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

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: January 10, 2006

MEMORANDUM

Subject: EPA File Symbol: 2596-RLU - HARTZ REFERENCE 121
DP Barcode: D318905
Decision No.: 357507
PC Code: 044312 DINOTEFURAN (CAS #165252-70-0)

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

Byron T. Backus 1-10-2006
SUR 1/13/2006

To: Rita Kumar/Daniel Kenny RM 01
Insecticide-Rodenticide Branch
Registration Division (7505C)

Applicant: The Hartz Mountain Corporation

FORMULATION DECLARATION FROM LABEL:

Chamber A

Permethrin (CAS #52645-53-1).....45.00%
Other Ingredients:.....55.00%

Chamber B

Dinotefuran (CAS #165252-70-0).....14.85%
[Sumilarv] 2-[1-Methyl-2-(4-phenoxyphenoxy)
ethoxy] pyridine (CAS #95737-68-1).....1.48%
Other Ingredients:.....83.67%

ACTION REQUESTED:

The Risk Manager requests: "Please review two companion animal safety studies to support registration of this new dog spot-on product with multiple active ingredients. This is a new use for dinotefuran. MRID #46552707 [and #46552708]..."

BACKGROUND:

This package includes two companion animal safety studies (adult dogs and 7-week-old puppies) in MRIDs 46552707 and 46552708, respectively.

COMMENTS AND RECOMMENDATIONS:

1. The companion animal safety study with adult dogs (MRID 46552707) has been classified as acceptable, while the companion animal safety study with 49-55 day old puppies (MRID 46552708) is currently classified as not acceptable. The proposed use on dogs of 6 months or older is supported, although it is noted that the doses for each of the weight classes were not specified on the labels received by TRB. The registrant should indicate the appropriate doses for each of these weight classes, and these should be consistent with and supported by the doses used in the two companion animal safety studies for this formulation.

2. With respect to the companion animal safety study (OPPTS 870.7200) in MRID 46552708 with 49-55 day old puppies, TRB has concluded that this study may demonstrate an adequate margin of safety (5x or reasonably close to 5x, as the tremors and ataxia observed in two of the three 5x puppies were reasonably transient). Currently, this study does not support the proposed use of this product at this dosage level on 8-week old puppies. as a number of points need to be addressed by the registrant, as indicated in the executive summary which follows:

   This companion animal safety study (MRID 46552708) utilized beagle puppies (source: Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland; males: 49-55 days on Day 0, 1.5-2.6 kg on Day 0; females: 49-55 days on Day 0, 1.2-2.9 kg on Day 0). There were 4 groups; Group 1 (controls) contained 7 males and 5 females; Groups 2, 3 and 4 (1X, 3X and 5X, respectively) each contained 6 males and 6 females. To facilitate pup supply and to replace pups that were withdrawn, it was necessary to have four batches of puppies with four separate test item/control item administration dates (Dec. 1, 2004, Dec. 29, 2004, Dec. 30, 2004 and Jan. 20, 2005).

   Each Group 1 puppy received 5 separate applications (spaced about 1 hour apart), with each application consisting of 0.70 mL control item 1 followed by 0.30 mL control item 2. Each Group 2 puppy received a single application (0.70 mL test item 1 followed by 0.30 mL test item 2); each group 3 puppy received 3 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals. Each group 4 puppy received 5 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals.

   Test item 1 (Chamber A on the proposed label) has a label declaration of 45% permethrin, and test item 2 (Chamber B on the proposed label) has a label declaration of 14.85% dinotefuran and 1.48% Sumilarv. Taking into account the specific gravities and chemical analyses of these two test items, each 1X puppy in this study was exposed to 343.5 mg

permethrin, 47 mg dinotefuran and 5 mg sumilarv (nylar).

Adverse nervous system effects (ataxia and tremors) were seen in three of the twelve pups assigned to Group 4 (5X). Tremors were present in two of these puppies at +4 hours after the final treatment and had resolved 2 hours later. Ataxia was also present in one of these puppies at the +5 hours assessment but had resolved at the +6 hours assessment. The third Group 4 puppy (#56857) had ataxia at the morning assessment on Study Day 1, which had resolved at the morning assessment on Study Day 3; this puppy also also showed lethargy at the am and pm observations on both Days 1 and 2, and at the am for Days 3 and 5. Ataxia was also observed in one Group 2 puppy on one occasion (Study Day 0 at +2 hours after treatment). There were no other incidences of ataxia or tremors detected in any other animal during the study.

The same Group 4 puppy (#56857) showing ataxia and lethargy also had a sporadically abnormal site of spot-on application from Day 1 through Day 6, and also had post treatment pruritus during this period of time.

Ocular discharge was a relatively common finding, and was seen in a number of puppies before exposure. Post exposure, this was observed most frequently in control puppies, with a possible negative dose relationship (number of post treatment occasions observed: Group 1: 89; Group 2: 47; Group 3: 63; Group 4: 28).

Abnormal (presumably loose) faeces were observed for some puppies from all groups even before exposure to the test materials. However, after treatment there was a dose relationship, as this finding was observed most frequently in Group 4 puppies with the following group incidences: Group 1 (controls): 38; Group 2: 35; Group 3: 44; Group 4: 111. There were increased incidences of abnormal faeces in Group 4 immediately following treatments and continuing sporadically throughout during the subsequent 14-day observation period (incidences from Day 1 through 14: Group 1: 33; Group 2: 29; Group 3: 40; Group 4: 95). This reviewer notes that a similar finding was not observed in the adult dog study with this product.

There was no indication of an effect on any of the hematology or clinical chemistry parameters, and there were no symptoms or other indications of systemic toxicity.

The proposed label received by this reviewer indicates this product will be applied at three dosage rates depending on body weight: one application rate will be for dogs or puppies weighing less than 10 lbs; a second will be for dogs/puppies weighing 11 to 20 lbs; and a third for dogs/puppies weighing 21 to 55 lbs. However, the proposed label does not specify application rates. The 1X application rate in this study was 0.7 mL of Test Material 1 (Chamber A, label declaration of 45% permethrin) and 0.3 mL Test Material 2 (Chamber B, label declaration of 14.85% dinotefuran and 1.48% Sumilarv). The 1X application rate in the adult dog study (MRID 46552707) was 2.5 mL of Test Material 1 and 1.0 mL of Test Material 2 [a 0.71 to 0.29 ratio, reasonably similar to the 0.70 to 0.30 in the puppy study] for dogs ranging from 10.0 to 21.9 kg (22 to 48 lbs). This leaves open the question of the application rate for dogs/puppies weighing 11 to 20 lbs.

TRB concludes that this companion animal safety study (OPPTS 870.7200) <u>may</u> demonstrate an adequate margin of safety (5x or reasonably close to 5x, as the tremors and ataxia observed in two of the three 5x puppies were reasonably transient). However, the

following points need to be addressed by the registrant:

TRB's major concern is the report of ataxia in Group 2 (1X) puppy 48334 at two hours postdose. One thing that could be helpful would be any additional observational information regarding this occurrence, particularly what was seen by the observer(s) that resulted in this diagnosis.

The increased incidence (relative to other groups) of loose faeces in the 5x puppies should be clarified (for example, were the faeces simply loose - which may be normal for puppies of this age and under these conditions - or were they watery or otherwise indicative of possible toxicity or pathology).

Puppy #15101 was in Group 4 (5X), and was diagnosed with gastroenteritis and euthanised on Study Day 5. This puppy was evidently replaced with batch 4 puppy #08858. However, the statement is made (p. 39) that: "Three pups, which did not complete the study on health grounds, were replaced with three pups from batch 4." There is no information as to why the two other puppies (one, in Group 1, was replaced with puppy #32512; the other, in Group 3, was replaced with puppy #15298) were removed from this study. Relevant information on all 3 puppies should be provided, including any observational data such as responses following treatment.

The statement is made (p. 31) regarding clinical assessments that: "Where adverse reactions were observed at the +4 hour clinical assessment on Study Day 0...then further assessments [were] continued on that particular animal at hourly intervals [actually up to +6 hours] until no further reactions were observed..." Two Group 2 puppies (#59295 and #48334) had extended assessments to 6 hours; #59295 had pruritus at +4 hours, but it is not evident from the clinical assessment sheets (p. 46-95 of MRID 46552708) why observations continued to be made on #48334 (pruritus was recorded at +5 hours for this puppy, but not at +4 hours; and this was also the 1X puppy which showed ataxia at +2 hours). Since this puppy was showing no symptoms at +4 hours, why were additional observations made on it?

TRB concludes that this companion animal safety study (OPPTS 870.7200) <u>may</u> demonstrate an adequate margin of safety (slightly below 5X) between the exposure associated with the dosages used in this study (0.7 mL of a 45% Permethrin formulation and 0.3 mL of a 14.85% S-1638 and 1.5% Nylar formulation) for 8-week old puppies weighing approximately 2.0 kg. However, the questions indicated above need to be adequately addressed by the registrant. Currently, this study does not support the proposed use of this product at this dosage level on 8-week old puppies.

3. The companion animal safety study with adult dogs (MRID 46552707) has been classified as acceptable. However, the proposed label received by TRB indicates three weight classes of dogs and puppies (< 10 lbs, 11- 20 lbs, 21- 55 lbs), but does not specify dosages. The registrant should indicate the appropriate doses for each of these weight classes, and these should be supported by the doses used in the two companion animal safety studies for this formulation.

   The following is the executive summary from the DER for this study:

   In a companion animal safety study (MRID 46552707) with adult Beagle dogs, 4 groups, each with 12 (6/sex) adult (age: 12-100 months) dogs (source: Charles River Laboratories

BioLabs Europe's colony at Glenamoy, Ireland; Males: 13.7-21.9 kg; Females: 10.0-17.6 kg); Dogs were treated at 0X (5 applications of 2.5 mL of control item 1 and 1.0 mL of control item 2); 1X (1 application each of 2.5 mL of test item 1 and 1.0 mL of test item 2); 3X (3 applications of 2.5 mL of test item 1 and 1.0 mL of test item 2); and 5X (5 applications 2.5 mL of test item 1 and 1.0 mL of test item 2). The tip of the syringe was used to part the dog's hair so that the sample was applied to the skin at each of the three spots. Each administration of both test items was 1 hour apart.

Most dogs were dosed with Permethrin (45%) batch no. TS12441 (assaying 44.32% total Permethrin) and S-1638 /Nylar batch no. TS12462 (assaying 15.45% S-1638 and 1.56% Nylar). However, as there was insufficient material received by the laboratory, two 3X dogs were dosed with Permethrin (45%) batch no. TS12478 (44.82% total Permethrin) and S-1638 /Nylar TS 12479 (15.21% S-1638 and 1.57% Nylar).

In order to facilitate study management, the animals were divided into two sets, each with 28 dogs (14 per sex), with two separate test/control item administration dates (Set 1- 25 Aug 2004; Set 2- 26 Aug 2004). Animal Nos 07049 and 14818 were dosed on 1 Sep 2004 since there was not enough test item available to dose all animals in Set 2. Once the control or test item was applied, each dog was held in an upright position for at least 2 minutes to prevent any potential loss of applied material.

Clinical assessments were carried out on each dog prior to the first treatment, and at approximately 1, 2, 3 and 4 and 5 hours where applicable after the final treatment on study day 0. Dogs receiving more than one treatment were also evaluated 10 minutes before each treatment.

According to study directions test items 1 and 2 were applied to three sites, namely Site 1- the intrascapular area, Site 2- the anterior lumbar area, and Site 3- the posterior lumbar area.

Individual body weights were measured before feeding on Days -15, -2, 0, 7 and 14. Individual food consumption was measured on a daily basis for Days -7, -6, -4 and then daily thereafter through Day 14 (values represented the amount of food consumed during the previous 24 hours). Blood samples were taken from each animal on Study Day -14, Study Day 1, and Study Day 7. Blood samples were collected on Study Day 14 for Animal #11289 (2x), Animal # 50342 (3x), Animal # 50573 (3x) and Animal # 89074 (5x). This is a deviation from the study plan for which there was no amendment. All blood samples were collected following an overnight fast.

There was no mortality. Clinical observations seen included abnormal (loose) faeces, abnormal site of spot-on application and vomiting.

A number of dogs (including seven of 12 in the control group and seven of 12 in the 5X group) had very low food consumption values (< 30g) for Day 1. As the controls and 5X groups showed the greatest reduction in mean food consumption, this may have been an effect associated with exposure to one or more inerts in this formulation, and/or to the stress of handling during five applications. Three of the 1X dogs showed a similar reduction in food consumption (< 30g) for Day 1. While some individual puppies in the puppy study (MRID 46552708) showed a reduced food consumption for Day 1, with no indication of a dose-related effect, adult dogs showed reduced mean food consumption on Day 7 (presumably

associated with fasting and/or the stress of blood collection). The Day 7 effect was not as pronounced as that of Day 1.

Cosmetic effects were observed in all dogs, and included matting, greasy appearance, clumping, spiking, discolouration, and deposits. The cosmetic effects had disappeared by Study Day 5 (Group 1, control), Study Day 5 (Group 2, 1x) except for Animal No. 89782 which had a greasy appearance until Study Day 12, Study Day 8 (Group 3, 3x), Study Day 11 (Group 4, 5x).

Overall, a number of dogs had minor weight changes from between Study Day -15 and Study Day 14 (ranging from a loss of about 0.1 kg to 1.8 kg), with no indication of any dose-related trend. Similar weight loss has been observed previously in adult dogs that are individually housed. There was no significant difference in body weight between animals assigned to any of the groups.

There were no significant differences ($P>0.05$) between the treatment groups and control group with respect to clinical chemistry and/or hematological parameters. There were no symptoms or other indications of systemic toxicity.

TRB concludes that this companion animal safety study (OPPTS 870.7200) demonstrates an adequate margin of safety (at least 5X) between the exposure associated with a use application of 2- 5 mL test material (45% Permethrin) and 1.0 mL test material 2 (14.85% Dinotefuran and 1.48% Nylar) for this formulation in adult dogs weighing between 10.0 and 21.9 kg and that at which significant systemic effects may occur. It is noted that the proposed label received by TRB indicates three weight classes of dogs and puppies (< 10 lbs, 11- 20 lbs, 21- 55 lbs), but does not specify dosages. The registrant should indicate the appropriate doses for each of these weight classes, and these should be consistent with and supported by the doses used in the two companion animal safety studies for this formulation.

EPA Primary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Technical Review Branch, Registration Division (7505C) Date 1-10-2006
EPA Secondary Reviewer: John Redden, M.S. Signature:
Technical Review Branch, Registration Division (7505C) Date 1/13/2006

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - 7-Week Old Puppies OPPTS 870.7200
PC CODES: 109701 (Permethrin), 044312 (Dinotefuran), 129032 (Nylar)
DP BARCODE: D318905 RISK MANAGER: (EPA): 01 DECISION NO.: 357507

PRODUCT AND TEST MATERIALS: According to the proposed label received by TRB, the product as proposed for registration consists of the liquid contents of two chambers. Chamber A contains a 45% permethrin formulation (test item 1), and Chamber B contains an S1638 (14.85%) + Nylar (1.48%) formulation (test item 2). In this study an application for 1X animals (7 to 8 week old puppies weighing 1.2 to 2.9 kg) consisted of 0.7 mL test item 1 (a clear yellow liquid with a specific gravity of 1.11 g/mL assaying 44.21% Peremthrin) and 0.3 mL test item 2 (a colorless liquid with a specific gravity of 1.08 assaying 14.59% S-1638 [Dinotefuran] and 1.52% Nylar).

CITATION: Doherty, P. (2005) Tolerance of an Experimental Flea and Tick Dermal Treatment when Topically Administered to Pups Seven Weeks (49-55 Days) Old, at 1 x, 3 x and 5 x the Recommended Dose. Study No. USA004\04-003; Hartz Test #1767. Unpublished study prepared by Charles River Laboratories BioLabs Europe, Ireland. Study Completion Date: 13 April 2005; MRID 46552708.

SPONSOR: The Hartz Mountain Corporation

EXECUTIVE SUMMARY: This companion animal safety study (MRID 46552708) utilized beagle puppies (source: Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland; males: 49-55 days on Day 0, 1.5-2.6 kg on Day 0; females: 49-55 days on Day 0, 1.2-2.9 kg on Day 0). There were 4 groups; Group 1 (controls) contained 7 males and 5 females; Groups 2, 3 and 4 (1X, 3X and 5X, respectively) each contained 6 males and 6 females. To facilitate pup supply and to replace pups that were withdrawn, it was necessary to have four batches of puppies with four separate test item/control item administration dates (Dec. 1, 2004, Dec. 29, 2004, Dec. 30, 2004 and Jan. 20, 2005).

Each Group 1 puppy received 5 separate applications (spaced about 1 hour apart), with each application consisting of 0.70 mL control item 1 followed by 0.30 mL control item 2. Each Group 2 puppy received a single application (0.70 mL test item 1 followed by 0.30 mL test item 2); each group 3 puppy received 3 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals. Each group 4 puppy received 5 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals.

Test item 1 (Chamber A on the proposed label) has a label declaration of 45% permethrin, and test item 2 (Chamber B on the proposed label) has a label declaration of 14.85%

dinotefuran and 1.48% Sumilarv. Taking into account the specific gravities and chemical analyses of these two test items, each 1X puppy in this study was exposed to 343.5 mg permethrin, 47 mg dinotefuran and 5 mg sumilarv (nylar).

Adverse nervous system effects (ataxia and tremors) were seen in three of the twelve pups assigned to Group 4 (5X). Tremors were present in two of these puppies at +4 hours after the final treatment and had resolved 2 hours later. Ataxia was also present in one of these puppies at the +5 hours assessment but had resolved at the +6 hours assessment. The third Group 4 puppy (#56857) had ataxia at the morning assessment on Study Day 1, which had resolved at the morning assessment on Study Day 3; this puppy also also showed lethargy at the am and pm observations on both Days 1 and 2, and at the am for Days 3 and 5. Ataxia was also observed in one Group 2 puppy on one occasion (Study Day 0 at +2 hours after treatment). There were no other incidences of ataxia or tremors detected in any other animal during the study.

The same Group 4 puppy (#56857) showing ataxia and lethargy also had a sporadically abnormal site of spot-on application from Day 1 through Day 6, and also had post treatment pruritus during this period of time.

Ocular discharge was a relatively common finding, and was seen in a number of puppies before exposure. Post exposure, this was observed most frequently in control puppies, with a possible negative dose relationship (number of post treatment occasions observed: Group 1: 89; Group 2: 47; Group 3: 63; Group 4: 28).

Abnormal (presumably loose) faeces were observed for some puppies from all groups even before exposure to the test materials. However, after treatment there was a dose relationship, as this finding was observed most frequently in Group 4 puppies with the following group incidences: Group 1 (controls): 38; Group 2: 35; Group 3: 44; Group 4: 111. There were increased incidences of abnormal faeces in Group 4 immediately following treatments and continuing sporadically throughout during the subsequent 14-day observation period (incidences from Day 1 through 14: Group 1: 33; Group 2: 29; Group 3: 40; Group 4: 95). This reviewer notes that a similar finding was not observed in the adult dog study with this product.

There was no indication of an effect on any of the hematology or clinical chemistry parameters, and there were no symptoms or other indications of systemic toxicity.

The proposed label received by this reviewer indicates this product will be applied at three dosage rates depending on body weight: one application rate will be for dogs or puppies weighing less than 10 lbs; a second will be for dogs/puppies weighing 11 to 20 lbs; and a third for dogs/puppies weighing 21 to 55 lbs. However, the proposed label does not specify application rates. The 1X application rate in this study was 0.7 mL of Test Material 1 (Chamber A, label declaration of 45% permethrin) and 0.3 mL Test Material 2 (Chamber B, label declaration of 14.85% dinotefuran and 1.48% Sumilarv). The 1X application rate in the adult dog study (MRID 46552707) was 2.5 mL of Test Material 1 and 1.0 mL of Test Material 2 [a 0.71 to 0.29 ratio, reasonably similar to the 0.70 to 0.30 in the puppy study] for dogs ranging from 10.0 to 21.9 kg (22 to 48 lbs). This leaves open the question of the application rate for dogs/puppies weighing 11 to 20 lbs.

TRB concludes that this companion animal safety study (OPPTS 870.7200) <u>may</u>

demonstrate an adequate margin of safety (5x or reasonably close to 5x, as the tremors and ataxia observed in two of the three 5x puppies were reasonably transient). However, the following points need to be addressed by the registrant:

TRB's major concern is the report of ataxia in Group 2 (1X) puppy 48334 at two hours postdose. One thing that could be helpful would be any additional observational information regarding this occurrence, particularly what was seen by the observer(s) that resulted in this diagnosis.

The increased incidence (relative to other groups) of loose faeces in the 5x puppies should be clarified (for example, were the faeces simply loose - which may be normal for puppies of this age and under these conditions - or were they watery or otherwise indicative of possible toxicity or pathology).

Puppy #15101 was in Group 4 (5X), and was diagnosed with gastroenteritis and euthanised on Study Day 5. This puppy was evidently replaced with batch 4 puppy #08858. However, the statement is made (p. 39) that: "Three pups, which did not complete the study on health grounds, were replaced with three pups from batch 4." There is no information as to why the two other puppies (one, in Group 1, was replaced with puppy #32512; the other, in Group 3, was replaced with puppy #15298) were removed from this study. Relevant information on all 3 puppies should be provided, including any observational data such as responses following treatment.

The statement is made (p. 31) regarding clinical assessments that: "Where adverse reactions were observed at the +4 hour clinical assessment on Study Day 0...then further assessments [were] continued on that particular animal at hourly intervals [actually up to +6 hours] until no further reactions were observed..." Two Group 2 puppies (#59295 and #48334) had extended assessments to 6 hours; #59295 had pruritus at +4 hours, but it is not evident from the clinical assessment sheets (p. 46-95 of MRID 46552708) why observations continued to be made on #48334 (pruritus was recorded at +5 hours for this puppy, but not at +4 hours; and this was also the 1X puppy which showed ataxia at +2 hours). Since this puppy was showing no symptoms at +4 hours, why were additional observations made on it?

TRB concludes that this companion animal safety study (OPPTS 870.7200) <u>may</u> demonstrate an adequate margin of safety (slightly below 5X) between the exposure associated with the dosages used in this study (0.7 mL of a 45% Permethrin formulation and 0.3 mL of a 14.85% S-1638 and 1.5% Nylar formulation) for 8-week old puppies weighing approximately 2.0 kg. However, the questions indicated above need to be adequately addressed by the registrant. Currently, this study does not support the proposed use of this product at this dosage level on 8-week old puppies.

<u>COMPLIANCE:</u> Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements were present.

I. MATERIALS

A. MATERIALS

1. Test material 1: Permethrin (label declaration 45%), containing (assay date Nov. 4, 2004; see p. 200 of MRID 46552708) 44.21% Permethrin.
   Description: A colorless liquid with a specific gravity of 1.11 g/mL.
   Sample ID.: 2071/43/2 T.S. 12529
   Storage: Stored between 14 and 21°C in the dark.

   Control Item 1: A formulation without Permethrin (see p. 198 of MRID 46552708)
   Description: A colorless liquid
   Batch No.: 2071/43/1 T.S. 12527
   Storage: Stored between 14 and 21°C in the dark.

   Test material 2: S-1638 (label declaration 14.85%) and Sumilarv (also known as Nylar with a label declaration of 1.48%), containing (assay date Nov. 4, 2004; see p. 201 of MRID 46552708) 14.59% S-1638 and 1.52% Nylar.
   Description: A colorless liquid with a specific gravity of 1.08 g/mL.
   Sample ID.: 2071/43/4 T.S. 12530
   Storage: Stored between 14 and 21°C in the dark.

   Control Item 2: A formulation without S-1638 and without Nylar
   Description: A colorless liquid
   Batch No.: 2071/43/1 T.S. 12528
   Storage: Stored between 14 and 21°C in the dark.

2. Administration: Topical (spot-on) on Day 0. An application consisted of 0.70 mL Test Material 1 followed by 0.30 mL Test Material 2 (or, in the case of controls: 0.70 mL Control Item 1 followed by 0.30 mL Control Item 2). If more than one application was administered then they were spaced at one hour intervals.

3. Test animals
   Species: Dog (puppy)
   Breed: Beagle
   Ages and weights at study initiation (Day 0): Males: 49-55 days; 1.5 to 2.5 kg on Day -2; Females: 49-55 days; 1.2 to 2.9 kg.
   Source: Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland
   Housing: From p. 20 of MRID 46552708: "Pups were housed with the dam until Study Day -4 when pups were individually housed... From Study Day -7 to Study Day -5 inclusive pups were separated from the dam for a period of two to three hours per day to prepare them for weaning."
   Diet: Standard commercially available pup food [Pedigree pup food] at the recommended rate of approximately 400 g/pup/day. From p. 20 of MRID 46552708: "Between Study Day -14 and Study Day -5 inclusive pups were fed three times daily... The 400 g was divided between the three feeds. From

Study Day -4 to the end of the study pups assigned to Batch 1 [Day of dosing: Dec. 1, 2004] were fed once daily in the afternoon between 12:00 and 15:00... For Batches 2, 3 and 4 [Dates of dosing: Dec. 29, 2004; Dec. 30, 2004; and Jan. 20, 2005] pups were fed, from Study Day -4 to the end of the study, twice daily, once in the morning between 09:30 and 10:30 and once in the afternoon between 15:30 and 16:30. .. The diet not consumed was weighed and recorded...to give the total quantity of diet not consumed for each twenty four hour period... Diet was withdrawn from all animals, at least 5 hours on Study Days 0 and 6, in order to facilitate fasting prior to blood sampling. Diet was withdrawn, for at least 5 hours on Study Day 13 from any animals due to be blood sampled on Study Day 14. In all these cases the weight of the diet withdrawn was recorded as diet not consumed for that day."

Water: "potable water," *ad libitum* via nipple drinkers.

Environmental conditions:

Temperature: 18-22°C

Humidity: 39-60%

Air changes: Not specified.

Photoperiod: Not specified.

Acclimation period: At least 14 days.

II. STUDY DESIGN

A. IN LIFE DATES

Dosing: 1 Dec. 2004 (Batch 1); 29 Dec. 2004 (Batch 2); 30 December 2004 (Batch 3); 20 Jan. 2005 (Batch 4). Last Observational Day (Study Day 14): 15 Dec. 2004 (Batch 1); 12 Jan. 2005 (Batch 2); 13 Jan. 2005 (Batch 3); 03 Feb. 2005 (Batch 4).

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

From p. 15: "To facilitate pup supply and to replace pups that were withdrawn, it was necessary to have four batches with four separate test item/control item administration dates..."

Pups were assigned to the study groups on Study Day -2. From p. 18: "All pups that met the inclusion criteria were ranked within sex in order of decreasing bodyweight. Where pups of the same bodyweight occurred they were ranked in order of decreasing animal number... The male pups in each batch were listed in replicates of four consecutively-ranked pups as far as possible. Within each replicate, one pup was assigned to each of the four study groups using random order numbers..." Table 1, below (taken from information on p. 43 of MRID 46552708) shows the numbers of puppies/sex/dose/batch:

TABLE 1. Number of Puppies/Sex/Dose/Batch					
		Number of Puppies	Number of Males	Number of Females	Date of Application (Study Day 0)
Placebo Control (1)	Batch 1	4	2	2	Dec. 1, 2004
	Batch 2	4	2	2	Dec. 29, 2004
	Batch 3	3	2	1	Dec. 30, 2004
	Batch 4	1	1	0	Jan. 20, 2005
	Total	12	7	5	
Group 2 (1X)	Batch 1	4	2	2	Dec. 1, 2004
	Batch 2	4	2	2	Dec. 29, 2004
	Batch 3	4	2	2	Dec. 30, 2004
	Batch 4	0	0	0	Jan. 20, 2005
	Total	12	6	6	
Group 3 (3X)	Batch 1	4	2	2	Dec. 1, 2004
	Batch 2	4	2	2	Dec. 29, 2004
	Batch 3	3	2	1	Dec. 30, 2004
	Batch 4	1	0	1	Jan. 20, 2005
	Total	12	6	6	
Group 4 (5X)	Batch 1	3	2	1	Dec. 1, 2004
	Batch 2	4	1	3	Dec. 29, 2004
	Batch 3	4	2	2	Dec. 30, 2004
	Batch 4	1	1	0	Jan. 20, 2005
	Total	12	6	6	

Information derived from Table 1, p. 43, of MRID46552708.

The statement is made (p. 39 of MRID 46552708) that: "Three pups, which did not complete the study on health grounds, were replaced with three pups from batch 4." As there were only three puppies in batch 4, these represented replacements.

TABLE 2. Study design						
		Number of Puppies	Number of Applica-tions	Mean puppy weight ± S.D. (Kg)[a]	Cumulative Amount of Test Material 1 Applied (mL)	Cumulative Amount of Test Material 2 Applied (mL)
Placebo Control (1)	Male puppies	7	5	1.91 ± 0.18	3.5[b]	1.5[c]
	Female puppies	5	5	1.96 ± 0.36	3.5[b]	1.5[c]
	Combined sexes	12	5	1.93 ± 0.25	3.5[b]	1.5[c]
Group 2 (1X)	Male puppies	6	1	2.05 ± 0.31	0.7	0.3
	Female puppies	6	1	1.95 ± 0.33	0.7	0.3
	Combined sexes	12	1	2.00 ± 0.31	0.7	0.3
Group 3 (3X)	Male puppies	6	3	2.15 ± 0.47	2.1	0.9
	Female puppies	6	3	1.88 ± 0.54	2.1	0.9
	Combined sexes	12	3	2.02 ± 0.50	2.1	0.9
Group 4 (5X)	Male puppies	6	5	1.97 ± 0.41	3.5	1.5
	Female puppies	6	5	1.93 ± 0.55	3.5	1.5
	Combined sexes	12	5	1.95 ± 0.47	3.5	1.5

Individual body weights given on p. 44 of MRID 46552708; the means (with standard deviations) were calculated by this reviewer from these data.

[a] Based on Day 0 bodyweights.

[b] Control Item 1

[c] Control Item 2

Application was as follows (from p. 27 of MRID 46552708): "At each dosing timepoint for Group 1(Control), Group 2 (1x), Group 3 (3x) and Group 4 (5x) the control item combination or test item combination as appropriate was applied by the following method: One 1 mL syringe was filled with 0.70 mL of control item 1/test item 1 and one 1 mL syringe was filled with 0.30 mL of control item 2/test item 2. There were 3 sites of application. Site 1 was the intrascapular area, site 2 was the anterior lumbar region and site 3 was the posterior lumbar region. Test items/control items were applied to each site in the following order; approximately 0.25 mL of control item 1/test item 1 was applied to site 1, approximately 0.25 mL of control item 1/test item 1 was applied to site 2 and approximately 0.20 mL of control item 1/test item 1 was applied to site 3. After successful application of control item 1/test item 1, approximately 0.1 mL of control item 2/test item 2 was applied to site 1, 2 and 3 in that order. The tip of the syringe was used to part the pups' hair so that the sample was applied to the skin at each of the tree spots. Contact with the pups' eyes and mouth was avoided. This route of application is the route which will be used for the final product. Once the control items/test items had been applied, the pup was held in an upright position for a period of at least 2 minutes, to prevent any potential loss of applied material..."

C. DOSE SELECTION RATIONALE

From p. 29 of MRID 46552708: "The objective of the study was to evaluate the tolerance of an experimental flea and tick dermal treatment when topically administered to pups seven weeks (49 to 55 days) old, at 1 x, 3 x and 5 x the recommended dose."

The proposed label received by this reviewer indicates this product will be applied at three dosage rates depending on body weight: one application rate will be for dogs or puppies weighing less than 10 lbs; a second will be for dogs/puppies weighing 11 to 20 lbs; and a third for dogs/puppies weighing 21 to 55 lbs. However, the proposed label does not specify application rates. The 1X application rate in this study was 0.7 mL of Test Material 1 (Chamber A, label declaration of 45% permethrin) and 0.3 mL Test Material 2 (Chamber B, label declaration of 14.85% dinotefuran and 1.48% Sumilarv). The 1X application rate in the adult dog study (MRID 46552707) was 2.5 mL of Test Material 1 and 1.0 mL of Test Material 2 [a 0.71 to 0.29 ratio, reasonably similar to the 0.70 to 0.30 in the puppy study] for dogs ranging from 10.0 to 21.9 kg (22 to 48 lbs). This leaves open the question of the application rate for dogs/puppies weighing 11 to 20 lbs.

The proposed directions for use include the statement: "Do not reapply for 30 days."

D. EXPERIMENTAL DESIGN

From p. 30 of MRID 46552708: "Each pup was examined by a Veterinary Surgeon on Study Day -14 (-13, Batch 4 only) and on Study Day -2/-3. Only healthy pups as assessed by these veterinary examinations were included in the study... General health observations were carried out on each pup twice daily (morning and afternoon) from Study Day -14 to Study Day -1, with at least 4 hours between each observation... No general health observations were performed from Study Day 0 to Study Day 14 inclusive, as clinical assessments were performed...

"Clinical assessments were carried out on all pups prior to the first treatment and at 1 hour (± 5 minutes), 2 hours (± 10 minutes), 3 hours (± 10 minutes), 4 hours (± 10 minutes), 5 hours (± 10 minutes) and 6 hours (± 10 minutes), where applicable, after the final treatment on Study Day 0, by a veterinary surgeon. For pups and assigned to Groups 1, 3 and 4, clinical assessments were also carried out by a veterinary surgeon within 10 minutes prior to the second, third, fourth and fifth dosings, where relevant. Where adverse reactions were observed at the +4 hour clinical assessment on Study Day 0, in any group, then further assessments continued on that particular animal at hourly intervals until no further reactions were observed...

From p. 32: "In addition, clinical assessments were performed by a veterinary surgeon twice daily on all pups assigned to Groups 1, 2, 3 and 4 from Study Day 1 to Study Day 14 inclusive, once in the morning and once in the afternoon, with at least 4 hours between assessments... The veterinary surgeon performing the clinical assessments observed each animal for at least 1 minute for the presence (score "1") or absence (score "0") of the following parameters: ...Lethargy, ataxia, recumbency, paralysis, coma, pruritus, hyperactivity, tremors, convulsions, abnormal mydriasis, abnormal miosis, corneal opacity, dyspnoea, tachypnoea, coughing, abnormal

salivation, vomiting, abnormal mucous membranes, ocular discharge, nasal discharge, cardiovascular changes, abnormal faeces (where present), abnormal urine (where present), abnormal coat condition, abnormal site of spot-on application. For every parameter given a score of "1", a brief comment, where applicable, was made to describe the abnormal assessment (e.g. abnormalities at the site of spot-on application may include oedema, erythema, alopecia, pruritus). If the abnormality did not change or deteriorate the initial comment was deemed sufficient.

"When the site of spot-on application showed any cosmetic effect after treatment, this was considered clinically normal. Cosmetic effects included any discolouration, stiffness causing the hair to stand up (spiking), stiffness with the hair sticking together (clumping), tangling of the hair (matting), greasy appearance or any deposits. Where cosmetic effects were present, they were recorded."

From p. 33: "Each animal was weighed, before feeding, on Study Days -14 (-13, Batch 4 only...), -2, 0, 7 and 14."

From p. 20 of MRID 46552708: "Between Study Day -14 and Study Day -5 inclusive pups were fed three times daily... The 400 g was divided between the three feeds. From Study Day -4 to the end of the study pups assigned to Batch 1 [Day of dosing: Dec. 1, 2004] were fed once daily in the afternoon between 12:00 and 15:00... For Batches 2, 3 and 4 [Dates of dosing: Dec. 29, 2004; Dec. 30, 2004; and Jan. 20, 2005] pups were fed, from Study Day -4 to the end of the study, twice daily, once in the morning between 09:30 and 10:30 and once in the afternoon between 15:30 and 16:30. .. The diet not consumed was weighed and recorded...to give the total quantity of diet not consumed for each twenty four hour period... Diet was withdrawn from all animals, at least 5 hours on Study Days 0 and 6, in order to facilitate fasting prior to blood sampling. Diet was withdrawn, for at least 5 hours on Study Day 13 from any animals due to be blood sampled on Study Day 14. In all these cases the weight of the diet withdrawn was recorded as diet not consumed for that day."

E. PATHOLOGICAL PARAMETERS

Blood samples were collected from each animal at the following time points: Study Days: -14, (-13, Batch 4 only), +1, and 7. Based on the results of blood samples collected on Study Days +1 and 7, it was deemed necessary to collect blood samples from a number of each pups from each group on Study Day 14 (and Study Day 15 in the case of one pup in Group 4). The following number of pups/group had blood collected for hematology and/or clinical chemistry on Day 14/15: Group 1: 2; Group 2: 6; Group 3: 9; and Group 4: 3.

The CHECKED (X) parameters were measured:

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)*
	Platelet count		Absolute reticulocytes
	Blood clotting measurements		Percent reticulocytes
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time [PT])*		
X	(Activated partial thromboplastin time [APTT])*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Blood creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*	X	Urea
X	Potassium*		Total Cholesterol
X	Sodium*	X	Globulin (Glob)*
		X	Glucose (Gluc)*
	ENZYMES	X	Total bilirubin (T Bil)*
X	Alkaline phosphatase(ALPor ALK)*	X	Direct bilirubin (D Bil)*
	Cholinesterase(ChE)	X	Total serum protein (TP)*
	Creatine kinase		Triglycerides
	Lactic acid dehydrogenase(LDH)		Serum protein electrophoresis
X	Serum alanine aminotransferase (ALT or SGPT)*		Albumin/Globulin (A/G) ratio
X	Serum aspartate aminotransferase(AST or SGOT)*		Lipase
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

A statistical summary report is found in Appendix 5, running from pages 202 to 248 of MRID 46552708.

From p. 138: "On completion of the biological phase of the study, the data will be entered into an Excel® file... All entries in this file will be subjected to a 100% check by the Study Director or a person designated by the Study Director... After completion of all quality control checks to verify the accuracy of the Excel® file, it will be transferred electronically to the statistician who will enter it into the statistical software SAS...

"Clinical chemistry and haematology, diet consumption and bodyweight data will be analysed using a mixed model repeated measures analysis of covariance including replicate and its interaction with day as random effects, and sex, treatment, sampling day, and interaction of sex and treatment with day as fixed effects. The covariate for each variable will be the applicable baseline measurement. The average value, or the final pretreatment value, will be used for more than one baseline measurement...

"If the overall treatment by time interaction is significant at α=0.05, then the pairwise comparison of each dose group mean against the control group mean at each evaluation period will be tested. These pairwise comparisons will be obtained from linear contrasts on the time by treatment group interaction. These interactions will be tested at α=0.05...

"The adequacies of all statistical models will be checked by analysing the residuals, at the discretion of the Statistician. It is anticipated that if the best-fitting variance-covariance structure is chosen, the model fit will be adequate...

G. DISPOSITION OF ANIMALS

Not stated. According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals." One puppy (#15101, Group 4 or 5x) was euthanized for ethical reasons of Study Day 4 and was necropsied.

H. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

From information supplied by the registrant, the density of Test Material 1 is 1.11 g/mL, and for Test Material 2 is 1.08 g/mL. Using these densities, each 1X puppy was exposed to 343.5 mg permethrin, 47 mg dinotefuran and 5 mg sumilarv (nylar). The following were the mean exposure levels/group/sex/kg/ for each of these 3 actives:

TABLE 3. Cumulative Dosage Rates					
		Mean puppy weight ± S.D. (Kg)[a]	Mean Permethrin Exposure (mg/kg)	Mean Dinotefuran Exposure (mg/kg)	Mean Sumilarv Exposure (mg/kg)
Placebo Control (1)	Male puppies	1.91 ± 0.18	0	0	0
	Female puppies	1.96 ± 0.36	0	0	0
	Combined sexes	1.93 ± 0.25	0	0	0
Group 2 (1X)	Male puppies	2.05 ± 0.31	168	22.9	2.44
	Female puppies	1.95 ± 0.33	176	24.1	2.56
	Combined sexes	2.00 ± 0.31	172	23.5	2.50
Group 3 (3X)	Male puppies	2.15 ± 0.47	479	65.6	6.98
	Female puppies	1.88 ± 0.54	548	75.0	7.98
	Combined sexes	2.02 ± 0.50	510	69.8	7.43
Group 4 (5X)	Male puppies	1.97 ± 0.41	872	119.3	12.7
	Female puppies	1.93 ± 0.55	890	121.8	13.0
	Combined sexes	1.95 ± 0.47	881	120.5	12.8

Individual body weights given on p. 44 of MRID 46552708; the means (with standard deviations) were calculated by this reviewer from these data.

B. MORTALITY

From information on p. 35 of MRID 46552708: "Animal No. 15101 Group 4 (5x) was euthanased for ethical reasons on Study Day 4 and was subjected to a post mortem examination on Study Day 5 by a veterinary surgeon and the Sponsor noted as soon as possible...:

No animal 15101 is listed in Table 1 (p. 43 of MRID 46552708), so apparently this animal was subsequently replaced.

C. CLINICAL SIGNS

From p. 40: "Adverse events associated with the nervous system (i.e. ataxia and tremors) were detected in three of the twelve pups assigned to Group 4 (5x). Tremors were detected in Animal No. 61286 and 66122 [Group 4 (5x)] at +4 hours after the final treatment and had resolved at the +6 hours assessment. [From p. 84 both #61286 and #66122 had tremors at +4 and +5 hours after the final treatment; this is consistent with the number of occasions - 4 - indicated for this adverse effect on p. 96]. Ataxia was detected in Animal No. 61286 [Group 4 (5x)] at the +5 hours assessment but this had resolved at the +6 hours assessment. Animal No. 56857 [Group 4(5x)] was first detected with ataxia at the morning assessment on Study Day 1. It had resolved at the morning assessment on Study Day 3 [note by reviewer: from p. 69: ataxia was present for this puppy at the am and pm observations on both Days 1 and 2]. Ataxia was detected in Animal No. 48334 [Group 2 (1x), weight on Day 0:

1.7 kg] on one occasion on Study Day 0 at +2 hours after treatment. There were no other incidences of ataxia or tremors detected in any other animal during the study" [note by reviewer: from p. 96 there were 5 post-treatment occurrences in Group 4 puppies for ataxia, and one for a Group 2 puppy; ataxia was not observed in any of the other animals. Tremors were observed only for Group 4 puppies, with a total of 4 occurrences].

Puppy 56857 [Group 4, 5X] showed lethargy at the am and pm observations on both Days 1 and 2, and at the am observations of Days 3 and 5. [see p. 63].

From p. 71, puppy 56857 also showed an abnormal site of spot-on application on Day 1 (pm observation), Day 2 (pm observation only), Day 3 (am and pm), and Days 5 and 6 (am observations only on both days). Puppy 71007 (also Group 4. 5X) showed an abnormal site of spot-on application at pretreatment no. 4. These account for the 7 occasions on which Group 4 puppies showed this adverse reaction.

From p. 96, post treatment pruritus was observed the following number of times: Group 1: 4; Group 2: 4; Group 3: 0; Group 4: 11. All occurrences in Groups 1 and 2 were on Day 0 (day of application), as were 6 occurrences (involving 3 puppies) in Group 4; 4 Day 0 Group 4 occurrences involved one puppy (#56857), and 4 (Day 3 at both am and pm, and at the am observations on Days 5 and 6) of the 5 subsequent occurrences involved this same puppy.

Group 4 puppy 56857, the only puppy in the study showing ataxia after Study Day 0, and also the only one showing lethargy (also with an abnormal site of spot-on application and pruritus in this period) was the only puppy in Group 4 which lost weight (0.2 kg; from 2.1 to 1.9 kg) in the period from Day 0 to 7.

From p. 76-77 ocular discharge was a relatively common finding, and was observed in a number of puppies before exposure to the test materials. Post exposure, this was observed most frequently in control puppies, with a possible negative dose relationship (from p. 96: number of post treatment occasions observed ocular discharge: Group 1: 89; Group 2: 47; Group 3: 63; Group 4: 28).

On p. 41 it is stated that there was a higher incidence of abnormal faeces in Group 4 (5x) than in any of the other three groups. From p. 92-93 abnormal faeces (presumably the same as loose; on p. 92 a score of "1" is defined as "Abnormal faeces present" while on p. 93 a score of "1" is defined as "Loose Faeces present") were present in some puppies from all groups even before exposure to the test materials. However, after treatment there was a dose relationship, as this was observed more frequently in Group 4 puppies. From Table 4(z) on p. 96 the following post treatment occurrences were noted: Group 1 (controls): 38; Group 2: 35; Group 3: 44; Group 4: 111. There were increased incidences of abnormal faeces in Group 4 immediately following treatments and continuing sporadically throughout during the subsequent 14-day observation period:

TABLE 4. Incidences of Abnormal Faeces (on Day of Treatment)											
Group	Pre-treat-ment	Pre 2nd treat-ment	Pre 3rd treat-ment	Pre 4th treat-ment	Pre 5th treat-ment	1 hr post treat-ment	2 hrs post treat-ment	3 hrs post treat-ment	4 hrs post treat-ment	5 hrs post treat-ment	6 hrs post treat-ment
1 - 0X	3/12	0/12	1/12	1/12	0/12	1/12	0/12	2/12	2/12	-	-
2 - 1X	4/12	-	-	-	-	3/12	1/12	2/12	2/12	0/2	-
3 - 3X	5/12	1/12	0/12	-	-	0/12	2/12	0/12	0/12	0/1	-
4 - 5X	5/12	3/12	1/12	1/12	2/12	1/12	4/12	4/12	4/12	0/2	0/2

TABLE 5. Incidences of Abnormal Faeces (on Days 1 through 6 Following Treatment)												
Group	Day 1 am	Day 1 pm	Day 2 am	Day 2 pm	Day 3 am	Day 3 pm	Day 4 am	Day 4 pm	Day 5 am	Day 5 pm	Day 6 am	Day 6 pm
1 - 0X	2/12	2/12	1/12	1/12	1/12	3/12	1/12	1/12	1/12	2/12	2/12	1/12
2 - 1X	0/12	1/12	3/12	3/12	1/12	1/12	0/12	0/12	2/12	1/12	0/12	2/12
3 - 3X	1/12	0/12	3/12	2/12	2/12	2/12	1/12	2/12	2/12	2/12	2/12	3/12
4 - 5X	2/12	2/12	6/12	5/12	5/12	4/12	5/12	2/12	2/12	2/12	4/12	3/12

TABLE 6. Incidences of Abnormal Faeces (on Days 7 through 12 Following Treatment)												
Group	Day 7 am	Day 7 pm	Day 8 am	Day 8 pm	Day 9 am	Day 9 pm	Day 10am	Day 10pm	Day 11am	Day 11pm	Day 12am	Day 12pm
1 - 0X	3/12	3/12	1/12	2/12	2/12	1/12	1/12	0/12	0/12	0/12	0/12	0/12
2 - 1X	2/12	2/12	3/12	2/12	1/12	2/12	1/12	1/12	0/12	0/12	0/12	0/12
3 - 3X	3/12	1/12	3/12	2/12	2/12	2/12	1/12	0/12	0/12	0/12	0/12	1/12
4 - 5X	6/12	2/12	4/12	3/12	3/12	4/12	7/12	2/12	3/12	3/12	3/12	3/12

From information on p. 93 total (cumulative) occurrences of abnormal ("loose") feces from the am observation on Day 1 through the pm observation on Day 14 were: Group 1: 33; Group 2: 29; Group 3: 40; Group 4: 95.

From p. 96 post-treatment vomiting was noted once in Group 3 and 4 times in Group 4. There were no occurrences on Day 0; vomiting was noted for one Group 3 puppy at the am observation on Day 7, and in two Group 4 puppies, #08858 and #25122. For #08858 it was noted at the am observations on Days 2 and 3, and for #25122 it was noted at both the am and pm observations on Day 7.

D. BODY WEIGHT AND WEIGHT GAIN

From p. 205 for body weight: "There was no significant interaction of sex and treatment or sex , treatment and post-treatment day, no significant interaction of treatment and day, no significant interaction of treatment and day, and no significant overall treatment effect..."

TABLE 7. Mean Body Weights ± S.D. (Kg)

		Day -14	Day -2	Day 0	Day 7	Day 14
Placebo Control Group 1	Male puppies	1.41 ± 0.21	1.86 ± 0.19	1.91 ± 0.18	2.06 ± 0.30	2.53 ± 0.31
	Female puppies	1.44 ± 0.23	1.94 ± 0.36	1.96 ± 0.36	2.04 ± 0.38	2.38 ± 0.37
	Combined sexes	1.43 ± 0.21	1.89 ± 0.26	1.93 ± 0.25	2.05 ± 0.32	2.47 ± 0.33
Group 2 (1X)	Male puppies	1.58 ± 0.19	1.95 ± 0.34	2.05 ± 0.31	2.15 ± 0.40	2.57 ± 0.36
	Female puppies	1.42 ± 0.33	1.92 ± 0.33	1.95 ± 0.33	2.12 ± 0.26	2.55 ± 0.28
	Combined sexes	1.50 ± 0.27	1.93 ± 0.32	2.00 ± 0.31	2.13 ± 0.33	2.56 ± 0.31
Group 3 (3X)	Male puppies	1.50 ± 0.21	2.05 ± 0.43	2.15 ± 0.47	2.33 ± 0.44	2.72 ± 0.46
	Female puppies	1.45 ± 0.33	1.90 ± 0.55	1.88 ± 0.54	1.98 ± 0.56	2.33 ± 0.55
	Combined sexes	1.48 ± 0.27	1.98 ± 0.48	2.02 ± 0.50	2.16 ± 0.51	2.53 ± 0.52
Group 4 (5X)	Male puppies	1.60 ± 0.24	1.93 ± 0.39	1.97 ± 0.41	2.10 ± 0.48	2.40 ± 0.58
	Female puppies	1.37 ± 0.35	1.90 ± 0.57	1.93 ± 0.55	2.10 ± 0.54	2.45 ± 0.60
	Combined sexes	1.48 ± 0.31	1.92 ± 0.31	1.95 ± 0.47	2.10 ± 0.49	2.43 ± 0.56

Individual body weights given on p. 44 of MRID 46552708; the means (with standard deviations) were calculated by this reviewer from these data.

All groups (including controls) showed relatively slight mean (reduced) weight gains in the period from Day 0 to 7. For example, the males in the control group showed a mean weight gain of 0.5 kg in the period from Day -14 to Day 0 (or 0.25 kg/week), then had only a mean weight gain of 0.14 kg from Day 0 to 7, but then had a mean weight gain of 0.47 kg from Day 7 to 14. This may have been not only from handling as well as exposure to the test material (or placebo control), but also the blood drawing on Days 1 and 7, and the associated fasting periods to these drawings. An additional factor (see p. 30) was that a number of Batch 1 puppies from all 4 groups were observed as being thin and not thriving on Study Day 1, and the veterinary surgeon recommended raw beef supplements to their diet. This supplementation was made from Study Day 7 to the end of the study; a reassessment after study completion indicated these animals were normal. Overall, there was no indication of a dose-related effect involving body weight gains (see Table 8, below).

TABLE 8. Mean Body Weight Gains ± S.D. (Kg)					
		Day -14 to -2	Day -2 to 0	Day 0 to 7	Day 7 to 14
Placebo Control Group 1	Male puppies	0.44 ± 0.17	0.06 ± 0.05	0.14 ± 0.22	0.47 ± 0.16
	Female puppies	0.50 ± 0.14	0.02 ± 0.08	0.08 ± 0.16	0.34 ± 0.21
	Combined sexes	0.47 ± 0.16	0.04 ± 0.07	0.12 ± 0.19	0.42 ± 0.19
Group 2 (1X)	Male puppies	0.37 ± 0.22	0.10 ± 0.06	0.10 ± 0.21	0.42 ± 0.15
	Female puppies	0.50 ± 0.20	0.03 ± 0.05	0.17 ± 0.16	0.43 ± 0.15
	Combined sexes	0.43 ± 0.21	0.07 ± 0.07	0.13 ± 0.18	0.43 ± 0.14
Group 3 (3X)	Male puppies	0.55 ± 0.27	0.10 ± 0.06	0.18 ± 0.12	0.38 ± 0.08
	Female puppies	0.45 ± 0.27	-0.02 ± 0.15	0.10 ± 0.13	0.35 ± 0.21
	Combined sexes	0.50 ± 0.26	0.04 ± 0.12	0.14 ± 0.12	0.37 ± 0.15
Group 4 (5X)	Male puppies	0.33 ± 0.25	0.03 ± 0.08	0.13 ± 0.20	0.30 ± 0.14
	Female puppies	0.53 ± 0.23	0.03 ± 0.05	0.17 ± 0.12	0.35 ± 0.15
	Combined sexes	0.43 ± 0.25	0.03 ± 0.07	0.15 ± 0.16	0.33 ± 0.14

Mean body weight gains were calculated from information on p. 44 of MRID 46552708 by this reviewer.

E. FOOD CONSUMPTION

From information on p. 107, a number of puppies had reduced food consumption values on Day 0. These included control male 26542 (61.0 g), group 2 female 59295 (52.0 g), group 3 female 00635 (15.0 g), and group 4 male 74062 (26.0 g). It is not immediately evident from the text whether fasting before drawing blood on Day 1 involved withdrawal of diet on Day 0 (resulting in reduced food consumption for Day 0) or was simply a delay in feeding on Day 1 (from p. 38 diet was offered on Days 1 and 7 between 11:46 and 11:53 as blood was drawn during this period). Most of the reductions in food consumption were for Day 0, although a few (including Group 4 puppies #56857 and #08858) had lower food consumption values on Day 1 rather than Day 0. A somewhat similar situation exists with respect to Days 6 and 7.

From p. 247:"There was no significant sex-by-treatment or sex-by-treatment-by-day interaction ($p > 0.05$); thus, the changes in Mean Daily Feed Consumption (g) during the study were similar between treatment groups for each sex and over time. There was no significant treatment-by-day interaction; thus the changes in Mean Daily Feed Consumption were similar between treatment groups over time. There were no significant differences among the treatment groups for Mean Daily Feed Consumption."

TABLE 9. Mean Food Consumption Values (g ± S.D./puppy) for Certain Study Days						
		Day -1	Day 0	Day 1	Day 2	Day 6
Placebo Control Group 1	Male puppies	166.5 ± 49.5	193.3 ± 115.8	303.4 ± 126.5	240.9 ± 117.9	243.9 ± 140.0
	Female puppies	214.1 ± 75.3	206.4 ± 69.7	258.9 ± 56.6	292.6 ± 82.8	240.8 ± 91.3
	Combined sexes	186.3 ± 63.2	198.8 ± 95.5	284.9 ± 102.1	262.4 ± 103.8	242.6 ± 117.2
Group 2 (1X)	Male puppies	270.4 ± 63.6	224.8 ± 119.6	298.0 ± 145.6	292.2 ± 102.4	269.2 ± 136.2
	Female puppies	300.0 ± 59.9	213.2 ± 117.1	360.9 ± 34.9	316.2 ± 70.1	288.8 ± 100.6
	Combined sexes	285.2 ± 60.9	219.0 ± 113.0	329.4 ± 106.1	304.2 ± 84.6	279.0 ± 114.7
Group 3 (3X)	Male puppies	282.1 ± 111.8	265.7 ± 146.5	316.4 ± 76.2	328.5 ± 74.4	321.7 ± 83.3
	Female puppies	180.4 ± 128.0	138.7 ± 140.4	249.7 ± 145.1	219.7 ± 153.2	211.3 ± 140.6
	Combined sexes	231.3 ± 126.3	202.2 ± 152.0	283.1 ± 115.8	274.1 ± 128.1	266.5 ± 124.4
Group 4 (5X)	Male puppies	174.8 ± 120.9	121.7 ± 82.9	236.7 ± 147.2	230.7 ± 121.7	230.2 ± 139.9
	Female puppies	246.5 ± 118.0	189.2 ± 114.7	323.5 ± 64.5	311.2 ± 70.4	244.7 ± 99.9
	Combined sexes	210.6 ± 119.9	155.4 ± 101.7	280.1 ± 117.5	270.9 ± 103.7	237.4 ± 116.2

Individual daily diet consumption values given on p. 107-108 of MRID 46552708. Means ± S.D. by group/sex/day given on p. 245-246.

F. HEMATOLOGY

There were no indications of any dose-related effects involving White Blood Cell (WBC) counts [refer to p. 206-207], Red Blood Cell (RBC) counts [refer to p. 207-208], Hemoglobin (HGB) concentrations [p. 208-209], Hematocrit [HCT; refer to p. 209-210], Mean Corpuscular Volume [MCV); refer to p. 210-211], Mean Corpuscular Hemoglobin [MCH; refer to p. 211-212], or Mean Corpuscular Hemoglobin Concentration [MCHC; refer to p. 212-213].

From p. 214 for percentage Mature Neutrophils (MN) (%): "There was a significant ($p<0.05$) sex-by-treatment-by-day interaction of sex. However, none of the pairwise comparisons of medicated [treated?] groups to the controls within day were significant ($p>0.05$)..."

There were no indications of any dose-related effects involving Mature Neutrophil (MN) (10^3/µL) counts (p. 215-216), percentage Band Neutrophils (BP)(%) (p. 216), Band Neutrophil (BP)(10^3/µL) counts (p. 217).

From p. 217-218: For percentage Lymphocytes (LC)(%) there was a significant interaction of sex and treatment. Males treated at 5X had significantly ($p<0.05$) lower Lymphocytes (%) than the controls on Day 7; there were no other significant differences between treatment groups for males, and none for females.

TABLE 10. Mean % LC ± S.D.				
		Day -14	Day 1	Day 7
Placebo Control Group 1	Male puppies	32.1 ± 10.6	40.0 ± 11.8	43.6 ± 8.2
	Female puppies	25.8 ± 10.0	38.4 ± 11.0	40.4 ± 14.2
	Combined sexes	29.5 ± 10.4	39.3 ± 11.0	42.3 ± 10.6
Group 2 (1X)	Male puppies	32.0 ± 7.8	32.0 ± 11.9	49.7 ± 18.4
	Female puppies	36.3 ± 8.5	37.7 ± 20.4	32.8 ± 15.1
	Combined sexes	34.2 ± 8.1	34.8 ± 16.2	41.3 ± 18.3
Group 3 (3X)	Male puppies	34.5 ± 8.8	37.0 ± 15.5	35.0 ± 10.3
	Female puppies	31.2 ± 5.8	42.7 ± 19.4	40.2 ± 13.5
	Combined sexes	32.8 ± 7.3	39.8 ± 17.0	37.6 ± 11.8
Group 4 (5X)	Male puppies	29.7 ± 9.2	36.3 ± 13.9	30.3[b] ± 12.5
	Female puppies	44.2 ± 13.7	33.7 ± 13.3	36.7 ± 13.2
	Combined sexes	36.9 ± 13.5	35.0 ± 13.0	33.5 ± 12.7

[a]Means and standard deviations calculated by this reviewer from individual data on p. 99-101 of MRID 46552708.
[b]Reported as significantly (p=0.0353) lower than control value.

Although Group 4 (5x) male puppies had a significantly lower mean value for % Lymphocytes relative to the control on Day 7, the mean was not significantly lower from the Group 4 mean values on Days -14 and 1, and was not significantly lower from the Day -14 control mean. In addition, the laboratory's normal reference range for this parameter is given as (see p. 99-101) as 12-30%. The Group 4 (5X) male mean on Day 7 was then closer to the normal range than was the Group 1 (placebo) male mean value on Day 7. It is concluded then that there is no biological relevancy to this occurrence of statistical significance for this parameter.

There were no indications of any dose-related effects involving Lymphocyte (LC) (10^3/µL) counts (p. 219), percentage Monocytes (MC)(%) (p. 220), Monocytes (MC) (10^3/µL) counts (p. 221), percentage Eosinophils (EP)(%) (p. 222), Eosinophil (EP) (10^3/µL) counts (p. 223), percentage Basophils (BP)(%) or Basophil (BP)(10^3/µL) counts (p. 224), Prothrombin Time (PT)(sec) (p. 225), or Activated Partial Thromboplastin Time (sec)(p. 226).

G. CLINICAL CHEMISTRY

There were no indications of any dose-related effects involving Alanine Aminotransferase (ALT) (IU/L) (p. 227-228) or Aspartate Aminotransferase (AST)(IU/L) (p. 228-229),

From p. 229-230 for Urea (mmol/L) there was a significant interaction of sex and treatment. Pairwise comparison indicated that Group 3 (3X) males had significantly lower (p=0.012) lower urea averaged over post-treatment days 1 and 7 than did control males. However, Group 4 (5X) males did not show a similar effect (on Day 7

the mean urea values ± S.D. [mmol/L] for males by group were the following [calculated by this reviewer from data on p. 105]: Group 1: 3.3 ± 0.6; Group 2: 3.2 ± 0.8; Group 3: 2.3 ± 0.3; and Group 4: 3.1 ± 0.4). However, all individual values for this parameter on Days 1 and 7 were within the laboratory's reference range of 1.2-7.9 mmol/L, and, in any case, pathology would usually involve an elevated urea level, rather than a reduced one. It is concluded that this finding was without biological relevance.

From p. 230-231 for Blood Urea Nitrogen (BUN) (mg/dL) there was a significant interaction of sex and treatment. Pairwise comparison indicated that Group 3 males had significantly (p=0.0117) lower BUN averaged over post-treatment days 1 and 7 than did control males. However, Group 4 95X) males did not show a similar effect (on Day 7 the mean BUN values ± S.D. [mg/dL] for males by group were the following [calculated by this reviewer from data on p. 105]: Group 1: 9.4 ± 1.8; Group 2: 8.9 ± 2.4; Group 3: 6.5 ± 1.0; and Group 4: 8.8 ± 1.2). The laboratory's reference range for this parameter is 3.36-22.1 mg/dL; all individual values (for both males and females) on Days 1 and 7 were within this range. BUN levels correlate with urea measurements and the comments given above for urea are also appropriate here.

There were no indications of any dose-related effects involving Creatinine (CREAT (µmol/L) (p. 232), Total Protein (T.PROT)(g/L) (p. 233), Albumin (ALB) (g/L) (p. 234), Globulin (GL) (g/L) (p. 235), Total Bilirubin (T.BIL) (µmol/L) (p. 236), Glucose (GLU) (mmol/L) (p. 237), Sodium (Na) (mmol/L) (p. 238), Potassium (K) (mmol/L) (p. 239), Chloride (Cl) (mmol/L) (p. 240), Calcium (Ca) (mmol/L) (p. 241), Phosphorus (phos) (mmol/L) (p. 242), Alkaline Phosphatase (ALP) (IU/L) (p. 243), or Direct Bilirubin (D.BIL) (µmol/L) (p. 244).

H. NECROPSY FINDINGS

From p. 31: "Animal 15101 (Group 4, 5X) was diagnosed with gastroenteritis on Study Day 3 by the veterinary surgeon... The pup was observed as being weak, dehydrated and lethargic with bloody diarrhoea and vomit. Approximately 50 mLs of Hartmann's solution was administered by intravenous infusion. The pup was reassessed on the morning of Study Day 4. This animal was observed to be in sternal recumbency, with dehydration, vomiting and diarrhoea... The pup was reassessed in the afternoon and it was decided to euthanse the pup on welfare grounds. A post mortem was performed on Study Day 5..."

From p. 35: "The results of the post mortem showed an intussusception in the colon which was considered not to be related to treatment. Other findings at post mortem consisted of black gastric contents in the stomach, evidence of inflammation in the ileum, liver appeared pale and blood stained runny faeces in the colon distal to the intussusception..."

Additional information (body weights, food consumption, symptoms, hematology, clinical chemistry) for #15101 does not appear in the report, as this puppy was replaced by Batch 4 puppy #08858.

IV. DISCUSSION

This companion animal safety study (MRID 46552708) utilized beagle puppies (source: Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland; males: 49-55 days on Day 0, 1.5-2.6 kg on Day 0; females: 49-55 days on Day 0, 1.2-2.9 kg on Day 0). There were 4 groups; Group 1 (controls) contained 7 males and 5 females; Groups 2, 3 and 4 (1X, 3X and 5X, respectively) each contained 6 males and 6 females. To facilitate pup supply and to replace pups that were withdrawn, it was necessary to have four batches of puppies with four separate test item/control item administration dates (Dec. 1, 2004, Dec. 29, 2004, Dec. 30, 2004 and Jan. 20, 2005).

Each Group 1 puppy received 5 separate applications (spaced about 1 hour apart), with each application consisting of 0.70 mL control item 1 followed by 0.30 mL control item 2. Each Group 2 puppy received a single application (0.70 mL test item 1 followed by 0.30 mL test item 2); each group 3 puppy received 3 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals. Each group 4 puppy received 5 applications (0.70 mL test item 1 followed immediately by 0.30 mL test item 2) at one hour intervals.

Test item 1 (Chamber A on the proposed label) has a label declaration of 45% permethrin, and test item 2 (Chamber B on the proposed label) has a label declaration of 14.85% dinotefuran and 1.48% Sumilarv. Taking into account the specific gravities and chemical analyses of these two test items, each 1X puppy in this study was exposed to 343.5 mg permethrin, 47 mg dinotefuran and 5 mg sumilarv (nylar).

Adverse nervous system effects (ataxia and tremors) were seen in three of the twelve pups assigned to Group 4 (5X). Tremors were present in two of these puppies at +4 hours after the final treatment and had resolved 2 hours later. Ataxia was also present in one of these puppies at the +5 hours assessment but had resolved at the +6 hours assessment. The third Group 4 puppy (#56857) had ataxia at the morning assessment on Study Day 1, which had resolved at the morning assessment on Study Day 3; this puppy also also showed lethargy at the am and pm observations on both Days 1 and 2, and at the am for Days 3 and 5. Ataxia was also observed in one Group 2 puppy on one occasion (Study Day 0 at +2 hours after treatment). There were no other incidences of ataxia or tremors detected in any other animal during the study.

The same Group 4 puppy (#56857) showing ataxia and lethargy also had a sporadically abnormal site of spot-on application from Day 1 through Day 6, and also had post treatment pruritus during this period of time.

Ocular discharge was a relatively common finding, and was seen in a number of puppies before exposure. Post exposure, this was observed most frequently in control puppies, with a possible negative dose relationship (number of post treatment occasions observed: Group 1: 89; Group 2: 47; Group 3: 63; Group 4: 28).

Abnormal (presumably loose) faeces were observed for some puppies from all groups even before exposure to the test materials. However, after treatment there was a dose relationship, as this finding was observed most frequently in Group 4 puppies with the following group incidences: Group 1 (controls): 38; Group 2: 35; Group 3: 44; Group 4: 111. There were increased incidences of abnormal faeces in Group 4 immediately following treatments and continuing sporadically throughout during the subsequent 14-day observation

period (incidences from Day 1 through 14: Group 1: 33; Group 2: 29; Group 3: 40; Group 4: 95).. This reviewer notes that a similar finding was not observed in the adult dog study with this product.

There was no indication of an effect on any of the hematology or clinical chemistry parameters, and there were no symptoms or other indications of systemic toxicity.

The proposed label received by this reviewer indicates this product will be applied at three dosage rates depending on body weight: one application rate will be for dogs or puppies weighing less than 10 lbs; a second will be for dogs/puppies weighing 11 to 20 lbs; and a third for dogs/puppies weighing 21 to 55 lbs. However, the proposed label does not specify application rates. The 1X application rate in this study was 0.7 mL of Test Material 1 (Chamber A, label declaration of 45% permethrin) and 0.3 mL Test Material 2 (Chamber B, label declaration of 14.85% dinotefuran and 1.48% Sumilarv). The 1X application rate in the adult dog study (MRID 46552707) was 2.5 mL of Test Material 1 and 1.0 mL of Test Material 2 [a 0.71 to 0.29 ratio, reasonably similar to the 0.70 to 0.30 in the puppy study] for dogs ranging from 10.0 to 21.9 kg (22 to 48 lbs). This leaves open the question of the application rate for dogs/puppies weighing 11 to 20 lbs.

TRB concludes that this companion animal safety study (OPPTS 870.7200) may demonstrate an adequate margin of safety (5x or reasonably close to 5x, as the tremors and ataxia observed in two of the three 5x puppies were reasonably transient). However, the following points need to be addressed by the registrant:

TRB's major concern is the report of ataxia in Group 2 (1X) puppy 48334 at two hours postdose. One thing that could be helpful would be any additional observational information regarding this occurrence, particularly what was seen by the observer(s) that resulted in this diagnosis.

The increased incidence (relative to other groups) of loose faeces in the 5x puppies should be clarified (for example, were the faeces simply loose - which may be normal for puppies of this age and under these conditions - or were they watery or otherwise indicative of possible toxicity or pathology).

Puppy #15101 was in Group 4 (5X), and was diagnosed with gastroenteritis and euthanised on Study Day 5. This puppy was evidently replaced with batch 4 puppy #08858. However, the statement is made (p. 39) that: "Three pups, which did not complete the study on health grounds, were replaced with three pups from batch 4." There is no information as to why two other puppies (one, in Group 1, was replaced with puppy #32512; the other, in Group 3, was replaced with puppy #15298) were removed from this study. Relevant information on all 3 puppies should be provided, including any observational data such as responses following treatment.

The statement is made (p. 31) regarding clinical assessments that: "Where adverse reactions were observed at the +4 hour clinical assessment on Study Day 0...then further assessments [were] continued on that particular animal at hourly intervals [actually up to +6 hours] until no further reactions were observed..." Two Group 2 puppies (#59295 and #48334) had extended assessments to 6 hours; #59295 had pruritus at +4 hours, but it is not evident from the clinical assessment sheets (p. 46-95 of MRID 46552708) why observations continued to be made on #48334 (pruritus was recorded at +5 hours for this puppy, but not at +4 hours; and this was also the 1X puppy which showed ataxia at +2 hours). Since this

puppy was showing no symptoms at +4 hours, why were additional observations made on it?

TRB concludes that this companion animal safety study (OPPTS 870.7200) may demonstrate an adequate margin of safety (slightly below 5X) between the exposure associated with the dosages used in this study (0.7 mL of a 45% Permethrin formulation and 0.3 mL of a 14.85% S-1638 and 1.5% Nylar formulation) for 8-week old puppies weighing approximately 2.0 kg. However, the questions indicated above need to be adequately addressed by the registrant. Currently, this study does not support the proposed use of this product at this dosage level on 8-week old puppies.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D318905
2. **PC CODES:** 109701 (Permethrin), 044312 (Dinotefuran), 129032 (Nylar)
3. **CURRENT DATE:** January 4, 2006
4. **TEST MATERIAL:** In this study an application for 1X animals (7 to 8 week old puppies weighing 1.2 to 2.9 kg) consisted of 0.7 mL test item 1 (a clear yellow liquid with a specific gravity of 1.11 g/mL assaying 44.21% Peremthrin) and 0.3 mL test item 2 (a colorless liquid with a specific gravity of 1.08 assaying 14.59% S-1638 [Dinotefuran] and 1.52% Nylar). According to the proposed label received by TRB, the product as proposed for registration consists of the liquid contents of two chambers. Chamber A contains a 45% permethrin formulation (test item 1), and Chamber B contains an S1638 (14.85%) + Nylar (1.48%) formulation (test item 2).

Study/Species/ Lab Study #/ Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal/49 to 55-day old beagle puppies/Charles River BioLabs Europe, Ireland/ Study No. USA004\04-003; Hartz Test #1767 /13-April-2005	46552708	4 groups (each with 6M & 6F) of 49 to 55-day-old beagle puppies (source: Charles River Laboratories BioLabs; males: 1.5-2.6 kg; females: 1.2-2.9 kg on day 0) were tested: Group 1 (controls) received five applications, each consisting of 0.7 mL control item 1 (test item 1 without Permethrin) and 0.3 mL of control item 2 (test item 2 without Dinotefuran and without Nylar); Group 2 (1X) received a single application of 0.7 mL test item 1 followed by 0.3 mL test item 2; Group 3 (3X) received 3 applications of the combination of 0.7 mL test item 1 and 0.3 mL test item 2; and Group 4 (5X) received 5 applications of the combination of 0.7 mL test item 1 and 0.3 mL test item 2. Puppies getting more than one application received them at 1-hr intervals. Adverse nervous system effects (ataxia and/or tremors) were seen in 3/12 puppies in Group 4; one of these puppies had ataxia at AM observation on Day 1 with resolution by Day 3, same puppy also had lethargy several times, the last being at the AM observation on Day 5. Ataxia was also seen in one Group 2 puppy on Study Day 0 at 2 hrs after treatment. Abnormal (loose?) faeces were seen in some puppies from all groups even before exposure. However, after treatment there was a dose relationship (observational incidences from Day 1 through 14 were: Group 1: 33; Group 2: 29; Group 3: 40; Group 4: 95). A dose-related effect for this was not observed in the adult study (MRID 46552707). There was no indication of an effect on any of the hematology or clinical chemistry parameters, and there were no symptoms or other indications of systemic toxicity other than those described above. This study may demonstrate an adequate margin of safety (5X, or reasonably close to 5X, as the observed tremors and ataxia in two of the three puppies were reasonably transient). However, registrant needs to address several points: 1) report of ataxia in one Group 2 puppy at 2 hrs postdose; 2) increased incidence of loose faeces in the 5X puppies; 3) information as to why 2 puppies (one in Group 1, was replaced with #32512; the other, in Group 3, was replaced with #15298; 4) why were additional observations made on Group 2 puppy #48334 at 5 and 6 hours, when this puppy was showing no symptoms at 4 hours?	N/A	Not Acceptable but probably upgradeable

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

EPA Primary Reviewer: Princess Campbell, D.V.M. Signature:
RIMUERB, Registration Division (7505C) Date 1/10/2006
EPA Secondary Reviewer: Byron Backus, Ph.D. Signature:
Registration Division (7505C) Date 1-10-2006
EPA Tertiary Reviewer: John Redden, M.S. Signature:
Registration Division (7505C) Date 01/13/2006

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Dogs OPPTS 870.7200
PC CODES: 109701 Permethrin; 044312 (Dinotefuran; S1638); 129032 (Nylar; Sumilarv)
DP BARCODE: D 318905 **RISK MANAGER: (EPA): 01** **DECISION NO.: 357507**

PRODUCT AND TEST MATERIAL: Permethrin (45.00%), S1638 (14.85%), Nylar (1.48%); a clear yellow liquid consistent with the proposed product [Hartz Reference 1749]. The product proposed for registration consists of two chambers. Chamber A contains 0.8 mL of a 45% permethrin formulation, and Chamber B contains 0.3 mL of an S1638 (14.85%) + Nylar (1.48%) formulation.

CITATION: Doherty, P. (2005) Tolerance of an Experimental Flea and Tick Dermal Treatment when Topically Administered to Adult Dogs at 1X, 3X and 5X the Recommended Dose. Study No. USA004\05-002; Hartz Test #1749. Unpublished study prepared by Charles River Laboratories BioLabs Europe, Ireland. Study Completion Date: 1 April 2005; MRID 46552707.

SPONSOR: The Hartz Mountain Corporation

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46552707) with adult Beagle dogs, 4 groups, each with 12 (6/sex) adult (age: 12-100 months) dogs (source: Charles River Laboratories BioLabs Europe's colony at Glenamoy, Ireland; Males: 13.7-21.9 kg; Females: 10.0-17.6 kg); Dogs were treated at 0X (5 applications of 2.5 mL of control item 1 and 1.0 mL of control item 2); 1X (1 application each of 2.5 mL of test item 1 and 1.0 mL of test item 2); 3X (3 applications of 2.5 mL of test item 1 and 1.0 mL of test item 2); and 5X (5 applications 2.5 mL of test item 1 and 1.0 mL of test item 2). The tip of the syringe was used to part the dog's hair so that the sample was applied to the skin at each of the three spots. Each administration of both test items was 1 hour apart.

Most dogs were dosed with Permethrin (45%) batch no. TS12441 (assaying 44.32% total Permethrin) and S-1638 /Nylar batch no. TS12462 (assaying 15.45% S-1638 and 1.56% Nylar). However, as there was insufficient material received by the laboratory, two 3X dogs were dosed with Permethrin (45%) batch no. TS12478 (44.82% total Permethrin) and S-1638 /Nylar TS 12479 (15.21% S-1638 and 1.57% Nylar).

In order to facilitate study management, the animals were divided into two sets, each with 28 dogs (14 per sex), with two separate test/control item administration dates (Set 1- 25 Aug 2004; Set 2- 26 Aug 2004). Animal Nos 07049 and 14818 were dosed on 1 Sep 2004 since there was not enough test item available to dose all animals in Set 2. Once the control or test item was applied, each dog was held in an upright position for at least 2 minutes to prevent any potential loss of applied material.

Clinical assessments were carried out on each dog prior to the first treatment, and at approximately 1, 2, 3 and 4 and 5 hours where applicable after the final treatment on study day 0. Dogs receiving more than one treatment were also evaluated 10 minutes before each treatment.

According to study directions test items 1 and 2 were applied to three sites, namely Site 1- the intrascapular area, Site 2- the anterior lumbar area, and Site 3- the posterior lumbar area.

Individual body weights were measured before feeding on Days -15, -2, 0, 7 and 14. Individual food consumption was measured on a daily basis for Days -7, -6, -4 and then daily thereafter through Day 14 (values represented the amount of food consumed during the previous 24 hours). Blood samples were taken from each animal on Study Day -14, Study Day 1, and Study Day 7. Blood samples were collected on Study Day 14 for Animal #11289 (2x), Animal # 50342 (3x), Animal # 50573 (3x) and Animal # 89074 (5x). This is a deviation from the study plan for which there was no amendment. All blood samples were collected following an overnight fast.

There was no mortality. Clinical observations seen included abnormal (loose) faeces, abnormal site of spot-on application and vomiting.

A number of dogs (including seven of 12 in the control group and seven of 12 in the 5X group) had very low food consumption values (< 30g) for Day 1. As the controls and 5X groups showed the greatest reduction in mean food consumption, this may have been an effect associated with exposure to one or more inerts in this formulation, and/or to the stress of handling during five applications. Three of the 1X dogs showed a similar reduction in food consumption (< 30g) for Day 1. While some individual puppies in the puppy study (MRID 46552708) showed a reduced food consumption for Day 1, with no indication of a dose-related effect, adult dogs showed reduced mean food consumption on Day 7 (presumably associated with fasting and/or the stress of blood collection). The Day 7 effect was not as pronounced as that of Day 1.

Cosmetic effects were observed in all dogs, and included matting, greasy appearance, clumping, spiking, discolouration, and deposits. The cosmetic effects had disappeared by Study Day 5 (Group 1, control), Study Day 5 (Group 2, 1x) except for Animal No. 89782 which had a greasy appearance until Study Day 12, Study Day 8 (Group 3, 3x), Study Day 11 (Group 4, 5x).

Overall, a number of dogs had minor weight changes from between Study Day -15 and Study Day 14 (ranging from a loss of about 0.1 kg to 1.8 kg), with no indication of any dose-related trend. Similar weight loss has been observed previously in adult dogs that are individually housed. There was no significant difference in body weight between animals assigned to any of the groups.

There were no significant differences (P>0.05) between the treatment groups and control group with respect to clinical chemistry and/or hematological parameters. There were no symptoms or other indications of systemic toxicity.

TRB concludes that this companion animal safety study (OPPTS 870.7200) demonstrates an adequate margin of safety (at least 5X) between the exposure associated with a use application of 2- 5 mL test material (45% Permethrin) and 1.0 mL

test material 2 (14.85% Dinotefuran and 1.48% Nylar) for this formulation in adult dogs weighing between 10.0 and 21.9 kg and that at which significant systemic effects may occur. It is noted that the proposed label received by TRB indicates three weight classes of dogs and puppies (< 10 lbs, 11- 20 lbs, 21- 55 lbs), but does not specify dosages. The registrant should indicate the appropriate doses for each of these weight classes, and these should be consistent with and supported by the doses used in the two companion animal safety studies for this formulation.

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

I. MATERIALS

A. MATERIALS

1. Test item 1: Permethrin (label declaration 45.00%), containing (see p. 161 and p. 166 of MRID 46552707) 44.32% /44.82% Permethrin.

Description:	A colorless liquid with a specific gravity of 1.11 g/mL.	
Sample ID.:	2039/90/2	2071/7/1
Batch No.:	T.S. 12441	T.S. 12478
Assay Date:	July 8, 2004	Aug 26, 2004
Storage:	Stored between 14 and 21°C/ 16 and 25°C in the dark.	

Control Item 1: A formulation without Permethrin (see p. 162 of MRID 46552707)

Description:	A colorless liquid
Batch No.:	2039/90/1 T.S. 12439
Storage:	Stored between 16 and 25°C in the dark.

Test item 2: S-1638 (label declaration 14.85%) and Nylar (with a label declaration of 1.48%), containing (see p. 163 and p. 165 of MRID 46552707) 15.45% S-1638 and 1.56% Nylar/ S-1638 15.21 and 1.57 Nylar.

Description:	A colorless liquid with a specific gravity of 1.08 g/mL.	
Sample ID.:	2039/100	2071/7/2
Batch No.:	T.S. 12462	T.S. 12479
Assay Date:	Aug. 9, 2004	Aug. 26, 2004
Storage:	Stored between 16 and 25°C in the dark.	

Control Item 2: A formulation without S-1638 and without Nylar

Description:	A colorless liquid
Batch No.:	2039/98/1 T.S. 12453
Storage:	Stored between 17 and 21°C in the dark.

2. Administration: Topical (spot-on) on Day 0. An application consisted of 0.80 mL of control item 1/ test item 1 applied to Site 1, followed by 0.80 mL control item 1/ test item 1 to Site 2 and the remaining approximately 0.9 mL of control item 1/ test item 1 to Site 3. Then approximately 0.3 mL of control item 2/ test item 2 was applied to Site 1, a further 0.3 mL of control item 2/ test item 2 was applied to Site 2 and the remaining approximately 0.4 mL

of control item 2/test item 2 was applied to Site 3. The dog was held in an upright position for at least two minutes to prevent any loss of applied material. If there was more than one application then there was a one hour interval between them.

3. Test animals
   Species: Canine (adult dogs)
   Breed: Beagle
   Ages and weights at study initiation (Day 0): On Day -2 Males: 12-100 months; 13.7 to 21.9 kg; Females: 9-98 months; 10.0 to 17.6 kg.
   Source: Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland.
   Housing: From p. 15 of MRID 46552707: "For the duration of the study dogs were housed in Unit 17 at Charles River Laboratories BioLabs Europe's beagle colony located at Glenamoy, Co. Mayo, Ireland." From p 17 of MRID 46552707: "The dogs were housed in stainless steel pens, lined with wood, measuring approximately 1.7 m x 1.4 m (lxw), one per pen."
   Diet: Adults: Standard commercially available dog food at the recommended rate of were fed once daily in the afternoon between 12:00 and 15:00.
   Water: "potable water," *ad libitum*
   Environmental conditions:
      Temperature: 17-22°C
      Humidity: 52-70%
      Air changes: Not specified.
      Photoperiod: Not specified.
   Acclimation period: 15 days.

II. STUDY DESIGN

A. IN LIFE DATES

Start of Dosing: 25 Aug 2004 (Set 1); 26 Aug 2004 (Set 2); Last Observational Day: 8 Sept 2004 (Set 1); 9 Sept 2004 (Set 2). Two 3X dogs were treated on 1 September 2004, so their last observational day was 15 September 2004.

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

There were a total of 12 (6 males & 6 females) adult dogs per dosage group. For group assignment, dogs were ranked within sex in order of decreasing bodyweight. On treatment Day 0 each adult dog in the [Placebo] Control group (Group 1) was treated with five 2.5 mL applications of control item 1, (containing the same "inert" ingredients but lacking the active - Permethrin - present in the test item 1), and 1.0 mL of control item 2 (containing the same "inert" ingredients but lacking the two actives). Administration of both control items was spaced at one hour intervals. Dogs in Group 2 (1X) were treated with a single application of 2.5mL of test item 1 and 1.0 mL of test item 2. Dogs assigned to Group 3 (3X) were treated with 3 applications (spaced at one-hour intervals) of 2.5 mL test item 1 and 1.0 mL of test item 2; Dogs in Group 4 (5X) were treated with 5 applications (spaced at one-hour intervals) of 2.5 mL test item 1 and 1.0 mL of test item 2.

There were three sites of application. Site 1 was the intrascapular area. Site 2 was

the anterior lumbar region. Site 3 was the posterior lumbar region.

TABLE 1. Study design

		Number of Animals	Number of Applica-tions	[1]Mean dog weight ± S.D. (Kg)	Cumulative Amount of Test Item1 Applied (mL)	[4]Mean dose (mg) of Permethrin	Cumulative Amount of Test Item 2 Applied (mL)	[5]Mean dose (mg) of S1638
Placebo Control (1)	Adult Males	6	5	16.5 ± 1.6	[2]12.5	0	[3]5.0	0
	Adult Females	6	5	12.9 ± 1.7	[2]12.5	0	[3]5.0	0
Group 2 (1X)	Adult Males	6	1	16.5 ± 1.6	2.5	1241	1.0	160.8
	Adult Females	6	1	12.3 ± 1.8	2.5	1241	1.0	160.8
Group 3 (3X)	Adult Males	6	3	17.7 ± 2.7	7.5	3723	3.0	482.5
	Adult Females	6	3	12.0 ± 1.6	7.5	3723	3.0	482.5
Group 4 (5X)	Adult Males	6	5	16.5 ± 1.8	12.5	6205	5.0	804
	Adult Females	6	5	13.1 ± 2.7	12.5	6205	5.0	804

The means (with standard deviations) were given on p. 170 of MRID 46552707
[1]Based on Day 0 body weights
[2]Control item 1
[3]Control item 2
[4]Based on a specific gravity of 1.11 g/mL for Test item 1 and 44.32% permethrin
[5]Based on a specific gravity of 1.08 g/mL for Test item 2 and 15.45% S-1638\Nylar

C. DOSE SELECTION RATIONALE

From p. 28 of MRID 46552707: "The route of administration (topical) is the proposed route of administration for normal use of the final product. The dose levels used were the normal recommended dose for this product on adult dogs weighing between10kg and 21.9 kg, three times the normal recommended dose and five times the normal recommended dose as per the health effects test guidelines (OPPTS 870.7200)."

According to the proposed label this product will consist of single applications to dogs or puppies weighing 21 to 55 lbs. No amounts were stated for each unit package. "Outer packaging may contain up to 150 units." The product claim is that it "Kills 98-100% of the fleas on dogs within 2 hours and continues to prevent infestation for four weeks." The precautionary statements include the following: "Do not apply more than once every 30 days."

D. EXPERIMENTAL DESIGN

From p. 30 of MRID 46552707: "Each dog was examined by a Veterinary Surgeon on Study Day -15 and on Study Day -2. Only healthy adult dogs as assessed by these veterinary examinations were included in the study... General health observations were carried out on each dog twice daily (morning and afternoon) from Study Day -14 to Study Day -1, with at least 4 hours between each observation. No general health observations were performed from Study Day 0 to...14 inclusive, as clinical assessments were performed..."

"Clinical assessments were carried out on all dogs prior to the first treatment and at 1 hour (± 5 minutes), 2 hours (± 10 minutes), 3 hours (± 10 minutes) and 4 hours (± 10 minutes), after the final treatment on Study Day 0, by a veterinary surgeon. For dogs assigned to Groups 1, 3 and 4, clinical assessments were also carried out by a veterinary surgeon within 10 minutes prior to the second, third, fourth and fifth dosings, where relevant. Vomit was observed at the 4 hour clinical assessment on Study Day 0 for Animal No. 50573 (Group 3, 3x). A further assessment was performed on this animal at 5 hours (±10 minutes) after dosing. No other animal in any group required further assessments.

"In addition, clinical assessments were performed by a veterinary surgeon twice daily on all dogs assigned to Groups 1, 2, 3 and 4 from Study Day 1 to Study Day 14 inclusive, once in the morning and once in the afternoon, with at least 4 hours between assessments." Each animal was observed for at least 1 minute for the presence (score of 1) or absence (score of zero) for the following parameters:

"Lethargy, ataxia, recumbency, paralysis, coma, pruritus, hyperactivity, tremors, convulsions, abnormal mydriasis, abnormal miosis, corneal opacity, dyspnea, tachypnoea, coughing, abnormal salivation, vomiting, abnormal mucous membranes, ocular discharge, nasal discharge, cardiovascular changes, abnormal faeces (where present), abnormal urine (where present), abnormal coat condition, abnormal site of spot-on application. For every parameter given a score of "1", a brief comment, where applicable, was made to describe the abnormal assessment (e.g. abnormalities at the site of spot-on application may include oedema, erythema, alopecia, pruritus)."

"...Where the site of spot-on application showed cosmetic effects after treatment, this was considered clinically normal. Cosmetic effects included any discolouration, stiffness causing the hair to stand up (spiking), stiffness with the hair sticking together (clumping), tangling of the hair (matting), greasy appearance or any deposits. Where cosmetic effects were present, they were recorded."

"Each animal was weighed, before feeding, on Study Days -15, -2, 0, 7 and 14."

From p. 17: "...Dogs were fed once daily in the afternoon between 12:00 and 15:00. The quantity of diet offered was weighed and recorded daily from Study Day -14 to Study Day 13 inclusive. Between Study Day -13 and Study Day 14 diet not consumed from the previous day was removed from each dog's cage between 10:00 and 12:00 and subsequently weighed, with the following exceptions. Diet was withdrawn from all animals, between 16:00 and 17:00 on Study Days -15, 0 and 6 in order to facilitate fasting prior to blood sampling. The weight of the diet withdrawn was recorded as diet

not consumed for the following day."

E. PATHOLOGICAL PARAMETERS

Blood samples were collected from each animal at the following Study Days: -14, +1, and 7. Blood samples were collected on Study Day 14 for the following animals: Animal No. 11289 (Group 2, 1x) for RBC, HGB and HCT, Animal No. 50342 (Group 3, 3x) for T. Bil and D. Bil, Animal No. 50573 (Group 3, 3x) for T. Bil, and Animal No. 89074 (Group 4, 5x) for ALT.

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	Absolute reticulocytes
	Blood clotting measurements	X	Percent reticulocytes
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time [PT])*		
X	(Activated partial thromboplastin time [APTT])*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Blood creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*	X	Urea
X	Potassium*		Total Cholesterol
X	Sodium*	X	Globulin (Glob)*
		X	Glucose (Gluc)*
	ENZYMES	X	Total bilirubin (T Bil)*
X	Alkaline phosphatase(ALPor ALK)*	X	Direct bilirubin (D Bil)*
	Cholinesterase(ChE)	X	Total serum protein (TP)*
	Creatine kinase		Triglycerides
	Lactic acid dehydrogenase(LDH)		Serum protein electrophoresis
X	Serum alanine aminotransferase (ALT or SGPT)*		Albumin/Globulin (A/G) ratio
X	Serum aspartate aminotransferase(AST or SGOT)*		Lipase
	Gamma glutamyl transferase(GGT)		
	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

A statistical summary report is found in Appendix 5, from page 167 to 217 of MRID 46552707.

From p. 169: "Each variable was analyzed using analysis of covariance for a repeated measures design. The repeated factor was post-treatment day. Fixed effects were sex and treatment, and replicate (block of four animals of the same sex) was a random effect. First order interactions were included in the model, as was the three-way interaction of sex, treatment, and post-treatment day. For the hematology and blood chemistry variables, the corresponding Day -14 value was used as covariate. Mean weight on Days -15, -2, and 0 was used as the covariate for post-treatment weight. Mean daily consumption from Day -14 to Day 0 was used as the covariate for mean daily post-treatment feed consumption. Feed consumption was averaged over Days 1- 7 and Days 8- 14 for analysis.

G. DISPOSITION OF ANIMALS

Not stated. According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals." No animals died in the course of this study.

H. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

From information supplied by the registrant, the density of Test Material 1 is 1.11 g/mL, and for Test Material 2 is 1.08 g/mL. Using these densities, each 1X dog was exposed to 1241 mg permethrin,160.8 mg dinotefuran, and 16.8 mg sumilarv (nylar).

B. MORTALITY

None of the animals died.

C. CLINICAL SIGNS

From p. 38: Clinical observations seen included abnormal (loose) faeces, abnormal site of spot-on application and vomiting.

Between Study Day 8 and Study Day 12: " Vomitus was detected at one time point for two animals and two time points for one animal out of twelve animals assigned to Group 1 (control). Vomitus was detected at one time point for five animals and four time points for one animal out of twelve animals assigned to Group 2 (1x). Vomitus was detected at one time point for one animal and two time points for one animal out of twelve animals assigned to Group 3 (3x). Vomitus was detected at one time point for three animals out of twelve animals assigned to Group 4 (5x). None of the incidences of loose faeces or vomiting could be classified as being treatment related as they occurred in the control and treatment groups at various time points."

D. BODY WEIGHT AND WEIGHT GAIN

From p. 37: "Between Study Day -15 and Study Day 14 (Study Day 20 for Animal No's 14818 and 07049, Group 3, 3x) a number of animals lost weight. The remaining animals either maintained or gained body weight over the same period. Similar weight loss has been observed previously in adult dogs that are individually housed. There was no significant difference (P>0.05) in body weight between animals assigned to any of the groups on any of the study days where body weight was recorded. No gender effect was detected (P>0.05)."

E. FOOD CONSUMPTION

From p. 43, " There was no significant difference detected between the animals assigned to the treatment groups (Group 2, 3, and 4) compared with animals assigned to Group 1 (control) when mean daily food consumption was analysed. There were no gender differences detected."

TABLE 4. Mean Food Consumption Values (g/animal) for Certain Study Days

Group	g ± S.D.				
	Day -1	Day 0	Day 1	Day 2	Day 7
Control Males	487.2 ± 31.4	470.6. ± 72.8	195.6 ± 245.1	480.8 ± 47.5	351.5 ± 119.1
Control Females	375.8 ± 192.7	347.5 ± 130.0	173.3 ± 253.1	481.7 ± 44.7	320.2 ± 208.2
1X Males	480.6 ± 35.9	444.6 ± 88.9	265.3 ± 259.6	500.1± 0.6	308.8 ± 175.1
1X Females	391.7 ± 103.2	462.5 ± 92.3	164.7 ± 172.4	416.0 ± 136.0	288.0 ± 182.9
3X Males	500.0 ± 0.0	500.2 ± 0.2	266.7 ± 182.7	499.9 ± 0.3	304.1 ± 132.1
3X Females	465.8 ± 62.7	440.1 ± 92.8	342.3 ± 208.2	489.6 ± 26.0	305.3 ± 207.7
5X Males	488.8 ± 27.5	372.0 ± 118.4	65.9± 112.2	486.2 ± 34.2	183.5 ± 121.2
5X Females	443.4 ± 89.9	376.7 ± 153.9	190.0 ± 215.9	447.8 ± 90.3	325.5 ± 176.5

Values obtained from data on p. 214-215 of MRID 46552707

A number of dogs (including seven in the control group and seven in thr 5X group had very low food consumption values (< 30 g) for Day 1. As the control and 5X groups showed the greatest reductions in food consumption, this may have been an effect of exposure to one or more inerts in the formulation, or to the stress of handling during five applications. Three of the 1X dogs showed a similar reduction (< 30g) in food consumption for Day 1, which was more pronounced than on Day 7.

F. HEMATOLOGY

There were no significant differences (P>0.05) between the treatment groups and control group with respect to hematological parameters.

Female animals assigned to Group 2 (1x), Group 3 (3x), and Group 4 (5x) had significantly lower RBC counts and hematocrit (HCT) levels compared to animals assigned to Group 1 (control) on Study Day 1. On Study Day 7 one animal (#11289) had RBC and HCT values (4.54×10^6 µL and 32% respectively) which were below the laboratory's reference range. However, HCT and RBC mean results for all groups

were within the reference range (RBC 4.61- 9.39 10^6 µL; HCT 33.5- 66.2%) for animals at this laboratory at all timepoints. On study Day 14 Animal # 11289 had hematology values which were within the laboratory's reference range (RBC 5.30 x10^6 µL, HCT 38.0 %)

For Prothrombin Time (PT) male animals assigned to Group 3 (3x) had significantly lower (P=0.0329) PT averaged over Study Days 1 and 7 compared with male animals assigned to Group 1 (control). All individual animal results for PT were within the reference range (9- 25 secs) for the laboratory at all timepoints. It is concluded that there was no dose-related effect involving this parameter.

For Activated Partial Thromboplastin Time (APTT) it is stated (p. 41) that: "Animals assigned to Group 2 (1x) had a significantly lower (P=0.0114) APTT compared with animals assigned to Group 1 (control) averaged over Study Days 1 and 7. There were no gender or other differences detected." On Study Day 1 three animals in Group 2 (Males # 13090, and 58542, and Female # 36332), one animal in Group 3 (#50342), and one animal in Group 4 (#26878) had APTT values of 11 secs which were slightly lower than the laboratory's reference range (12- 45 secs). It is concluded then that there was no dose-related effect involving this parameter.

G. CLINICAL CHEMISTRY

From page 41: "There were no significant differences (P>0.05) detected between the treatment groups and the control group for the following parameters: alanine aminotransferase, aspartate aminotransferase, urea, blood urea nitrogen, creatinine, potassium, phosphorus, and direct bilirubin."

For values (mmol/L) of Glucose (GLU) it is stated (p. 42) that: "Animals assigned to Group 4 (5x) had significantly lower glucose levels (P=0.0482) than animals assigned to Group 1 (control) or Group 3 (3x), and Group 1 (control). Reduced glucose levels can be seen in animals with, for example, reduced appetite due to general illness, hypoadrenocorticism, and hepatic disease. No clinical signs of these conditions were evident in any of the animals on this study." All individual animal GLU values were within the laboratory's reference range (3.27- 6.75 mmol/l). It is concluded then that exposure to the test material had no significant effect involving glucose levels.

On Study Day -14, one female assigned to Group 3 (3x) and and two female animals assigned to Group 4 (5x) had significantly higher levels of sodium (P=0.0008 and 0.0237 respectively) than female animals assigned to Group 1 (control).There were no indications of any dose-related effects involving values (mmol/L) for Sodium (p. 42). On Study Days 1 and 7 all individual animal results were within the laboratory's reference range (114.6- 151.0).

For values (mmol/L) of Chloride (Cl) it is stated (p. 42) that: "Animals assigned to Group 2 (1x), Group 3 (3x), and Group 4 (5x) had significantly higher (P=<0.0001, 0.0079, and 0.0068 respectively) levels of chloride on Study Day 1 compared with animals assigned to Group 1 (control). On Study Day 7 only animals assigned to Group 2 (1x) had higher chloride levels (p=0.0014) compared with animals assigned to Group 1 (control). There were no clinical signs of illness, and these values are not deemed to be clinically significant. Also, except for animal # 89779 (104.7 mmol/l) all individual animal results were within the laboratory's reference range (106- 119

mmol/l) on Study Days 1 and 7.

H. NECROPSY FINDINGS

As there were no mortalities, no necropsies were necessary.

IV. DISCUSSION

In a companion animal safety study (MRID 46552707) with adult Beagle dogs, 4 groups, each with 12 (6/sex) adult (age: 12-100 months) dogs (source: Charles River Laboratories BioLabs Europe's colony at Glenamoy, Ireland; Males: 13.7-21.9 kg; Females: 10.0-17.6 kg); Dogs were treated at 0X (5 applications of 2.5 mL of the control item 1 and 1.0 mL of control item 2); 1X (1 application each of 2.5 mL of test item 1 and 1.0 mL of test item 2); 3X (3 applications of 2.5 mL of test item 1 and 1.0 mL of test item 2); and 5X (5 applications 2.5 mL of test item 1 and 1.0 mL of test item 2). The tip of the syringe was used to part the dog's hair so that the sample was applied to the skin at each of the three spots. Each administration of both test items was 1 hour apart.

Test item 1 was a colorless liquid containing 44.32% (batch TS 12441) or 44.82% (batch TS 12478) permethrin (only two 3X dogs were treated with this). Test item 2 was a colorless liquid containing 15.45% S-1638 and 1.56% Nylar (batch TS 12462), or 15.21% S-1638 and 1.57% Nylar (batch TS 12479). Only two 3X dogs were treated with this second batch.

Clinical assessments were carried out on each dog prior to the first treatment, and at approximately 1, 2, 3 and 4 hours following the last application. Dogs receiving more than one treatment application were also evaluated 10 minutes before each treatment.

According to study directions test materials 1 and 2 were applied to three sites along the dog's back (intrascapular, anterior lumbar, and posterior lumbar).

Individual body weights were measured on Days -15, -2, 0, 7 and 14. Individual food consumption was measured on a daily basis for Days -7, -6, -4 and then daily thereafter through Day 14 (values represented the amount of food consumed during the previous 24 hours). Blood samples were taken on Day -14, Day 1, and Day 7, following an overnight fast.

There was no mortality. Clinical observations seen in one or more groups included abnormal (loose) faeces, abnormal site of spot-on application, and vomiting.

There were reductions in individual animal food consumption across all groups on Day 1 and Day 7. This could be associated with fasting prior to blood sampling. However, there was no significant difference between controls and treatment groups for mean daily feed consumption.

Cosmetic effects were observed in all dogs, and included matting, greasy appearance, clumping, spiking, discolouration and deposits. The cosmetic effects had disappeared by Study Day 5 (Group 1, control), Study Day 5 (Group 2, 1x) except for Animal No. 89782 which had a greasy appearance until Study Day 12, Study Day 8 (Group 3, 3x), Study Day 11 (Group 4, 5x).

A number of dogs (including seven of 12 in the control group and seven of 12 in the 5X group) had very low food consumption values (< 30g) for Day 1. As the controls and 5X

groups showed the greatest reduction in mean food consumption, this may have been an effect associated with exposure to one or more inerts in this formulation, and/or to the stress of handling during five applications. Three of the 1X dogs showed a similar reduction in food consumption (< 30g) for Day 1. While some individual puppies in the puppy study (MRID 46552708) showed a reduced food consumption for Day 1, with no indication of a dose-related effect, adult dogs showed reduced mean food consumption on Day 7 (presumably associated with fasting and/or the stress of blood collection). The Day 7 effect was not as pronounced as that of Day 1.

Overall, a number of dogs had minor weight changes from between Study Day -15 and Study Day 14 (ranging from a loss of about 0.1 kg to 1.8 kg), with no indication of any dose-related trend. Similar weight loss has been observed previously in adult dogs that are individually housed. There was no significant difference in body weight between animals assigned to any of the groups.

There were no significant differences (P>0.05) between the treatment groups and control group with respect to clinical chemistry and/or hematological parameters. There were no symptoms or other indications of systemic toxicity.

TRB concludes that this companion animal safety study (OPPTS 870.7200) demonstrates an adequate margin of safety (at least 5X) between the exposure associated with a use application of 2- 5 mL test material (45% Permethrin) and 1.0 mL test material 2 (14.85% Dinotefuran and 1.48% Nylar) for this formulation in adult dogs weighing between 10.0 and 21.9 kg and that at which significant systemic effects may occur. It is noted that the proposed label received by TRB indicates three weight classes of dogs and puppies (< 10 lbs, 11- 20 lbs, 21- 55 lbs), but does not specify dosages. The registrant should indicate the appropriate doses for each of these weight classes, and these should be consisted with and supported by the doses used in the two companion animal safety studies for this formulation.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D318905
2. **PC CODE:** 109701 (Permethrin)
3. **CURRENT DATE:** Dec 2, 2005
4. **TEST MATERIAL:** Permethrin (44.32% -TS 12441 or 44.82% TS 12478); a colorless liquid containing 15.46% of the active S-1638, and Nylar (1.5%) consistent with the proposed product [Hartz Reference 1749] with a label declaration of 45% Permethrin (CAS #165252-70-0).

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal/Beagle/Charles River BioLabs Europe, Ireland/Study No. USA004\04-002; Hartz Test #1749 Aug 2004	46552707	4 groups of adult Beagle dogs each with 12 (6/sex) were treated at 0X (5 applications of 2.5 and 1.0 mL formulation without active), 1X (1 application of 2.5 and 1.0 of proposed product), 3X (3 applications of 2.5 and 1.0 mL proposed product) or 5X (5 applications of 2.5 and 1.0-mL proposed product). Dogs receiving more than one application got them at 1-hr intervals. Application was to 3 sites: site 1 intrascapular area; site 2 anterior lumbar region; site 3 posterior lumbar region. There was a 14-day post-exposure observation period. There was no mortality. There were reductions in individual animal food consumption across all groups on Day 1 and Day 7. This could be associated with fasting prior to blood sampling. However, there was no significant difference between controls and treatment groups for mean daily feed consumption. Clinical observations seen in one or more groups included abnormal (loose) faeces, abnormal site of spot-on application, and vomiting. Effects observed at application site were mainly cosmetic effects, observed in all dogs, and included matting, greasy appearance, clumping, spiking, discolouration and deposits. The cosmetic effects had disappeared by Study Day 5 (Group 1, control), Study Day 5 (Group 2, 1x) except for Animal No. 89782 which had a greasy appearance until Study Day 12, Study Day 8 (Group 3, 3x), Study ay 11 (Group 4, 5x). Overall, a number of dogs had minor weight changes from between Study Day -15 and Study Day 14 (ranging from a loss of about 0.1 kg to 1.8 kg), with no indication of any dose-related trend. There was no indication of an effect on any of the hematology or clinical chemistry parameters, and there were no symptoms of systemic toxicity. Study demonstrates 5X margin of safety for application to adult dogs weighing between 10 kg and 21.9 kg.	N/A	Acceptable

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