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

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

February 12, 1999

MEMORANDUM

Subject: EPA Reg. No. 2382-150
DP Barcode: D249947
Case No: 060911

From: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus Feb. 12, 1999
Technical Review Branch
Registration Division (7505C)

To: Joseph Tavano/Susan Lewis, PM 03
Insecticide Branch
Registration Division (7505C)

Registrant: VIRBAC INC.

ACTION REQUESTED: "Review Companion Animal Safety data and label. MRIDs 441283-07 & 08."

BACKGROUND: The Companion Animal Safety study in MRID 44128307 was conducted on nine-week old puppies; the study in MRID 44128308 was conducted on nine-week old kittens. Both studies were conducted at Professional Laboratory & Research Services in Corapeake, North Carolina. The studies were conducted on different formulations (the puppy study in MRID 44128307 was conducted on a liquid containing 0.252% pyriproxyfen [nylar] and 1.006% permethrin; the kitten study in MRID 44128308 was conducted on a formulation containing 0.25% pyriproxyfen [nylar], 0.15% prallethrin, 0.15% pyrethrins, 1.5% piperonyl butoxide and 1.5% n-octyl bicycloheptene dicarboximide). The product contains 0.01% Pyriproxyfen [nylar] as sole active ingredient.

RECOMMENDATIONS:

1. The Companion Animal Safety study (conducted on 9-week old puppies) in MRID 44128307 has been classified as acceptable in support of the use of this product, which contains 0.01% Pyriproxyfen as sole active ingredient, on puppies. The following is the executive summary from the DER for this study:

   In a companion animal safety study (MRID 44128307), a permethrin-pyriproxyfen shampoo (Active Ingredients: permethrin 1.006%; pyriproxyfen, 0.252%) was applied one time to a group of 2 male and 4 female mixed breed puppies approximately nine weeks old. The mean dose rate was 37.7 g/kg equivalent to 379.5 mg/kg of permethrin and 95.1 mg/kg of pyriproxyfen. For a target safety factor of 5X, it was reported that this dose would support a maximum label dose rate of 7.5 g/kg or 0.6 fl. oz./lb body weight of the formulated product. Controls were treated with water alone. Blood samples were obtained on the Days -14, 1 and 7 for hematology and clinical chemistry measurements. Animals were observed for 7 days.

   No mortality was observed and there were no treatment-related, biologically-significant effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the test animals when compared to pretreatment values, these changes were not considered to be biologically significant. No neurological abnormalities were observed in any of the animals. No concurrent medications or treatments were given to these puppies during the course of the study.

   This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently, certain of the guideline requirements were not followed. Only six animals were used in the treatment group instead of the required six animals per sex per treatment group. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours after treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr and daily thereafter, but for only 7 days. These deviations from the guidelines are not considered to be critical in the final evaluation of the product safety, and because a 5X margin of safety has been demonstrated for puppies 60 days old (for a recommended label dose rate of 7.5 g/kg), the study is considered **acceptable**.

2. The Companion Animal Safety study (conducted on 9-week old kittens) in MRID 44128308 has been classified as unacceptable, and cannot be used as supporting data for the use of this product to kittens. The following is the executive sumary from the DER for this study:

   In this companion animal safety study (MRID 44128308), a pyrethrin-pyriproxyfen

shampoo (From the confidential appendix the active ingredients were: pyrethrins, 0.15%; pyriproxyfen, 0.25%; prallethrin, 0.15%: piperonyl butoxide, 1.5%; n-octyl bicycloheptene dicarboximide, 1.5%; however, the presence - and percentage - of prallethrin is not indicated in the listing of active ingredients on p. 41) was dermally applied one time to a group of 2 male and 3 female kittens (approximately 9 weeks old) at a dose rate of 50 g/kg, reported to be equivalent to 5X the recommended label dose of 10 g/kg. Controls were treated with water alone. Blood samples were obtained on Days -14, 1 and 7 for hematology and clinical chemistry measurements. Animals were observed for 7 days.

No mortality was observed and there were no treatment-related, biologically-significant effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the treatment group when compared to pretreatment values, these changes were not considered to be biologically significant. No significant clinical signs or neurological abnormalities were reported in the treated animals, although one male kitten in Group A was observed shaking its hind leg at 2 hr after treatment.

This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently certain of the guideline requirements were not followed. Only five animals were used in the treatment group whereas the study guidelines require that six animals per sex per treatment group be used. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours after treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr, and daily thereafter, but for only 7 days. These deviations from the guidelines are not considered to be critical in the final evaluation of the product safety. However, the kittens were infected with coccidia at the time they were received, and all had been under treatment. According to the text, all kittens were treated starting on Day -14 (some had been treated starting Day -18) first using Sulfatrim suspension, and then Di-Trim tablets "because kittens were reacting to the liquid suspension." This treatment with the Di-Trim continued until Day 0. Because of this concomitant exposure the study is classified as **unacceptable**.

Refer to the additional comments from the HED Companion Animal Safety Committee (see the attached memorandum dated February 9, 1999) with respect to these studies.

DATA EVALUATION REPORT

SAFETY - INS 48.2

STUDY TYPE: COMPANION ANIMAL SAFETY – DOG (OPPTS 870.7200)

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

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	Date:	_____
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Cheryl B. Bast, Ph.D., D.A.B.T.	Signature:	_____
	Date:	_____
Robert H. Ross, M.S., Group Leader	Signature:	_____
	Date:	_____
Quality Assurance:		
Bobette Nourse, Ph.D.	Signature:	_____
	Date:	_____

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: _____
Technical Review Branch(7505C)
EPA Work 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: D249947; MRID NUMBER: 44128307

TEST MATERIAL: Permethrin-pyriproxyfen shampoo

STUDY NUMBER: PLRS 9560

TESTING FACILITY:Professional Laboratory and Research Services, Inc., Route 1, Box 3AA, Corapeake, NC 27926

SPONSOR: Virbac, P.O. Box 162059, Fort Worth, TX 76161

TITLE OF REPORT: Safety- INS 48.2

AUTHOR: Larry R. Cruthers

REPORT ISSUED: May 1, 1996

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 44128307), a permethrin-pyriproxyfen shampoo (Active Ingredients: permethrin 1.006%; pyriproxyfen, 0.252%) was applied one time to a group of 2 male and 4 female mixed breed puppies approximately nine weeks old. The mean dose rate was 37.7 g/kg equivalent to 379.5 mg/kg of permethrin and 95.1 mg/kg of pyriproxyfen. For a target safety factor of 5X, it was reported that this dose would support a maximum label dose rate of 7.5 g/kg or 0.6 fl. oz./lb body weight of the formulated product. Controls were treated with water alone. Blood samples were obtained on the Days -14, 1 and 7 for hematology and clinical chemistry measurements. Animals were observed for 7 days.

No mortality was observed and there were no treatment-related, biologically-significant effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the test animals when compared to pretreatment values, these changes were not considered to be biologically significant. No neurological abnormalities were observed in any of the animals. No concurrent medications or treatments were given to these puppies during the course of the study.

This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently, certain of the guideline requirements were not followed. Only six animals were used in the treatment group instead of the required six animals per sex per treatment group. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours after treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr and daily thereafter, but for only 7 days. These deviations from the guidelines are not considered to be critical in the final evaluation of the product safety, and because a 5X margin of safety appears to have been demonstrated for puppies 60 days old (for a recommended label dose rate of 7.5 g/kg), the study is considered **acceptable**.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present. On p. 39 there is a "MEMO TO FILE" which appears to be relevant only to PLRS 9561 (a concurrent kitten study).

I. MATERIALS

A. TEST MATERIAL: Permethrin-pyriproxyfen shampoo

Description: Liquid
Lot/Batch No.: Ins 48.30/ 06.14.95
Active Ingredients: Permethrin, 1.006%; pyriproxyfen, 0.252%.
Storage Conditions: Stored at room temperature and in the dark

B. ADMINISTRATION: Dermal

The following was the application method (from p. 12 of MRID 44128307):

1. Haircoat was wetted
2. Shampoo was applied and lathered
3. Lather was allowed to stand for approximately 5 minutes
4. The haircoat was rinsed thoroughly
5. The haircoat was dried using hand-held dryer and warm air

C. VEHICLE AND/OR POSITIVE CONTROL

Vehicle: Water
Positive control: none

D. TEST ANIMALS

Species: Dog
Breed: Mongrels
Age and weight at study initiation (Day 0): 61-65 days old; mean body weight for Group A (4 females and 2 males) 6.7 lb (range 5.6 to 7.8 lb or 2.5-3.5 kg); mean body weight for Group B (3 females and 3 males) 6.9 lb (range (5.0 to 8.2 lb).
Source: HRP, Inc., Denver, PA
Housing: House individually in stainless steel cages
Diet: Commercial puppy chow (Joy Puppy Food)
Water: daily according to PLRS-AHC-90-09-04
Environmental conditions:
Temperature: Not reported
Humidity: Not reported
Acclimation period: Approximately 14 days

II. STUDY DESIGN

A. IN LIFE DATES

Start: August 4, 1995; end: May 1, 1996

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

On test Day -4, 12 of the 13 pretest puppies were placed into one of two groups based on sex and ranked according to body weight. They were then randomly allocated to one of two test groups (Table 1). Group A was treated with one application of the product (haircoat was wetted, shampoo applied and lathered, lather was allowed to stand for about 5 min, the haircooat was rinsed, and the haircoat was dried using warm air). Group B, the control group, was treated in a similar manner, but with water alone. The target dose for the Group A animals was 37.5 g of test product/kg.

Table 1. Experimental Design

Group	No. of animals		Treatment	Number of applications
	Male	Female		
A	2	4	Test product	1
B	3	3	Control	1

Data taken from p. 11, MRID 44128307.

C. DOSE SELECTION RATIONALE

The amount of shampoo applied to the test animals (mean dose 37.7 g/kg bw) was reported to be 5 times the recommended application rate.

D. EXPERIMENTAL DESIGN

Clinical observations were conducted daily (7 days/wk) for any signs of adverse reactions. Each puppy received a complete physical examination by an attending veterinarian on Days -14, 1 and 7. Body weights were recorded Day 0 and Day 7. The test animals were evaluated for general appearance, food consumption, fecal consistency, urination, integument (skin), eyes /pupils, ears and respiration. An examination of the skin and haircoat was conducted for any treatment-related abnormalities. The animals were also monitored for tremors, unsteady gait (ataxia), depression, salivation, vomiting, mydriasis, diarrhea, convulsions and death.

E. PATHOLOGICAL PARAMETERS

Blood samples were obtained for hematology and clinical chemistry on Days -14, 1 and 7. 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		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)*		
	(Activated partial thromboplastin time)*		
	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
x	Lactic acid dehydrogenase(LDH)		
x	Serum alanine amino- transferase (also		
x	SGPT)*		
x	Serum aspartate amino- transferase(also		
x	SGOT)*		
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F STATISTICS

Information given in Appenix II of submission MRID 44128307 indicates that Student's "t" test was used for Group A vs. Group B comparisons for the hematology and clinical chemisty data. A probability value of equal to or less than 0.05 was considered significant.

G. DISPOSITION OF ANIMALS

The test animals were returned to the animal colony at PLRS at the end of the study.

H. COMPLIANCE

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

III. RESULTS

A. EXPOSURE LEVELS

Table II and Appendix II of submission MRID 44128307 indicate that the mean test substance dose rate was 37.7 g/kg, equivalent to 380 mg permethrin and 95 mg pyriproxyfen per kg. It was reported that this dose rate would support a maximum label dose rate of formulated product of 7.5 g/kg or 0.6 fl. oz./lb body weight.

B. MORTALITY

No puppies died during the study.

C. CLINICAL SIGNS

Clinical observations are presented in Table 2. In addition, one puppy had a mild nail infection on Day 1 and another had a small ulcerated wound on the right upper lip on Day 1. There were no reported neurological abnormalities.

TABLE 2*. Clinical observations

Treatment group	Sex	Day	Observation
Group A (treated)	F	1	Loose feces
	M	3	Loose feces
	F	3	Loose feces
	M	7	Loose feces
	F	7	Slight eye discharge
Group B (controls)	M	1	Loose feces
	M	3	Loose feces
	F	3	Loose feces
	M	4	Loose feces
	F	7	Loose feces
	M	7	Slight eye discharge

* Data taken from p. 18, MRID 44128307.

D. BODYWEIGHT AND WEIGHT GAIN

All test animals gained weight throughout the study. At Day 7, the mean body weight for Group A was 7.3 lb and that for Group B was 7.6 lb.

E. FOOD CONSUMPTION

Post treatment food consumption was reported as normal.

F. HEMATOLOGY

It was reported that within-group sequential variations were considerable between Day -14, Day 1 and Day 7. Several hematology values varied over this period and it is stated (p. 41 of MRID 44128307) that these occurred "in both principals and controls as the animals grew and/or were presumably exposed to new extraneous microbiological insults in the kennel environment."

G. CLINICAL CHEMISTRY

It was reported that within-group sequential variations were considerable between Day -14, Day 1 and Day 7. Several clinical chemistry values changed over this period, but it was reported that the observed changes occurred in both the test and control groups and were similar in magnitude and were attributed to the young age of the test animals.

H. NECROPSY FINDINGS

No necropsies were performed.

IV. DISCUSSION

A group of 2 male and 4 female mixed breed puppies was treated with 37.5 g/kg of a shampoo containing 0.252% pyriproxyfen and 1.006% permethrin. The test dose was reported to be equivalent to 5X the recommended treatment level. A control group was treated with water alone. No mortality was observed and there were no treatment-related, biologically-significant effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the Group A when compared to pretreatment values, these changes were not considered to be biologically significant. No neurological abnormalities were observed in the treated animals

This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently, certain of the guideline requirements were not followed. Only six animals were used in the treatment group instead of the required six animals per sex per treatment group. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours after treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr and daily thereafter, but for only 7 days. These deviations from the guidelines are not considered to be critical in the final evaluation of the product safety, and because a 5X margin of safety appears to have been demonstrated (if the recommended dose rate is 7.5 g of product/kg bw), the study is considered **acceptable** . The product label included with the submission is for a 0.01% pyriproxyfen shampoo without permethrin or

other active ingredients. The recommended dose rate for this product is 7.5 g/kg bw.

NOTE: Several errors are present in MRID 44128307 including a listing of the product test dose as 37.5 mg/kg on page 18. The title of the Appendix II and III refers only to pyriproxyfen and not to permethrin, and the label that was included also referred specifically to a product containing only 0.01% pyriproxyfen. The concentrations of active ingredients are incorrectly listed in Table 2 of Appendix II as 0.252% permethrin and 1.006% pyriproxyfen. A memo on page 39 refers to kittens and not to puppies. On page 10 it is stated that blood samples were taken on Day -14; however, in Appendix II, reference is made to Day -15 samples. It would appear that the initial text of Appendix II was lifted from another report and used in MRID 44128307 without careful scrutiny.

DATA EVALUATION REPORT

SAFETY - INS 54.3

STUDY TYPE: COMPANION ANIMAL SAFETY – CAT (OPPTS 870.7200)

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

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Cheryl B. Bast, Ph.D., D.A.B.T.	Signature:	_____
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Robert H. Ross, M.S., Group Leader	Signature:	_____
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Quality Assurance:		
Bobette Nourse, Ph.D.	Signature:	_____
	Date:	_____

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: _____
Technical Review Branch(7505C)
EPA Work Manager: John Redden, M.S. _____ Date: _____
Registration Division (7505C)

DATA EVALUATION RECORD

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

EPA I.D. NUMBERS: DP BARCODE: D249947; MRID NUMBER: 44128308C

TEST MATERIAL: Pyrethrin-pyriproxyfen shampoo

STUDY NUMBER: PLRS 9561

TESTING FACILITY: Professional Laboratory and Research Services, Inc., Route 1, Box 3AA, Corapeake, NC 27926

SPONSOR: Virbac, P.O. Box 162059, Fort Worth, TX 76161

TITLE OF REPORT: Safety- INS 54.3

AUTHOR: Larry R. Cruthers

REPORT ISSUED: May 1, 1996

EXECUTIVE SUMMARY:

In this companion animal safety study (MRID 44128308), a pyrethrin-pyriproxyfen shampoo (From the appendix the active ingredients were: pyrethrins, 0.15%; pyriproxyfen, 0.25%; prallethrin, 0.15%: piperonyl butoxide, 1.5%; n-octyl bicycloheptene dicarboximide, 1.5%; however, the presence - and percentage - of prallethrin is not indicated in the listing of active ingredients on p. 41) was dermally applied one time to a group of 2 male and 3 female kittens (approximately 9 weeks old) at a dose rate of 50 g/kg, reported to be equivalent to 5X the recommended label dose of 10 g/kg. Controls were treated with water alone. Blood samples were obtained on Days -14, 1 and 7 for hematology and clinical chemistry measurements. Animals were observed for 7 days.

No mortality was observed and there were no treatment-related, biologically-significant effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the treatment group when compared to pretreatment values, these changes were not considered to be biologically significant. No significant clinical signs or

neurological abnormalities were reported in the treated animals, although one male kitten in Group A was observed shaking its hind leg at 2 hr after treatment.

This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently certain of the guideline requirements were not followed. Only five animals were used in the treatment group whereas the study guidelines require that six animals per sex per treatment group be used. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours after treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr, and daily thereafter, but for only 7 days. These deviations from the guidelines are not considered to be critical in the final evaluation of the product safety. However, the kittens were infected with coccidia at the time they were received, and all had been under treatment. According to the text, all kittens were treated starting on Day -14 (some had been treated starting Day -18) first using Sulfatrim suspension, and then Di-Trim tablets "because kittens were reacting to the liquid suspension." This treatment with the Di-Trim continued until Day 0. Because of this concomitant exposure the study is classified as **unacceptable**.

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

I. MATERIALS

A. TEST MATERIAL: pyrethrin-pyriproxyfen shampoo

Description: Liquid
Lot/Batch No.: Ins 51.10/10.30.95
Active Ingredients: Pyrethrins, 0.15%; pyriproxyfen, 0.25%; prallethrin, 0.15%: piperonyl butoxide, 1.5%; n-octyl bicycloheptene dicarboximide, 1.5% [note: the presence (and percentage) of prallethrin is not indicated on p. 41].
Storage Conditions: Stored at room temperature and in the dark

B. ADMINISTRATION: Dermal

C. VEHICLE AND/OR POSITIVE CONTROL

Vehicle: Water
Positive control: none

D. TEST ANIMALS

Species: Cat
Breed: Domestic shorthair.
Age and weight at study initiation: 63-67 days old; mean body weights, 0.89 kg for Group A (3 females and 2 males), 0.86 kg for Group B (3 females and 2 males).
Source: Liberty Research Inc., Waverly, NY.
Housing: House individually in stainless steel cages
Diet: Purina Kitten Chow
Water: daily according to PLRS-AHC-90-10-05
Environmental conditions:
Temperature: Not reported
Humidity: Not reported
Acclimation period: Approximately 21 days

II. STUDY DESIGN

A. IN LIFE DATES

Start: August 4, 1995; end: May 1, 1996

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

On test Day -1, 10 of the 14 pretest kittens were placed into one of two groups based on sex and ranked according to body weight. They were then randomly allocated to one of two test groups (Table 1). Group A was treated with one application of the test product; the haircoat was wetted, shampoo applied, lather was allowed to stand for about 5 min, the haircoat was rinsed and then dried using warm air. Group B, the control group, was treated in a similar manner, but with water alone. The target dose for the Group A animals was 50 g of test product/kg (erroneously reported as 50 mg/kg on page 17 of MRID 44128308). The amount of shampoo applied ranged from 40.3 to 50.7 g (erroneously reported as 40.3-50.7 mg on page 17 of MRID 44128308).

Table 1. Experimental Design*

Group	No. of animals		Treatment	Number of applications
	Male	Female		
A	2	3	Test product	1
B	2	3	Water (control)	1

* Data taken from p. 11-12, MRID 44128308.

C. DOSE SELECTION RATIONALE

Page 45 of Appendix II of MRID 44128308 indicates that the test dose was selected to evaluate a 5X margin of safety for the test product.

D. EXPERIMENTAL DESIGN

Clinical observations were conducted every 2 hours after the application of the test material for the remainder of the workday, and daily thereafter for 7 days. Each kitten received a complete physical examination by an attending veterinarian on Days -14, 1 and 7. Body weights were recorded Day 0 and Day 7. The test animals were evaluated for general appearance, food consumption, fecal consistency, urination, integument, gait (ataxia), eyes/pupils, ears, respiration, tremors, depression, salivation, vomiting, mydriasis, diarrhea, convulsions and death.

E. PATHOLOGICAL PARAMETERS

Blood samples were obtained for hematology and clinical chemistry on Days -14, 1 and 7. 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		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)*		
	(Activated partial thromboplastin time)*		
	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 (TP)*
x	Alkaline phosphatase (ALK)*		Triglycerides
	Cholinesterase (ChE)		*Serum protein electrophoresis*
	Creatine kinase		
x	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino- transferase (also		
x	SGPT)*		
x	Serum aspartate amino- transferase (also		
x	SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F STATISTICS

Information given in Appendix II of submission MRID 44128308 indicates that the Student's "t" test was used for Group A vs. Group B comparisons for the hematology and clinical chemisty data. A probability value of equal to or less than 0.05 was considered significant.

G. DISPOSITION OF ANIMALS

The test animals were returned to the animal colony at PLRS at the end of the study.

H. COMPLIANCE

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

III. RESULTS

A. EXPOSURE LEVELS

According to Table 2 of Appendix II of MRID 44128308, the mean test product dose rate was 50 g/kg, equivalent to 76.0 mg/kg of pyrethrins, 75.5 mg/kg of

prallethrin, 753.4 mg/kg of piperonyl butoxide, 753.4 mg/kg of n-octyl bicycloheptene dicarboximide, and 126.0 mg/kg of pyriproxyfen. The 1X dosage was reported to be 10.0 g/kg of test product.

B. MORTALITY

No kittens died during the study.

C. CLINICAL SIGNS

Clinical observations are presented in Table 2. The kittens were infected with coccidia and some were treated with Sulfatrim suspension beginning on Day -18. From information on p. 17 "all kittens began treatment on Test Day -14 first using the Sulfatrim suspension which was replaced by Di-Trim tablets (trimethoprim 20 mg; sulfadiazine 100 mg) because kittens were reacting to the liquid suspension. The last day of treatment for cocciodiosis was Test Day 0." From information on p. 19 the course of the cocciodiosis was protracted "since treatment had to be discontinued for approximately four days (due to the fact that the kittens reacted to the Sulfatrim Suspension) until the Di-Trim tablets were received from the distributor."

TABLE 2*. Clinical observations

Treatment group	Sex	Day	Observation
Group A (treated)	M	1	One kitten was observed shaking its hind legs at +2 hr.
Group B (controls)	F	1	One kitten was observed to be slightly depressed.

* Data taken from pp. 18 and 161, MRID 44128308.

D. BODYWEIGHT AND WEIGHT GAIN

All test animals gained weight throughout the study, and it was reported that weight gain was normal for all treated animals (statistical analysis not provided). For Group A, the mean body weights were 1.44, 1.84, 1.96, and 2.32 lb on Days -14, -1, 0, and 7.

E. FOOD CONSUMPTION

In Group A, food consumption was described as "normal" for 26 observations, "most consumed" for 17 observations, and "some consumed" for 2 observations. Quantitative data were not provided.

F. HEMATOLOGY

Measured hematology values were "generally comparable" between the test and control groups at Days 1 and 7, and also comparable with pretreatment values. It was noted by the author that the hematology values reflected the young age of the animals and the protracted coccidia infection.

G. CLINICAL CHEMISTRY

Measured clinical chemistry values were "generally comparable" between the test and control groups at Days 1 and 7, and also comparable with pretreatment values. It was noted that these values reflected the young age of the animals and the protracted coccidia infection.

H. NECROPSY FINDINGS

No necropsies were performed.

IV. DISCUSSION

A group of 2 male and 3 female kittens was treated with a pyrethrin-pyriproxyfen shampoo at a dose rate of 50 g/kg bw, reported to be equivalent to 5X the recommended label dose of 10 g/kg. The label included with the submission, however, was for a 0.01% pyriproxyfen shampoo without any other active ingredients, and the recommended dose rate for this product was 7.5 g/kg bw. Controls were treated with water alone. Blood samples were obtained on the Days -14, 1 and 7 for hematology and clinical chemistry measurements. Animals were observed for 7 days.

No mortality was observed and there appeared to be no treatment-related effects on body weight, clinical biochemistry, or hematology. Although some statistically-significant changes in clinical chemistry and hematology values were recorded in the treatment group when compared to pretreatment values, few of these values were significantly different from concurrent controls at Day 1 or 7; therefore, there are not considered to be toxicologically significant. There were no clinical or neurological signs of treatment-related effects, although one male kitten in Group A was observed shaking its hind leg at 2 hr after treatment.

This study was conducted before the guidelines for a companion animal safety study (OPPTS 870.7200) were promulgated and, consequently certain of the guideline requirements were not followed. Only five animals were used in the treatment group whereas the study guidelines require that six animals per sex per treatment group be used. Furthermore, the guidelines require a minimum 14-day observation period, including hourly observations for the first 4 hours afetr treatment. In this study the animals were observed at 2 hr intervals for the first 4 hr, and daily thereafter, but for only 7 days. However, the kittens were infected with coccidia at the time they were received, and all had been under treatment. According to the text, all kittens were treated starting on Day -14 (some had been

treated starting Day -18) first using Sulfatrim suspension, and then Di-Trim tablets "because kittens were reacting to the liquid suspension." This treatment with the Di-Trim continued until Day 0. Because of this concomitant exposure the study is classified as **unacceptable**.

NOTE: Several errors are present in submission MRID 44128308, including a listing of the title of Appendix II and III as " Pyriproxyfen IGR Residual Ovisterilant Shampoo #1. In addition, on page 17 the target dose is reported as 50 mg/kg; and on page 45 of Appendix II reference is made to puppies, a Day -15 sampling period, and a significant difference in phosphorus values between control and treated groups on Day -15. None of this information agrees with that given on the Tables. It appears that parts of the text of this Appendix were extracted from another report and used in MRID 44128308 without careful scrutiny.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D249947
2. **PC CODES**: 129032, 109701
3. **CURRENT DATE:** February 12, 1999
4. **TEST MATERIAL**: a permethrin-pyriproxyfen shampoo (active ingredients: permethrin 1.006%; pyriproxyfen 0.252%).

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety/dog (9 wk old puppy)/Professional Laboratory & Research Services/PLRS 9560/MAY-1-1996	44128307	Shampoo was applied one time to a group of 2 male and 4 female mixed breed pups. Mean dose rate was 37.7 g/kg equivalent to 379.5 mg/kg of permethrin and 95.1 mg/kg of pyriproxyfen. For a target safety factor of 5X, this dose would support a maximum label dose rate of 7.5 g/kg or 0.6 fl. oz./lb body weight. Animals were observed for 7 days. No mortality was observed and there were no treatment-related and/or biologically significant effects on body weight, clinical biochemistry and hematology values. No neurological abnormalities were observed in any of the animals. No concurrent medications or treatments were given to these puppies during the course of the study.	–	A

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

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D249947
2. **PC CODE:** 129032, 069001, 067501, 057001, 128722(?)
3. **CURRENT DATE:** February 12, 1999
4. **TEST MATERIAL:** a pyrethrin-pyriproxyfen shampoo: active ingredients: pyrethrins 0.15%; pyriproxyfen 0.25%; piperonyl butoxide 1.5%; n-octyl bicycloheptene dicarboximide 1.5%, and (possibly) prallethrin 0.15%

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety/cat (9 wk old kitten/Professional Laboratory & Research Services/PLRS 9561/MAY-1-1996	44128308	Shampoo was applied one time to a group of 2 male and 3 female kittens, approx. 9 weeks old, at a dose rate of 50 g/kg, reported to be equivalent to 5X the recommended label dose of 10 g/kg. Controls were treated with water alone. Animals were observed for 7 days. No mortality was observed, and there were no treatment-related and/or biologically significant effects on body weight, clinical biochemistry or hematology. No significant clinical signs or neurological abnormalities were reported in treated animals, although one male kitten exposed to the test material was observed shaking its hind leg at 2 hr after treatment. According to text all kittens were infected with coccidia when they were received, and all had been under treatment, including with Di-Trim tablets to Day 0. Because of this concomitant exposure the study is classified as unacceptable.	-	U

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