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

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

October 21, 2002

MEMORANDUM

Product Name: FRONTLINE® PLUS FOR DOGS
EPA Reg. No.: 65331-5
DP Barcode: D282205
Case No: 066320
Submission: S613600
Chemicals: 129121 Fipronil
105402 S-methoprene

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

10/28/2002

To: Ann Sibold/Arnold Layne, PM 03
Insecticide Branch
Registration Division (7505C)

Registrant: MERIAL LIMITED

ACTION REQUESTED: "Please review the attached label and data and determine if they are acceptable..."

BACKGROUND: This registered product has the following label ingredient declaration:
Active Ingredients:
Fipronil.....9.8%
(S)-methoprene.....8.8%
Inert Ingredients:.....81.4%

According to the revised labeling which TRB has received the registrant is proposing the following additional uses: 1) "For Dogs & Puppies 8 weeks or older and up to 22 lbs." (Previous labeling did not include the use of this product on dogs and puppies weighing less than 22 lbs) and 2) FRONTLINE PLUS can also be used for the treatment and control of flea, tick and chewing lice infestations on breeding, pregnant and lactating bitches." To support these additional uses, the registrant has submitted two studies. The first (in MRID 45612702) is formidably titled: "TO INVESTIGATE THE SAFETY OF A TOPICAL FORMULATION CONTAINING ML-2,095,988 509T IN DOGS WEIGHING APPROXIMATELY 2 KG (+/- 1.5 KG) AT ONE, THREE AND FIVE TIMES THE MAXIMUM LABEL RECOMMENDED DOSAGE." The second (in MRID 45620507) is titled "ML-2,095,988 509T/Dog/Solution/Topical/Safety/Tolerance/ Reproduction, Reactions."

COMMENTS AND RECOMMENDATIONS:

1. The study titled: "TO INVESTIGATE THE SAFETY OF A TOPICAL FORMULATION CONTAINING ML-2,095,988 509T IN DOGS WEIGHING APPROXIMATELY 2 KG (+/- 1.5 KG) AT ONE, THREE AND FIVE TIMES THE MAXIMUM LABEL RECOMMENDED DOSAGE" (MRID 45612702) has been reviewed by TRB. It is concluded that the findings of this study adequately support the proposed use of this product at the label-specified dosage rate of 0.67 mL/application on dogs weighing less than 10 kg with applications at no less than 30-day intervals. Refer to the first attached DER for a complete review and executive summary.

2. The study titled: ""ML-2,095,988 509T/Dog/Solution/Topical/Safety/Tolerance/ Reproduction, Reactions" has also been reviewed by TRB. It is concluded that the findings of this study adequately support the proposed use of this product at the label-specified dosage rates on breeding, pregnant and lactating bitches with applications at no less than 28-day intervals (the label specifies 30-day intervals). Refer to the second attached DER for a complete review and executive summary.

Reviewer: Masih Hashim, DVM., Ph.D. MH

STUDY TYPE: Companion Animal Safety Dog (OPPTS 870.7200)

EPA I.D. NUMBERS: DB Barcode: D282205; MRID: 456127-02

TEST MATERIAL: ML-2,095,988 509T P.C. Codes 129121 (Fipronil) and 105402 (S -Methoprene)

STUDY NUMBER: (Covance) 1686/12

TESTING FACILITY: Covance laboratories, Otley Road, Harrogate, North Yorkshire, England.

SPONSOR: Merial Limited, Athens, Georgia, 30601-1649

TITLE OF REPORT: To Investigate the Safety of a Topical Formulation Containing ML-2,095,988 509T in Dogs Weighing Approximately 2 KG (+/- 1.5 KG) at One, Three, and Five Times the Maximum Label Recommended Dosage

AUTHORS: S Nolan-Smith

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 456127-02), Frontline Plus (active ingredients:10% w/v fipronil and 9% w/v {S}-methoprene) was topically applied (between the base of the skull and the shoulder blades at one or more spots) to eight week old dogs weighing less than 11 lbs at 1x, 3x and 5x the prepackaged use (0.67 mL/ puppy) dosage. Forty-eight beagle dogs (24/sex) were randomly allocated to one control and 3 treatment groups with 6 male and 6 female animals in each group. Controls received no treatment. The formulation was delivered from the marketed (0.67 mL) pipettes having 67 mg of fipronil and 60.3 mg of S-methoprene as 1x dosage. Puppies were exposed to the formulation only on Day 1, when they were at an average age of 57 (50 to 63) days and weighed 1.67 to 3.57 kg. All the test and control animals were isolated for 6 hours after treatment and observed at timely intervals (10 minutes in the 1st hour, then each hour on the hour). Any reaction (erythema, edema, alopecia, hair coat condition, and pruritus) at the application site, clinical parameters including rectal temperature, condition of the eyes (nystagmus, congestion, discharge, visual impairment); muscular disturbances (tremors, paralysis and atony); gastrointestinal disturbances (vomiting, consistency of stools); color of mucus membranes; and general behavior and general physical condition, were evaluated during the acclimatization period, on the day of treatment (Day l), Days 2, 4, 8, 15, 22 and 29 (termination) . Body weights were measured weekly and blood samples were collected for clinical pathology (hematology and clinical chemistry) on Days 2, 15 and 28. Animals were sacrificed upon termination for gross and histopathological evaluations.

Statistical analyses were conducted for rectal temperature, body weight and for clinical pathology data.

There were no deaths on the study, no local reaction on the application site and no treatment-related clinical signs in the measured parameters. There were no effects on rectal temperature, body weight, food and/or milk consumption, hematology or clinical chemistry.

Gross necropsy and histopathology revealed no abnormalities, with possible exception of a few dermal effects at the treated sites (red area on the treated site in a few dogs which in one 3x male was 30 mm in diameter, and in another one 3x male was 10 mm in diameter, pages 84, 174, 176).

A single topical application of fipronil (10% w/v) and (S)-methoprene (9% w/v) in a solution at 1x, 3x and 5 times the maximum recommended dose, when given to eight week old dogs (approx.1.5-3.5 kg) showed no treatment related effects.

This study (870-7200) is classified **Acceptable** in demonstrating a 5x margin of safety in dogs 8 weeks old or older and weighing less than 11 lbs.

COMPLIANCE: The study has Data Confidentiality (p.2), Good Laboratory Practice Compliance statements (p.3) and Quality Assurance Statement (p.4) are included.

1. MATERIALS

A. Test Material: ML-2,095,988 509T 10% w/v fipronil and 9% w/v (S)-methoprene

Description: They are combined in a topical solution to be used for treatment and prevention of flea and tick infestations in dogs and cats. Supplied in pre-packaged boxes containing 3 X 0.67 ml pipettes.
Lot/Batch No.: R01012BR-F (page16)
Active Ingredients: (10% w/v fipronil and 9% w/v (S)-methoprene)
Storage Conditions: Room temperature.

B. Administration: The test article was applied once to eight week old dogs, weighing aproximately 1.5-2.0 kg , at 1x, 3x and 5x the maximum recommended dosage when delivered from marketed (0.67 mL) pipettes.

C. Control

There was no reference control item used in the study.

D. Test animals

Species: Dog
Breed: Beagle
Age and weight at study initiation: Av of 8 weeks
mean weight; male 1.67-3.57 kg, female 1.79-3.45 kg
Source: Harlan UK, Loughborough, UK
Housing: Groups of 3 of the same sex
Diet: Initially reconstituted milk (Milkivit Lamb Plus), and Harlan Tekland 9680 (28% Puppy Diet) ad libitum
Water: Mains water initially in bowls ad libitum, then to automatic drinkers
Environmental conditions:
Temperature: 24-26°C[stated in the report on p. 18]
Humidity: 9-40%
Photoperiod: 12 hr dark/light
Acclimation period: 11 days prior to test article application. Immunization: Page 19.

II. STUDY DESIGN

A. In life dates

Study initiation 2-26-01, Study completion 9-27-01

B. Animal assignment/Dosage and Administration

"A total of 52 pure-bred beagle dogs (26 male and 26 female) were obtained from Harlan UK Ltd (Loughborough, UK). Twenty four animals of each sex were used for the study; on delivery, they were approximately six weeks of age, they were uniquely identified by the supplier's tattoo and a subcutaneous electronic implant. At treatment, the age of the animals was approximately 56 days +/- 7 days (range 50 to 63, with an overall average of 57 days). The bodyweight range at treatment was 1.67 to 3.57 kg. Each dog was clinically assessed by a veterinarian once between Days -7 and -1; the examination included a clinical evaluation of the general health of the dog and an assessment of the proposed application site. Dogs were deemed acceptable for study based on the clinical assessment of the dogs and their baseline clinical pathology. One female animal (31F) was judged unhealthy by the Study Director and the veterinarian, so was excluded and replaced with a suitable spare animal before dosing."

TABLE 1. Experimental design					
Group	No. of animals		Dose Level	mg/dog	
	Male	Female		methoprene	fipronil
1	6	6	Placebo Collar (0X)	-	-
2	6	6	1X	67	60.3
3	6	6	3X	201.0	180.9
4	6	6	5x	335.0	301.5

Note: size of the pipette 0.67 ml

C. Dose selection rationale

The dose selected was 1x, 3x and 5x the prescribed dose. The puppies were dosed only on Day 1. Controls were not dosed.

D. Experimental design

This was a one time topical application of the test article was at 1x,3x and 5x the label specified dose. "The study design is a randomised block. Forty-eight dogs were used. Between Day -14 and Day -7, twelve replicates of four dogs each were formed within sex, based on order of presentation from the supplier of similarly aged suitable dogs. Within replicates, dogs were randomly allocated to one of the four treatment groups. Within group and sex, three dogs were allocated to a pen sequentially through replicates and pen numbers."

E. Clinical parameters

Clinical evaluations were conducted on the day after arrival, on Day 1 i.e., the day of treatment prior to dosing and Days 2, 4, 8, 15, 22 and termination, before necropsy (Day 29). These clinical evaluations consisted of the following:

a. Evaluation of skin reaction at application site: At each examination, skin was evaluated for erythema, edema alopecia (none, slight, moderate, extensive), haircoat condition (good, poor -description) and pruritus (none, slight, moderate, severe). Erythema and oedema were assessed according to a numerical system based on that of Draize (Draize, JH (1965), Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials ofthe United States, Topeka, Kansas).

b. Rectal temperatures: Rectal temperature measurements were recorded at approximately the same time on each clinical evaluation day.

c .Physical examination: "A physical examination was conducted with particular attention paid to locomotion, the eyes (eg nystagmus, congestion, discharge, signs of visual impairment), muscular disturbances (tremors, paralysis and atony), gastrointestinal disturbance (vomiting, consistency of stools, by pen when direct observation was not possible), colour of mucous membranes, and general behaviour. Any other abnormalities detected were also recorded. In addition to the specified clinical evaluations, daily observations of general health and behaviour of all dogs were conducted and recorded and on the day of treatment, the dogs were observed for post-dosing signs at 10 minutes after dosing and approximately every hour thereafter for six hours. "

F. Body weight

All animals were weighed after arrival, and then weekly from approximately Day -7, including the day of treatment (before dosing); reported from Day -7.

G. Clinical pathology

Blood samples were taken from the jugular vein of all animals once between Day -7 and Day -1 and on Days 2,15 and 28. The collected samples were divided into 1 mL tubes as follows:

Citrate anticoagulant ..... for clotting (prothrombin) time
EDTA anticoagulant ..... for haematology investigations
Lithium heparin anticoagulant..... for biochemical investigations
The haematology analyser was used for the following estimates :

a. Hematology

X	Hemoglobin (HGB)*	X	Leukocyte differential count*
X	Hematocrit (HCT)*	X	Mean corpuscular HGB (MCH)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count	X	Reticulocyte count
X	Blood clotting measurements (Thromboplastin time) (Clotting time) (Prothrombin time)*	X	Packed cell volume (PCV)

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHERS
X	Sodium	X	Total protein*
X	Potassium*	X	Albumin
X	Calcium*	X	Globulin*
X	Chloride*	X	Albumin
	Inorganic Phosphorus*	X	Globulin (calculated)
X	Potassium*		Albumin/globulin (A/G) ratio (calculated)
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total cholesterol
X	Aspartate aminotransferase (AST)	X	Blood creatinine*
X	Alanine amino transferase (ALT)	X	Blood urea nitrogen*
X	Alkaline phosphatase (AP)	X	Creatinine
X	Glutamic-oxaloacetic transaminase (GOT)		

*Recommended in OPPTS 870.7200 Guidelines.

H. Urinalysis

Urinalysis was not performed.

I. Necropsy and histopathology

Upon termination (Day 29) of study all animals were killed by intravenous overdose of sodium thiopentone. Necropsy was performed and ay lesions were recorded.
Following tissue samples were preserved in neutral buffered 10% formalin (except eyes, which were preserved in Davidson's fixative): adrenals, alimentary tract (including oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), aorta (arch and abdominal), brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum); eyes (with optic nerves); gall bladder, heart, kidneys, lacrimal gland, liver, lungs (with bronchi), lymph nodes (cervical, mediastinal and mesenteric), mammary gland, ovaries, pancreas, pituitary, prostate, salivary gland (submandibular), sciatic nerve, skeletal muscle, spinal cord (cervical, thoracic and lumbar

regions), spleen, sternum (with marrow), tattoo, testes (and epididymides), thymus, thyroids (and parathyroids), tongue, trachea, urinary bladder, uterus, vagina, macroscopically abnormal tissues. Two skin samples from the application site and one from an untreated area of the dorsal midline from all dogs, including controls, were sampled and preserved in neutral buffered 10% formalin: The skin samples were embedded in paraffin wax, sectioned at a nominal 5 pm, stained with haematoxylin and eosin. These samples were examined by the pathologist under light microscopy.

J. Statistics

a. Data analysis: The data was analyzed as follows.
"Where values below the limit of detection were observed (total bilirubin), the data were rank transformed prior to analysis. As specified in the protocol, white cell differential counts were transformed to radians using the arcsine square-root transformation. For all other variables analysed, Levene's test for heterogeneity of variances among groups, between sexes and their interaction was performed. Where this showed evidence of heterogeneity between sexes ($P<0.01$), even after log transformation, the data were analysed separately for each sex. Where there was evidence of heterogeneity among groups ($P<0.01$), even after log transformation, the data were rank transformed prior to analysis. "

"Haematology and clinical chemistry variables were analysed using repeated measures Analysis of Covariance (ANCOVA), using the pre-treatment values as covariate. Where the test for equality of slopes (i.e. the COVARIATE x GROUP interaction) was significant ($P<0.05$), the effect was retained in the model and the treatment comparisons of interest tested at the 25th, 50 th and 75th percentiles of the covariate. Where the test for equality of slopes was not significant (P>_0.05) the effect was removed from the model. Where the covariate was significant ($P<0.05$), the effect was retained in the model. Where the covariate was not significant, the effect was removed from the model and the repeated measures Analysis of Variance (ANOVA) model used. The repeated measures ANOVA model was also used for rectal temperatures."

For both ANOVA and ANCOVA models, the following factors were included
Sex, Group, Sex x Group interaction. Day, sex xDay, Group x Day, Group x Sex x Day.
In addition, PEN BLOCK was fitted as a random effect.

"The interaction terms of GROUP x SEX and of GROUP x SEX x DAY were tested at the 5% level of significance. Where a significant "sex" interaction term was found, the data were analysed for each sex separately. The interaction term of GROUP with DAY was tested at the 10% level of significance. Where a significant "time" interaction term was found, the data were presented graphically. Where the interaction terms were not significant, linear contrasts comparing each treated group with control were tested at the 10% level of significance." (page 24)

"Bodyweight gain from day 1 to 29 was analysed using two-way ANOVA. In the absence of a significant SEX x GROUP interaction (P>_0.05), linear contrasts comparing each treated group with control were tested at the 10% level of significance."

b. Data interpretation: All tests were interpreted with two-sided risk.

c. Collection and retention of data
"All raw data has been paginated and identified with the assigned PR&D number and CLE study number. All raw data has been signed and dated by the person making the observation (and by the person recording the information, if not the same person). All data (excluding that provided by the animal supplier) has been collected by Covance personnel. All original laboratory data records and documentation necessary to reconstruct and evaluate the study has been maintained."

K. Disposition of animals

Animals were killed and necropsied on Day 29.

L. Compliance

The study has Quality Assurance Statement (p.4), Data Confidentiality (p.2) and Good Laboratory Practice Compliance (p.3) statements are included.

III. RESULTS

A.Exposure levels

The animals were exposed to 3 doses., i.e., 1x, 3x and 5x the prescribed dose. The 1x dose was 0.67 mls having 67 mg of fipronil and 60.3 mg of (S)-methoprene.

B.Mortality

No dogs died on the study.

C. Clinical signs

Any findings of general health had similar incidence in all groups and were not (significantly) different from the aclimmatization period. There were no findings that colud be considered as treatment related.
"Three, one and three animals from groups 2, 3 and 4, respectively, were described as being thin for one or more (post-dosing) days of the study; with the exception of animal 8M (see Bodyweight section'), this was transient and, as this is a common finding in animals of this young age and the animals were otherwise healthy, the thin appearance of all of these animals is considered to be unrelated to treatment."

a. Post dosing observations
There were no adverse signs during the 6 hours after treatment.

b. Clinical evaluations

"There were no differences in rectal temperature between the groups and the only findings at the

application site occurred either before treatment or in a control animal. At the physical examination part of the clinical evaluation, one Group-2 male (8M) was described as thin (see also 'Bodyweight' below) on Day 2 and one Group-4 female was trembling on Day 4; these singular occurrences are considered incidental to treatment. All other findings were either seen pre-dosing (eg sparse haircoat) or at similar levels in treated and control groups (eg lacrimation)."

D. Body weight and weight gain

There appeared to be no treatment-related effect on body weights of animals in any of the groups. "Two males and a female for Group 2, and a male and female from Group 4, showed very small - body weight losses in the first week after treatment, otherwise, all animals gained weight throughout the study (Days 1 to 29)".

"Group mean body weight gain (sexes combined) over Days 1 to 29 (see Table S2 in Appendix 12) was slightly lower in Group 2 compared to the control, however, this value was greatly influenced by one animal (8M) which gained only 600 g over this period (for reference, the mean gain for all study animals was approximately 2.25 kg). As there were no statistically significant differences between the group mean body weight gains, and as Groups 3 and 4 compared favourably with the controls, the reduction in weight gain seen in Group 2 is considered to be unrelated to treatment".

E Food and milk consumption

"There was no treatment-related effect on food or milk consumption. Group mean food consumption was slightly lower in the Group-2 males; this is consistent with the group differences in body weight (see above); all other groups had similar values. Milk consumption varied from pen to pen; the biggest variation was seen in the control males and there was no treatment-related effect seen in the group mean values."

F. Hematology

There was little difference in the hematology pre versus post dose values. There are some sporadic changes with no biological significance.
"There were some overall post-treatment group mean values showing differences from control which were statistically significant, however, none of the differences was considered to be of biological significance. There were no dose-response relationships in the findings and none of the effects seen in haematology parameters were considered to be related to treatment.
For the following variables, a significant interaction with time ($P<0.10$) was found, although, there was no significant overall difference from control for any dose group"

G. Clinical Chemistry

There were a few variations in the clinical chemistry values in the post treatment particularly for total protein, urea, albumin, globulin, bilirubin and ALT. However, they have little biological significance. "There were a few minor fluctuations in clinical chemistry values and some overall post-treatment group mean values were statistically significantly different from the control group but none was considered either clinically significant or related to treatment. For most of these parameters the values had changed little, or not at all, from the predose values, for total protein, urea, albumin and globulin, there was very little variation in values across all the groups, making the statistical tests oversensitive. None of the differences present in more than one group showed any relevant dose relationship. Post-treatment total bilirubin was slightly increased for Group 1, and although the difference from control was statistically significant for the rank transformed data, it was only at the P<O.10 level. For ALT a significant interaction with time (P<0.10) was found but no significant overall difference between the control and any of the treatment groups; *the data are presented graphically*"(summary tables and graphs pages 221-226).

Table 2. Clinical Chemistry - Summary of Group means- Statistically Significant Selected Values

Parameters/ Sample Time	Males				Females			
	Control 0x	1x	3x	5x	0x	1x	3x	5x
Total Protein- Wk 1	45	43	44	48	45	45	44	45
Total Protein- Post Treatment (2,15,28 days)	46	43*	44*	45*	46	46	48*	47
Albumin g/L -Wk 1	24	24	24	24	26	26	26	26
Albumin- Post Treatment (2,15,28 days)	26	26	26	26	26	26	28*	28*

* Statistically significant difference from control (P<0.10)

H. Necropsy and Histopathology

a. Gross pathology: There were no gross pathological findings suggestive of either local or systemic toxicity due to the test article administration, other than some local effects. At necropsy, one 3x male animal had a 30 mm diameter red area at the treatment site, and another 3x male had a 10 mm red area at the treatment site (refer page 174+176).

b. Microscopic findings : Several animals had slight focal epidermatitis or dermato-epidermatitis, but there was no dose-response relationship. Similar focal epidermal changes were found in the untreated skin sites. In general these findings at the skin application site were of little significance and generally of a minor nature. There were no erosion, ulceration or dermatitis, suggesting local toxicity due to test article administration.

IV DISCUSSION

All animals survived to study termination. There were no major differences in any of the parameters evaluated which includes body weights, food consumption, clinical evaluations. There were a few fluctuations in clinical chemistry values i.e., overall post-treatment group mean values were significantly different from the control group but none was considered either clinically or biologically significant For most of these parameters the values had changed a little from pretreatment e.g., total protein, urea, albumin and globulin. However, this variation seen was across the groups. None of the differences present in more than one group showed any dose response. Post-treatment total bilirubin was slightly increased for Group 1, and although the difference from control was statistically significant (for the rank transformed data), it was only at the $P<O.10$ level. For ALT there was significant interaction with time ($P<0.10$) , but no significance (overall) in between the control and any of the treatment groups.

Urinalysis was not conducted for this study. However, this may not affect the outcome of the study. There are several deviations mentioned in the study notebook (page 26). These deviations do not affect the outcome of the study.

A previous study 65331-L with topical application of the formulation (fipronil and methoprene) with topical application on Day 1 and Day 29 was classified by TRB as supplementary. This study MRID 456127-02, Fipronil Plus for dogs, with Fipronil 10% and (S)-methoprene 9% is **Acceptable** in accordance with the Sub-Division F guidelines. An adequate safety margin at 1x, 3x and 5x has been established.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D282205
2. **PC CODE:** 129121, 105402
3. **CURRENT DATE:** 9-27-02
4. **TEST MATERIAL:** Fipronil 10% and (S)-methoprene 9%

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety/Dogs/ Covance 1686/12 9-27-01	456127-02	No mortality in the dogs by topical application of Fipronil plus at 1x 3x and 5x the proposed use. There were no dose related effects in parameters including food consumption, body weight gains, hematology, and clinical chemistry. The study demonstrated (1, 3, and 5x) margin of safety in dogs associated with the proposed use.	-	*A*

Core Grade Key: A =Acceptable, S = Supplementary, U = Unacceptable, V = Self-Validated

Reviewer:Byron T. Backus, Ph.D. Byron T. Backus 10/24/2002
Secondary Reviewer:

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Special/Pregnant Dog

EPA I.D. NUMBERS: DP BARCODE: D; MRID NUMBER: 45620507

TEST MATERIAL: EPA Reg. No. 65331-X; ML-2,095,988 509T (10% w/v fipronil and 9% w/v (S)-methoprene)

STUDY NUMBER: WEL 99885, PR&D 0020201

TESTING FACILITY: White Eagle Toxicology Laboratory
2003 Lower State Road
Doylestown, PA 18901

SPONSOR: Merial Limited, Pharmaceutical Research and Development, 2100 Ronson Road, Iselin, NJ 08830-3077

TITLE OF REPORT: ML-2,095,988 509T/Dog/Solution/Topical/Safety/Tolerance/ Reproduction, Reactions

AUTHORS: Godin, C.S. & Alva, R.

STUDY COMPLETION DATE: June 22, 2000

EXECUTIVE SUMMARY: ***In a special (reproductive) companion animal safety study (MRID 45620507) ML-2,095,988 509T (a topical formulation containing 10% w/v fipronil and 9% w/v (S)-methoprene) was administered at 0X (Group 1; sham-treated controls), 1X or 0.133 mL/kg (Group 2) and 3X or 0.399 ml/jg (Group 3) to groups of 12 adult female beagle dogs (bitches) prior to and during pregnancy, through parturition and to weaning of the puppies. All bitches were proven breeders, as each had at least two previous litters. The bitches were dosed at the start (March 16, 1999) of the study, and at 28-day intervals thereafter until mating, then were treated within 24 hours of their first mating session and every 28 days thereafter until their puppies were 41-43 days old. Even if a bitch was mated a few days following an application treatment, she was treated again on the day following mating (example: Bitch #14 of Group 2 was treated on June 8, then again 3 days later on June 11 following a mating on June 10, then was treated again 28 days later on July 9, then on August 6, and finally on September 3.***

Bitches in Group 1 were sham treated. Bitches in Groups 2 and 3 were treated by parting

the hair and applying the formulation directly to the skin between the base of the skull and shoulder blades; bitches in Group 2 were dosed (0.133 mL/kg) at one spot; bitches in Group 3 were dosed at 0.399 mL/kg, with 3 applications of a approximately the same volume at 3 spots. The male studs that were used for breeding were not treated with the test material.

Clinical observations were conducted hourly for 6 hr following each treatment; on other days each bitch was observed at least once a day. Daily clinical observations were conducted on puppies beginning within 24 hours of parturition. Bitches were weighed on the day of or the day before each treatment, at 41-43 days post-mating and within 3 days post-parturition. Puppies were weighed individually within 24 hours of parturition, at 13-15, 27-29, and 41-43 days post-parturition.

All bitches except one in the 3X dose group mated during the study; all other bitches were mated once; however, three bitches in the control group and one in the 1X group mated but did not have puppies. At Day 93 after mating they were removed from the study. One bitch in the control group and one in the 1X group had cesarian sections. Their pups received a physical exam and then were removed from the study, as were the bitches.

The only dose-related effects observed in bitches were pink skin at the application site on days of treatment (recorded 15 times in Group 2, 37 times in Group 3), pink skin at the application site on the day following treatment (3 times in Group 3). There were no significant differences between the bitches of different groups with respect to mean body weights or for a number of parameters measured at weaning (mean rectal temperatures, mean respiratory rates, or mean heart rates).

There was no indication of an adverse effect on any reproductive parameters (including gestation index, incidence of cesarean section, incidences of stillborn pups, incidences of pups dying between birth and weaning). Although there were somewhat greater percentages of males at birth and subsequently in Groups 2 and 3 relative to Group 1 (At birth: Group 3: 58.2%; Group 2: 54.1%; Group 1: 52.1%) these incidences did not appear to be statistically significant.

There were no significant differences between puppies from the 3 groups with respect to physiological parameters (mean body weights, mean rectal temperatures, mean respiratory rates, mean heart rates) measured on post-parturition days 14, 28 and 42 .

Group 2 bitches weighed from 8.4 to 13.4 kg (18.48 - 29.48 lbs) and those in Group 3 weighed from 9.6 to 14.7 kg (21.12 to 32.34 lbs). From information in the administrative materials for EPA Reg. No. 65331-3 (9.7% fipronil as sole active) and EPA Reg. No. 65331-5 (with the two actives fipronil at 9.8% and (S)-methoprene at 8.8%) the dosage rate for both products for dogs weighing 23-44 lbs (approx. 10-20 kg) is 1.34 mL/application so the label use application rate is approximately 0.067 - 0.134 mL/dog for dogs in this size range. In this study the 12 dogs in Group 2 (1X) had 0.133 mL/kg of the formulation

applied directly to the skin at one spot between the base of the skull and the shoulder blades, while each of the 12 females in Group 3 (3X) received a total 0.399 mL/kg,

Although no dogs were dosed at 5X level, and no blood was taken for clinical chemistry and hematology measurements, the purpose of this study was to allow a label claim for use of at least two registered products (EPA Reg. Nos. 65331-3 and 65331-5) on breeding, pregnant and lactating bitches. TRB has previously reviewed a companion animal (dog) study for this formulation which included a 5X dosage group and measurements of clinical chemistry and hematology parameters

This study is classified as acceptable. The findings of this study are adequate to support the use of EPA Reg. Nos. 65331-3 and 65331-5 at the specified label use rates (varying according to the weight of dog) at 28-day intervals on breeding, pregnant and lactating bitches.

COMPLIANCE: Signed and dated Quality Assurance (p. 4-6), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included.

I. MATERIALS:

A. Test material: ML-2,095,988 509T containing 10% w/v fipronil and 9% w/v (S)-methoprene. The test material was a liquid, supplied by the sponsor in amber screw-top vials, ready for use.
Lot/Batch No.: Not specified, but perhaps included in the numeric designation of this test material (ML-2,095,988 509T 003) as specified on p. 15 of MRID 45620507.
Active Ingredients: Fipronil: 10% w/v; (S)-methoprene Permethrin: 9 w/v
Storage Conditions: in a locked cabinet at ambient temperature

B. Placebo: None (From information on p. 15 of MRID 45620507 control animals were not treated but were otherwise handled in the same manner as treated animals).

C. Administration: Individual bitches were dosed every 28 days, starting on day 0 (first treatment date: March 16, 1999). Subsequent pre-mating treatments were administered to bitches every 28 days; each bitch received one to eight doses prior to mating. One day after the first day of mating (a bitch would be mated to a stud on one day, would receive a treatment the following day, then would be remated again to the same stud on next day, and two and four days after that) and subsequent treatments for this dog would follow at 28-day intervals; as an example bitch #14 of Group 2 was treated on June 8, then again 3 days later on June 11 following a mating on June 10, then was treated again 28 days later on July 9, then on August 6, and finally on September 3. Gestation intervals ranged from 60-73 days. Mated bitches which had litters usually received four post-mating treatments (post-mating days 1, 29, 57 and 85, although in two cases in which there was a late delivery and the puppies were less than 42 days old there was an additional treatment on day 113), with no further treatments administered after the puppies were 41-43 days old. Those which had a cesarian section were treated only up

to day 57 (they were removed from the study after the cesarian section).

There were 3 groups: the 12 bitches in Group 1 (controls) were sham-treated; the 12 in Group 2 (1X) had 0.133 mL/kg of the formulation applied directly to the skin at one spot between the base of the skull and the shoulder blades. Each of the 12 females in Group 3 (3X) received a total 0.399 mL/kg, applied as three approximately equal aliquots of 0.133 mL/kg to 3 different spots between the base of the skull and shoulder blades.

D. Test animals

Species: dog *(Canis familiaris);* females only were dosed
Breed: Beagle
Age and weight at study initiation: 2.0-7.8 years (birthdates ranged from 14-May-1991 to 16-March-1997). Each bitch had previously had from 2 to 6 litters; it is stated on p. 16 of MRID 45620507 that each had delivered at least 2 litters of at least 3 pups each with no congenital malformations in any pup. From p. 25 of MRID 45620507 Day 0 body weights (ages in parenthesis) in the control group (Group 1) ranged from 9.7-13.0 kg (3.4-7.8 years old); for the 1X dogs (Group 2) from 8.4-13.4 kg (2.0-7.7 years old); and for the 3X dogs (Group 3) from 9.6-14.7 kg (3.5-7.8 years old). From information on p. 16 of MRID 45620507 the ten males used as studs had each previously sired at least 2 litters of at least 3 pups each with no congenital malformations in any pup; from information on p. 26 they ranged from 10.5-13.8 kg (ages from 2.3-7.8 years).

Source: White Eagle Laboratories, Doylestown, PA. From p. 16 of MRID 45620507: "The dogs were obtained from White Eagle Toxicology Laboratories and were identified by a unique tattoo in the ear. The dogs were acclimated to the research facility for 7 days prior to treatment..."
Housing: Individual, in raised metal cages. "Males were housed in the same room as the females but not in direct contact with them, other than for mating."
Diet: Certified Canine Diet. "Bitches received approximately 300 grams once daily until 10 days prior to their scheduled parturition date. During the remainder of their pregnancy and throughout lactation, bitches were fed approximately 300 grams twice daily. Beginning at approximately 21-28 days of age, puppies were fed wetted food mixed with powdered milk replacer twice daily..."
Water: tap water, *ad libitum*

Environmental conditions: On page 17 of MRID 45620507 it is stated that: "The housing facility was temperature and humidity controlled."

Photoperiod: From p. 17: "Lighting was controlled to give 12 to 14 hours of light and 10 to 12 hours of darkness during a 24 hour period." On p. 23 of MRID 45620507 it is stated (as a deviation from protocol) that (effective 2 June 1999): "...The protocol indicated that lighting would be controlled to give 12 hours of light and 12 hours of darkness during a 24 hour period. However, lighting was controlled to give 14 hours of light and 10 hours of darkness during a 24 hour period... Reason: To increase the frequency of bitches coming into heat."

Acclimation period: 7 days prior to treatment. From p. 14 of MRID 45620507 for Day -7: "Began acclimation and daily observation of animals; observed females for signs of estrus on Mondays, Wednesdays, and Fridays until they were bred." The first treatment (Day 0) was on March 16, 1999.

Medications, therapies and immunizations: There is a listing in Table 24 (p. 63-64) of MRID 45620507 of the concurrent medications for bitches. IM injections of 0.75 mL oxytocin were administered to 3 bitches (one - #3 in the control group - received 2 injections, one on September 9 and the other on September 10, 1999) to aid contractions during delivery; all bitches received a 1 mL injection dose of oxytocin post-whelping "to clear placenta and/or remnants from the uterus." From time to time a number of bitches were orally treated with amoxicillin trihydrate/clavulanate potassium tablets for such conditions as swollen mammary gland, vaginal discharge and mastitis.

II. STUDY DESIGN

A. In life dates

From information on p. 14 of MRID 45620507: Start: 4 March, 1999 (start of acclimation), 16 March, 1999 (first application of test material); "the in-life phase of the study for each bitch ended when her pups were 41 to 43 days old. The experimental termination date was 16 December 1999."

B. Animal assignment/ Dosage and Administration

Only dogs that were proven breeders were used. Each bitch had previously delivered at least 2 litters with at least 3 pups in each, with no congenital malformations. Ten male dogs were selected as studs; each had sired at least 2 litters of at least 3 pups with no congenital malformations in any pup. From p. 16 of MRID 45620507: "...thirty-six bitches were selected for the study. Twelve replicates of 3 bitches were formed based on body weight and parity. The 36 bitches were ranked by parity from highest to lowest number of litters. Bitches with the same number of litters were ranked by number of pups in descending order. Once the bitches were ranked by parity, two equal stratums were formed. The first stratum consisted of the 18 bitches with the highest parity and the second stratum consisted of the 18 bitches with the lowest parity. Within each stratum,

the dogs were ranked based on descending Day -5 body weight. The three heaviest dogs in stratum 1 were assigned to replicate 1, the next three heaviest dogs were assigned to replicate 2, and so on, until six replicates were formed. The same procedure was followed for the second stratum. Within each replicate, bitches were allocated to one of three treatment groups using a computer generated random permutation table. Studs were assigned to breeding order (1 to 10) using a computer generated random permutation table. The first estrus female was presented to the first male in the mating sequence. If mating was observed, the next estrus female was presented to the second male dog in the mating sequence. If the inbreeding coefficient was >0.025, the female was presented to the next available male in the mating sequence. Mating continued in this manner until all females were mated with one male dog. One female (Study Animal Number (SAN) 28) in Group 3 never showed signs of estrus, and after 8 treatments, was dropped from the study."

Each dog was identified by a unique ear tattoo.

Table 1 - animal (bitch) assignment:

Bitch ID	Tattoo	Treatment group	Birth Date	Age (years)	# Previous Litters	Day -5 Body Weight (kg)	Stud Mated To
1	4036	1	5 Aug. 1991	7.6	5	13.0	96AIB1
2	4173	1	31 Oct. 1992	6.4	4	12.1	94AQM2
3	4137	1	27 June 1992	6.7	4	11.0	94AVC1
4	4004	1	12 June 1991	7.8	4	10.7	92AQR3
5	4098	1	28 Feb. 1992	7.0	4	10.2	94AVB4
6	4114	1	14 Apr. 1992	6.9	6	10.1	96AIB1
7	4250	1	7 Oct. 1995	3.4	3	12.5	96AIB1
8	4222	1	31 May 1994	4.8	3	12.4	94AVA3
9	4191	1	2 June 1993	5.8	3	12.2	91BEL2
10	4255	1	4 Nov. 1995	3.4	3	10.8	91AKR7
11	4239	1	8 Nov. 1994	4.4	3	9.9	91BEL2
12	4246	1	16 Sep. 1995	3.5	2	9.7	94AVA3
13	4083	2	21 Nov. 1991	7.3	5	12.6	94AVB4
14	4099	2	28 Feb. 1992	7.0	5	11.9	91AKR7
15	4095	2	24 Jan. 1992	7.1	5	11.2	94AQM2
16	4228	2	16 Aug. 1994	4.6	4	10.7	94AVC1
17	4016	2	29 June 1991	7.7	5	10.3	92AQR3
18	4146	2	24 July 1992	6.6	4	9.8	94AVA3
19	4192	2	3 June 1993	5.8	3	13.4	93ANA2
20	4281	2	16 Mar. 1997	2.0	2	12.3	92BCG1
21	4259	2	27 Dec. 1995	3.2	2	11.6	94AQM2
22	4261	2	27 Dec. 1995	3.2	3	11.5	92AQR3
23	4267	2	11 May 1996	2.8	2	10.4	93ANA2
24	4260	2	27 Dec. 1995	3.2	2	8.4	94AVB4
25	4015	3	27 June 1991	7.7	6	14.7	92BCG1
26	4140	3	17 July 1992	6.7	4	11.7	92BCG1
27	4100	3	1 Mar. 1992	7.0	4	10.7	94AQM2
28	4006	3	14 June 1991	7.8	4	10.6	did not mate
29	4138	3	10 July 1992	6.7	6	10.5	92BCG1
30	4145	3	24 July 1992	6.6	5	10.1	94AVC1
31	4206	3	19 Apr. 1993	5.9	3	13.6	93ANA2
32	4226	3	31 May 1994	4.8	3	12.4	91BEL2
33	4197	3	12 July 1993	5.7	3	11.8	93ANA2
34	4205	3	23 Aug. 1993	5.6	3	10.7	91AKR7
35	4247	3	16 Sep. 1995	3.5	2	10.7	94AVA3
36	4207	3	22 Oct. 1993	5.4	3	9.6	91BEL2

All bitches were initially dosed on March 16, 1999. They were then dosed at 28 day intervals until they came into estrus. They were then treated the day following the first day of mating, and at 28-day intervals thereafter. Even if a bitch was mated a few days following an application, then she was retreated on the day following the first day of mating (example: no. 14 of group 2 was treated on March 16 1999, again on April 13 (28 days after the first treatment), again on May 11 (56 days after the first treatment), and again on June 8 (84 days after first treatment). She subsequently went into estrus, her first day of mating was June 10, and she was treated with the formulation on June 11. Subsequent applications were at 28-day intervals from June 11 (July 9, August 6, September 3). Each bitch received at least one, but no more than 7 treatments prior to mating (bitch #28 received 8 treatments, but did not mate).

The test article was used as supplied. The 1X dose rate was **0.133 mL/kg**, while the 3X dose rate was **0.399 mL/kg**. According to the proposed labels for Frontline Top Spot (EPA Reg No. 65331-3, containing fipronil at 9.7% as sole active ingredient) and Frontline Plus For Dogs (EPA Reg. No. 65331-5, containing fipronil at 9.8% and (S)-methoprene at 8.8% as actives) these products are packaged as 0.67, 1.34, 2.68 and 4.02 mL applications for single treatments of dogs. These application rates are for dogs weighing up to 22 lbs, dogs 23-44 lbs, dogs 45-88 lbs, and dogs 89-132 lbs respectively.

TABLE 2. Experimental design

Group	Number of Females	Dosage	Number of Applications[a]
1	12	0	7-10[b]
2	12	1X	6-11
3	12	3X	5-11

Data taken from information in Table 2 on pp. 27-33 of MRID 45620507.
[a]Each bitch received at least one, but no more than 7 treatments prior to mating (except for #28, which received 8, but did not mate). Treatments were on days 0 and at 28-day intervals thereafter until mating; then there was treatment at 24 hours after first mating and at 28-day intervals thereafter until the puppies were 41-43 days old.
[b]Nothing was really applied to the animals ("sham-treated").

C. Study objective and dose rationale

From p. 12 of MRID 45620507: "The objective of this study was to investigate the safety of a topical formulation containing ML-2,095,988 509T (10% w/v fipronil and 9% w/v (S)-methoprene) in adult female dogs during pregnancy and lactation at one and three times the maximum recommended dosage."

D. Physical Examinations

From p. 18 of MRID 45620507: "Each bitch had a physical examination prior to mating and at the end of the trial when her pups were 41 to 43 days old. Each stud also had a pre-breeding physical examination. The physical examination included evaluations of the following areas or systems:

Mental status (behavior, depression)
Equilibrium/coordination (gait, posture, ataxia)
*Ocular (pupillary light response, nystagmus, menace reflex)
Muscular (general body condition)
Integument
Application sites
Gastrointestinal (palpation; absence of vomiting, diarrhea)
Cardiovascular (heart rate; mucous membranes; absence of arrhythmias, murmurs)
Respiratory (rate, sounds)
Behavior
Appetite
Body weight
Rectal temperature
General health

*For puppies beginning 28 days post-parturition

"Within 24 to 48 hours of parturition, all live pups were given a physical examination, with the exception of heart and respiration rate and rectal temperature. Each pup was given a physical examination 13 to 15, 27 to 29 and 41 to 43 days post-parturition. The sex of each pup was recorded."

E. Clinical Examinations

From p. 18 of MRID 45620507: "On treatment days, each female was observed approximately hourly for 6 hours after treatment and at least once a day throughout the study. Abnormalities or the absence of abnormalities were recorded. Clinical 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, vomition, abnormal feces and abnormal urine."

"Daily clinical observations were conducted on puppies beginning within 24 hours of parturition."

E. Parturition

From p. 19 of MRID 45620507: "Female dogs were monitored for any signs of whelping. While in labor, dogs were observed periodically and abnormal findings were recorded. If a dog failed to deliver a pup in over 1 hour, if signs of dystocia were present, or if the pup was very large, then manual assistance was used to deliver the pup.

E. Reproductive indices

The report includes information as to the number of puppies born in each litter, the number of live puppies born in each litter, the number of stillborn puppies in each litter, puppies with abnormalities, individual puppy body weights at birth and gains to weaning, weaning index (number of puppies weaned/number of pups born alive), as well as clinical observations.

F. Blood chemistry and hematology parameters

Blood was not taken, and these parameters were not measured.

G. Statistics

From p. 20 of MRID 45620507: "The incidence of clinical observations that affected five or more bitches, or five or more litters, was analyzed using Fisher's Exact test. For the bitches and pups, some observations were pooled into broader categories: dermatological observations at the treatment application site, dermatological observations elsewhere, mastitis and mammary gland observations, hungry pup and abnormal appetite, and so forth.

"In all analyses, each treated group was compared to the control group. A two-sided significance level of 0.10 was used. Data for bitches or litters removed from the study were included in statistical analyses up to the point the animals were removed, except as noted above..."

H. Disposition of animals

From p. 20 of MRID 45620507: "All studs, bitches in Groups 1, 2 and 3, and puppies from Group 1 were returned to the White Eagle colony. Puppies 23-5, 16-2 and 21-1 from Group 2, and puppies 25-8, 26-1, 35-7, and 35-8 from Group 3 were adopted. All other live puppies from Groups 2 and 3 were euthanized."

I. Compliance

Signed and dated Quality Assurance (p. 4, with Q.A.U. inspection and report dates on p. 4-6), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included within the report.

III. RESULTS

A. Exposure levels

Individual dosages were based on body weights and set at 0.133 mL/kg for females in Group 2 and 0.399 mL/kg for females in Group 3. Individual doses ranged from 1.12 to 2.35 mL/dog in Group 2 and from 3.63 to 7.70 mL/dog in Group 3. According to information in the administrative materials for EPA Reg. No. 65331-3 (containing 9.7% fipronil as sole active) and EPA Reg. No. 65331-5 (with the two actives fipronil at 9.8% and (S)-methoprene at 8.8%) the dosage rate for both products for dogs weighing 23-44 lbs (10-20 kg) is 1.34 mL/application, so the label use application rate is approximately 0.067 - 0.134 mL/dog for dogs in this size range.

B. Cesarean Sections

One bitch [#1] in the control group and one [#20] in the 1X group had a cesarean section. "Their pups were given a physical exam and then the litters were removed from the study. The bitches were weighed and removed from the study."

C. Mortality and necropsy findings

The following are the number of stillborn puppies per group, as well as those which did not survive to weaning:

Table 3: Number of stillborn puppies per group and number of puppies/group dying between birth and weaning.

Group	# of puppies born[a]	# of puppies stillborn	# of puppies born alive	# of puppies dying between birth and weaning	incidence of litters with all puppies born surviving to weaning
1	62[a]	0	62[a]	4	5/8[a]
2	64[a]	4[b]	60[a]	7	7/10[a]
3	79	1	78	6	8/11

[a]Does not include puppies or litters from bitches (#1, #20) which had cesarean sections. It is noted that for bitch #1 (Group 1) 2 puppies (1-5 & 1-6) out of 9 died shortly after birth; bitch #20 (Group 2) had a total of 10 puppies.
[b]Includes 1 pup (19-11) which had a heart beat at birth but was completely atelectic, did not breathe (from information on p. 60 of MRID 45620507). This is consistent with information on p. 48 which indicates 2 puppies from this litter were stillborn.
Data taken from Table 10, pp. 48-49 of MRID 45620507, with additional information from Table 23, pp. 58-62.

The following table gives a listing of all stillborn puppies:

Table 4: Listing of all stillborn puppies with necropsy findings.

Female (and total # of puppies born)	# of stillborn puppies	Numerical designation of stillborn puppy	Necropsy findings from the puppy
18(6)	1	18-6	0.22 kg. Stillborn, no gross lesions, lungs atelectic, indicating pup was born dead.
19(11)	2	19-9	0.288 kg. Stillborn, lungs completely atelectic, did not breathe. Cause of death not visible on gross examination. 5 cc blood-tinged fluid in abdominal cavity probably post-mortem change.
		19-11	0.197 kg. Found with heart beat present at birth; unable to revive, pup died several minutes after birth. Lungs completely atelectic, did not breathe. Cause of death not evident on gross examination. All tissues appeared grossly normal.
24(10)	1	24-7	0.22 kg. Lungs atelectic, had not inflated. Stillborn, no significant gross lesions.
31(10)	1	31-7	0.27 kg. Lungs dark red, atelectic, had not inhaled. Stillborn, no significant gross changes other than atelectic lungs.

Data taken from Table 10, pp. 48-49, and Table 23, pp. 58-62 of MRID 45620507.

Several puppies (4 from Group 1, 7 from Group 2, and 6 from Group 3) died between birth and weaning. The following are the necropsy results from these puppies:

Table 5: Listing of all puppies dying between birth and weaning with necropsy findings

Bitch (and group)	Numerical designation of puppy which died	# days after birth puppy died	Necropsy findings from the puppy
6(1)	6-6	0	0.20 kg. Interventricular septal defect in the heart (congenital) which resulted in circulatory insufficiency.
10(1)	10-9	10	0.22 kg. Malnourishment from maternal neglect. Findings consistent with not eating and malnutrition. Pup refused feeding by technician.
12(1)	12-6	1	Weight not given: tiny pup, umbilical opening not completely closed. Intestinal and umbilical adhesion had resulted in obstruction of the lumen of the ileum.
12(1)	12-11	3	0.13 kg. Small pup, lungs dark red with moist consistency (edema). Cause of death: acute pulmonary edema.
16(2)	16-1	4	0.270 kg. Pup was lethargic, hand-fed. Bitch had mastitis. Cause of death given as pulmonary edema.
16(2)	16-6	3	0.224 kg. Found dead in cage. Cause of death: pulmonary edema.
16(2)	16-7	1	0.200 kg. Pup lethargic and cool, had been hand fed. Cause of death given as pulmonary edema.
16(2)	16-8	2	0.182 kg. Listless since birth, cool, had been hand fed. Cause of death given as pulmonary edema.
19(2)	19-7	3	0.220 kg. Not nursing, was tube fed, vocalizing, euthanized. Pup was weak and would not nurse, was tube fed; lost righting reflex and was vocalizing. Pup was euthanized; diagnosis: not able to nurse properly.
19(2)	19-8	2	0.190 kg. Pup found dead. It appeared pup had not nursed. The cause of death could have been from dehydration or metabolic imbalance from not eating. A lung change was probable terminal and agonal edema.
24(2)	24-8	0	0.22 kg. Blood stain around the mouth and nose; free blood in the abdominal cavity. Cause of death: abdominal hemorrhage.
25(3)	25-5	7	Had been lethargic, had been hand fed. Death from pulmonary edema.
25(3)	25-9	3	Had been lethargic, had been hand fed. Death from pulmonary edema.
31(3)	31-6	0	0.27 kg. Bitch very rough with pup, which had intestinal & subcutaneous hemorrhage, probably from trauma. Euthanized.
33(3)	33-4	1	0.25 kg. Umbilicus chewed too close; jejunum and ileum missing. Acute peritonitis from patent umbilicus.
33(3)	33-5	2	0.32 kg. Strangulation due to burlap string wrapped around neck.
33(3)	33-8	3	0.20 kg. Peritonitis in umbilical area with diffuse reddening and distention of the small intestines.

Data taken from Tables 10 and 23, pp. 48-49 and pp. 58-62 of MRID 45620507.

C. Clinical signs

Selected clinical observations for the bitches are presented in Table 6. The only findings which correlated with exposure to the test material were dermatological observations

(including pink and/or red skin) at the application site (refer to Table 3, p. 34, Table 26, pp. 71-73, and Table 28, p. 98 of MRID 45620507). It is noteworthy that Table 3 (p. 34) reports 0 dermatological observations at the application site for Group 1; 5 for Group 2 and 11 for Group 3. However, these were apparently the total number of bitches affected per group;

Table 6: Selected Clinical Observations in Bitches

Clinical Observation	Untreated Control	1X	3X
Number of bitches showing "dermatological observation at application site" at one or more times during the study[a]	0	5*	11*
Total number of reported incidences of treatment site skin slightly pink or pink[b]	0	15	42
Total number of incidences of treatment site skin slightly pink or pink on treatment days[c]	0	15	39
Total number of incidences of treatment skin site slightly pink or pink on days other than treatment days[d]	0	0	3
Number of bitches showing vomition during study	10	9	10
Number of bitches showing vaginal discharge during study	6	1	5
Number of bitches showing abnormal feces during study	12	12	12
Number of bitches showing mastitis/swollen mammary during study	3	3	3

*Reported as significantly ($p<0.10$) different from control group by Fisher's Exact Test.
Data taken from Table 3 p. 34, Table 26 pp. 71-73 and Table 28 p. 98 of MRID 45620507.

D. Bodyweights:

From p. 21 of MRID 45620507: "No significant ($p>0.10$) differences in body weights of bitches were found before treatment (Day -1), at 42 days after mating, or withing 3 days of parturition." The following are the reported means:

Table 7: Mean Body Weights for Bitches

Group	Pretreatment (Day -1)(kg)	Day 42 post-mating (kg)	≤3 days post-parturition
1	11.27	13.18*	12.13*
2	11.18	12.77*	12.73*
3	11.46	12.77*	12.46*

*Excludes bitches that did not mate or mated and did not whelp.
Data taken from Table 3, p. 34 of MRID 45620507.

There were no significant differences between groups with respect to pup body weights. The following are the mean body weights of the puppies of the different groups at the weighing times:

Table 8: Mean Body Weights for Puppies

Group	Sex	Day 0-1 mean body weight in kg (S.D.)	Day 14 mean body weight in kg (S.D.)	Day 28 mean body weight in kg (S.D.)	Day 42 mean body weight in kg (S.D.)
1	M	0.278 (0.0397)	0.662 (0.1067)	1.190 (0.2151)	2.036 (0.3345)
2	M	0.277 (0.0430)	0.658 (0.1111)	1.176 (0.1861)	2.1316 (0.3537)
3	M	0.279 (0.0395)	0.693 (0.1496)	1.280 (0.2520)	2.158 (0.3553)
1	F	0.256 (0.0511)	0.660 (0.1361)	1.161 (0.2497)	1.988 (0.3274)
2	F	0.268 (0.0517)	0.640 (0.1103)	1.139 (0.1929)	1.901 (0.2739)
3	F	0.257 (0.0342)	0.644 (0.1239)	1.195 (0.2212)	1.997 (0.3158)

Data calculated by this reviewer from information in Table 8, pp. 41-46 of MRID 45620507.

E. Food consumption

Food consumption was not measured.

F. Clinical chemistry

Clinical chemistry parameters were not measured.

G. Other observational parameters

For the bitches, there were no significant differences between groups with respect to rectal temperatures, respiratory rate, or heart rate at weaning.

Table 9: Other Observational Parameters in Bitches

Group	Mean Rectal Temp. Day -5 (S.D.)	Mean Rectal Temp. at Weaning (S.D.)	Mean Respiratory Rate Day -5 (S.D.)	Mean Respiratory Rate at Weaning (S.D.)	Mean Heart Rate Day -5 (S.D.)	Mean Heart Rate at Weaning (S.D.)
1	102.1(0.91)	101.7(0.45)	45.5(15.87)	62.3(11.97)	121.0(33.93)	123.8(18.68)
2	102.3(0.73)	101.4(0.51)	47.5(11.57)	57.0(16.31)	107.0(19.29)	111.0(19.44)
3	102.3(0.41)	101.8(0.35)	50.5(16.67)	67.1(19.50)	113.5(34.08)	133.6(19.37)

Data calculated by this reviewer from information in Table 5, p. 36 of MRID 45620507.

For the puppies, there were no significant differences between groups with respect to mean rectal temperatures, mean respiratory rates, or mean heart rates (taken on post-parturition days 14, 28, and 42):

Table 10: Mean Rectal Temperatures in Puppies

Group	Mean Rectal Temp. Day 14 (with S.D.)	Mean Rectal Temp. Day 28 (with S.D.)	Mean Rectal Temp. Day 42 (with S.D.)
1	99.07 (0.458)	100.27 (0.492)	101.25 (0.469)
2	99.47 (0.352)	100.53 (0.414)	101.07 (0.441)
3	99.23 (0.383)	100.74 (0.643)	101.13 (0.323)

Data calculated by this reviewer from information in Table 8, pp. 41-46 of MRID 45620507.

Table 11: Mean Respiratory Rates in Puppies

Group	Mean Respiratory Rate Day 14 (with S.D.)	Mean Respiratory Rate Day 28 (with S.D.)	Mean Respiratory Rate Day 42 (with S.D.)
1	119.38 (33.861)	103.03 (26.361)	107.38 (29.665)
2	124.30 (36.504)	115.02 (36.148)	105.28 (23.045)
3	131.08 (36.610)	111.42 (19.883)	107.25 (24.041)

Data calculated by this reviewer from information in Table 8, pp. 41-46 of MRID 45620507.

Table 12: Mean Heart Rates in Puppies

Group	Mean Heart Rate Day 14 (with S.D.)	Mean Heart Rate Day 28 (with S.D.)	Mean Heart Rate Day 42 (with S.D.)
1	190.6 (28.81)	199.9 (19.44)	188.8 (29.29)
2	195.1 (39.90)	203.2 (26.52)	194.1 (21.13)
3	200.8 (33.40)	201.40 (22.06)	200.1 (24.07)

Data calculated by this reviewer from information in Table 8, pp. 41-46 of MRID 45620507.

Although there were somewhat greater proportions of males in Groups 2 and 3 relative to Group 1, the incidences did not appear to be statistically significant.

Table 13: Sex Ratio in Puppies by Group:

Group	Number of Males/Females - All Puppies Including Stillborns (proportion males)	Number of Males/Females Born Alive (proportion males)	Number of Living Males/Females at Weaning (proportion males)
1	37/34 (0.521)	37/34 (0.521)	30/28 (0.517)*
2	40/34 (0.541)	38/32 (0.543)	31/22 (0.585)**
3	46/33 (0.582)	45/33 (0.577)	42/30 (0.583)

*Does not include 9 puppies (6M, 3F) from Bitch 1 which were delivered by cesarean section
**Does not include 10 puppies (3M, 7F) from Bitch 20 which were delivered by cesarean section..
Data calculated by this reviewer from information in Table 8, pp. 41-46 of MRID 45620507.

IV. DISCUSSION:

In a special (reproductive) companion animal safety study (MRID 45620507) ML-2,095,988 509T (a topical formulation containing 10% w/v fipronil and 9% w/v (S)-methoprene) was administered at 0X (Group 1; sham-treated controls), 1X (Group 2) and 3X (Group 3) the recommended [use] dose (0.5 mL/cat) to groups of 12 adult female beagle dogs (bitches) prior to and during pregnancy, through parturition and to weaning of the puppies. All bitches were proven breeders, as each had had at least two previous litters. The bitches were dosed at the start (March 16, 1999) of the study, and at 28-day intervals thereafter until mating, then were treated within 24 hours of their first mating session and every 28 days thereafter until their puppies were 41-43 days old. Even if a bitch was mated a few days following an application treatment, she was treated again on the day following mating (example: Bitch #14 of Group 2 was treated on June 8, then again 3 days later on June 11 following a mating on June 10, then was treated again 28 days later on July 9, then on August 6, and finally on September 3.

Bitches in Group 1 were sham treated. Bitches in Groups 2 and 3 were treated by parting the hair and applying the formulation directly to the skin between the base of the skull and shoulder blades; bitches in Group 2 were dosed (0.133 mL/kg) at one spot; bitches in Group 3 were dosed at 0.399 mL/kg, with 3 applications of a approximately the same volume at 3 spots. The male studs that were used for breeding were not treated with the test material.

Clinical observations were conducted hourly for 6 hr following each treatment; on other days each bitch was observed at least once a day. Daily clinical observations were conducted on puppies beginning within 24 hours of parturition. Bitches were weighed on the day of or the day before each treatment, at 41-43 days post-mating and within 3 days post-parturition. Puppies were weighed individually within 24 hours of parturition, at 13-15, 27-29, and 41-43 days post-parturition.

All bitches except one in the 3X dose group mated during the study; all other bitches were mated once; however, three bitches in the control group and one in the 1X group mated but did not have puppies. At Day 93 after mating they were removed from the study. One bitch in the control group and one in the 1X group had cesarian sections. Their pups received a

physical exam and then were removed from the study, as were the bitches.

The only dose-related effects observed in bitches were pink skin at the application site on days of treatment (recorded 15 times in Group 2, 37 times in Group 3), and pink skin at the application site on the day following treatment (3 times in Group 3). There were no significant differences between the bitches of different groups with respect to mean body weights or for a number of parameters measured at weaning (mean rectal temperatures, mean respiratory rates, or mean heart rates).

There was no indication of an adverse effect on any reproductive parameters (including gestation index, incidence of cesarean section, incidences of stillborn pups, incidences of pups dying between birth and weaning). Although there were somewhat greater percentages of males at birth and subsequently in Groups 2 and 3 relative to Group 1 (At birth: Group 3: 58.2%; Group 2: 54.1%; Group 1: 52.1%) these incidences did not appear to be statistically significant.

There were no significant differences between puppies from the 3 groups with respect to physiological parameters (mean body weights, mean rectal temperatures, mean respiratory rates, mean heart rates) measured on post-parturition days 14, 28 and 42 .

Group 2 bitches weighed from 8.4 to 13.4 kg (18.48 - 29.48 lbs) and those in Group 3 weighed from 9.6 to 14.7 kg (21.12 to 32.34 lbs). From information in the administrative materials for EPA Reg. No. 65331-3 (9.7% fipronil as sole active) and EPA Reg. No. 65331-5 (with the two actives fipronil at 9.8% and (S)-methoprene at 8.8%) the dosage rate for both products for dogs weighing 23-44 lbs (approx. 10-20 kg) is 1.34 mL/application so the label use application rate is approximately 0.067 - 0.134 mL/dog for dogs in this size range. In this study the 12 dogs in Group 2 (1X) had 0.133 mL/kg of the formulation applied directly to the skin at one spot between the base of the skull and the shoulder blades, while each of the 12 females in Group 3 (3X) received a total 0.399 mL/kg,

Although no dogs were dosed at 5X level, and no blood was taken for clinical chemistry and hematology measurements, the purpose of this study was to allow a label claim for use of at least two registered products (EPA Reg. Nos. 65331-3 and 65331-5) on breeding, pregnant and lactating bitches. TRB has previously reviewed a companion animal (dog) study for this formulation which included a 5X dosage group and measurements of clinical chemistry and hematology parameters

This study is classified as acceptable. The findings of this study are adequate to support the use of EPA Reg. Nos. 65331-3 and 65331-5 at the specified label use rates (varying according to the weight of dog) at 28-day intervals on breeding, pregnant and lactating bitches.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D282205
2. **PC CODES:** 129121 Fipronil; 105402 (S)-methoprene
3. **CURRENT DATE:** October 21, 2002
4. **TEST MATERIAL:** ML-2,095,988 509T, a topical formulation containing 10% w/v fipronil and 9% w/v (S)-methoprene, consistent with EPA Reg. No. 65331-5 FRONTLINE PLUS FOR DOGS

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Special/Breeding, Pregnant & Nursing Dog/WEL 99885/JUN-22-2000	45620507	There were 3 Groups, each containing 12 female beagle dogs which were proven breeders (each with at least two previous litters). Group 1 was sham treated; Group 2 was treated at 1X (0.133 mL/kg/application) and Group 3 at 3X (0.399 mL/kg/application). Bitches were dosed at start of the study and then at 28-day intervals, until mating, when they were treated again and every 28 days thereafter until their puppies were weaned. All bitches except one in the 3X group were mated; however, 3 in Group 1 and one in the 1X group did not have puppies. Only dose-related effects in bitches were pink skin at the application site on days of treatment (recorded 15 times for Group 2, 37 times for Group 3) and pink skin at the application site on the day following treatment (3 times in Group 3). There was no indication of an adverse effect on any reproductive parameter (including gestation index, incidence of cesarean section, incidences of stillborn puppies, incidences of puppies dying between birth and weaning). There were slightly greater percentages of males at birth and subsequently in Groups 2 & 3 relative to Group 1 (At birth: Group 3: 58.2%; Group 2: 54.1%; Group 1: 52.1%) but these incidences did not appear to be statistically significant. There were no significant differences between puppies from the 3 groups with respect to physiological parameters (mean body weights, mean rectal temperatures, mean respiratory rates, mean heart rates) on post-parturition days 14, 28 and 42. Findings of this study are adequate to support the use of this formulation at the specified label use rates for EPA Reg. No. 65331-5 at 28-day intervals on breeding, pregnant and nursing bitches.	N/A	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**