a] Extracted from pages 20-21 of MRID 47914237.
Adm. = administered

* Except in two cases. One female in the 1X group was overdosed by 0.67 mL Frontline Plus® and 0.40 mL amitraz at her 2nd post-breeding treatment. Another female in the 1X group was overdosed by 0.4 mL amitraz at her 2nd post-breeding treatment.

B. OBSERVATIONS:

1. **Clinical signs of toxicity**: There were no clinical signs of toxicity in treated females or their pups at 1X and 3X the recommended dose during the observations or at any time after each treatment administration. Concomitant medications were used in several animals. One female in the 1X group was given oxytocin to stimulate contractions and Esbilac® for calcium supplementation during parturition before one large pup was manually extracted from the birth canal. The female also had post-parturtion treatment with antibiotics. Another female was given antibiotics and fluids subcutaneously for mastitis. Another female was initially treated with antibiotics but was euthanized due to an open pyometra.

2. **Application site examination**: No application site examinations were reported.

3. **Mortality**: One female in the 1X group was euthanized 7 days after parturition due to a severe bacterial infection of the uterus (pyometra).

C. **BODY WEIGHT AND WEIGHT GAIN**: Body weight data for females are presented in Table 3. Body weight was statistically significantly decreased compared to the control group at Day 42 post-partum for females in the 1X group. This is not considered treatment-related since body weight was not decreased in the 3X group. Body weight gain over the course of the study was decreased in both the 1X and 3X groups compared to the control group but there was no dose-response relationship.

TABLE 3: Body weight and body weight gain in breeding females (n=12)[a]			
	Control	1X	3X
Body weight (kg)			
Pre-treatment (Day -4/-1)	11.22	10.28	11.34
Day 7 PP	11.52	10.12	11.86
Day 42 PP	12.88	10.92*	12.70
Body weight gain (kg)[b]			
Pre-treatment to Day 7 PP	0.3	-0.16	0.52
Day 7 PP to Day 42 PP	1.36	0.8	1.36
Pre-treatment to Day 42 PP	1.66	0.64	1.36

[a] Extracted from page 81, MRID 47914237.
[b] Calculated by the reviewer; no statistical analysis was conducted.
PP = post-partum
* Significantly different from control group, $p<0.10$, analysis of variance

D. FOOD CONSUMPTION: Food consumption data were not recorded.

E. CLINICAL PATHOLOGY ANALYSES: Clinical pathology analyses were not conducted.

F. REPRODUCTION DATA: Summary data are presented in Table 4. There were no treatment-related effects on reproduction parameters.

TABLE 4: Reproduction parameters[a]			
	Control	1X	3X
Number of females	12	12	12
Number of females observed in estrus	12	12	12
Number of females observed mating	5	2	5
Mating index[1]	5/12	2/12	5/12
Gestation index A[2]	10/10	9/9	11/12
Gestation index B[3]	10/12	9/12	11/12
Delivery rate[4]	10/12	9/12	12/12
Failure to deliver[5]	2/12	3/12	0/12

[a] Extracted from page 81, MRID 47914237.
[1] Number of females with at least one mating observed/number paired.
[2] Number of females with live pups born/number of females pregnant, assuming females that were placed with a male but did not whelp were not pregnant.
[3] Number of females with live pups born/number of females pregnant, assuming females that were placed with a male but did not whelp were pregnant.
[4] Number of females that delivered any pup (live or dead)/number of females that were placed with a male.
[5] Number of females that did not deliver any pup (live or dead)/number of females that were placed with a male.

G. LITTER AND WEANING DATA: Litter and weaning data are summarized in Table 5. 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. No congenital abnormalities were detected in the 1X group

pups, resulting in a significantly ($p<0.01$) lower proportion of pups with abnormalities per litter in this group compared to the control. In the control group, one stillborn pup was grossly deformed, with a brachiocephalic muzzle and large cleft palate. Another pup in the control group had a bent tail tip ("broken tail") and three pups had overbites ("brachygnathia" or "parrot mouth"). 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 animals in the colony that were not involved in toxicology studies. Another pup (stillborn) in the 3X group had a congenital diaphragmatic hernia; no pups in the breeding colony exhibited this finding in the previous year. Other congenital findings in the 3X group included double rear dewclaws in one pup, underbite in one pup, small reducible hernia in one pup, and "broken tail" in two pups.

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. In one litter of the 3X group, 3 out of 8 pups had slight unilateral or bilateral serous ocular discharge during their final examination on Day 42. One pup in the 3X group had normal growth on Days 7 and 14 but lost weight on Day 28 post-partum and remained the smallest of the litter until weaning. She also had labored breathing and an enlarged abdomen. She was found dead 4 days after weaning. On necropsy, bronchopneumonia was diagnosed and *Bordetella bronchiseptica* was isolated.

TABLE 5: Litter and weaning parameters[a]

	Control	1X	3X
Number of litters	10	9	12
Total born	62	57	64
Number of pups born/litter[1]	6.2	6.3	5.3
Number of pups born alive	60	56	62
Number of live pups born/litter[1]	6.0	6.2	5.2
Number of stillborn pups	2	1	2
Number of litters with stillborn pups	1	1	2
Stillborn pups/litter, %[1,2]	0.3	0.2	2.6
Number of abnormal pups	5	0	7
Pups with abnormalities/litter, %[1,3]	3.6	0.0*	3.8
Number of pups weaned	32	40	56
Weaning index, %[1,4]	61.8	81.3	92.7
Number of live male pups	28	32	30
Sex ratio, %[1,5]	45.7	56.7	40.0
Pup live body weight (kg)/litter[1]			
post-parturition (day 7)	0.50	0.45*	0.49
gain (day 7-42) (weaning)	1.73	1.78	1.64

[a] Extracted from pages 61-65, 82 and 102, MRID 47914237.
[1] Least square means
[2] Retransformation of arcsine square root of (number of stillborn pups/number of pups born)
[3] Retransformation of arcsine square root of (number of abnormal pups/number of pups born)
[4] Retransformation of arcsine square root of (number of pups weaned/number of pups born alive)
[5] Retransformation of arcsine square root of (number of live male pups/number of pups born alive)
* Significantly different from control group, $p<0.10$, analysis of variance

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the combination of Frontline Plus® and amitraz did not cause any adverse effect in breeding females or pups when administered topically to the females at 1X and 3X the recommended dose at intervals of 28 days or less before breeding and throughout breeding, pregnancy and lactation until weaning. All doses of the active ingredients were well tolerated, even when administered 2.5, 6 or 7 days apart at the 3X dose around conception time. The treatment of breeding females with the Frontline Plus® and amitraz combination is therefore safe for both females and pups during all phases of reproduction.

B. **REVIEWER COMMENTS:** In this non-guideline study of breeding female beagle dogs, there were no treatment-related effects on mortality, clinical signs of toxicity or body weight/body weight gain when the proposed combination of products (Frontline Plus® and amitraz) was applied at a maximum dose of 3X the recommended dose. No treatment-related effects on the reproduction parameters were reported. 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. No historical control data on stillborn/litter incidence for the testing laboratory or animal source were provided. Body weight and body weight gain in pups were unaffected by treatment. Vital signs in pups on Days 14, 28 and 42 were comparable between control and treated groups. In the 3X group, one female pup was euthanized; at 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 this finding in the previous year. The Agency concern is that the major congenital abnormality findings in the 3X group may have been treatment-related. The inclusion of a 5X treatment group in the study might have confirmed or refuted that these findings at 3X were treatment-related; however, the Agency does not have a specific protocol for reproductive safety studies in companion animals. This study used a protocol based on FDA Guideline 33 (which has been superseded by VICH GL43).

Because of Agency concerns regarding the occurrence of the major congenital abnormalities observed in two puppies in the 3X group, this companion animal safety study in female breeding beagle dogs is currently classified as Supplementary/Non-Guideline. The registrant should address the issue of these abnormalities. If possible, any historical control data (including incidences) from the laboratory, as well as information from the literature for these types of abnormalities in beagles should be provided to the Agency. If this issue is adequately addressed, the study can be upgraded to acceptable.

C. STUDY DEFICIENCIES:

The following minor deficiencies were noted:

1. The females should not have been housed together during treatment. The dogs could have had additional exposure to the treatment either orally or dermally.

2. The application sites should have been examined and the findings reported since CERTIFECT® for dogs is a slight skin irritant and a potential skin sensitizer according to "Safety Summary for CERTIFECT® for dogs (Fipronil + (S)-Methoprene + Amitraz) Topical Spot-On" (MRID 47914234).

1. **DP BARCODE:** DP 377589
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 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. Because of Agency concerns regarding the occurrence of the major congenital abnormalities observed in two puppies in the 3X group, this companion animal safety study in female breeding beagle dogs is currently classified as Supplementary/Non-Guideline. The registrant should address the issue of these abnormalities. If possible, any historical control data (including incidences) from the laboratory, as well as information from the literature for these types of abnormalities in beagles should be provided to the Agency. If this issue is adequately addressed, the study can be upgraded to acceptable.	N/A	S (potentially upgradeable)

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