

US EPA ARCHIVE DOCUMENT



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

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

February 24, 2010

MEMORANDUM

Subject: Name of Pesticide Product: ETOFENPROX/NYLAR SPOT-ON FLEA AND
TICK FOR DOGS AND PUPPIES
EPA Reg. No. /File Symbol: 2517-RGG
DP Barcode: DP 371676
Decision No.: 420097
Action Code: R310
PC Codes: 128965 (Etofenprox: 55%)
129032 (Pyriproxyfen: 2.2%)

From: Byron T. Backus, Ph.D., Toxicologist *Byron T. Backus*
Technical Review Branch *02-24-2010*
Registration Division (7505P) *DM*

To: Kevin Sweeney/Richard Gebken, RM 13
Insecticide Branch
Registration Division (7505P)

Registrant: SERGEANT'S PET CARE PRODUCTS, INC.

FORMULATION FROM LABEL:

| | | |
|------------------------------|--------------|----------------|
| <u>Active Ingredient(s):</u> | | <u>By wt.</u> |
| 128965 Etofenprox | | 55.00% |
| 129032 Pyriproxyfen | | 2.20% |
| <u>Other Ingredient(s):</u> | | <u>42.80%</u> |
| | TOTAL | 100.00% |

ACTION REQUESTED: The Risk Manager requests:

“...Please review the attached study and documentation.”

BACKGROUND:

The material received includes a companion animal safety study (in MRID 47849609) titled: "Companion animal safety evaluation on dogs of spot-on products containing multiple toxicants and insect growth regulators" as well as a CSF and a proposed label for the product which would be applied to small and/or toy breed dogs weighing 4-20 lbs. The label dosage rate is given as 2.0 mL/dog/application.

COMMENTS AND RECOMMENDATIONS:

1. An Agency contractor, Oak Ridge National Laboratory, conducted the primary review of the companion animal safety study. TRB performed the secondary review and made changes as necessary.

2. The study is not acceptable to support the proposed use(s) on adult dogs and puppies and cannot be upgraded. Test groups consisted of 6 (3 male and 3 female) adult dogs or puppies. The 870.7200 Companion Animal Safety Guidelines specify that at least 6 animals per sex should be used at each dosage level. In addition, the adult dogs that were treated with test substance II (containing 55.16% w/w etofenprox, 8.78% w/w methoprene, and 2.31% w/w pyriproxyfen) at 5X the dosage rate weighed (on day -1) 4.06, 7.00, 7.98, 8.78, 8.94 and 9.10 kg. Since only one dog in this group weighed less than 9 lbs (4.06 kg = 8.95 lbs), the data could not be used to support the use of this formulation on adult dogs weighing 4-9 lbs, particularly as the ratio of body surface area to mass could be a factor in absorption and toxicity. Cumulative dosage rates were 3.08, 3.21, 2.82, 2.56, 2.52 and 2.47 mL/kg (the 4.06 kg dog received a cumulative amount of 12.5 mL test material; the others each received 22.5 mL), with a mean of 2.78 mL/kg; dividing this by 5 (for the 5X margin of exposure) gives 0.555 mL/kg, and dividing 2.0 mL (the proposed dose) by 0.555 mL/kg gives 3.60 kg (= 7.94 lbs), which is also substantially above the proposed 4 lb lower weight limit. For the puppies, it is impossible to compare and interpret differences in body weight gains between groups when there was such a range in ages (10-15 weeks, with no information as to specific ages for individual animals), the dogs were mixed breed and the two heaviest puppies (CC7 692 and CD4 7EC), which would be expected to show the greatest weight gains, were both in the control group.

3. TRB considers the behavioral changes (running and circling immediately after application of the test substance) in 7 animals (one adult, 6 puppies) as an adverse effect. It is noted that the animals showing behavioral changes were all exposed to test material IA (8.20% cyphenothrin, 9.8% fipronil, either in combination with material III, 8.90% methoprene and 2.43% pyriproxyfen, for the one adult, or in combination with material II, 55.16% etofenprox, 8.78% methoprene and 2.31% pyriproxyfen for the puppies). Although the four puppies in group A2 (receiving 5 applications of material IA and 5 of II) showed running and circling only after the first application of the test substance and not after the second, third, fourth or fifth application, this may simply be an indication that the application area had been numbed.

4. Refer to the attached DER for additional comments.

DATA EVALUATION RECORD

**CYPHENOTHHRIN, FIPRONIL, ETOFENPROX, METHOPRENE,
PYRIPROXIFEN
COMPANION ANIMAL SAFETY STUDY- DOGS/PUPPIES – (OPPTS 870.7200)
MRID 47849609**

Prepared for

Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

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Disclaimer

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

Oak Ridge National Laboratory managed and operated by UT_Battelle, LLC., for the U.S. Department of Energy under Contract No. DE_AC05_00OR22725.

EPA Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: 02-24-2010
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- dogs/puppies – OPPTS 870.7200

PC CODE: 128965- Ethofenprox, 129032- Pyriproxyfen,

DP BARCODE: 371676

TEST MATERIAL (PURITY): Cyphenothrin (8.2% w/w), Fipronil (9.8% m/w), Etofenprox (55.16% w/w), S-Methoprene (8.78% w/w), Methoprene (8.90% w/w) and Pyriproxifen (2.31% and 2.43% w/w)

TRADE NAME: Not provided

CITATION: Delport, P.C. (2009) Companion animal safety evaluation on dogs of spot-on products containing multiple toxicants and insect growth regulators. ClinVet International (Pty) Ltd., Bloemfontein, Republic of South Africa. Study No. CV 08/551, August 6, 2009. MRID 47849609. Unpublished.

SPONSOR: Sergeant's Pet Care Products, Plano, Texas

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47849609), groups of random-source adult (> 6 months of age) and juvenile (10-15 weeks of age) dogs were topically administered 3 combinations of insecticides and insect growth regulators or one inert substance. Two groups of three male and three female juvenile dogs were administered test substance IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil; Batch No. 012003-09) and test substance II (55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen; Batch No. 011901-09) at either 1X (recommended dose) or 5X (recommended dose hourly for 5 applications). The test substances were applied simultaneously as adjacent stripes along the dorsum of the dog from behind the ears caudally to the base of the tail. Two groups of three male and three female adult dogs weighing 9-20 kg were administered the test substance IA and test substance III (8.90% w/w methoprene and 2.43% w/w pyriproxifen; Batch No. 011902-09) at either 1X (recommended dose) or 5X (recommended dose hourly for 5 applications). The test substances were applied as previously described. A group of three male and three female adult dogs weighing less than 9 kg was administered test substance II at 5X (recommended dose hourly for 5 applications). A group of three male and three female juvenile dogs and another group of three male and three female adult dogs were administered control substance IVA (Batch No. 012004-09) containing inert ingredients (not otherwise stated) at 5X the recommended dose. The groups and test materials they received are shown in the table below:

| Group | | | |
|-------|--|--|--|
| A | Group A1 (n=6) Puppies (10-15 weeks) Single application of substance IA Single application of substance II | Group A2 (n=6) Puppies (10-15 weeks) Five applications of substance IA Five applications of substance II | |
| B | Group B1 (n=6) Adults (9-20 kg) Single application of substance IA Single application of substance III | Group B2 (n=6) Adults (9-20 kg) Five applications of substance IA Five applications of substance III | Group B3 (n=6) Adults (<9 kg) Five applications of substance II |
| C | Group C1 (n=6) Puppies (10-15 weeks) Five applications control substance IVA | Group C2 (n=6) Adults (9-20 kg) Five applications control of substance IVA | |

Substance IA contains 8.2% w/w cyphenothrin and 9.8% m/w fipronil.

Substance II contains 55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen.

Substance III contains 8.90% methoprene, 2.43% w/w pyriproxifen.

Control substance IVA contains inert control substances.

The animals were observed for clinical signs of toxicity and dermal irritation for four hours post-application and then twice daily for the duration of the study (14 days post-application). Body weight, body weight gain, food consumption and clinical pathology parameters (hematology and clinical chemistry) were measured at the required intervals.

All animals survived to the end of the study. Immediately after treatment, one adult dog in Group B1 (1X applications of test substances IA and III) showed behavioral changes (running, circling). This behavioral change was not considered treatment-related in adults by the sponsor since it was not observed at 5X the recommended dose. Immediately after treatment, two puppies in Group A1 (1X application of test substances IA and II) showed behavioral changes (running, circling). Four puppies in Group A2 (5X application of test substances IA and II) showed similar behavioral changes after the first application but not after subsequent applications. These behavioral changes are considered to be an adverse effect. White crystallization was observed on the hair tips of many adult and juvenile dogs in the treated and control groups. The application sites of all dogs in Group B3 (5X application of test substance II) had an oily or wet appearance. The sign disappeared from one dog on Day +14 but was present on all others at the end of the study.

All adult dogs and puppies gained weight from Day -1 to Day 14. However, mean weight gain in pups in the treated groups (A1 and A2) was 15% lower than the control group pups. The mean weight gain in the treated adult animals (Groups B1, B2 and B3) was decreased (30-99% as compared to the control group). The difference relative to controls was largest in the smaller adult dogs in Group B3 (99% decrease as compared to the control group). Food consumption was not affected by treatment. No treatment-related hematology or clinical chemistry changes were observed.

It is noted that the 7 animals (one adult, 6 puppies) that showed behavioral changes (running and circling) immediately after application of the test substance were all exposed to test material IA (8.20% cyphenothrin, 9.8% fipronil, either in combination with material III, 8.90% methoprene and 2.43% pyriproxifen, for the one adult, or in combination with material II, 55.16% etofenprox, 8.78% methoprene and 2.31% pyriproxifen for the puppies). Although the four puppies in group A2 (receiving 5 applications of material IA and 5 of II) showed running and circling only after the first application of the test substance and not after the second, third, fourth or fifth application, this may simply be an indication that the application area had been numbered.

Test groups consisted of 6 (3 male and 3 female) adult dogs or puppies. The 870.7200 Companion Animal Safety Guidelines specify that at least 6 animals per sex should be used at each dosage level. In addition, the adult dogs that were treated with test substance II (containing 55.16% w/w etofenprox, 8.78% w/w methoprene, and 2.31% w/w pyriproxyfen) at 5X the dosage rate weighed (on day -1) 4.06, 7.00, 7.98, 8.78, 8.94 and 9.10 kg. Since only one dog in this group weighed less than 9 lbs (4.06 kg = 8.95 lbs), the data could not be used to support the use of this product/formulation on adult dogs weighing 4-9 lbs, particularly as the ratio of body surface area to mass could be a factor in absorption and toxicity. Also, it was not possible to compare body weight changes between adult group B3 (which received the etofenprox-containing formulation) and the control (C2) group because initial mean body weights were so different. For the puppies, it is impossible to compare and interpret differences in body weight gains between groups when there was such a range in ages (10-15 weeks, with no information as to specific ages for individual animals), the dogs were mixed breed and the two heaviest puppies (CC7 692 and CD4 7EC), which would be expected to show the greatest weight gains, were both in the control group.

In addition, it is concluded that the margin of safety in puppies administered simultaneous topical application of test substances IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and II (55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen) was less than 1X the recommended dose, based on clinical signs of toxicity (behavioral changes immediately post-application).

This companion animal safety study in male and female random-source adult dogs and puppies is **Unacceptable/Guideline** and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the dog and cannot be upgraded for either adults or puppies. See Study Deficiencies section for details. Major deficiencies include too few animals per group, failure to test the end-use product and lack of some required reporting.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided for ClinVet International. Pathcare Veterinary Laboratory, which conducted the hematology and clinical chemistry analyses, is not GLP accredited but is ISO 15189 certified.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

1a. Test substance 1 (code name IA)

Description: Not provided
Batch #: 012003-09
Purity: 8.2% w/w cyphenothrin and 9.8% m/w fipronil*
Storage: At room temperature
Compound Stability: Expiration date: January 20, 2011
CAS #: Not provided

* Batch 2008-Fip.D3, expiration date August 19, 2010, packed in foil and stored at ambient temperature, inside carton box, dry

1b. Test substance 2 (code name II)

Description: Not provided
Batch #: 011901-09
Purity: 55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen
Storage: At room temperature
Compound Stability: Expiration date: January 19, 2011
CAS #: Not provided

1c. Test substance 3 (code name III)

Description: Not provided
Batch #: 011902-09
Purity: 8.90% w/w methoprene, 2.43% w/w pyriproxifen
Storage: At room temperature
Compound Stability: Expiration date: January 19, 2011
CAS #: Not provided

1d. Control substance (code name IVA)

Description: Not provided
Batch #: 012004-09
Purity: Inert control substance (no purity provided)
Storage: At room temperature
Compound Stability: Expiration date: January 20, 2011
CAS #: Not provided

2. Vehicle control: See control substance described in 1d. above.

3. Test animals:

| | |
|----------------------------------|---|
| Species: | Dog |
| Strain: | Described as “indifferent” |
| Age/weight | Groups A1 and A2: 10-15 weeks on day 0 (2.12 – 7.32 kg or 4.67-16.14 lbs) Groups B1 and B2: ≥ 6 mos on day 0 (> 9 kg ≤ 20 kg or >19.84 lb ≤ 44.09 lbs) Group B3: ≥ 6 mos on day 0 (≤ 9 kg or ≤ 19.84 lb) Group C1: 10-15 weeks on day 0 (2.88 – 9.67 kg or 6.35-21.32 lbs) Group C2: ≥ 6 mos on day 0 (> 9 kg ≤ 20 kg or >19.84 lb ≤ 44.09 lbs) |
| Source: | ClinVet International (Pty) Ltd. Colony |
| Housing: | Individually housed in pens |
| Diet: | Adults (>12 months) were fed a commercial dry dog food (example: ULTRADOG SUPERWOOF DOG FOOD); puppies (< 12 months) were fed an “appropriate puppy food” (example: Eukanuba puppy and junior medium breed) |
| Water: | Tap water, <i>ad libitum</i> |
| Environmental conditions: | |
| Temperature: | 20 ± 4° C |
| Humidity: | Not provided |
| Air changes: | 12-15 air changes/hr |
| Photoperiod: | 12 hours light/12 hours dark |
| Acclimation period: | Fourteen days |

B. STUDY DESIGN:

1. **In life dates:** Start: March 19, 2009; End: April 16, 2009
2. **Animal assignment:** Forty-two animals were assigned to the study. The genders were separated and the dogs allocated to study groups (Table 1) within the body weight requirements using randomization through minimization with body weight as the only criterion. For medium sized adult dogs, within each gender, animals were blocked into three blocks of three dogs each. The three heaviest dogs formed block 1; the next three heaviest dogs formed block 2 and the next three formed block 3. Within blocks, dogs were allocated to one of three groups, 1, 2 or 3. The first animal of block 1 was assigned to group 1, the second to group 2 and the third to group 3. The first animal of block 2 was assigned to group 3, the second to group 2 and the third to group 1. The first animal of block 3 was assigned to group 1, the second to group 2 and the third to group 3. Groups 1, 2 and 3 were then randomly assigned to the actual groups B1, B2 and C using Excel randbetween. Puppies were similarly assigned to groups A1, A2 and C1. The six dogs in group B3 were allocated; no randomization was possible.

| Group | | | |
|-------|--|--|--|
| A | Group A1 (n=6) Puppies (10-15 weeks) Single application of substance IA Single application of substance II | Group A2 (n=6) Puppies (10-15 weeks) Five applications of substance IA Five applications of substance II | |
| B | Group B1 (n=6) Adults (9-20 kg) Single application of substance IA Single application of substance III | Group B2 (n=6) Adults (9-20 kg) Five applications of substance IA Five applications of substance III | Group B3 (n=6) Adults (<9 kg) Five applications of substance II |
| C | Group C1 (n=6) Puppies (10-15 weeks) Five applications control substance IVA | Group C2 (n=6) Adults (9-20 kg) Five applications control of substance IVA | |

Substance IA contains 8.2% w/w cyphenothrin and 9.8% m/w fipronil.

Substance II contains 55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen.

Substance III contains 8.90% methoprene, 2.43% w/w pyriproxifen.

Control substance IVA contains inert control substances.

3. Dose selection rationale: The dose rates for test substance IA are based on fipronil and methoprene on the Frontline Plus label and pyriproxifen in Sergeant's Gold for Dogs. For test substance II, the study report states that the maximum etofenprox small dog dose rate is 758 mg/kg. For test substance III, the dose of pyriproxifen and s-methoprene is based on Sergeant's Gold for Dogs and Frontline Plus label. However, none of the dose rates used is representative of the proposed label.

4. Preparation and treatment: The test and control substances were applied as measured doses from syringes (without needles) to a furrow in the hair on the back, starting high on the neck behind the ears and extending caudally towards the base of the tail. The fur was not shaved or clipped. Technicians applying the substances were blinded to the test material. Test substances IA, II and III were applied separately as "terminally adjacent stripes, applying test substance IA anterior to test substance II or test substance IA anterior to test substance III." The placebo control formulation IVA was applied separately. Substances IA, II and III were not mixed.

Test substances IA and II were applied as single doses, once only, to the pups in test group A1 and five times consequently at hourly intervals, each time as a single dose, to the pups in test group A2. Substances IA and II were not mixed.

Test substances IA and III were applied as single doses, once only, to the adult dogs in group B1, five times consequently at hourly intervals, each time as a single dose, to the adult dogs in group B2. Substances IA and III were not mixed. Test substance II was applied five times consequently at hourly intervals, each time as a single dose, to the adult dogs in group B3.

The control substance IVA was applied five times consequently at hourly intervals, each time as a single dose, to pups and dogs in groups C1 and C2, respectively.

The dose volumes of the test and control substances were determined by the animal's weight on Day -1 (Table 2).

| Table 2: Doses | | |
|----------------|----------------|-------------------------------------|
| Group | Test substance | Total dose administered |
| A1 (puppies) | IA | 0.83 – 2.0 mL (1X) |
| | II | 3.1 – 7.5 mL (1X) |
| A2 (puppies) | IA | 5.0 – 13.30 mL (1.0 – 2.66 mL x 5) |
| | II | 18.8 – 50.0 mL (3.76 – 10.0 mL x 5) |
| B1 (adults) | IA | 1.34 mL (1X) |
| | III | 1.34 mL (1X) |
| B2 (adults) | IA | 3.4 - 6.7 mL (0.68 – 1.34 mL x 5) |
| | III | 3.4 - 6.7 mL (0.68 – 1.34 mL x 5) |
| B3 (adults) | II | 12.5 - 22.5 mL (2.5 – 4.5 mL x 5) |
| C1 (puppies) | IVA | 13.10 mL (2.62 mL x 5) |
| C2 (adults) | IVA | 5.0 mL (1.0 mL x 5) |

Substance IA contains 8.2% w/w cyphenothrin and 9.8% m/w fipronil.

Substance II contains 55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen.

Substance III contains 8.90% methoprene, 2.43% w/w pyriproxifen.

Control substance IVA contains inert control substances.

5. **Statistics:** The emphasis of the statistical analyses was the change from baseline of each of the hematology and clinical chemistry parameters. Individual hematology and clinical chemistry values on test days were tabulated separately for each variable and each group, together with the following descriptive statistics: mean, standard deviation (SD), coefficient of variation (CV %), geometric mean, geometric SD, minimum, maximum and the number of observations in the group. The individual changes and percentage changes from baseline (test day -3) for each of the post-treatment days were calculated and tabulated for each variable and each group. The number of post-treatment values that fell outside the reference range was calculated. The post-treatment values were compared to the baseline values in an intra-treatment comparison by means of an analysis of variance (ANOVA) with an animal and observation time as effects. An inter-treatment comparison with respect to the changes from baseline was also performed. The post-treatment values of each the three test treatment groups (B1, B2 and B3) were compared to the control group (C2) for adult dogs. The post-treatment values of each of the two test treatment groups (A1 and A2) were compared to the two control groups (C1) for puppies. An analysis of covariance (CO-ANOVA) with a treatment effect and the baseline values as covariate was used for the analyses. In addition, the change from baseline values of each of the three treatment groups (B1, B2 and B3) were compared to those of the control group (C2) for dogs and the two test groups (A1 and A2) were compared to those of the control group (C1) for puppies by means of an ANOVA with a treatment effect. Groups A1 and A2 were compared in a similar way.

C. METHODS:

1. Observations:

- a. **General health observations:** The animals were observed daily for general health. Specific health observations were observed hourly on the day of application until four hours after application of the test material and twice daily, thereafter, for the duration of the study. Observations included, but were not limited to, changes in skin and fur, eyes and mucous membranes, nervous signs and behavior pattern as well as vomiting and diarrhea. Local tolerance observations were also included.

b. **Veterinary examinations:** Physical examinations were conducted by a veterinarian on Days -14, -3, +1, +7 and +14. The examinations included but were not limited to body temperature, pulse rate, auscultation of the heart and lungs, mucous membranes and skin, joints, eyes, ears, mouth and genital organs.

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

3. **Food consumption:** The amount of food offered was measured and the food consumption was recorded daily and scored using the following scoring system.

Fc 1 - 0- 25%

Fc 2 - >25- 50%

Fc 3 - >50-75%

Fc 4 - >75-100%

4. **Hematology and clinical chemistry:** Blood was collected from the jugular vein for hematology and clinical chemistry assessments on fasted animals on the following days: - 3, +1, +7 and +14. The tests were conducted by Pathcare, Bloemfontein, South Africa, because ClinVet International did not have the expertise and apparatus to perform the analyses. The CHECKED (X) parameters were examined.

a. **Hematology**

| | | | |
|---|------------------------------|---|--------------------------------|
| X | Hematocrit (HCT)* | X | Leukocyte differential count* |
| X | Hemoglobin (HGB)* | X | Mean corpuscular HGB (MCH)* |
| X | Leukocyte count (WBC)* | X | Mean corpusc. HGB conc.(MCHC)* |
| X | Erythrocyte count (RBC)* | X | Mean corpusc. volume (MCV)* |
| X | Platelet count* | X | Reticulocyte count |
| | Blood clotting measurements* | | |
| X | (Thromboplastin time) | | |
| | (Fibrinogen) | | |
| X | (Prothrombin time) | | |

*Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. Clinical chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|------------------------------|
| X | Calcium* | X | Albumin* |
| X | Chloride* | X | Creatinine* |
| | Magnesium | X | Urea nitrogen* |
| X | Phosphorus * | | Total Cholesterol |
| X | Potassium* (K) | X | Globulins* |
| X | Sodium* (NA) | X | Glucose* |
| | ENZYMES (more than 2 hepatic enzymes, eg., *) | X | Total bilirubin * |
| X | Alkaline phosphatase (AP)* | X | Total protein* |
| | Cholinesterase (ChE) | | Triglycerides |
| | Creatine kinase | | Albumin/Globulin ratio |
| | Lactic acid dehydrogenase (LDH) | X | Direct bilirubin* |
| X | Alanine aminotransferase (ALT/also SGPT)* | | Indirect bilirubin |
| X | Aspartate aminotransferase (AST/also SGOT)* | | BUN/Creatinine ratio |
| | Gamma glutamyl transferase (GGT) | | TCO ₂ Bicarbonate |
| | Amylase | | |
| | Sorbitol dehydrogenase | | |

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200

5. **Urinalysis:** Urinalysis was not conducted.

6. **Sacrifice and pathology:** The study did not have a scheduled necropsy.

II. RESULTS

A. **ACTUAL DOSES ADMINISTERED:** The mg/kg doses of active ingredients were not calculated. Based on calculations by the EPA reviewer, the mL/kg doses to puppies were significantly higher than those to adults. For example, adults in Group B1 weighing 11.3 to 19.38 kg received 1.34 mL each of test substances IA and III for a 0.12 to 0.07 mL/kg dose of each substance. Puppies in Group A1 weighing 2.12 to 5.56 kg received 0.83 to 2.0 mL of test substance IA for a 0.36 to 0.39 mL/kg dose, approximately 5 times the adult dose. Puppies in Group IA received 3.1 to 7.5 mL of test substance II for a 1.34 to 1.46 mL/kg dose, approximately 19 times the adult dog dose.

B. OBSERVATIONS:

1. **Clinical signs of toxicity:** Immediately after treatment, one adult dog in Group B1 showed behavioral changes (running, circling). Another adult dog in Group B3 had skin irritation on the shaved area of the neck (assumed to be area shaved for blood collection). In Group C2, one dog had loose feces three hours post-application, one dog had loose mucoid feces with blood on Day +2 and another dog had mucoid feces on Day +2. Minor clinical signs observed in adult dogs after treatment included the following: swollen mammary gland (Group C2, Days +7 and +14); scab on scrotum (Group C2, Day +14); eczema on neck (Group B3, Day +7); hair loss on chin (Group B3, Days +7 and +14); and right eye lacrimation (Group B2, Day +7). A female in Group B1 came into estrus on Day +1.

Immediately after treatment, two puppies in Group A1 showed behavioral changes (running, circling). Four puppies in Group A2 showed similar behavioral changes after the first application but not after subsequent applications. One pup in Group A1 had loose feces on Day +1 with blood on Day +4. A pup in Group A2 had loose feces on Day +13. Minor clinical signs in puppies after treatment included the following: thinning of hair in one pup in Group A2 on Day +7 which was diagnosed as demodicosis on Day +14; thinning of hair (Group A2, Day +14); and scales (two pups in Group C1, Day +14). While the demodicosis (mange) was not life threatening, its occurrence suggests that the overall health of the dogs and puppies may have been compromised.

2. **Application site examination:** White crystallization was observed on the hair tips of adult dogs in Groups B1 [four dogs at one hour post-application and one dog on Day +1], B2 (three dogs at one hour post-application, one dog on Day +1 and two dogs on day +2), B3 (two dogs at one hour post-application, four dogs at three hours post-application and one dog at Day +2) and C2 (three dogs at two hours post-application, one dog at 3 hours post-application, one dog at four hours post-application and one dog at Day +2). The application sites of all dogs in Group B3 had an oily or wet appearance. The sign disappeared from one dog on Day +14 but was present on all other dogs at the end of the study.

White crystals were observed on the hair tips of pups in Groups A1 (two pups at one hour post-application and one pup at three hours post-application), A2 (one pup each at one hour and two hours post-application) and C1 (three pups at one hour post-application and one pup at two hours post-application).

3. **Mortality:** All adult and juvenile dogs survived to the end of the study.
- B. BODY WEIGHT AND WEIGHT GAIN:** Body weight data are presented in Table 3. All adult and juvenile dogs gained weight from Day -1 to Day 14. However, mean weight gain in pups in the treated groups (A1 and A2) was 15% lower than the control group pups. The mean weight gain in the treated adult animals was decreased (30-99% as compared to the control group). The change was largest in the smaller adult dogs in Group B3 (99% decrease as compared to the control group).

| Group | Body weight (kg) | | | | Body weight gain (kg) | | |
|-------------|------------------|---------------------|---------------------|---------|---------------------------|---------------------------|-------------------------|
| | Day -1 | Day +1 ^b | Day +7 ^b | Day +14 | Day -1 to +1 ^b | Day -1 to +7 ^b | Day -1 to +14 |
| A1 (pups) | 4.24 | 4.30 | 4.78 | 5.44 | 0.06 | 0.54 | 1.20 (15%) ^c |
| A2 (pups) | 4.94 | 5.06 | 5.54 | 6.15 | 0.12 | 0.60 | 1.21 (15%) ^c |
| C1 (pups) | 5.24 | 5.50 | 5.89 | 6.66 | 0.26 | 0.65 | 1.42 |
| B1 (adults) | 16.07 | 16.41 | 16.63 | 16.62 | 0.34 | 0.56 | 0.55 (30%) ^c |
| B2 (adults) | 15.56 | 15.48 | 15.75 | 15.80 | -0.08 | 0.19 | 0.24 (70%) ^c |
| B3 (adults) | 7.64 | 7.62 | 7.62 | 7.65 | -0.02 | -0.02 | 0.01 (99%) ^c |
| C2 (adults) | 16.05 | 16.30 | 16.93 | 16.84 | 0.25 | 0.88 | 0.79 |

^a N = 6/group, except where noted.

^b Calculated by the reviewer from data on pages 46-47, MRID 47849609

^c Decrease compared to control group, calculated by the reviewer

Group A1 treated at 1X with Substance IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and Substance II (55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen)

Group A2 treated at 5X with Substance IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and Substance II (55.16% w/w etofenprox, 8.78% w/w s-methoprene, 2.31% w/w pyriproxifen)

Groups C1 and C2 treated at 5X with Control substance IVA

Group B1 treated at 1X with Substance IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and Substance III (8.90% methoprene, 2.43% w/w pyriproxifen)

Group B2 treated at 5X with Substance IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and Substance III (8.90% methoprene, 2.43% w/w pyriproxifen)

C. FOOD CONSUMPTION: No treatment-related effects were reported.

D. CLINICAL PATHOLOGY ANALYSES:

- 1. Hematology:** No treatment-related changes were observed in adults or pups. An increase (as compared to the control group) in monocyte counts in Group B1 adult dogs on Day +1 was not considered toxicologically significant.
- 2. Clinical Chemistry:** No treatment-related effects were reported in adults or pups. A decrease (as compared to the control group) in creatinine in Group B3 on Day +7 and a decrease in AST in Group B3 on Day +14 were not considered toxicologically significant. The mean glucose value in Group A2 was increased from baseline (Day -3) to Days +1 and +14. The mean chloride values in Groups A1 and A2 were increased from baseline to Day +7. These deviations are not considered toxicologically significant.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that two test substances, blend IA containing 8.20% w/w cyphenothrin and 9.80% m/w fipronil and blend II containing 55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen, were safe to use under the conditions of the study when applied simultaneously as a spot-on to puppies at the recommended dose as well as five times (5X) the recommended dose.

Blend II containing 55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen, was safe to use under the conditions of the study when applied topically to adult dogs at five times the recommended dose.

Two test substances, blend IA containing 8.20% w/w cyphenothrin and 10.07% w/w fipronil and blend III containing 8.90% w/w methoprene and 2.43% w/w pyriproxifen, were safe to use under the conditions of the study when applied simultaneously as a spot-on to adult dogs at the recommended dose as well as at five times the recommended dose.

B. REVIEWER COMMENTS: All animals survived to the end of the study. Immediately after treatment, one adult dog in Group B1 (1X applications of test substances IA and III) showed behavioral changes (running, circling). Immediately after treatment, two puppies in Group A1 (1X application of test substances IA and II) showed similar behavior. Four puppies in Group A2 (5X application of test substances IA and II) showed the same behavior after the first application but not after subsequent applications. The behavioral signs are considered treatment-related in puppies since they occurred in multiple animals both at 1X and 5X the recommended dose. The behavioral change in adult dogs is considered treatment-related since it also occurred in puppies.

White crystallization was observed on the hair tips of many adult and puppies in the treated and control groups. The application sites of all dogs in Group B3 (5X application of test substance II) had an oily or wet appearance. The sign disappeared from one dog on Day +14 but was present on all other dogs at the end of the study. Since there was no skin irritation, the signs are considered cosmetic. However, the persistence of test substance II on the hair of dogs for more than 14 days is of concern due to potential human exposure of owners to pets.

All adult and juvenile dogs gained weight from Day -1 to Day 14. However, mean weight gain in pups in the treated groups (A1 and A2) was 15% lower than the control group pups. The change is considered treatment-related. The mean weight gain in the treated adult animals (Groups B1, B2 and B3) was decreased (30-99% as compared to the control group). The change was largest in the smaller adult dogs in Group B3 (99% decrease as compared to the control group). The animals were considered adults if they were >6 months of age. Adult dogs usually are not expected to change weight in a 14-day period; however, dogs as young as 6 months may still be gaining weight. The birth dates of the animals were not provided so their exact age is unknown. Since the decreases from the control group were large, the decreased body weight gain is considered treatment-related in adults. Food consumption was not affected by treatment. No treatment-related hematology or clinical chemistry changes were observed.

It is concluded that the margin of safety in puppies administered simultaneous topical applications of test substances IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and II (55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen) was less than 1X the recommended dose, based on clinical signs of toxicity (behavioral changes immediately post-application).

It is concluded that the margin of safety in adult dogs (9-20 kg) administered simultaneous topical application of test substances IA (8.2% w/w cyphenothrin and 9.8% m/w fipronil) and III (8.90% w/w and 2.43% w/w pyriproxifen) was less than 1X the recommended dose, based on clinical signs of toxicity (circling and running in one dog after application).

It is concluded that no margin of safety in adult dogs (< 9 kg) administered a topical application of test substance II (55.16% w/w etofenprox, 8.78% w/w s-methoprene and 2.31% w/w pyriproxifen) could be established from the findings of this study.

This study is unacceptable and cannot be upgraded for either adult dogs or puppies.

C. STUDY DEFICIENCIES:

- a. The study report states that the purpose of the study is to determine the safety of a combination of three test formulation spot-on products containing three toxicants and two insect growth regulators. The Companion Animal Safety Guideline (OPPTS 870.2700) requires that a study be conducted with the end-use product using the label dose and directions for use, although there is a provision for use of a max/tox product containing multiple toxicants at the maximum levels which would be anticipated in formulated topical products. However, this does not appear to have been the objective of the study. It is also unclear why different combinations of products were tested in adult and juvenile animals. Pups were administered test substances IA and II, whereas large adult dogs were administered test substances IA and III and smaller dogs were administered test substance II alone.
- b. Other Companion Animal Safety Guideline non-compliances are as follows:
 1. The study used three dogs/sex/group, whereas six dogs/sex/group are required.
 2. Individual animal data for clinical signs were not provided.
 3. Group mean values for body weight were not calculated for Days -14, -7, +1 and +7.
 4. The control formulation is described as "inert control substance". However, it is not certain for which of the test formulations was this the "inert control substance," as no analysis was provided.
 5. The date of birth of the animals was not provided.
 6. The mg/kg dose of active ingredients was not calculated.
 7. The laboratory which conducted the hematology and clinical chemistry testing is not GLP accredited.
 8. The Guideline allows for five hourly applications of a 1X dose to achieve the 5X dose. However, with the large volumes of test material used on some of the smaller animals, it is likely that the material ran off and was not available for absorption.
 9. The study report states that test substances IA, II and III were applied separately as terminally adjacent stripes, applying test substance IA anterior to test substance II or test substance IA anterior to test substance III. It is unclear how the second substance could be applied anteriorly when both were administered from the neck to the base of the tail.
 10. A certificate of analysis was not provided for each of the test substances. The study report states that the concentration of cyphenothrin in test substance IA should have been 15% as stated in the protocol but a certificate of analysis indicated that it was 8.2%.

11. The study author's conclusion (page 56) gives different percentages of fipronil in test substance IA when discussing the findings in puppies (9.8% m/w) and adults (10.07% w/w).

1. **DP BARCODE:** 371676
2. **PC CODES:** 128965 (Etofenprox); 129032 (Pyriproxyfen)
3. **CURRENT DATE:** February 24, 2010
4. **TEST MATERIALS:** Blend IA: 8.20% w/w cyphenothrin, 9.80% w/w fipronil; Blend II: 55.16% w/w etofenprox, 8.78% w/w methoprene, 2.31% w/w pyriproxyfen; Blend III: 8.90% w/w methoprene, 2.43% w/w pyriproxyfen; Blend IVA: inert control substance

| Study/Species/Lab Study # / Date | MRID | Results | Tox. Cat. | Core Grade |
|---|----------|--|--------------|---------------|
| Companion Animal Safety Study/Dogs & Puppies ClinVet International (Pty) Ltd; Bloemfontein, South Africa Study CV 08/551 / August 6, 2009. | 47849609 | Four groups (each 6M & 6F) of adult dogs; 3 groups (each 6M & 6F) of puppies were used. Adults: Group B1 1X IA&III; Group B2: 5X 1A&III, Group B3: 5X II; Group C2 5X IVA. Puppies: Group A1 1X IA&II; Group A2: 5X IA&II; Group C1: 5X IVA. Not possible to adequately interpret data from design of study. | N/A | U |

Core Grade Key: A = Acceptable, S = Supplementary, U = Unacceptable, W = Waived