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

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

May 5, 2000

MEMORANDUM

EPA File Symbol: 65331-L FRONTLINE PLUS FOR DOGS
DP Barcode: D261551
Case No: 066320
PC Codes: 129121 (Fipronil); 105402 (S-methoprene)

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

Byron T. Backus 05-05-2000

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

Registrant: MERIAL LIMITED

ACTION REQUESTED: "Please review the attached domestic animal safety study, label, and CSF for a new formulation -- fipronil/methoprene pet care products..."

BACKGROUND: This package, as received by this reviewer, contains a Companion Animal Safety Study on beagle puppies (MRID 44942104) for a formulation with a label declaration of 9.8% Fipronil and 8.8% (S)-methoprene.

COMMENTS AND RECOMMENDATIONS: The following is the executive summary for the study in MRID 44942104:

In a companion animal safety study (MRID 44942104) Frontline Plus [Active Ingredients: fipronil:10% w/v; (S)-methoprene:9% w/v] was topically applied (between the base of the skull and the shoulder blades) at dose levels of 0.133 mL/kg, 0.399 mL/kg, and 0.665 mL/kg, to groups of 6 male and 6 female beagle puppies (52-60 days old at initiation) on test Day 1 and again on test Day 29. Controls received no treatment.

Clinical evaluations were conducted on approximately Day -14 (or soon after arrival if this was later than Day -14), and on Days 1 and 29 just prior to treatment. Following each treatment, the puppies were isolated for 6 hr and observed for the first 10 min, hourly for 6 hr and thereafter twice daily. Clinical evaluations were also conducted at 1, 3, 7, 14, and 21 days after each treatment. Clinical evaluations included: skin reaction at the application site (erythema, edema, alopecia, haircoat condition, and pruritus); 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. Body weights were recorded on arrival, and thereafter weekly from approximately Day-7, and on the day of each treatment. Blood samples were obtained from fasted animals via the jugular vein once between Day -7 and Day-1 and on Days 15, 29 (prior to second treatment) and 43.

No mortality was observed during the study period, and there were no statistically significant, dose-related effects on body weight, clinical biochemistry, or hematology. Some control and test animals in all groups exhibited fecal abnormalities both before and during treatment (e.g., soft, creamy, or mucoid feces and red discharge in feces). Although these signs were considered by the study authors "not to be unusual for puppies", it is possible that they represent an abnormal condition such as a parasitic infection. According to the report text, the puppies had received anthelmintic treatments prior to arrival, and received further treatments at the laboratory. However, the schedule of treatments (as well as the material administered) is not reported. One male in the high-dose group exhibited clinical signs (body weight loss early in the study (after fasting for blood collection) and subdued behavior on Day 18), and changes in hematology (stress leukogram, microcytosis and hypochromasia and anisocytosis on Days 15 and/or 29) and clinical chemistry (reduction in albumin and albumin: globulin ratio on Days 15, 29 and 43, and elevated AST and ALT on Day 15). Most of the hematological and clinical chemistry changes observed in this one animal were of a transitory nature; however, the reduction in albumin level persisted throughout the study. Decreased albumin was also observed in one female in the 1X group on Day 15. Because the study was still ongoing when the interim report was released, it is recommended that the final condition of the one high-dose male be ascertained (including any necropsy findings, if a necropsy is conducted). It is also recommended that laboratory reference values for the hematological and clinical chemistry parameters be included in the final report.

Overall, the observed clinical signs and changes in hematological and clinical chemistry parameters did not indicate significant treatment related adverse effects. The package labeling and application instructions indicate that the product is to be used on dogs or puppies 8 weeks or older and should not be used more frequently then once every 30 days. However, the package label does not specify the volume of product to be applied, and because the amounts applied in the study were indicated only as volume of product per kilogram body weight, it was not possible to verify that the amounts used in the study conformed to 1X, 3X and 5X of the amounts on the product label.

This study deviated from the companion animal safety study Guidelines (OPPTS 870.7200), in that blood samples were collected prior to treatment on Day -1 and on Day 29, but not at 24 hrs following treatment, as specified by the Guidelines. However, the HED Companion Animal Safety Committee suggested that a similar study conducted on kittens with a similar fipronil-methoprene formulation could be classified as acceptable for the following reasons: 1) the remainder of the study was conducted according to the guidelines; 2) there was no evidence of toxicity in any of the animals; 3) 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, insofar as the Committee is aware, there is no evidence that it interacts with these other chemicals, although the proposed 8.8% concentration in this product may be somewhat higher than that of most of the other products listed in REFS.

It is noted that doses are not stated on the proposed label. TRB concludes that the final dosages, as indicated on the label and as packaged in the applicator tubes, must be consistent with (no more than) the 1X application rate in this study (0.133 mL/kg).

It is also noted that this is an interim report, and there is insufficient information relating to the schedule of individual anthelmintic treatments (as well as the material administered), which should be provided in a final report (or as an amendment to this interim report). For these reasons, the study is currently classified as **Supplementary/Guideline** as a companion animal safety study (OPPTS 870.7200) in dogs (puppies).

DATA EVALUATION REPORT

FRONTLINE PLUS FOR DOGS
[Fipronil/(S)Methoprene Topical Solution]
STUDY TYPE: COMPANION ANIMAL SAFETY – DOG [OPPTS 870.7200]
MRID 44942104

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Primary Reviewer:
Dennis M. Opresko, Ph.D.

Signature:
Date: MAR 0 7 2000

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T.

Signature:
Date: MAR 0 7 2000

Robert H. Ross, M.S., Group Leader

Signature:
Date: MAR 0 7 2000

Quality Assurance:
Donna L. Fefee, D.V.M.

Signature:
Date: MAR 0 7 2000

Disclaimer

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

Managed by Lockheed Martin Energy Research Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer:Byron T. Backus, Ph.D. _____ Date: _____

EPA Work Assignment Manager:John Redden, M.S_____ Date: _____
Registration Division (7505C)

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/Dog [OPPTS 870.7200]

EPA I.D. NUMBERS: DP BARCODE: D261551; MRID NUMBER: 44942104

TEST MATERIAL: Frontline Plus

STUDY NUMBER: Covance Study #1686/2

TESTING FACILITY: Covance Laboratories Ltd, Otley Road, Harrogate, North Yorkshire HG3 1PY, England

SPONSOR: Merial Limited, 2100 Ronson Road, Iselin, NJ 08830-3077

TITLE OF REPORT: Fipronil/S-Methoprene Topical Solution: Target Species Safety Study in the Dog (puppies)

AUTHOR: S. Nolan-Smith

REPORT ISSUED: September 17, 1999

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 44942104) Frontline Plus [Active Ingredients: fipronil:10% w/v; (S)-methoprene:9% w/v] was topically applied (between the base of the skull and the shoulder blades) at dose levels of 0.133 mL/kg, 0.399 mL/kg, and 0.665 mL/kg, to groups of 6 male and 6 female beagle puppies (52-60 days old at initiation) on test Day 1 and again on test Day 29. Controls received no treatment.

Clinical evaluations were conducted on approximately Day -14 (or soon after arrival if this was later than Day -14), and on Days 1 and 29 just prior to treatment. Following each treatment, the puppies were isolated for 6 hr and observed for the first 10 min, hourly for 6 hr and thereafter twice daily. Clinical evaluations were also conducted at 1, 3, 7, 14, and 21 days after each treatment. Clinical evaluations included: skin reaction at the application site (erythema, edema, alopecia, haircoat condition, and pruritus); 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. Body weights were recorded on arrival, and thereafter weekly from approximately Day-7, and on the day of each treatment. Blood samples were obtained from fasted animals via the jugular vein once between Day -7 and Day-1 and on Days 15, 29 (prior to second treatment) and 43.

No mortality was observed during the study period, and there were no statistically significant, dose-related effects on body weight, clinical biochemistry, or hematology. Some control and test animals in all groups exhibited fecal abnormalities both before and during treatment (e.g., soft, creamy, or mucoid feces and red discharge in feces). Although these signs were considered by the study authors "not to be unusual for puppies", it is possible that they represent an abnormal condition such as a parasitic infection. According to the report text, the puppies had received anthelmintic treatments prior to arrival, and received further treatments at the laboratory. However, the schedule of treatments (as well as the material administered) is not reported. One male in the high-dose group exhibited clinical signs (body weight loss early in the study (after fasting for blood collection) and subdued behavior on Day 18), and changes in hematology (stress leukogram, microcytosis and hypochromasia and anisocytosis on Days 15 and/or 29) and clinical chemistry (reduction in albumin and albumin: globulin ratio on Days 15, 29 and 43, and elevated AST and ALT on Day 15). Most of the hematological and clinical chemistry changes observed in this one animal were of a transitory nature; however, the reduction in albumin level persisted throughout the study. Decreased albumin was also observed in one female in the 1X group on Day 15. Because the study was still ongoing when the interim report was released, it is recommended that the final condition of the one high-dose male be ascertained (including any necropsy findings, if a necropsy is conducted). It is also recommended that laboratory reference values for the hematological and clinical chemistry parameters be included in the final report.

Overall, the observed clinical signs and changes in hematological and clinical chemistry parameters did not indicate significant treatment related adverse effects. The package labeling and application instructions indicate that the product is to be used on dogs or puppies 8 weeks or older and should not be used more frequently then once every 30 days. However, the package label does not specify the volume of product to be applied, and because the amounts applied in the study were indicated only as volume of product per kilogram body weight, it was not possible to verify that the amounts used in the study conformed to 1X, 3X and 5X of the amounts on the product label.

This study deviated from the companion animal safety study Guidelines (OPPTS 870.7200), in that blood samples were collected prior to treatment on Day -1 and on Day 29, but not at 24 hrs following treatment, as specified by the Guidelines. However, the HED Companion Animal Safety Committee suggested that a similar study conducted on kittens with a similar fipronil-methoprene formulation could be classified as acceptable for the following reasons: 1) the remainder of the study was conducted according to the guidelines; 2) there was no evidence of toxicity in any of the animals; 3) 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, insofar as the Committee is aware, there is no evidence that it interacts with these other chemicals, although the proposed 8.8% concentration in this product may be somewhat higher than that of most of the other products listed in REFS.

It is noted that doses are not stated on the proposed label. TRB concludes that the final dosages, as indicated on the label and as packaged in the applicator tubes, must be consistent with (no more than) the 1X application rate in this study (0.133 mL/kg).

It is also noted that this is an interim report, and there is insufficient information relating to the schedule of individual anthelmintic treatments (as well as the material administered), which should be provided in a final report (or as an amendment to this interim report). For these reasons, the study is currently classified as **Supplementary/Guideline** as a companion animal safety study (OPPTS 870.7200) in dogs (puppies).

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

I. MATERIALS

A. Test material: Frontline Plus for Dogs [Fipronil/(S)-methoprene topical solution]

Description: Liquid
Lot/Batch No.: Lot No. ML-2,095,988 509T003 (expiry date Aug. 15, 1999)
Active Ingredients: Fipronil 10% w/v; (S)-methoprene 9% w/v
Storage Conditions: Stored in a refrigerator; warmed up overnight prior to dosing

B. Administration: Topical (spot on)

C. Vehicle and/or positive control

Vehicle: None
Positive control: none

D. Test animals

Species: Dog
Breed: Beagle (purebred)
Age and weight at study initiation: 56 ±4 days (average 55.4 days); males:1.41 to 3.31 kg; females: 1.17-2.93 kg.
Source: Harlan UK, Ltd., Bicester, UK
Housing: In groups of 3 of the same sex and treatment group in pens of 4.5 m^2. Housed individually for 6 hr following each treatment
Diet: Shepherdess Milk Substitute once daily and Harlan Teklad 9680 puppy diet, *ad libitum*
Water: Mains water, *ad libitum*
Environmental conditions:
Temperature: 22-28°C
Humidity: 40-80%
Air changes: ≥10/hr
Acclimation period:11 days or more

II. STUDY DESIGN

A. In life dates

Start: March 11, 12, 15, 18, 19, 22, 1999; end: it was reported that this interim report included only investigations, findings, and statistical analyses up to Day 43, two weeks after the second dose.

B. Animal assignment/ Dosage and Administration

Between Day-21 and Day-14, 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 distributed to one of the four experimental groups (Table 1). Groups of 6 dogs/sex were topically treated (between the base of the skull and the shoulder blades) with 0.133, 0.399, or 0.665 mL/kg (1, 3 or 5 spots, respectively) of the test material on test Day 1 and test Day 29. Controls received no treatment.

TABLE 1. Experimental design

Group	No. of animals		Treatment (mL/kg)	Number of treatments[a]
	Male	Female		
1	6	6	0	0
2	6	6	0.133	2
3	6	6	0.399	2
4	6	6	0.665	2

Data taken from p. 16, MRID 44942104.
[a] One treatment on Day 1 and one treatment on Day 29.

C. Dose selection rationale

The rationale for dose levels was to establish the margin of safety and potential dermal and systemic toxicity of 1X, 3X, and 5X the recommended topical application. However, the package label does not specify the volume of product to be applied, and because the amounts applied were indicated only as volume of product per kilogram body weight, it was not possible to verify that the amounts used in the study conformed to the product labelling.

D. Experimental design

Clinical evaluations were conducted on approximately Day -14 (or soon after arrival if this was later than Day -14), and on the day of treatment prior to dosing. Following dosing the dogs were isolated for 6 hr and observed for the first 10 min, hourly for 6 hr and thereafter twice daily. Clinical evaluations were conducted 1, 3, 7, 14 and 21 days after each treatment. Clinical evaluations included: skin reaction at the application site (erythema, edema, alopecia, haircoat condition, and pruritus); 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. Body weights were recorded on arrival, and thereafter weekly from approximately Day-7, and on the day of each treatment.

E. Pathological parameters

Blood samples were obtained from the jugular vein of fasted animals once between Day -7 and Day-1 and on Days 15, 29 (prior to second treatment) and 43. The CHECKED (X) parameters were examined.

a. Hematology

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

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*		Total Cholesterol
x	Potassium*	x	Globulin*
x	Sodium*	x	Glucose*
		x	Total and direct bilirubin*
	ENZYMES	x	Total serum protein*
x	Alkaline phosphatase(ALK)*		(TP)
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)	x	Albumin/Globulin ratio
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase(also SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. Statistics

Where values below the limit of detection were observed, the data were rank transformed prior to analysis. White cell differential counts were transformed to radians using the arcsine square-root transformation. For all other variables analyzed, Levene's test for heterogeneity of variances among groups, between sexes and their interaction was performed. When this showed evidence of heterogeneity between sexes ($p<0.01$), even after log transformation, the data were analyzed separately for each sex. Hematology and clinical chemistry variables were analyzed using repeated measures Analysis of Covariance (ANCOVA) using the pretreatment values as covariate. In all cases, the test for equality of slopes (i.e., the COVARIATE x GROUP interaction) was not significant ($p\geq0.05$) and the effect was removed from the model. When the covariate was significant, 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 was used. The repeated measures ANOVA model was also used for rectal temperatures. Body weight gains were analyzed using two-way ANOVA. All tests were interpreted with two-sided risk.

G. Disposition of animals

All animals were still on study at the time of the interim study completion.

H. Compliance

Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

III. RESULTS

A. Exposure levels

In the text the exposure levels were reported to be 0.133, 0.399 and 0.665 mL/kg body weight; however, in many of the tables and Appendices the doses were given as 0.133, 0.399 and 0.665 mg/kg, respectively. It was indicated that 0.133 mL/kg is equivalent to 13 mg fipronil/kg and 12 mg methoprene/kg.

B. Mortality

No dogs died during the study.

C. Clinical signs

Clinical observations for selected individuals are presented in Table 2. In addition, some control and test animals in all groups exhibited fecal abnormalities both before and during treatment (e.g., soft, creamy, or mucoid feces and red discharge in feces). Although these signs were considered by the study authors "not to be unusual for puppies," it is possible

that they represent an abnormal condition such as a parasitic infection. It was reported that there were no treatment related effects at the application site; however, one male in the low dose group had slight alopecia and or thin haircoat at the site on Days 4 and 8, and another male in the high dose group was observed to be scratching the test site between 4 and 6 minutes after the second dose (Day 29). Mean values for rectal temperature were similar for all groups.

TABLE 2. Clinical observations in dogs given Fipronil/S-Methoprene topical solution

Treatment group	Sex	Day	Observation
Control	M	-7, 22-32	Distended abdomen in 1 animal Thinning fur in 1 animal
	M	29-43	Sporadic tearing of eyes in 1 animal
1X-Treatment	F	<33	Intermittent eversion of the gland at the base of the nictitating membrane in 1 animal (gland removed on Day 33)
	M	4 and 8	Slight alopecia and/or thin haircoat at the test site in 1 animal
	M	31-42 6-13	Sporadic tearing of eye/s in 1 animal Thinning fur - body in same animal
	F	1-8, 12-32, 26-35	1 animal frequently described as thin; Same animal had thinning fur - body
	F	12-16; 17-43	Hair loss, head, both ears, in 1 animal; Sporadic tearing of eyes of same animal
3X- Treatment	F	2	1 animal appeared thin
	F	26-39	Sporadic tearing of eyes in 5 animals
5X - Treatment	F	-2, -1	Subdued behavior in 1 animal
	F	2	1 animal appeared thin
	F	8-11	1 animal appeared thin
	F	31-58	Sporadic tearing of eyes in 5 animals
	M	18	1 animal had subdued behavior
	M	29	1 animal scratching at the test site
	M	39	Small focal opacity on the cornea and protrusion of the nictitating membrane in 1 animal

Data taken from pp 26-27, MRID 44942104.

D. Bodyweight and weight gain

Overall, there were no significant treatment related effects on body weight or body weight gain; however, individual animals in several of the treatment groups had reduced body weights at certain time periods. Three females in the low-dose group and one male in the mid-dose group showed reduced weight during the first few weeks of the study. In addition, one female in the low-dose group and one female and one male in the high-dose group showed body weight loss during the first two weeks of the study and had reduced body weight gain over the full six weeks. The early body weight loss in the high-dose

male (after fasting for blood collection) coincided with clinical signs, thin appearance and subdued behavior on Day 18.

E. Food consumption

Food consumption was not reported.

F. Hematology

Overall, there were no effects on hematology that were considered to be related to treatment, although changes in some parameters were observed in some groups at certain times periods (see Table 3). In both treated animals and controls there was a general increase in hemoglobin levels, red cell counts and packed cell volume from Day-7 to Day 43, which were considered to be age-related. Animals in the low-dose group exhibited a significantly ($p < 0.10$) smaller increase in reticulocyte levels up to Day 29 and showed significantly lower mean cell hemoglobin concentrations ($p < 0.10$) as compared to controls, but because these changes were not seen in the mid- and high-dose animals, they are not considered to be treatment related. Total white cell counts generally decreased across the study; however, in males this parameter was higher than pretreatment values on Day 15; on Days 29 and 43 the values for all male groups were similar. Changes in cell morphology were seen in individual animals; i.e., slight microcytosis, slight hypochromasia and anisocytosis, but were not considered to be treatment related.One male in the high-dose group with reduced body weight and subdued behavior on Day 18, displayed a stress leukogram on Day 15; i.e., neutrophil number increased slightly and lymphocyte number decreased, but total white cell count was normal.

TABLE 3. Selected changes in hematology parameters in dogs given Fipronil/S-Methoprene topical solution [a]

Parameter / Study day	Males				Females			
	Controls	1X	3X	5X	Controls	1X	3X	5X
Hb/Day 15 (g/dL)	8.0	8.8	8.8	8.3	8.5	9.0	7.8	8.6
Hb/Day 29 (g/dL)	9.6	9.9	9.7	9.8	8.9	9.5	8.7	9.6
Hb/Day 43 (g/dL)	11.1	11.0	10.6	11.3	11.2	10.9	10.5	11.1
RBC/Day 15 (mil/cmm)	4.27	4.63	4.76	4.46	4.75	4.94	4.59	4.86
RBC/Day 43 (mil/cmm)	5.45	5.46	5.38	5.71	5.78	5.45	5.69	5.64
PCV/Day 15 (%)	26.5	29.2	29.2	27.6	28.6	29.5	26.1	29.1
PCV/Day 43 (%)	35.7	35.2	34.2	36.4	36.4	34.7	33.7	35.7
RETIC/Day 15 (%)	1.4	1.4	1.5	1.5	1.6	1.2	1.4	1.6
RETIC/Day 29 (%)	2.4	2.0*	2.4	2.6	2.8	1.8*	2.3	2.8
WBC/Day 15 (thds/mm^3)	15.5	17.0	18.5	18.2	17.8	15.6	17.8	15.5
WBC/Day 29 (thds/mm^3)	15.3	15.4	14.9	16.1	17.3	14.4	15.2	15.3
WBC/Day 43 (thds/mm^3)	14.3	13.6	15.1	14.0	14.1	12.9	13.4	15.0

Data taken from Table 3, pp. 37-52, MRID 44942104.
[a]Mean values
* $p < 0.10$

G. Clinical chemistry

One male in the high-dose group that showed clinical signs (reduced body weight in the first two weeks of the study and subdued behavior on Day 18) also exhibited changes in clinical chemistry (reduction in albumin levels and albumin:globulin ratios on Days 15, 29 and 43, and elevated AST and ALT on Day 15). On Day 15 AST activity in the high-dose male was 71 IU/L compared to 26-33 IU/L in the male controls, and ALT activity was 58 IU/L vs. 20-34 IU/L in the male controls. The albumin level was 17 g/L on Day 15 (19-25 g/L in controls), 17 g/L on Day 29 (19-27 g/l in controls) and 16 g/L on Day 43 (21-28 g/L in controls). Albumin levels were also reduced on Days 15 and 29 in one male in the control group; the value on both days was 19 g/L, vs 23-27 g/L in the remaining controls. Laboratory reference values were not included in the report; therefore, it cannot be determined whether the reported values for albumin, AST and ALT were outside the normal ranges. Mean post-treatment total bilirubin levels in the mid and high-dose groups (1.8 and 1.7 umol/L) were statistically ($p < 0.10$) higher than controls (1.4 μg/L); however, individual values were within the pretreatment range.

H. Necropsy findings

No necropsies were performed.

IV. DISCUSSION

Groups of 6 male and 6 female beagle dogs (puppies) were treated topically with 0.133 mL/kg, 0.399 mL/kg, or 0.665 mL/kg of Frontline Plus (Active Ingredients: Fipronil:10% w/v; (S)-methoprene:9% w/v). [NOTE: these exposure levels were reported in the text; however, in many of the tables and Appendices the doses were given as 0.133, 0.399 and 0.665 mg/kg, respectively]. It was indicated that 0.133 mL/kg is equivalent to 13 mg fipronil/kg and 12 mg methoprene/kg Controls were not dosed. Animals were treated on Day 1 and again on Day 29.

No mortality was observed and there were no statistically significant, dose-related, effects on body weight, clinical biochemistry, or hematology. Some control and test animals in all groups exhibited fecal abnormalities both before and during treatment (e.g., soft, creamy, or mucoid feces and red discharge in feces). Although these signs were considered by the study authors "not to be unusual for puppies", it is possible that they represent an abnormal condition such as a parasitic infection. According to the report text (p. 18), the puppies had received anthelmintic treatments prior to arrival, and received further treatments at the laboratory. However, the schedule of treatments (as well as the material administered) is not reported. One male in the high-dose group exhibited clinical signs (body weight loss early in the study (after fasting for blood collection) and subdued behavior on Day 18), and changes in hematology (stress leukogram, microcytosis and hypochromasia and anisocytosis on Days 15 and/or 29) and clinical chemistry (reduction in albumin and albumin:globulin ratio on Days 15, 29 and 43, and elevated AST and ALT on Day 15). Most of the hematological and clinical chemistry changes observed in this one animal were of a transitory nature; however, the reduction in albumin level persisted throughout the study. Decreased albumin was also observed in one female in the 1X group on Day 15. Because the study was still ongoing when the interim report was released, it is recommended that the final condition of the one high-dose male be ascertained (including any necropsy findings, if a necropsy is conducted). It is also recommended that laboratory reference values for the hematological and clinical chemistry parameters be included in the final report.

Overall, the observed clinical signs and changes in hematological and clinical chemistry parameters did not indicate significant treatment related adverse effects. The package labeling and application instructions indicate that the product is to be used on dogs or puppies 8 weeks or older and should not be used more frequently then once every 30 days. However, the package label does not specify the volume of product to be applied, and because the amounts applied in the study were indicated only as volume of product per kilogram body weight, it was not possible to verify that the amounts used in the study conformed to 1X, 3X and 5X of the amounts on the product label. This study deviated from the companion animal safety study Guidelines (OPPTS 870.7200), in that blood samples were collected prior to treatment on Day -1 and on Day 29, but not at 24 hrs following treatment, as specified by the Guidelines. However, the HED Companion Animal Safety Committee suggested that a similar study conducted on kittens with a similar fipronil-methoprene formulation could be classified as acceptable for the following reasons: 1) the remainder of the study was conducted according to the guidelines; 2) there was no evidence of toxicity in any of the animals; 3) 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, insofar as the Committee is aware, there is no evidence that it interacts with these other chemicals, although the proposed 8.8% concentration in this product may be somewhat higher than that of most of the other products listed in REFS.

It is noted that doses are not stated on the proposed label. TRB concludes that the final dosages, as indicated on the label and as packaged in the applicator tubes, must be consistent with (no more than) the 1X application rate in this study (0.133 mL/kg).

It is also noted that this is an interim report, and there is insufficient information relating to the schedule of individual anthelmintic treatments (as well as the material administered), which should be provided in a final report (or as an amendment to this interim report). For these reasons, the study is currently classified as **Supplementary/Guideline** as a companion animal safety study (OPPTS 870.7200) in dogs (puppies).

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D261551
2. **PC CODES:** 129121, 105402 (Fipronil, S-Methoprene)
3. **CURRENT DATE:** May 5, 2000
4. **TEST MATERIAL:** Frontline Plus for Dogs [Fipronil/(S)-methoprene topical solution]; a liquid containing 10% w/v Fipronil and 9% w/v (S)-methoprene.

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety (interim report)/ beagle puppies/Covance Laboratories Ltd, England/#1686/2 /SEP-17-1999	44942104	Test material was dermally applied at dose levels of 0.133, 0.399 & 0.665 mL/kg to groups of 6M & 6F beagle puppies (52-60 days old at initiation) on Test Days 1 & 29. Controls received no treatment. No significant dose-related effects were noted. Blood samples were not collected at 24 hrs following treatment, as specified by the Guidelines, but remainder of the study generally conformed to Guidelines. Some control and test animals had fecal abnormalities (soft, creamy or mucoid feces) prior to and during the treatment period, possible indications of a parasitic infection. According to report text, puppies had received antihelmintic treatments prior to arrival and had received treatments in the laboratory, but the schedule of treatments is not reported. Because study was still ongoing when this interim report was written, and there is insufficient information relating to the schedule of individual antihelmintic treatments (as well as the material administered for these treatments), the study is currently classified as supplementary.	–	S

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