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EPA Reviewer: Byron T. Backus, Ph.D. **Signature**: _____
Technical Review Branch, Registration Division (7505P) **Date:** _____
Template version 02/2006

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Puppies; OPPTS 870.7200

PC CODES: 129032; 044312 **DP BARCODE:** 358752

TEST MATERIAL (PURITY): DTE; Batch No. H-988-018; (22.074% Dinotefuran, 3.099% Nylar), described as a clear, colorless liquid

SYNONYMS: SVP9

CITATION: Gupta, S. (2008) Tolerance of an Experimental Flea Dermal Treatment when Topically Administered to Approximately 7 Week Old Pups at 5 Times the Recommended Dose. Project Number: USA011/08-003, USA011/08/081107. Unpublished study prepared by Charles River Laboratories BioLabs Europe. 134 p. MRID 47592901.

SPONSOR: Summit VetPharm, Fort Lee, N.J.

EXECUTIVE SUMMARY: In a 14-day companion animal (puppy) safety study (MRID 47592901), two groups, each consisting of 4 males and 4 females, were treated on Day 0 with five 1.3 mL applications of either DTE blank (Batch No. H-988-016, described as a colorless liquid) or DTE (Batch No. H-988-018, containing 22.0% Dinotefuran and 3.02% Nylar). The puppies were beagles from Charles River Laboratories Preclinical Services Ireland Ltd.'s colony. On Day 0 the males ranged in age from 47 to 55 days, and the females from 54 to 55 days. On Day -1 males weighed from 2.4 to 3.0 kg; females weighed from 2.1-2.6 kg.

The DTE (or the DTE blank) was applied topically to the skin at the base of the skull. A total of 5 applications were made, spaced approximately 1 hour (± 5 minutes) apart. Each individual application was dispensed at one spot. The puppies were treated on study day 0, and no repeat treatment was carried out on subsequent days.

There were no deaths or treatment-related effects on body weight, food consumption, hematology, or clinical chemistry. Adverse events were limited to loose feces in one DTE-treated puppy at pretreatment on Day 0 and at +1 hour on Day 0, and loose feces (with slightly bloody diarrhea) in a DTE blank-treated puppy on Study Day 1; the latter was found to be negative for internal parasites including coccidia and giardia. Cosmetic effects were observed in all puppies; these had resolved for all in the DTE-treated group by the morning of Study Day 2.

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Two control females (animal numbers 62577 and 71785) had noticeably reduced food consumption on Day 1 (82 and 220 g), as did two 5X DTE-treated females (13069 and 67592, 99 and 250 g respectively). All other puppies consumed the full 400 g ration on that date. The reduced food consumption in some females on Day 1 may have been an adverse effect from exposure to the solvent(s) in the formulation.

All puppies were treated vaccinated and wormed prior to acclimation, with no vaccinations or worming medications administered during the study period.

This study is classified as minimally acceptable, and in conjunction with a previously submitted puppy study (in MRID 47246601, with additional information in MRID 47519901) adequately addresses the companion animal safety study (OPPTS 870.7200) data requirement for the use of this product on puppies of 8 weeks of age or older [as 6/8 of the puppies treated with the test material DTE were 54 or 55 days old on Study Day 0, this study does not provide adequate support for a use on puppies of 7 weeks of age] and weighing ≥ 2.1 kg (=4.6 lbs, the minimum day -1 weight among the puppies treated with 5X DTE). The proposed label should be revised to indicate use only on puppies from 8 weeks of age and weighing ≥ 4.6 lbs.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: DTE

Description:	Colorless liquid
Lot/Batch #:	H-988-018
Purity:	22.074% Dinotefuran, 3.099% Nylar
Compound Stability:	One year from date of manufacture at room temperature; Expiration date: Oct. 3, 2009
CAS #:	Pyriproxyfen (95737-68-1); Dinotefuran (165252-70-0)

2. Vehicle and/or positive control: "DTE blank" (Lot/Batch No. H-988-016) was used as a control. The ingredients [presumably the inerts in DTE] were not listed.

3. Test animals:

Species:	Puppies
Breed:	Beagles
Age/weight at study initiation:	Males: 47-55 days old; Females: 54-55 days old [Day -1] Males: 2.4-3.0 kg; Females: 2.1-2.6 kg
Source:	Charles River Laboratories Preclinical Services Ireland Ltd. colony
Housing:	Individually housed in pens measuring 1.7 m x 1.4 m.
Diet:	Pedigree pup food manufactured by Pedigree Masterfoods, Meltron Mowbray. Leicester, LE13)BR, U.K. ~400 g/pup/day, offered once daily between 12:00 and 15:00. From Study Day -1 to Study Day 14 diet not consumed the previous day was removed between !0:00 and 12:00 and weighed.
Water:	*Ad libitum* via stainless steel bowls

Environmental conditions:	**Temperature:**	16-19° C
	Humidity:	41-64%
	Air changes:	Not provided
	Photoperiod:	Not provided
Acclimation period:	7 days	

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B. STUDY DESIGN:

1. **In life dates:** Start: October 8, 2008; Administration of test item (Study Day 0): October 15, 2008; End of animal phase (Study Day 14): October 29, 2008.

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups using random order numbers derived from Fisher and Yates tables. Animals were listed by gender and within each gender animals were listed in order of decreasing body weight recorded on Study Day -1. Where two animals had the same weight they were listed in order of decreasing microchip number. The first two puppies formed a block and were randomly allocated, one to each group. This process was continued for each block until 16 animals were allocated into two groups, each consisting of 8 animals (4 males and 4 females).

TABLE 1. Study design [a]

Test Group	Treatment	Number of males	Number of females
1. Control	5 applications of a 1.3 mL volume of the control	4	4
2. 5X	5 applications of a 1.3 mL volume of the test material	4	4

[a] Data taken from the table on p. 20, MRID 47592901.

3. **Dose selection rationale:** According to the study report, a 1.3 mL dosing volume is the normal recommended dose for this product for puppies; according to the label dosage rates are 1.3 mL for dogs and puppies weighing from 2.5 to 10 lbs (1.13 to 4.53 kg); 2.0 mL for dogs and puppies from 11-20 lbs (4.99-9.07 kg); 4.0 mL for 21-55 lbs (9.53-24.95 kg), 6.0 mL for 55-100 lbs (24.95-45.36 kg) and 7.4 mL for >100 lbs (>45.36 kg). The doses used in the study are consistent with 5X that specified for dogs (and puppies) of 2.1-3.0 kg, and the exaggerated doses were achieved via multiple applications of the end-use product (see below). The control substance was also administered at 5 times the normal recommended dosing volume of the product. It is unknown whether the control substance contained the inert ingredients of the formulation at identical levels as would be found in the 1X formulation as this was not stated in the study report.

4. **Treatment:** The control or test material, as appropriate, was applied topically, using a 2-mL syringe, at a dosing volume of 1.3 mL (per application). For each application, the tip of the syringe was positioned at the base of the animal's head and used to part the animal's hair to apply the contents directly to the skin. The entire contents of the syringe were then dispensed at one spot, avoiding contact with the eyes and mouth. Dosing volume was confirmed visually immediately prior to administration, and each syringe was checked after administration to ensure none of the contents remained.

   Five dosing periods were conducted at approximately hourly intervals (generally within ±5 minutes).

5. **Statistics:** From p. 68 of MRID 47592901: "Descriptive statistics (mean and standard

deviation [SD]) for each continuous variable were calculated at each time point for each of the two treatment groups. All descriptive statistics are presented for males and females and overall… These statistics, and all other analyses, were performed using programs in SAS®/STAT (SAS® v8.2).

"Each continuous 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 two animals of the same sex as described above) 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 -6 value was used as a covariate. Day -1 body weight was used as the covariate for post-treatment body weight for the puppies. Mean daily feed consumption from Day -1 to Day 0 (2 daily values) was used as the covariate for mean daily post-treatment feed consumption; feed consumption was averaged over Days 1-7 (7 daily values) and Days 8-14 (7 daily values) for analysis.

"When a main effect or interaction term involving Treatment was statistically significant at p 0.05, pairwise comparison of the control group to the medicated group was performed at the highest-order significant interaction terms. Thus, when the three-way interaction of sex, treatment and post-treatment day was significant, treatments were compared within sex and day, and significant two-way interactions and treatment effect were ignored. When the three-way interaction was not significant, but the two-way interaction of treatment and post-treatment day was significant, treatments were compared within day, averaged over sex, and a significant treatment effect was ignored… Finally, when none of the interactions were significant, and the overall test of treatments was significant, pairwise comparison of treatments was done, averaged over sex and post-treatment day.

"…because there were only two post-treatment sampling days for body weight, hematology and clinical chemistry in the study, and the feed consumption was averaged over Days 1-7… and Days 8-14 to two post-treatment values for analysis…there were…only three different VCV [variance-covariance structures] to compare. The best-fitting VCV, based on Akaike's Information Criterion (AIC), is noted for each variable in the ANCOVA table…"

C. METHODS:

1. Observations:

1a. General health observations: Each animal was observed once daily from Study Day -7 to -1.

1b. Clinical assessments: Clinical assessments were conducted on all animals prior to the first treatment, approximately 10 minutes prior to each of the second through fifth dosing periods, and at approximately 1, 2, 3 and 4 hours after the

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final treatment on Study Day 0. During the remainder of the study (days 1 through 14), a veterinarian conducted clinical assessments on all animals twice daily, once in the morning and once in the afternoon, with at least 4 hours between examinations. Clinical assessments consisted of observing each animal for at least one minute and recording the presence or absence of the following: lethargy, ataxia, recumbency, paralysis, coma, pruritus, hyperactivity, tremors, convulsions, abnormal mydriasis, abnormal miosis, corneal opacity, dyspnea, tachypnea, coughing, abnormal salivation, vomiting, abnormal mucous membranes, ocular discharge, nasal discharge, cardiovascular changes, abnormal feces (when feces were present), abnormal urine (when urine was present), abnormal coat condition, abnormal site of spot-on application. When feces or urine were present they were removed after each assessment. When the spot-on application site showed a cosmetic effect after treatment this was considered clinically normal. Cosmetic effects included discoloration, spiking of hair, clumping of hair, tangling (matting) of hair, greasy appearance or any deposits. When cosmetic effects were present, they were recorded.

2. **Body weight:** The animals were weighed on days -7, -1, 7, and 14.

3. **Food consumption:** Each animal was given a weighed (400 g) quantity of food. The amount remaining in the bowl the following day was weighed, and the quantity consumed was calculated and recorded.

4. **Hematology and clinical chemistry:** Baseline blood samples were collected on study day
-6, and post-treatment blood samples were collected on study days 1 and 7. There is no indication within the report as to whether or not the puppies were fasted prior to blood collection. The report did not include mention of the particular venipuncture site or sites used. 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		Reticulocyte count
	Blood clotting measurements		
X	(Activated partial thromboplastin time)*		
X	(Prothrombin time)*		

* Recommended for companion animals safety evaluation based on OPPTS 870.7200

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b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen (BUN)*
X	Phosphorus*		Cholesterol
X	Potassium*	X	Globulin*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)		Indirect bilirubin
	Creatine phosphokinase	X	Total protein (TP)*
	Lactic acid dehydrogenase (LDH)		Triglycerides
X	Alanine aminotransferase (ALT/also SGPT)*		Serum protein electrophoresis
X	Aspartate aminotransferase (AST/also SGOT)*		Albumin/globulin ratio
	Sorbitol dehydrogenase		Bicarbonate (TCO_2)
	Gamma glutamyl transferase (GGT)	X	Urea
	Glutamate dehydrogenase		

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

5. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** The reported clinical signs data are given in Table 2. According to the text on p. 26 of MRID 47592901: "Animal no. 13069 assigned to Group 2 (Test Item) was observed to have loose feces at the pre-treatment clinical assessment on Study Day 0 and was also observed to have loose feces along with normal feces in its pen at +1 hour clinical assessment on Study Day 0… Animal no. 08537 assigned to Group 1 (Control) was observed to have loose feces along with normal feces in its pen during the morning clinical assessment on Study Day 1… Slightly bloody diarrhea was also observed in the pen… The feces of this animal was [sic] collected for examination and found negative for internal parasites including coccidian / giardia… At all other timepoints, both animals had normal feces, and all remaining animals had normal feces during the course of the study."

TABLE 2. Number of Reported Post-Treatment Clinical Signs [a]

Observation	Control		Control	
	Males	Females	Males	Females
Abnormal feces	1*	0	1	0

[a] Taken from the text on p. 26 of MRID 47592901
* "Slightly bloody diarrhea."

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2. **Cosmetic effects:** Cosmetic effects (such as spiking, clumping, matting, greasy appearance and/or deposits) were observed in all animals from the pre-treatment no. 2 time point [this would be following the first application] on Study Day 0 until the +4 hour time point on Study Day 0. These cosmetic effects had resolved for all Group 1 (Control) puppies on the morning of Study Day 1, and for all Group 2 (DTE-treated) puppies on the morning of Study Day 2.

3. **Mortality:** There were no deaths or moribund sacrifices.

B. BODY WEIGHT AND WEIGHT GAIN:

Body weight data are given in Table 3. There were no treatment-related effects on body weight or body weight gain.

TABLE 3: Body weight data from puppies treated topically with DTE (H-988-018) [a]

Parameter/ Study day or interval		Dosage			
		Control	5X DTE	Control	5X DTE
		Males		Females	
Body Weight (kg):	**Day -7**	2.35 ± 0.10	2.43 ± 0.26	2.28 ± 0.15	2.20 ± 0.20
	Day -1	2.73 ± 0.22	2.70 ± 0.24	2.43 ± 0.15	2.43 ± 0.22
	Day 7	2.90 ± 0.22	2.93 ± 0.29	2.55 ± 0.17	2.69 ± 0.24
	Day 14	3.10 ± 0.22	3.15 ± 0.19	2.80 ± 0.16	2.85 ± 0.26
BW gain (kg) [b]:	**Days -7 to -1**	0.38 ± 0.22	0.28 ± 0.10	0.15 ± 0.10	0.23 ± 0.05
	Days -1 to 7	0.18 ± 0.10	0.23 ± 0.05	0.13 ± 0.13	0.18 ± 0.13
	Days 7 to 14	0.20 ± 0.08	0.23 ± 0.10	0.25 ± 0.13	0.25 ± 0.06

[a] Calculated from data on p. 32, MRID 47592901. Values are Mean ± Standard Deviation, with n=4 for all groups.
[b] Calculated by reviewer using individual body weight values.

C. FOOD CONSUMPTION:

Selected mean food consumption data are given in Table 4. It was a common occurrence for the entire provided amount (400 g) to be eaten. It is noted that two control females had noticeably reduced food consumption on Day 1 (220 and 82 g) and two 5X DTE females also had noticeably reduced food consumption on Day 1 (250 and 99 g).

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TABLE 4: Mean daily food consumption (g) from puppies treated topically with DTE (H-988-018) [a]				
Study day or interval	Dosage			
	Control	5X DTE	Control	5X DTE
	Males		Females	
Day -1	400.0 ± 0.0	400.0 ± 0.0	400.0 ± 0.0	400.0 ± 0.0
Day 0	400.0 ± 0.0	400.0 ± 0.0	389.0 ± 22.0	374.5 ± 30.3
Day 1	396.0 ± 8.0	400.0 ± 0.0	275.5 ± 154.4	287.3 ± 144.0
Day 2	400.0 ± 0.0	380.5 ± 39.0	400.0 ± 0.0	336.3 ± 123.5
Day 3	400.0 ± 0.0	338.3 ±123.5	335.5 ± 129.0	337.3 ± 106.4
Day 4	400.0 ± 0.0	400.0 ± 0.0	367.8 ± 64.5	365.5 ± 49.0
Day 5	400.0 ± 0.0	400.0 ± 0.0	296.0 ± 173.3	400.0 ± 0.0
Day 6	400.0 ± 0.0	369.3 ± 61.5	301.0 ± 154.3	387.3 ± 25.5
Day 7	400.0 ± 0.0	400.0 ± 0.0	348.0 ± 75.5	400.0 ± 0.0
Day 8	400.0 ± 0.0	400.0 ± 0.0	372.5 ± 36.9	400.0 ± 0.0
Day 9[b]	400.0 ± 0.0	400.0 ± 0.0	383.0 ± 34.0	400.0 ± 0.0

[a] From data on p. 37 and 103 of MRID 47592901. Values are Mean ± Standard Deviation, with n=4 for all groups.

[b] From Day 10 through Day 14 the 5X DTE animals all consumed their entire ration of 400 g. One control male did not consume the entire ration on Day 13, while one or more control females did not consume the entire ration on Days 10, 11 and 12.

D. BLOOD ANALYSES:

1. **Hematology:** Pups treated with 5X DTE had significantly lower hemoglobin on Day 7 than their controls; however, the Day 7 values in the 5X DTE treated animals were higher than their Day -6 and +1 values (Day – 6 values: M: 10.3 g/dL; F: 10.1 g/dL; Day +1 values: M: 10.6 g/dL; F: 10.4 g/dL: Day +7 values: M: 11.3 g/dL; F: 10.7 g/dL). Statistically significant differences in mean monocyte counts for females and elevated mean numbers of eosinophils for 5X DTE-treated puppies of both sexes were not biologically relevant with the exception of the Day 7 eosinophil level in a single 5X DTE female (1.79 x 10^9/L, outside the reference range of 0.10-1.25 x 10^9/L), which may have been indicative of (among other possibilities) intestinal parasites.

2. **Clinical chemistry:** Puppies treated with 5X DTE showed higher mean chloride on Day 7 relative to their controls, but, given the reference range (97.3-144.2 mmol/L) the differences were not biologically relevant (Day 7 means were the following: control males: 112.0 mmol/L; F: 108.8 mmol/L; 5X DTE males: 113.3 mmol/L; F: 113.0 mmol/L).

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III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that treatment with DTE, when topically administered to pups at 5X the recommended dose is well tolerated both locally and systemically. There were short-term cosmetic effects at the site of administration, which were of little (if any) clinical significance.

B. REVIEWER COMMENTS:

The reviewer agrees that the application of the test material (or a 5X volume of the control) did not result in mortality or any evident adverse effects on body weight, hematology, or clinical chemistry. There may have been an effect on food consumption involving the solvent(s) in this formulation, as 2/4 females in both the control and 5X DTE-treated groups showed reduced food consumption on Day 1. Although the study utilized only 4 puppies/sex/dose, the findings, when taken in conjunction with previously submitted data (particularly the study in MRID 47246601), allow this puppy study to be classified as minimally acceptable. This study can be used to support the use of the proposed product on puppies $\geq$ 8 weeks of age and $\geq$ 2.1 kg (= 4.6 lbs, the minimum day -1 weight among the puppies treated with 5X DTE). The proposed label should be revised accordingly.

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