US EPA ARCHIVE DOCUMENT

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

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

September 23, 2002

MEMORANDUM

Product Name: FRONTLINE PLUS FOR CATS
EPA Reg. No.: 65331-4
DP Barcode: D282326
Case No: 066318
Submission: S613512
Chemicals: 129121 Fipronil
105402 S-methoprene

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

Byron T. Backus 9/23/2002
9/23/2002

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

Registrant: MERIAL LIMITED

ACTION REQUESTED: "Please review the attached label and data to determine if they are acceptable... This data is also being cited to support label amendments on two other products, and additional beans are attached for their review..."

BACKGROUND: This registered product has the following label ingredient declaration:
Active Ingredients:
Fipronil.....9.8%
(S)-methoprene.....11.8%
Inert Ingredients:.....78.4%

According to the revised labeling which TRB has received the registrant is proposing the following additional use: "Can also be used on breeding, pregnant and lactating queens." To support this additional use, the registrant has submitted a study (in MRID 45618502) titled: "ML-2,095,988 508Q/CAT/SOLUTION/TOPICAL SAFETY/ TOLERANCE/REPRODUCTION, REACTIONS."

COMMENTS AND RECOMMENDATIONS:

1. The study titled: "ML-2,095,988 508Q/CAT/SOLUTION/TOPICAL SAFETY/ TOLERANCE/REPRODUCTION, REACTIONS" (in MRID 45618502) has been reviewed by TRB. It is concluded that the findings of this study are adequate to support a label claim for use of this registered product at the dosage rate of 0.5 mL/cat at 28-day intervals on breeding, pregnant and nursing (lactating) female cats.

2. Refer to the attached DER for a complete review and executive summary .

Reviewer:Byron T. Backus, Ph.D.
Secondary Reviewer:

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Special/Pregnant Cat

EPA I.D. NUMBERS: DP BARCODE: D282326; MRID NUMBER: 45618502

TEST MATERIAL: EPA Reg. No. 65331-4; ML-2,095,988 508Q (10% w/v fipronil and 12% w/v (S)-methoprene)

STUDY NUMBER: WEL 99886, PR&D 0020301

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 508Q/CAT/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 45618502) ML-2,095,988 508Q (a topical formulation containing 10% w/v fipronil and 12% 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 cats (queens) prior to and during pregnancy, through parturition and to weaning of the kittens. There were 3 groups, each containing 12 queens (all proven breeders, as each had had at least two previous successful pregancies). The queens were dosed at the start (March 17, 1999) of the study, and then 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 kittens were weaned (ages 42-44 days). Even if a queen was mated a few days following an application treatment, she was treated again on the day following mating (example: Queen #17 of Group 2 was treated on March 17, then again 28 days later on April 14, again 28 days later on May 12, and then on May 26, one day after mating which happened to be 14 days after the previous treatment, followed by treatments on June 23, July 21, and August 18).***

Queens in Group 1 were sham treated. Queens 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; queens in Group 2 were dosed (0.5 mL) at one spot; queens in Group 3 received three 0.5 mL treatments at each of three sites. Based on the body weights, initial doses ranged from 0.0096 to 0.022 mL/kg for queens in Group 2 and from 0.32 to 0.54 mL/kg in Group 3. The toms 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 cat was observed at least once a day. Daily clinical observations were conducted on kittens beginning within 24 hours of parturition. Queens were weighed before each treatment, at 42 days post-mating and within 3 days post-parturition. Kittens were weighed individually within 24-48 hours of parturition, at 14-15, 26-28, and 42-44 days post-parturition.

All queens became pregnant, although some required more than one mating. Those queens which were rebred were exposed to more applications of the test material. Individual queens in Groups 2 and 3 received from one to seven exposures to the test material before a mating resulting in pregnancy. Two of the 12 queens in Group 1 required two breeding sessions, and one required three. Each of the 12 queens in Group 2 became pregnant as a result of one breeding session, while four of the 12 queens in Group 3 required two breeding sessions.

The only dose-related effects observed in queens were flaky skin at the application site (recorded 2 times in Group 1, 7 times in Group 2, and 10 times in Group 3), and slight to moderate salivation lasting from 1 to 5 minutes following dosage of the test material (not observed in Groups 1 or 2, observed 4 times in Group 3). There were no significant differences between queens from the different groups for mean body weights, or for a number of parameters measured on post-parturition day 42 (mean rectal temperatures, mean respiratory rates, or mean heart rates).

With respect to reproductive parameters, there was an increased incidence of stillborn kittens in Group 3 relative to the other two groups: Group 1: 2/40; Group 2: 2/36; and Group 3: 8 or 9/42 (one kitten in Group 3 from Queen #32 was found cannibalized from the waist up, and it was not possible to determine whether its lungs had ever expanded, and whether or not it had been stillborn). However, this increase was due in part to Queen #34, which had a gestation period of 72 days, compared to 63-69 days for the other 35 queens. Queen #34 delivered two stillborn kittens on day 72, then required a C-section to deliver two additional stillborn kittens. Queen #26 delivered two small stillborn kittens, at least one of which had been dead in utero, in addition to two normal kittens. These were sporadic findings which, in each case, were limited to one queen, and it is concluded then that there is no evidence that the increased incidence of stillborn kittens in Group 3 was a result of exposure to the test material. Also, it is noted that there was no indication of any post-natal adverse effect in Group 3 kittens (one Group 1 kitten died in the period between birth and weaning, as did two

Group 2 kittens, but all Group 3 kittens survived to weaning); and there were no significant differences between kittens 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 . While the sex ratio in Group 3 was somewhat skewed (27/42, or 0.643 of the kittens born were males), this was also incidental as the chances of having a ≥0.643 ratio of one sex out of 42 births, assuming an equal likelihood of male and female births, is approximately 8%, and there is no indication within the report of any kittens of ambiguous gender.

Although no queens 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 this registered product (EPA Reg. No. 65331-4) on breeding, pregnant and lactating female cats. TRB has previously reviewed (MRID 44942009; date of review: 12 April 2000) a companion animal (cat) study for this formulation which included a 5X dosage group and measurements of clinical chemistry and hematology parameters. The TRB review of 12 April 2000 was reviewed by the HED Companion Animal Safety Committee which noted, among other things that there was no evidence of toxicity in any of the animals and that "as fipronil is registered at this concentration in other products, there are CAS studies with no evidence of toxicity at 5X. In addition, methoprene is used with many other chemicals in flea and tick products, and, in so far as the Committee is aware, there is no evidence that it interacts with these other chemicals, although the proposed 12% concentration in this product may be somewhat higher than that of most of the other products listed in REFS."

This study is classified as acceptable. The findings of this study are adequate to support the use of this registered product at the dosage rate of 0.5 mL/cat at 28-day Intervals on breeding, pregnant and lactating female cats.

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 508Q containing 10% w/v fipronil and 12% w/v (S)-methoprene. The test material was a liquid, supplied by the sponsor in amber screw-top vials.
Description: The test material was a liquid, supplied by the sponsor in amber screw-top vials.
Lot/Batch No.: Not specified, but perhaps included in the numeric designation of this test material.
Active Ingredients: Fipronil: 10% w/v; (S)-methoprene Permethrin: 12% w/v
Storage Conditions: in a locked cabinet at ambient temperature

B. Placebo: None (From information on p. 15 of MRID 45618502 control animals were not treated but were otherwise handled in the same manner as treated animals).

C. Administration:
Individual queens were dosed every 28 days, starting approximately 24 hours after the initial placement of the queen into the tom's cage (i.e., 1, 29, 57, 85 days post-first mating day). The day 1 treatment occurred during an interval in which the breeding pair were separated; the queen was returned to the tom's cage once all post-treatment observations were completed. There were 3 groups: the 12 queens in Group 1 (controls) were sham-treated; the 12 queens in Group 2 (1X) had 0.5 mL 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 queens in Group 3 (3X) received a total of three 0.5 mL aliquots of the formulation; each of these aliquots was applied to a different spot (3 spots in all) between the base of the skull and shoulder blades.

D. Test animals

Species: Cat *(Felis domestica); females only were dosed*
Breed: short-hair
Age and weight at study initiation: 1.9-5.9 years (birthdates ranged from 5-May-1993 to 14-Apr-1997). Each queen had previously had from 2 to 8 litters; each had previously delivered at least 2 litters of at least 3 kittens each with no congenital malformations in any kitten. Day 0 body weights (ages in parenthesis) in the control group (Treatment Group 1) ranged from 2.6 to 4.2 kg (2.8 to 5.9 years); in the 1X group (Treatment Group 2) ranged from 2.3 to 5.2 kg (2.8 to 5.8 years); in the 3X group (Treatment Group 3) ranged from 2.8 to 4.7 kg (1.9 to 5.9 years).

Source: White Eagle Laboratories, Doylestown, PA. From p. 17 of MRID 45618502: "The cats were obtained from the White Eagle Laboratory colony and were identified by a unique ear tattoo. Liberty, Hillgrove, Buckshire and White Eagle genetic sources were used."
Housing: Individual, in raised metal cages, except for mating, when the queen was placed in the tom's cage.
Diet: Purina® Laboratory Feline Diet #5003. Queens received 150g of food once a day until 10 days prior to their scheduled queening date. During the remainder of the pregnancy and throughout lactation, queens were fed 150g twice daily. Kittens were given milk replacer as needed. Beginning at approximately 21-28 days of age, kittens were fed wetted food mixed with powdered milk replacer twice daily.
Water: tap water, *ad libitum*
Environmental conditions: On page 18 of MRID 45618502 it is stated that: "The housing facility was temperature and humidity controlled." However, the parameters are not reported.

Photoperiod: 12-13 hrs of light and 11-12 hrs darkness during a 24-hr period

Acclimation period: 7 days prior to treatment. From p. 14 of MRID 45618502 for Day -7: "Began acclimation and daily observation of animals; observed females for signs of estrus." The first treatment (Day 0) was on March 17, 1999.

Medications, therapies and immunizations: There is a listing in Table 23 (p. 58) of MRID 45618502 of the concurrent medications for queens. IM injections of 0.5 mL oxytocin were administered to 10 queens (3 in the control group, 2 in the 1X group, and 5 in the 3X group) "to aid parturition." One female (#25) in treatment group 3 received 10 mL mineral oil on two separate occasions (April 20, 1999 and April 29, 1999) for "absence of feces." Female #26 of treatment group 3 received two tablets/day of Clavamox in the period from March 19, 1999 to March 25, 1999, and again from May 12, 1999 to May 25, 1999. The same female was treated (for dehydration) with lactated ringers injection USP on May 10, 1999 and again on May 14, 1999, and with Viceton Chloramphenicol tablets (for red discharge present at vulva) in the period from May 5, 1999 through May 12, 1999. From information on p. 58 this animal was treated with oxytocin on August 30, 1999, but according to information on p. 55-56 the birth dates of two of the kittens were August 29, 1999.

II. STUDY DESIGN

A. <u>In life dates</u>

From information on p. 14 of MRID 45618502: Start: 5 March, 1999 (start of acclimation), 17 March, 1999 (first application of test material); experimental termination date: 27 December 1999.

B. Animal assignment/ Dosage and Administration

Only cats that were proven breeders were used. Each queen had previously delivered at least 2 litters with at least 3 kittens in each, with no congenital malformations. A total of 24 toms were also selected; each had sired at least 2 litters of at least 3 kittens with no congenital malformations in any kitten. According to the summary on p. 12 of MRID 45618502 "Twelve replicates of 3 similar queens were formed based on body weight, parity and genetic source. Within replicates, queens were randomly allocated to one of three treatment groups. Each replicate of three female cats was randomly assigned to two male cats, from a different genetic source,to form a breeding unit." From p. 16 of MRID 45618502: "A total of 40 queens were available for this study. Twelve replicates of 3 similar queens were formed based on body weight, parity and genetic source. Prior to the first treatment, thirty-six queens were selected for the study and grouped together by genetic source. The first group consisted of 18 queens from the Liberty genetic source. The second group consisted of 12 queens from the Hillgrove genetic source. The third group consisted of a mixture of 6 queens from the Buckshire, Liberty and White Eagle genetic sources. The three groups were ranked by parity from the highest to lowest number of litters. Queens with the same number of litters were ranked by the number of kittens in descending order."

"Males were grouped separately by genetic source and ranked by descending body weight. Each replicate of 3 female cats was randomly assigned 2 male cats, from a different genetic source, to form a breeding unit... If an incompatibility occurred between the primary male and a female or if the primary male was being used for mating with another female, then the secondary male in the breeding unit was used. If both of the toms in the breeding unit were in use or if a compatibility problem occurred, then a tom from another breeding unit was used. Mating continued in this manner until all queens were bred. Queens that returned to estrus were rebred."

Each cat was identified by a unique ear tattoo.

Table 1 - animal (queen) assignment:

Queen ID	Tattoo	Treatment group	Birth Date	Age (years)	# Previous Litters	# of breedings in this study	Last Tom Mated To
1	95AQL3	1	04/29/95	3.9	5	1	153
2	95APH4	1	04/20/95	3.9	6	1	932590
3	95APE4	1	04/19/95	3.9	5	1	942748
4	96QPC3	1	05/18/96	2.8	3	1	79C
5	96QNH5	1	05/07/96	2.9	2	2	997
6	96QPI5	1	05/20/96	2.8	3	2	932320
7	Z26	1	09/28/95	3.5	4	1	95AOE2
8	73I	1	08/23/95	3.6	4	1	95APF4
9	77N	1	01/21/96	3.2	2	1	95AUX3
10	125	1	02/04/96	3.1	2	1	95AMY1
11	932268	1	05/05/93	5.9	4	3	95ATS2
12	96QBQ3	1	01/12/96	3.2	2	1	74A
13	95APY4	2	04/25/95	3.9	4	1	153
14	95MEK4	2	03/03/95	4.0	4	1	932419
15	95APH2	2	04/20/95	3.9	8	1	942753
16	94BEF4	2	09/15/94	4.5	2	1	79C
17	96QPT3	2	05/30/96	2.8	3	1	X78
18	96QPH5	2	05/20/96	2.8	2	1	942958
19	Y96	2	09/05/95	3.5	2	1	95ATS2
20	O1D	2	08/29/95	3.6	2	1	95APF4
21	78A	2	01/31/96	3.1	2	1	95AUX3
22	155	2	02/25/96	3.1	2	1	95AL01
23	942937	2	04/19/94	4.9	4	1	95AMY1
24	932420	2	05/25/93	5.8	4	1	95AOE2
25	95APE2	3	04/19/95	3.9	5	2	Y09
26	95AOQ3	3	04/30/95	3.9	4	2	932419
27	95AJE3	3	03/07/95	4.0	5	1	942748
28	96QNJ4	3	05/08/96	2.9	3	2	79C
29	96QPX5	3	06/03/96	2.8	3	1	997
30	96QNG5	3	05/07/96	2.9	2	1	932320
31	Y49	3	08/05/95	3.6	2	1	95ATS2
32	011	3	09/01/95	3.5	3	1	95APF4
33	Z02	3	09/05/95	3.5	2	1	95MIG1
34	00L	3	08/14/95	3.6	2	2	95AMY1
35	932396	3	05/10/93	5.9	6	1	95AVE2
36	Q3	3	04/14/97	1.9	2	1	00Y

All queens were initially dosed on March 17, 1999. They were then dosed at 28 day intervals until they came into estrus. They were then treated the day following mating and at 28-day intervals thereafter. Even if a queen was mated a few days following an application, then it was retreated on the day following mating (example: queen no. 17 of group 2 was treated on March 17 1999, again on April 14 (28 days after the first treatment), again on May 12 (56 days after the first treatment), and then on May 26 (1 day after mating or 14 days after the previous treatment), followed by treatments on June 23, July 21 and August 18 (29, 57 and 85 days after mating). Each queen received at least one, but no more than five treatments prior to mating.

The test article was used as supplied. According to the proposed label received by TRB the product is packaged as 0.50 mL aliquots for single treatments. The 1X intended labeled use is: **0.5 mL**/cat; the 3X is **1.5 mL**/cat.

TABLE 2. Experimental design

Group	Number of Females	Dosage	Number of Applications[a]
1	12	0	0
2	12	1X	5-8
3	12	3X	5-11

Data taken from information on pp. 28-33 of MRID45618502.
[a]Each queen received at least one, but no more than 5 treatments prior to mating. Treatments were on days 0 and at 28-day intervals thereafter until mating; treatments was then within 24 hours of mating and at 28-day intervals thereafter until the kittens were weaned or until the female returned to estrus.

C. Study objective and dose rationale

From p. 12 of MRID 45618502: "The objective of this study was to investigate the safety of a topical formulation containing ML-2,095,988 508Q (10% w/v fipronil and 12% w/v (S)-methoprene) in adult female cats during pregnancy at one and three times the maximum recommended dosage."

D. Physical Examinations

From p. 18 of MRID 45618502: "Each queen had [a] physical examination prior to mating and at the end of the study when her kittens were 42 to 44 days old. Each tom 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 kittens beginning 28 days post-parturition

"Within 24 to 48 hours of parturition, all live kittens were given a physical examination, with the exception of heart and respiration rate and rectal temperature. Each kitten was also given a physical examination which included heart and respiratory rate and rectal temperature 14 to 15, 26 to 28 and 42 to 44 days post-parturition. The sex of each kitten was recorded."

E. Clinical Examinations

From p. 19 of MRID 45618502: "On treatment days, each queen was observed approximately hourly for 6 hours after treatment and at least once daily 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 kittens beginning within 24 hours of parturition."

E. Parturition

From p. 20 of MRID 45618502: "Queens were monitored for any signs of parturition. During parturition, each queen was observed periodically and abnormal findings were recorded. If necessary, oxytocin was administered to aid parturition. In some cases, abdominal palpation was used to determine if parturition was complete."

E. Reproductive indices

The report includes information as to the number of kittens born in each litter, the number of live kittens born in each litter, the number of stillborn kittens in each litter, kittens with abnormalities, individual kitten body weights at birth and gains to weaning, weaning index (number of live kittens born surviving to weaning), as well as clinical observations (primarily raw &/or red skin and dry scabs).

F. Blood chemistry and hematology parameters

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

G. Statistics

From p. 20 of MRID 45618502: "The incidence of clinical observations that affected five or more queens, or five or more litters, was analyzed using Fisher's Exact test. For the queens and kittens, some observations were pooled into broader categories: dermatological observations at the treatment application site, dermatological observations elsewhere, and ocular observations.

"In all analyses, each treated group was compared to the control group. A two-sided significance level of 0.10 was used. Data for queens 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. 21 of MRID 45618502: "All toms, all queens in Group 1, queens 23 and 24 (Group 2) and queens 33, 34 and 35 (Group 3) were returned to the White Eagle colony. Kittens 33-1 and 33-2 were donated. All other queens and kittens were euthanized."

I. Compliance

Signed and dated Quality Assurance (p. 4, with Q.A.U. inspection and report dates on p. 5-6), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements are included within the report.

III. RESULTS

A. Exposure levels

On Day 0 the queens in group 2 weighed from 2.3 to 5.2 kg, while the queens in group 3 weighed from 2.8 to 4.7 kg. Therefore, the initial dosage on a body weight basis for the queens in group 2 ranged from 0.0096 to 0.022 mL/kg and for the queens in group 3 from 0.32 to 0.54 mL/kg.

B. Mortality and necropsy findings

One queen (#34) in group 3 required a cesarean section; according to information on p. 20 of MRID 45618502, and "was immediately removed from the study." From information on p. 21 queen #34 was among those returned to the White Eagle colony, so presumably this cat was not sacrificed following the cesarean section, although it is noted that the statement is made on p. 33 for queen #34 that: "animal terminated on 5 Aug. 99 following C-section."

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

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

Group	# of kittens born	# of kittens definitely stillborn	# of kittens possibly stillborn	# of kittens definitely born alive	# of kittens dying between birth and weaning	# litters with all kittens born surviving to weaning
1	40	2	0	38	1	9/12
2	36	2	0	34	2	9/12
3	42	8	1[a]	33	0	6/12

Data taken from Table 10, pp. 45-46 of MRID 45618502.

[a]This kitten (from female 32) was cannibalized from the waist up, and it could not be determined whether it was born alive or stillborn.

The following table gives a listing of all stillborn kittens, including the 8 (possibly 9) in Group 3:

Table 4: Listing of all stillborn (and possibly stillborn) kittens with necropsy findings.

Female (and total # of kittens born)	# of kittens definitely stillborn	# of kittens possibly stillborn	Numerical designation of stillborn kitten	Necropsy findings from the kitten
4(6)	1	0	4-6	Male, 0.08 kg, still in sac. Lungs unexpanded.
11(1)	1	0	11-1	Female, 0.09 kg. Lungs not expanded.
15(2)	1	0	15-2	Female, 0.11kg. Chewed at both hind legs and tail. Lungs: dark, heavy and moist - not inflated.
23(5)	1	0	23-5	0.05 kg. Eaten from the waist down Lungs did not appear to have ever been inflated.
25(5)	1	0	25-5	Female, 0.07 kg. Found dead at birth. Lungs had not inflated. Kitten had not breathed, no gross lesions.
26(4)	2	0	26-1	Male, extremely small (0.022 kg), pale, obviously had been dead in utero and not completely developed; rear leg deformity (rear legs shorter than normal and rotated medially).
-	-	-	26-4	Female. Fetus was very small (0.029 kg) and immature. Abdominal cavity opened at umbilicus with partial cannibalization.
29(4)	1	0	29-1	Male, 0.08 kg. Found dead at birth. Lungs were unexpanded.
32(1)	0	1	32-1	Male, found dead, eaten from the waist up, remains weighed 0.05 kg. Not able to determine if kitten was stillborn.
34(4)	4	0	34-1	Male, 0.12 kg. Obtained from mother after C-section, dead
-	-	-	34-2	Male, 0.11 kg. Obtained from mother after C-section, dead
-	-	-	34-3	Female, 0.1 kg. Stillborn (found dead)
-	-	-	34-4	Female, 0.11 kg. Stillborn (found dead)

Data taken from Table 10, pp. 45-46 of MRID 45618502.

One reason for the high incidence of stillborn births in Group 3 involved Queen #34, which had a cesarean section and was removed from the study on August 5, 1999. According to Table 9 (p. 44) the queening date for this cat was August 5, and this date was the 72nd day of pregnancy (From information in Table 9 the other 35 queens had gestation periods of from 63 to 69 days; the other queens in Group 3 also had gestation periods ranging from 63-69 days). According to the report summary (p. 12 of MRID 45618502): "There was an increase in the number of stillborn kittens in the 3X group due primarily to a queen that was overdue and had a cesarean section with four stillborn kittens." This is consistent with information on page 46 that all four of the kittens from queen #34 were stillborn. On p. 81 of MRID 45618502 kittens 1 & 2 are listed as being stillborn and as being "obtained from mother after C-section, dead." whereas kittens 3 & 4 are simply listed as being stillborn; on p. 57 it is indicated that kittens 3 & 4 were "found dead in cage" and "found dead at birth" which indicates

they were actually born.

Female 26 in Group 3 had two extremely small kittens (0.022 and 0.029 kg) which were stillborn. From Table 22 (p. 55) one of the kittens "was small and pale and obviously had been dead in utero and not completely developed." From Table 22 (p. 56) the other stillborn kitten for 26 was "very small and immature. All tissues present were small and immature." However, from information in Table 7 (p. 41) the two kittens from this female which survived, 26-2 and 26-3 (both female) weighed 0.13 and 0.12 kg respectively on day 0-1. From information in Table 22 (p. 57) the stillborn kittens of Queen #34 each weighed from 0.1 to 0.12 kg, so they were of normal size.

Of the 8 or 9 stillborn kittens in Group 3, four were normal size kittens from one queen which had a late (day 72) delivery and two were immature (one dead in utero) kittens from another female. Occurrences of the other stillborn kittens were sporadic (one in a litter in which the other 4 kittens survived to maturity, one in a litter in which the other 3 kittens survived to maturity, and one in a litter in which it was the only kitten and it may or may not have been stillborn). Although there were more stillborn kittens in Group 3 than in either of the other 2 groups, these findings do not suggest a common causative mechanism. Additionally, all kittens in Group 3 that were alive on day 1 following birth survived to weaning.

Several kittens (possibly none from group 3, although the findings from 32-1 are ambiguous) died between birth and weaning. The following are those that definitely died between birth and weaning:

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

Female (and group)	Numerical designation of kitten which died	# days after birth kitten died	Necropsy findings from the kitten
6(1)	6-1	5	Female, 0.16 kg. Cause of death: diffuse peritonitis; unilateral renal agenesis (right kidney missing)
15(2)	15-1	18	Female, 0.17 kg. Last two days kitten was lethargic, cool; tried warming and hand feeding. Cause of death unknown.
16(2)	16-3	0	Female, 0.11 kg. Found dead (newborn). Queen licked intestines (small and large) through umbilicus and ate them (cord). Lungs inflated.

Data taken from Tables 7 and 22, pp. 39-41 and pp. 53-57 of MRID 45618502.

C. Clinical signs

Selected clinical observations for the queens are presented in Table 6. The only findings which correlated with exposure to the test material were flaky skin at the application site and slight to moderate salivation lasting 1-5 minutes following dosage of test material (observed in Group 3 queens only; refer to

Table 2, pp. 28-33 of MRID 45618502).

Table 6: Selected Clinical Observations in Queens

Clinical Observation	Untreated Control	1X	3X
Dermatological observation at application site (includes dry scab, hair loss, pink or red skin, red areas, scars, scratches, shedding)	2	2	4
Flaky skin at application site	2	7*	10*
Dermatological observation not at application site (includes dry scabs, flaky skin, hair loss, pink or red skin, red areas, scars, scratches, shedding, sores, swollen lips and thin hair).	8	5	7
Slight to moderate salivation lasting 1-5 minutes following dosage of test material	0	0	4
Vomition	4	5	6
Vaginal discharge	3	1	4
Abnormal feces	5	1	4
Ocular observations (includes glassy eye, watery eye, and crusty eye)	1	0	2

*Reported as significantly ($p<0.10$) different from control group by Fisher's Exact Test.
Data taken from Table 3 p. 34 and Table 2 p. 33 of MRID 45618502.

D. Bodyweights:

From p. 22 of MRID 45618502: "There were no significant ($p>0.10$) differences among treatment groups in body weights of queens before treatment (Day -5), at 42 days after the final mating, or within 3 days of parturition..." The following are the reported means:

Table 7: Mean Body Weights for Queens

Group	Pretreatment (kg)	Day 42 post-mating (kg)	≤3 days post-parturition
1	3.35	4.47	4.53
2	3.56	4.64	4.73
3	3.53	4.61	4.53*

*Queen 34 had a cesarean section; data from this cat not used in the calculation of this number.
Data taken from Table 3, p. 34 of MRID 45618502.

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

Table 8: Mean Body Weights for Kittens

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	0.113 (0.0126)	0.290 (0.0185)	0.441 (0.045)	0.667 (0.099)
2	M	0.126 (0.0199)	0.318 (0.031)	0.495 (0.050)	0.709 (0.087)
3	M	0.121 (0.0132)	0.302 (0.031)	0.449 (0.064)	0.720 (0.109)
1	F	0.110 (0.0146)	0.279 (0.026)	0.420 (0.045)	0.642 (0.073)
2	F	0.109 (0.0127)	0.281 (0.036)	0.429 (0.056)	0.618 (0.070)
3	F	0.114 (0.0225)	0.289 (0.031)	0.435 (0.050)	0.648 (0.050)

Data calculated by this reviewer from information in Table 7, pp. 39-41 of MRID 45618502.

E. Food consumption

Food consumption was not measured.

F. Clinical chemistry

Clinical chemistry parameters were not measured.

G. Other observational parameters

For the queens, there were no significant differences between groups with respect to rectal temperatures, respiratory rate, or heart rate on day 42.

Table 9: Other Observational Parameters in Queens

Group	Mean Rectal Temp. Day -5(S.D.)	Mean Rectal Temp. Day 42 (S.D.)	Mean Respiratory Rate Day -5 (S.D.)	Mean Respiratory Rate Day 42 (S.D.)	Mean Heart Rate Day -5 (S.D.)	Mean Heart Rate Day 42 (S.D.)
1	100.7(0.63)	101.6(0.90)	61.5(10.89)	66.5(15.23)	225.5(29.56)	264.5(33.89)
3	101.0(0.83)	101.4(0.77)	63.0(15.46)	57.5(13.41)	232.5(26.11)	261.0(30.97)
3	100.9(0.86)	101.2(0.82)	59.0(11.68)	61.6(13.44)	220.0(26.87)	243.5(16.37)

Data calculated by this reviewer from information in Table 5, p. 36 of MRID 45618502.

For the kittens, 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 Kittens

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	99.52 (0.594)	100.51 (0.438)	101.45 (0.610)
2	99.89 (0.584)	100.53 (0.434)	101.57 (0.554)
3	99.58 (0.403)	100.54 (0.539)	101.61 (0.531)

Data calculated by this reviewer from information in Table 7, pp. 39-41 of MRID 45618502.

Table 11: Mean Respiratory Rates in Kittens

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	105.2 (22.33)	99.9 (19.04)	99.2 (12.62)
2	100.9 (18.21)	100.9 (17.27)	96.8 (17.95)
3	95.3 (27.28)	100.3 (19.55)	98.7 (16.91)

Data calculated by this reviewer from information in Table 7, pp. 39-41 of MRID 45618502.

Table 12: Mean Heart Rates in Kittens

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	281.8 (44.96)	285.4 (31.32)	293.2 (32.96)
2	303.8 (43.70)	280.1 (45.22)	282.6 (33.05)
3	266.8 (59.16)*	278.1 (42.65)**	293.9 (38.95)

*Without the anomalous value (as reported: 66) from kitten 29-4.
**Without the anomalous values (as reported: 108 and 96) from kittens 28-1 and 28-2.
Data calculated by this reviewer from information in Table 7, pp. 39-41 of MRID 45618502.

There was a noticeably greater proportion of males in Group 3 relative to the other two Groups (64.3% for all kittens, including stillborns, vs. 42.9% in Group 2 and 52.5% in Group 1). However, this appears to be incidental (the chances of having a $\geq$0.643 ratio of one sex out of 42 births, assuming an equal likelihood of male and female births, is approximately 8%).

Table 13: Sex Ratio in Kittens by Group:

Group	Number of Males/Females - All Kittens Including Stillborns (proportion males)	Number of Males/Females Born Alive (proportion males)	Number of Living Males/Females at Weaning (proportion males)
1	21/19 (0.525)	20/18 (0.526)	20/17 (0.541)
2	15/20* (0.429)	15/19 (0.441)	15/17 (0.469)
3	27/15** (0.643)	22/11** (0.667)	22/11 (0.667)

*One additional kitten (23-5) was eaten from the waist down and its sex was not determined.
**Kitten 32-1 classified as stillborn (male, found dead, eaten from the waist up. Not able to determine if kitten was stillborn).
Data calculated by this reviewer from information in Tables 7 and 10, pp. 39-41 and 45-46 of MRID 45618502.

IV. DISCUSSION:

In a special (reproductive) companion animal safety study (MRID 45618502) ML-2,095,988 508Q (a topical formulation containing 10% w/v fipronil and 12% 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 cats (queens) prior to and during pregnancy, through parturition and to weaning of the kittens. There were 3 groups, each containing

12 queens (all proven breeders, as each had had at least two previous successful pregancies). The queens were dosed at the start (March 17, 1999) of the study, and then 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 kittens were weaned (ages 42-44 days). Even if a queen was mated a few days following an application treatment, she was treated again on the day following mating (example: Queen #17 of Group 2 was treated on March 17, then again 28 days later on April 14, again 28 days later on May 12, and then on May 26, one day after mating which happened to be 14 days after the previous treatment, followed by treatments on June 23, July 21, and August 18).

Queens in Group 1 were sham treated. Queens 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; queens in Group 2 were dosed (0.5 mL) at one spot; queens in Group 3 received three 0.5 mL treatments at each of three sites. Based on the body weights, initial doses ranged from 0.0096 to 0.022 mL/kg for queens in Group 2 and from 0.32 to 0.54 mL/kg in Group 3. The toms 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 cat was observed at least once a day. Daily clinical observations were conducted on kittens beginning within 24 hours of parturition. Queens were weighed before each treatment, at 42 days post-mating and within 3 days post-parturition. Kittens were weighed individually within 24-48 hours of parturition, at 14-15, 26-28, and 42-44 days post-parturition.

All queens became pregnant, although some required more than one mating. Those queens which were rebred were exposed to more applications of the test material. Individual queens in Groups 2 and 3 received from one to seven exposures to the test material before a mating resulting in pregnancy. Two of the 12 queens in Group 1 required two breeding sessions, and one required three. Each of the 12 queens in Group 2 became pregnant as a result of one breeding session, while four of the 12 queens in Group 3 required two breeding sessions.

The only dose-related effects observed in queens were flaky skin at the application site (recorded 2 times in Group 1, 7 times in Group 2, and 10 times in Group 3), and slight to moderate salivation lasting from 1 to 5 minutes following dosage of the test material (not observed in Groups 1 or 2, observed 4 times in Group 3). There were no significant differences between queens from the different groups for mean body weights, or for a number of parameters measured on post-parturition day 42 (mean rectal temperatures, mean respiratory rates, or mean heart rates).

With respect to reproductive parameters, there was an increased incidence of stillborn kittens in Group 3 relative to the other two groups: Group 1: 2/40; Group 2: 2/36; and Group 3: 8 or 9/42 (one kitten in Group 3 from Queen #32 was

found cannibalized from the waist up, and it was not possible to determine whether its lungs had ever expanded, and so whether or not it had been stillborn). However, this increase was due in part to Queen #34, which had a gestation period of 72 days, compared to 63-69 days for the other 35 queens. Queen #34 delivered two stillborn kittens on day 72, then required a C-section to deliver two additional stillborn kittens. Queen #26 delivered two small stillborn kittens, at least one of which had been dead in utero, in addition to two normal kittens. These were sporadic findings which, in each case, were limited to one queen, and it is concluded then that there is no evidence that the increased incidence of stillborn kittens in Group 3 was a result of exposure to the test material. Also, it is noted that there was no indication of any post-natal adverse effect in Group 3 kittens (one Group 1 kitten died in the period between birth and weaning, as did two Group 2 kittens, but all Group 3 kittens survived to weaning); and there were no significant differences between kittens 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 . While the sex ratio in Group 3 was somewhat skewed (27/42, or 0.643 of the kittens born were males), this was also incidental as the chances of having a $\geq$0.643 ratio of one sex out of 42 births, assuming an equal likelihood of male and female births, is approximately 8%, and there is no indication within the report of any kittens of ambiguous gender.

Although no queens 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 this registered product (EPA Reg. No. 65331-4) on breeding, pregnant and lactating female cats. TRB has previously reviewed (MRID 44942009; date of review: 12 April 2000) a companion animal (cat) study for this formulation which included a 5X dosage group and measurements of clinical chemistry and hematology parameters The TRB review of 12 April 2000 was reviewed by the HED Companion Animal Safety Committee which noted, among other things that there was no evidence of toxicity in any of the animals and that "as fipronil is registered at this concentration in other products, there are CAS studies with no evidence of toxicity at 5X. In addition, methoprene is used with many other chemicals in flea and tick products, and, in so far as the Committee is aware, there is no evidence that it interacts with these other chemicals, although the proposed 12% concentration in this product may be somewhat higher than that of most of the other products listed in REFS."

This study is classified as acceptable. The findings of this study are adequate to support the use of this registered product at the dosage rate of 0.5 mL/cat at 28-day intervals on breeding, pregnant and lactating female cats.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D282326
2. **PC CODES:** 129121 Fipronil; 105402 (S)-methoprene
3. **CURRENT DATE:** September 23, 2002
4. **TEST MATERIAL:** ML-2,095,988 508Q, a topical formulation containing 10% w/v fipronil and 12% w/v (S)-methoprene, consistent with EPA Reg. No. 65331-4 FRONTLINE PLUS FOR CATS

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Special/Breeding, Pregnant & Nursing Cat/WEL 99886/JUN-22-2000	45618502	3 Groups, each containing 12 female cats which were proven breeders (each previously had raised at least two litters). Group 1 was sham treated; Group 2 was treated at 1X (0.5 mL/cat/application) and Group 3 at 3X (1.5 mL/cat/application). Female cats 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 kittens were weaned. All queens became pregnant, although some required more than one mating (each of the 12 queens in Group 2 became pregnant as the result of one mating, while 4/12 queens in Group 3 required a second mating). Only dose-related effects in queens were flaky skin at the application site and slight to moderate salivation lasting from 1-5 minutes following dosage of test material (not observed in Groups 1 & 2; observed 4 times in Group 3). An increased incidence of stillbirths in Group 3 (8 or 9/42 births, compared with 2/40 in Group 1 and 2/36 in Group 2) was due to late delivery in one female (72 days; 4 stillbirths) and delivery of two immature kittens (along with two normal kittens) by another queen. No indication of any post-natal adverse effect in Group 3 kittens. Study acceptable to support use of this product at a dosage of rate of 0.5 mL/cat at 28-day intervals on breeding, pregnant and lactating female cats.	N/A	A

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