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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR No. 0052259

MEMORANDUM

DATE: 02/17 /2004

SUBJECT: Hartz Companion Animal Safety Study - Cats (OPPTS 870.7200)

DP Barcode:D296096
Submission #:N/A
PC Code:069005

PRAT Case#:
Tox. Chem. No.:
MRID No.:46118301

TO: Ann Sibold, PM and Marion Johnson, Chief
Insecticide Branch
Registration Division (7505C)

FROM: William Dykstra, Ph.D., Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

William Dykstra
2/18/04

THRU: Susan Hummel.
Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

Susan Hummel

Background and Request: Based on a recent companion animal study in adult cats with d-phenothrin, the Hartz Co. asserted in meetings that the neurotoxic clinical signs observed in the study were due to the fact that the cats were anemic. The final report of the Hartz Companion Animal Study in cats with d-phenothrin has been reviewed by HED at the request of Registration Division.. Based on weight-of-the evidence considerations, HED concludes that the clinical signs in normal cats (HCT>24%) occurred with similar incidence to anemic cats (HCT<24%) used in this study.

An Executive Summary for the study review is attached.

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46118301), Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens (TS#12260, 85.7% phenothrin; 2.9% methoprene) was topically administered to groups of 12 (5 males/7 females or

6 males/6 females) adult domestic shorthair cats at 0, 1, 3 or 5 X the label dose (1.0 mL). On the day of dosing (Day 0), Group I (placebo control) was administered safflower oil once hourly for 5 hours for a 5X dose. Group II was administered a 1X dose (1.0 mL) of the product on the dorsal aspect of the neck. Group III (3X) and Group IV (5X) were administered the label dose of the product hourly for either three or five applications. The animals were observed hourly for 12 hours following the final dosing and twice daily for the duration of the 14-day study. Body weights were measured pre-treatment and on Days 7 and 14. Food consumption was recorded pre-treatment and daily throughout the study. Hematology and clinical chemistry parameters were measured on Days -7, 1, 7 and 14 (hematocrits only).

All cats survived to study termination. Very slight ataxia was reported in one Group II (1X) female at the 9- and 10-hour observations on Day 0. Very slight unsteady gait (one cat, 8 and 10 hours), very slight to moderate trembling (four cats, 7-14 hours), very slight to moderate ataxia (three cats, 9-14 hours) and seizure (one cat, between 8-9 hours) were observed in the Group III (3X) animals. Clinical signs in Group IV (5X) animals included very slight to moderate unsteady gait (four cats, 8-10 hours), very slight to moderate trembling (ten cats, 8-16 hours) and very slight ataxia (ten cats, 9-16 hours). Very slight tremors were observed in two female Group III animals on Day 1; tremors were not reported in these cats on Day 0. Very slight to moderate tremors were observed in five Group IV cats (1M, 4F) on Days 1-3. Tremors were reported for 16 hours post-dosing on Day 0 in all these animals so the findings on Day 1 are likely a continuation of Day 0. In one animal, the tremors continued until the afternoon of Day 3.

There was no evidence of a treatment-related effect on body weight, body weight gain or hematology parameters. Food consumption was decreased in the 5X males and all treated female groups (no dose response) on Day 1 only. On Day 7, there was an increase in ALT levels in the 5X males which was outside the conducting laboratory's reference range. The increase was due to one male that had an elevated value pre-treatment which increased with each testing period. The AST value in this animal was also outside the reference range on Day 7. The increases in liver enzymes (ALT, AST) in this cat after dosing are possibly treatment-related.

It has not been demonstrated that 90% of the cats in the study which exhibited clinical signs were anemic. Reference range values from the conducting laboratory were provided for all the hematology and clinical chemistry parameters, except for hematocrit. For that parameter, the range (30-45%) was taken from the *Merck Veterinary Manual, Eighth Edition*. There is no explanation for this substitution. In general, hematocrit values are approximately three times hemoglobin levels. Hemoglobin levels listed in the reference range for the conducting laboratory were 8-16 g/dL. Therefore, the expected hematocrit values would be 24 - 48%, with a lower minimal value than that cited from the *Merck Veterinary Manual*. Values for hematocrit in other veterinary texts also have lower minimum levels. In *Veterinary Clinical Pathology, Fourth Edition*, the hematocrit and hemoglobin ranges are 24-45% and 8-15 gm/dL, respectively. In *Kirk's Current Veterinary Therapy, XIII*, hematocrit and hemoglobin ranges are 24-45% and 8-14 gm/dL, respectively. Based on weight-of-the-evidence considerations presented in this DER, HED concludes that some of the cats used in this study were anemic (HCT < 24%), although most animals were within the lower normal range for hematocrit from most sources (>24%). A comparison of the hematocrit values for individual cats displaying clinical signs shows that the following results for the various Groups: (Group II: 26.6%, Group III: 23.9, 21.6, 24.3, 29.4, 21.0, 26.7, 33.5%, Group IV: 20.2, 27.6, 25.8, 21.2, 27.6, 39.1, 25.6, 27.6, 36.3, 29.6%).

Therefore, it can be seen that both anemic (<24%) and normal cats (>24%) displayed clinical signs.

It is concluded that Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens produces clinical signs of toxicity in adult cats when applied at the recommended dose.

This companion animal safety study in the cat is **Unacceptable/guideline** and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the cat. A margin of safety was not established for cats treated according to label instructions.

DATA EVALUATION RECORD

HARTZ ADVANCED CARE BRAND FLEA & TICK DROPS PLUS+ FOR CATS AND KITTENS [PHENOTHRIN/(S)-METHOPRENE]

STUDY TYPE: COMPANION ANIMAL SAFETY - Cat - (OPPTS 870.7200)
MRID 46118301

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No.13-2004

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	Date:	JAN 2 2 2004
Quality Assurance: Lee Ann Wilson, M.A.	Signature:	
	Date:	JAN 2 2 2004

Disclaimer

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

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

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TXR#: 0052259

DATA EVALUATION RECORD

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

PC CODE: 069005 (phenothrin)

DP BARCODE: D296096
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): Hartz Advanced Care Brand Flea and Tick Drops+ For Cats and Kittens (EPA Reg. No. 2596-148) (Phenothrin, 85.7%; methoprene, 2.9%)

CITATION: Kuhn, J.O. (2003) Companion Animal Safety Study (EPA Guideline 870.7200) for Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens, a Production Spot-on Formulation Using Adult Cats. Stillmeadow Inc., Sugar Land, TX. Study number 7584-03, October 17, 2003. MRID 46118301. Unpublished.

SPONSOR: The Hartz Mountain Corporation, 400 Plaza Drive, Secaucus, New Jersey 07003

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46118301), Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens (TS#12260, 85.7% phenothrin; 2.9% methoprene) was topically administered to groups of 12 (5 males/7 females or 6 males/6 females) adult domestic shorthair cats at 0, 1, 3 or 5 X the label dose (1.0 mL). On the day of dosing (Day 0), Group I (placebo control) was administered safflower oil once hourly for 5 hours for a 5X dose. Group II was administered a 1X dose (1.0 mL) of the product on the dorsal aspect of the neck. Group III (3X) and Group IV (5X) were administered the label dose of the product hourly for either three or five applications. The animals were observed hourly for 12 hours following the final dosing and twice daily for the duration of the 14-day study. Body weights were measured pre-treatment and on Days 7 and 14. Food consumption was recorded pre-treatment and daily throughout the study. Hematology and clinical chemistry parameters were measured on Days -7, 1, 7 and 14 (hematocrits only).

All cats survived to study termination. Very slight ataxia was reported in one Group II (1X) female at the 9- and 10-hour observations on Day 0. Very slight unsteady gait (one cat, 8 and 10 hours), very slight to moderate trembling (four cats, 7-14 hours), very slight to moderate ataxia (three cats, 9-14 hours) and seizure (one cat, between 8-9 hours) were observed in the Group III (3X) animals. Clinical signs in Group IV (5X) animals included very slight to moderate unsteady gait (four cats, 8-10 hours), very slight to moderate trembling (ten cats, 8-16 hours) and very

slight ataxia (ten cats, 9-16 hours). Very slight tremors were observed in two female Group III animals on Day 1; tremors were not reported in these cats on Day 0. Very slight to moderate tremors were observed in five Group IV cats (1M, 4F) on Days 1-3. Tremors were reported for 16 hours post-dosing on Day 0 in all these animals so the findings on Day 1 are likely a continuation of Day 0. In one animal, the tremors continued until the afternoon of Day 3.

There was no evidence of a treatment-related effect on body weight, body weight gain or hematology parameters. Food consumption was decreased in the 5X males and all treated female groups (no dose response) on Day 1 only. On Day 7, there was an increase in ALT levels in the 5X males which was outside the conducting laboratory's reference range. The increase was due to one male that had an elevated value pre-treatment which increased with each testing period. The AST value in this animal was also outside the reference range on Day 7. The increases in liver enzymes (ALT, AST) in this cat after dosing are possibly treatment-related.

It has not been demonstrated that 90% of the cats in the study which exhibited clinical signs were anemic. Reference range values from the conducting laboratory were provided for all the hematology and clinical chemistry parameters, except for hematocrit. For that parameter, the range (30-45%) was taken from the *Merck Veterinary Manual, Eighth Edition*. There is no explanation for this substitution. In general, hematocrit values are approximately three times hemoglobin levels. Hemoglobin levels listed in the reference range for the conducting laboratory were 8-16 g/dL. Therefore, the expected hematocrit values would be 24 - 48%, with a lower minimal value than that cited from the *Merck Veterinary Manual*. Values for hematocrit in other veterinary texts also have lower minimum levels. In *Veterinary Clinical Pathology, Fourth Edition*, the hematocrit and hemoglobin ranges are 24-45% and 8-15 gm/dL, respectively. In *Kirk's Current Veterinary Therapy, XIII*, hematocrit and hemoglobin ranges are 24-45% and 8-14 gm/dL, respectively. Based on weight-of-the-evidence considerations presented in this DER, HED concludes that some of the cats used in this study were anemic (HCT < 24%), although most animals were within the lower normal range for hematocrit from most sources (>24%). A comparison of the hematocrit values for individual cats displaying clinical signs shows that the following results for the various Groups: (Group II: 26.6%, Group III: 23.9, 21.6, 24.3, 29.4, 21.0, 26.7, 33.5%, Group IV: 20.2, 27.6, 25.8, 21.2, 27.6, 39.1, 25.6, 27.6, 36.3, 29.6%). Therefore, it can be seen that both anemic (<24%) and normal cats (>24%) displayed clinical signs.

It is concluded that Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens produces clinical signs of toxicity in adult cats when applied at the recommended dose.

This companion animal safety study in the cat is **Unacceptable/guideline** and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the cat. A margin of safety was not established for cats treated according to label instructions.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Hartz Advanced Care Brand Flea and Tick Drops+ For Cats and Kittens (EPA Reg. No. 2596-148) - referred to as TS# 12260 in study

Description: clear amber liquid
Lot #: BL09431
Purity: Sumithrin (phenothrin), 85.7%; methoprene, 2.9%
Compound Stability: expiration date: April 2005
CAS #of TGAI: Not available
Structure: Not available

2. Placebo control:

safflower oil (lot # 111050) - referred to as TS# 12261 in study

3. Test animals:

Species: Cat
Strain: Domestic shorthair
Age/weight at study initiation: Approximately 6 mos - 6 yrs/ males: 3.0-5.1 kg; females: 2.2-4.0 kg.
Source: Liberty Research (Waverly, NY), Harlan Sprague Dawley (Madison, WI), Martin Creek Kennels (Wiliford, AR) and Sinclair Research (Columbia, MO)
Housing: Individually in stainless steel, suspended, wire bottom cages
Diet: PMI Feline Lab Diet # 5003
Water: Tap water *ad libitum*
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: 30-70%
Air changes: 10-12/hr
Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period: Two weeks

B. STUDY DESIGN:

1. **In life dates**: July 24 - August 7, 2003

2. **Animal assignment**: Animals were assigned using a computer-generated randomizing plan to dose groups in an effort to equalize mean body weights within sex (Table 1).

TABLE 1. Animal assignment[a]

Group	Article	Dosage per Application	Number of Applications	Phenothrin (mg)	Methoprene (mg)
I (5M. 7 F)	Placebo	1 mL	5	NA	NA
II (6 M. 6 F)	Test	1 tube (1 mL)	1	896.0	30.3
III (6 M. 6 F)	Test	1 tube (1 mL)	3	2688.0	90.9
IV (6 M. 6 F)	Test	1 tube (1 mL)	5	4480.0	151.5

[a] From Pages 11 and 16, MRID 46118301.

During the application, the tip of the tube was used to part the cat's hair at the base of the head. The contents were then dispensed to form a spot on the head, avoiding the cat's eyes and mouth. After the material was applied, the cat was held in an upright position for approximately two minutes to prevent loss of the applied material.

On Day 0, Group II animals were treated once with the test material. The test material was applied to Group III animals once hourly for 3 hours for a 3X dose and once hourly for 5 hours for a 5X dose. Group I cats (placebo) were dosed once hourly for 5 hours for a 5X dose. The placebo (safflower oil, 1.0 mL) was applied in the same manner as the test material using a syringe with no needle.

C. METHODS:

1. Observations:

1a. Cageside observations: On Day 0, animals with more than a single dosing were observed prior to each dosing for signs of pharmacological and/or toxicological effects. Each animal was then observed hourly for 12 hours following the final dosing and then twice daily for the duration of the study. Each observation lasted at least one minute minimum. The technicians watched especially for fine or coarse muscle tremors. The Companion Animal Safety Guideline (OPPTS 870.7200) requires hourly observations for at least four hours after the last treatment. Study modification was required in an agreement between the EPA and the Hartz Mountain Corporation due to safety concerns stemming from use of this product in cats and kittens. Reports of adverse reactions submitted to EPA ranged from minor adverse effects including skin irritation or hair loss at the application site and salivation to more serious effects on the nervous system, such as tremors and, in some circumstances, severe full body tremors (convulsion).[1] The time period between product application and onset of clinical signs in pet cats was as long as 12 hours in the adverse incident reports.

1b. Clinical examinations: Detailed physical examinations by a veterinarian were conducted on Day -8 to determine suitability for the study and then again on Day 1.

2. **Body weight:** Animals were weighed on Days -15, -7, -1, 7 and 14.

3. **Food consumption:** Food consumption was recorded daily beginning on Day -7.

4. **Hematology and clinical chemistry:** Blood was collected from all fasted animals on Days -7, 1, 7 and 14. The CHECKED (X) parameters were examined on all days, except on Day 14 when only hematocrit values were determined.

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[1]Questions & Answers: Label Instructions Tightened on Flea & Tick Control Products for Pets at www.epa.gov/pesticides/factsheets/hartzq_a.htm#4

a. **Hematology:**

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 estimate*		Reticulocyte count
	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Recommended for companion animal safety studies based on Guideline 870.7200

b. **Clinical chemistry:**

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin*
x	Chloride*	x	Creatinine*
	Magnesium	x	Urea nitrogen*
x	Phosphorus*		Total Cholesterol*
x	Potassium*	x	Globulins*
x	Sodium*	x	Glucose*
	ENZYMES	x	Total bilirubin*
	(more than 2 hepatic enzymes suggested)*	x	Direct bilirubin*
x	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	x	A/G Ratio
x	Alanine aminotransferase (also SGPT)*		
x	Aspartate aminotransferase (also SGOT)*		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for companion animal safety studies based on Guideline 870.7200

5. **Sacrifice and pathology**: Not required by Companion Animal Safety Study Guideline (OPPTS 870.7200).

6. **Statistics**: No statistical analyses were described.

II. RESULTS:

A. OBSERVATIONS:

1. **Exposure Levels**: The mean and range of the topical mg/kg doses for the treated groups are presented in Table 2.

TABLE 2. Mean topical dose (mg/kg) to treated animals*			
Group	Mean Body Weight (kg) Day -1	Phenothrin (mg/kg)	Methoprene (mg/kg)

II (1X)	3.4	273.7 (165.9-373.3)[b]	9.3 (5.6-12.6)[b]
III (3X)	3.5	800.3 (584.3-1120.0)[b]	27.1 (19.8-37.9)[b]
IV (5X)	3.3	1413.4 (1066.7-1792.0)[b]	47.8 (36.1-60.6)[b]

[a] Calculated by the reviewer from data on page 16, MRID 46118301.
[b] Dose range given in parentheses.

2. **Mortality**: All cats survived to study termination.

3. **Clinical Signs of Toxicity**: Selected findings on the day of treatment are summarized in Table 3. Spiked and/or greasy fur at the dose sites was noted in all animals at all observation times. Very slight ataxia was reported in one Group II (1X) female at the 9- and 10-hour observations. Wet fur (one cat), very slight unsteady gait (one cat, 8 and 10 hours), very slight to moderate trembling (four cats, 7-14 hours), slight salivation (one cat, following first and second doses), very slight to moderate ataxia (three cats, 9-14 hours) and seizure (one cat, between 8-9 hours) were observed in the Group III (3X) animals. Clinical signs in Group IV (5X) animals included very slight to moderate unsteady gait (four cats, 8-10 hours), very slight to moderate trembling (ten cats, 8-16 hours), very slight to slight salivation (two cats, 3-4 hours) and very slight ataxia (ten cats, 9-16 hours).

TABLE 3. Selected clinical signs on day of treatment[a]

Clinical sign	Number affected (sex) (first hour-last hour observed post-dosing)			
	Control	1X Dose	3X Dose	5X Dose
Ataxia	0	1(F) (9-10)	3 (3 F) (9-14)	10 (4M, 6F) (9-16)
Unsteady Gait	0	0	1 (1M) (8-10)	4 (2 M, 2F) (8-10)
Tremor	0	0	4 (1M, 3F) (7-14)	10 (4M, 6F) (8-16)
Seizure	0	0	1 (1F) (8-9)	0
Salivation	0	0	1 (1F) (1-2)	2 (1M, 1F) (3-4)

[a] Data from pages 17-20, MRID 46118301.

Selected clinical signs reported on Days 1 through 14 are summarized in Table 4. Very slight to moderate ocular discharge was observed in one animal each in Groups I and IV throughout the study. Spiked and/or greasy fur at the dose sites was reported in all Group I and IV animals through Day 11 and through Day 3 in Groups II and III. Slight soft feces were seen once in three Group III and two Group IV cats. Slight to moderate diarrhea was observed in one Group III cat sporadically throughout the study and in another on Day 1 only. Vomiting was reported once for two Group III cats (Days 2 and 3) and one Group IV cat (Day 1). Very slight tremors were observed in two female Group III animals on Day 1; tremors were not reported in these cats on Day 0. Very slight to moderate tremors were observed in five Group IV cats (1M, 4F) on Days 1-

3. Tremors were reported for 16 hours post-dosing on Day 0 in all these animals so the findings on Day 1 are likely a continuation of Day 0. In one animal, the tremors continued until the afternoon of Day 3. Polyuria was observed in three Group IV cats on Day 1.

TABLE 4. Clinical signs from Day 1 to Day 14 post-treatment[a]

Clinical sign	Number affected (sex)			
	Control	1X Dose	3X Dose	5X Dose
Tremors	0	0	2 (2F)	5 (1M, 4F)
Soft feces	0	0	3 (1M, 2F)	2 (2M)
Diarrhea	0	0	2 (1M, 1F)	0
Vomiting	0	0	2 (2F)	1 (1M)
Polyuria	0	0	0	3 (2M, 1F)

[a] Data from pages 21-25, MRID 46118301.

B. **BODY WEIGHT AND WEIGHT GAIN**: There was no evidence of a treatment-related effect on body weight or body weight gain during the study (Table 5).

TABLE 5: Mean body weight and body weight gain[a]

Time period	Dose Group							
	Males				Females			
	0	1X	3X	5X	0	1X	3X	5X
Body Weight (kg)								
Day -1[b]	3.9	3.9	4.0	3.6	3.4	2.9	3.0	2.9
Day 7	3.6 ± 0.3	3.7 ± 0.6	3.7 ± 0.5	3.4 ± 0.5	3.2 ± 0.4	2.5 ± 0.4	2.7 ± 0.4	2.8 ± 0.5
Day 14	3.6 ± 0.2	3.8 ± 0.6	3.9 ± 0.4	3.6 ± 0.5	3.1 ± 0.4	2.8 ± 0.3	2.9 ± 0.4	2.9 ± 0.5
Body Weight Gain (kg)[c]								
Day -1 to Day 7	-0.3	-0.2	-0.3	-0.2	-0.2	-0.4	-0.3	-0.1
Day -1 to 14	-0.3	-0.1	-0.1	0	-0.3	-0.1	-0.1	0

[a] Data from page 15, MRID 46118301.
[b] Calculated by the reviewer from data on page 16, MRID 46118301.
[c] Calculated by the reviewer from data on page 15, MRID 46118301.

C. **FOOD CONSUMPTION**: Mean food consumption in Group IV (5X) males was decreased (16%) in comparison to the control group on Day 1 but was increased or comparable to the control for the remainder of the study (Table 6). Food consumption was decreased in all treated females on Day 1 but there was no dose response and no evidence of a treatment-related effect for the study duration.

TABLE 6: Mean food consumption (in grams)[a]

Time period	Dose Group							
	Males				Females			
	0	1X	3X	5X	0	1X	3X	5X
Pretest	66.7 ± 14.7	65.3 ± 23.3	65.3 ± 38.1	69.3 ± 21.6	44.5 ± 18.7	48.0 ± 12.1	62.3 ± 8.3	53.5 ± 22.2
Day 0	60.8 ± 23.7	72.6 ± 15.6	74.2 ± 19.4	77.1 ± 23.4	46.5 ± 22.1	50.1 ± 29.1	58.8 ± 32.8	50.0 ± 17.7
Day 1	47.7 ± 13.0	47.0 ± 28.5	45.6 ± 30.1	40.0 ± 19.3 (84)[b]	38.3 ± 19.3	21.0 ± 18.6 (55)[b]	30.2 ± 16.1 (79)[b]	22.6 ± 22.2 (59)[b]
Day 7	68.7 ± 20.7	70.7 ± 22.1	62.5 ± 28.4	78.8 ± 21.5	55.9 ± 18.0	48.6 ± 17.9	55.1 ± 21.1	62.9 ± 24.3
Day 14	71.4 ± 14.1	68.1 ± 32.3	71.8 ± 34.2	76.0 ± 20.9	53.5 ± 24.0	54.6 ± 15.7	73.2 ± 20.1	83.6 ± 29.7

[a] Data from pages 27-29, MRID 46118301.
[b] (Percentage of control calculated by reviewer).

D. BLOOD ANALYSES:

1. **Hematology**: There was no evidence of a treatment-related effect. The study report notes that hemoglobin and hematocrit values were low in most of the animals throughout the study (Table 7).

TABLE 7: Selected Hematology Parameters[a]

Time Period	Dose Group							
	Males				Females			
	0	1X	3X	5X	0	1X	3X	5X
Hematocrit (%)								
Day -7	28.7 ± 6.4	26.6 ± 3.9	30.9 ± 5.4	25.9 ± 5.3	26.9 ± 3.2	28.3 ± 5.9	25.6 ± 2.5	30.8 ± 4.0
Day 1	25.8 ± 4.5	25.1 ± 4.4	30.6 ± 5.1	24.8 ± 4.3	26.4 ± 4.2	28.0 ± 4.6	25.2 ± 3.5	31.0 ± 5.4
Day 7	29.9 ± 9.2	29.1 ± 4.4	33.4 ± 4.4	24.3 ± 4.3	27.1 ± 4.2	27.8 ± 2.5	26.4 ± 2.2	32.2 ± 6.0
Day 14	28.4 ± 8.1	27.1 ± 4.2	33.4 ± 6.0	23.9 ± 3.7	27.4 ± 5.0	27.7 ± 2.8	24.6 ± 1.9	28.4 ± 7.6
Hemoglobin (g/dL)								
Day -7	12.9 ± 2.9	13.6 ± 1.4	14.7 ± 1.9	13.9 ± 1.8	12.9 ± 0.7	13.7 ± 1.1	12.6 ± 1.1	13.7 ± 1.2
Day 1	11.2 ± 2.3	12.1 ± 1.5	13.7 ± 1.5	12.8 ± 1.4	11.9 ± 1.7	12.7 ± 1.1	12.0 ± 1.3	12.7 ± 0.9
Day 7	10.6 ± 2.6	12.7 ± 1.8	14.2 ± 1.1	11.8 ± 0.9	11.8 ± 1.2	12.4 ± 1.3	12.1 ± 0.6	12.4 ± 1.7

[a] Data from pages 60-72, MRID 46118301.

2. **Clinical chemistry**: On Day 7, there was an increase in ALT (213 IU/L) in Group IV males which was >5x the control value and outside the reference range (28-76 IU/L). The AST value (40 IU/L) in this group was >3x the control value but was within the reference range (5-55 IU/L). Examination of the individual animal data showed that the increases were due to one animal, number 1531, which had an increased ALT (139 IU/L) on Day -7, Day 1 (177 IU/L) and Day 7 (1030 IU/L). AST values for this animal were within the reference range on Days -7 and 1 but were increased (165 IU/L) on Day 7. There were several other animals that had ALT values outside the reference range on Day -7, including two Group I females (80 and 93 IU/L), one Group II male (101 IU/L), one Group III male (78 IU/L), one Group III female (86 IU/L) and one Group IV female (88 IU/L). The value in the Group II male rose to 226 IU/L by Day 7 but values in the other animals remained essentially the same as pre-treatment.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study report concluded that, based on the findings from the in-life observations, the blood analyses, body weight and food consumption, the test substance produced tremors and/or unsteady gait in one or more animals treated at 1X, 3X or 5X the label dose rate, the incidence increasing with dose.

The study report states that 89% of the study animals that exhibited clinical symptoms were anemic with hematocrits below 30%. Glutathione is synthesized in RBCs and is essential for glucuronidation, which is believed to be the pathway for metabolizing pyrethroids. (Phenothrin is a synthetic pyrethroid.) Thus, low RBCs would result in low glucuronidation, which would make anemic animals have a higher probability of exhibiting adverse effects to pyrethroid formulations like Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens.

B. **REVIEWER COMMENTS:** On Day 0, there was evidence of a treatment-related and dose-responsive increase in clinical signs consistent with central nervous system (CNS) toxicity in all treated groups. Central nervous system toxicity is characteristic of excessive pyrethroid exposure. Ataxia was observed in one female at the recommended label dose (1X) at 9 and 10 hours post-dosing. At 3X and 5X the label dose, ataxia and other signs of CNS toxicity, including unsteady gait and tremors, were reported in increasing numbers and duration of effect. Ataxia and/or tremors were observed for the duration of the observation period (12 hours after the last dosing) in five animals in the 5X group and continued until Day 3 in one animal. Salivation on the day of dosing in the 3X and 5X animals was most likely due to oral exposure to the product. Other clinical signs observed from Day 1 to Day 14 in the 3X and 5X groups included soft feces, diarrhea, vomiting and polyuria. Most were isolated occurrences and there was no dose-responsive increase in incidence. It should be noted that tremors were not reported until a minimum of seven hours after the last dose was applied. The Companion Animal Safety Guideline (OPPTS 870.7200) requires hourly observations for at least four hours after the last treatment. Study modification was required in an agreement between the EPA and the Hartz Mountain Corporation due to safety concerns stemming from use of this product in cats and kittens. Reports of adverse reactions submitted to EPA ranged from minor adverse effects including skin irritation or hair loss at the application site and salivation to more serious effects on the nervous system, such as tremors and, in some circumstances, severe full body tremors (convulsion). The time period between application and onset of clinical signs in pet cats was as long as 12 hours in the adverse incident reports. A prior Companion Animal Safety Study (MRID 44864007C) with this Hartz product was classified as unacceptable. Only control and 5X groups were used; this complies with the Companion Animal Safety Study Guideline as long as no toxicity is observed at 5X. In the study, toxicity (excessive salivation, restlessness with signs of discomfort and scratching in adults; decreased body weight and food consumption in adults and kittens) was observed at 5X. Therefore, 1X and 3X doses should have been tested. No CNS signs were observed as in the present study; however, clinical observations on the day of dosing were not extended.. Therefore, signs could have been missed.

In the present study there was no evidence of a treatment-related effect on body weight or body weight gain. Food consumption was decreased in the 5X males and all treated female groups (no dose response) on Day 1 only. No treatment-related changes were noted in the

hematology parameters. On Day 7, there was an increase in ALT levels in the 5X males which was outside the conducting laboratory's reference range. The increase was due to one male (#1531) that had an elevated value pre-treatment which increased with each testing period. The AST value in this animal was also outside the reference range on Day 7. Several other cats in the control and treated groups had pre-treatment ALT values outside the reference range but most maintained that level throughout the study. The increases in liver enzymes (ALT, AST) in cat #1531 after dosing are possibly treatment-related.

The study report postulates that the cats in the study were more susceptible to the effects of the product because they were anemic (HCT <30%). It is proposed that glutathione is synthesized in RBCs and is essential for glucuronidation which is believed to be the pathway for metabolizing pyrethroids. Low RBCs would result in low glucuronidation and thus increase the risk of adverse reactions. This argument is unacceptable for the following reasons:

1. It has not been demonstrated that the cats in the study were anemic. Reference range values from the conducting laboratory were provided for all the hematology and clinical chemistry parameters, except for hematocrit. For that parameter, the range (30-45%) was taken from the *Merck Veterinary Manual, Eighth Edition*. There is no explanation for this substitution. In general, hematocrit values are approximately three times hemoglobin levels. Hemoglobin levels listed in the reference range for the conducting laboratory were 8-16 g/dL. Therefore, the expected hematocrit values would be 24 - 48%, with a lower minimal value than that cited from the *Merck Veterinary Manual*. Values for hematocrit in other veterinary texts also have lower minimum levels. In *Veterinary Clinical Pathology, Fourth Edition*, the hematocrit and hemoglobin ranges are 24-45% and 8-15 gm/dL, respectively. In *Kirk's Current Veterinary Therapy, XIII*, hematocrit and hemoglobin ranges are 24-45% and 8-14 gm/dL, respectively. Based on weight-of-the-evidence considerations presented in this DER, HED concludes that some of the cats used in this study were anemic (HCT < 24%), although most animals were within the lower normal range for hematocrit from most sources (>24%). A comparison of the hematocrit values for individual cats displaying clinical signs shows that the following results for the various Groups: (Group II: 26.6%, Group III: 23.9, 21.6, 24.3, 29.4, 21.0, 26.7, 33.5%, Group IV: 20.2, 27.6, 25.8, 21.2, 27.6, 39.1, 25.6, 27.6, 36.3, 29.6%). Therefore, it can be seen that both anemic and normal cats displayed clinical signs at comparable incidences.

2. No references were provided to confirm that a decrease in RBCs will alter glucuronidation metabolism of pyrethroids. Glutathione in erythrocytes functions to protect the red blood cell from oxidative stress.

3. The CNS effects observed in the study are consistent with pyrethroid toxicity in cats and have been reported as adverse reactions in pet cats. Analysis of reports of cats exposed to the Hartz product (Reg. No. 2596-148) showed that in 41% of all adverse incidents neurological signs (defined as tremors, seizures or convulsions) were

observed in cases with severity categories D-A (death), D-B (major effect) and D-C (moderate effect).[2]

It is concluded that Hartz Advanced Care Brand Flea and Tick Drops Plus+ for Cats and Kittens produces clinical signs of toxicity in adult cats when applied at the recommended dose.

[2] June 6, 2002 Memorandum from Virginia A. Dobozy to Marion Johnson/Ann Sibold.

C. STUDY DEFICIENCIES:

1. The original protocol required that hourly observations on the day of dosing would be continued until no adverse effects were observed. The protocol was amended on the day of dosing to discontinue the observations at 12 hours following the final dose. No explanation for this change was provided.

2. The Companion Animal Safety Study Guideline requires that a vehicle control containing 5X the inert ingredients should be tested. In the present study, a placebo control was used.

3. The study tested the product only on adult cats; it is registered for use on cats and kittens.

DATA FOR ENTRY INTO ISIS

Companion Animal Safety Study - cats (870.7200)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
069005	46118301	companion animal safety	cat	14 days	topical	topical	1X-5X label dose	0, 1X, 3X, 5X label dose	not established	1X	central nervous system	Toxicity