**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:** 345267

**TEST MATERIAL (PURITY):** DTE (X-6249-07); Lot AA9208; (22.0% Dinotefuran, 3.02% Nylar)

**SYNONYMS:** DTE Formula 1

**CITATION:** Neumann, N. (2007) DTE companion animal safety evaluation (7 week and older puppies). Cheri-Hill Kennel & Supply, Inc., Stanwood, Michigan. Study Number SVP07015, September 22, 2007. MRID 47246601. Unpublished.

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

**EXECUTIVE SUMMARY:** In a 14-day companion animal safety study (MRID 47246601), DTE (X-6249-07) (22.0% Dinotefuran, 3.02% Nylar; Lot/Batch # AA9208) was applied topically at the base of the skull to groups of six male and six female 50-60 day (specified as 7.4 to 8.3 week) old beagle puppies at 1X (1.3 mL), 3X (three applications of 1.3 mL), and 5X (five applications of 1.3 mL). **"**DTE blank" (X-6284-07) (Lot/Batch No. GLP-2122; otherwise not defined) was applied in identical manner to a control group of 6 male and 6 female 50-60 day old beagle puppies as five applications of 1.3 mL. Animals were treated on study day 0, and no repeat treatment was carried out. The puppies were supplied from Ridglan Farms, Mt. Horeb, WI 53672; on study day -1 males weighed from 3.1 to 6.2 lbs; females weighed from 2.8 to 5.1 lbs.

There were no deaths or treatment-related effects on body weight, food consumption, hematology, or clinical chemistry. Unspecified numbers of animals were treated for coccidia, giardia, upper respiratory signs, and/or conjunctivitis during acclimation, and treatment for the conjunctivitis continued into the main study. While the initial review of this study stated that: "Accurate interpretation of the clinical signs data is precluded by the presence of infectious disease and/or pre-existing illness in the animals," further evaluation has resulted in the tentative conclusion that the puppies may have been sufficiently healthy from day -1 so that the study can be reclassified as marginally acceptable. This is partially based on survival (all puppies survived to the end of the study), as well as the weight data from day -1 to 7 (with the exception of one 3X female all gained or maintained weight during this interval). Observations of tremors, lethargy/depression, and vomiting in females at the 5X treatment level may have been treatment-related or due to concurrent infectious disease, and high incidences of

"abnormal feces" in all groups due to coccidia or giardia, may easily have hidden a treatment-related effect on defecation. "Ocular discharge" noted in 1-7 animals from most groups at most of the examinations may have been related to the pre-exisisting conjunctivitis, and yet animals treated at 5X had a significantly greater incidence of ocular discharge than controls at the afternoon examination on day 2 and the morning observation on day 3 (0, 3-4, 1, and 5 control, 1X, 3X, and 5X animals affected, respectively). It is possible that the continued treatment for conjunctivitis may have lessened the duration or severity of treatment-related ocular effects, and that exposure to the test material at the 5X dose may have caused an increase in ocular discharge/conjunctivitis.

**The study is currently classified as unacceptable, although it may be reclassified to marginally acceptable provided data adequately addressing the deficiencies indicated below are provided:**

- The exact nature of the control material should be provided or stated.
- Individual animal data should be provided regarding the time of observation for each abnormal sign, its severity, and its subsequent course.
- A tabular summary of clinical signs data should be provided including a description of the observations and the time of onset. Clinical signs such as ocular discharge, abnormal feces, or abnormal urine should be further characterized.
- The study report should include mention of which particular puppy or puppies had "cherry eye" and required treatment with topical ophthalmic ointment over the entire study duration. All medications given to the puppies, even during acclimation, should be included in the study report.
- Any discrepancies or inaccuracies in the reporting of the clinical signs should be resolved. For example, the text (p. 26 of MRID 47246601) mentions one "situation" that occurred on day 13 and included tremors of the ears, followed by diaphragmatic spasms, and then vomiting, which in turn was followed by depression/lethargy, and disappearance of the tremors while the animal slept. However, according to Table 94 (p. 119) the only incident involving depression/lethargy occurred on day 4 in a 5X female; while tremors only occurred in a 5X female on day 13. In addition, one 5X female is reported (p. 129) to have vomited on day 4, and a 5X female (whether this was the same animal that vomited on day 4 is unknown) is reported to have vomited on day 5, while a control male vomited on day 8. There is no mention of a 5X female which vomited on day 13.

Without the information indicated above, this companion animal safety study in puppies **is classified as unacceptable and does not currently satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in puppies.

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

# I. MATERIALS AND METHODS

## A. MATERIALS:

**1. Test material:** DTE (X-6249-07)

| | |
|---|---|
| **Description:** | Colorless liquid |
| **Lot/Batch #:** | AA9208 |
| **Purity:** | 22.0% Dinotefuran, 3.02% Nylar |
| **Compound Stability:** | One year from date of manufacture at room temperature; Expiration date: April 17, 2008 |
| **CAS #:** | Pyriproxyfen (95737-68-1); Dinotefuran (165252-70-0) |

**2. Vehicle and/or positive control: "**DTE blank" (X-6284-07) (Lot/Batch No. GLP-2122) was used as a control. The ingredients were not listed.

**3. Test animals:**

| | | |
|---|---|---|
| **Species:** | Dog | |
| **Breed:** | Purpose-bred beagles | |
| **Age/weight at study initiation:** | 50-60 days old/<br>Males: 2.8-6.0 pounds; Females: 2.9-5.0 pounds | |
| **Source:** | Ridglan Farms, Inc., Mt. Horeb, Wisconsin | |
| **Housing:** | Individually in raised metal cages, measuring at least 2'x4'x3' (l x w x h), with polypropylene dividers. | |
| **Diet:** | Purina Hi-Pro®, 100 g/animal/day at the start of acclimation, gradually increased to 200 g/animal/day | |
| **Water:** | *Ad libitum* via sipper tubes | |
| **Environmental conditions:** | **Temperature:** | 19-24° C |
| | **Humidity:** | 33-69% |
| | **Air changes:** | Not provided |
| | **Photoperiod:** | Not provided |
| **Acclimation period:** | 7 or 8 days | |

**B. STUDY DESIGN:**

1. **In life dates:** Start: June 5, 2007; End: June 20, 2007

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups using stratified blocked randomization according to body weight. The study was conducted in two replicates (or sets), each containing three animals per sex per treatment group.

**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 | 6 | 6 |
| **2. 1X** | 1 application of a 1.3 mL volume of the test material | 6 | 6 |
| **3. 3X** | 3 applications of a 1.3 mL volume of the test material | 6 | 6 |
| **4. 5X** | 5 applications of a 1.3 mL volume of the test material | 6 | 6 |

[a] Data taken from Table I, p. 29, MRID 47246601.

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, with no mention of dosing according to body weight ranges. The doses used in the study were those specified in the guideline: 1X, 3X, and 5X the recommended dose, 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; this was not stated in the study report.

4. **Treatment:** The control or test material, as appropriate, was applied topically, using a 3-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, with some deviations). The control and 5X groups were dosed during all five dosing periods. The 1X group was dosed during the first dosing period and received "sham doses" during the second through fifth dosing periods, and the 3X group was dosed during the first through third dosing periods and received "sham doses" during the fourth and fifth dosing periods. "Sham dosing" consisted of bringing an animal to the treatment area and holding it there for approximately the same amount of time required for a treatment.

5. **Statistics:** For continuous data (body weight, food consumption, hematology, clinical chemistry, and coagulation parameters), descriptive statistics (mean and standard deviation) were calculated for each treatment group (separately by sex and combined)

at each time point. These data were analyzed using analysis of covariance for a repeated measures design, with post-treatment day as the repeated factor. Fixed effects included sex and treatment, and replicate was a random effect. Two-way (first-order) interactions were included in the model, as was the three-way interaction of sex, treatment, and post-treatment day. For hematology and blood chemistry parameters, the corresponding day -7 value was used as a covariate. The day -1 body weight was used as the covariate for post-treatment body weight. Mean daily food consumption from day -6 to day 0 was used as the covariate for mean daily post-treatment food consumption. The daily post-treatment food consumption values were averaged over days 1-7 and 8-14 for analysis.

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

Five variance-covariance structures were compared for each variable using the Akaike Information Criterion and the one with the lowest value was selected: first-order autoregressive; heterogeneous first-order autoregressive; compound symmetry; heterogeneous compound symmetry; and unrestricted.

Categorical data from the clinical assessments were analyzed using Fisher's Exact Test.

## C. METHODS:

### 1. Observations:

**1a. General health observations:** The animals were observed for mortality or abnormal clinical signs twice daily (at least four hours apart) during acclimation.

**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 final treatment. The study protocol stipulated ±5 minutes of variation for the timing of the 1 hour observation and ±10 minutes of variation for the observations at 2, 3, and 4 hours after the final dose, but there were a number of deviations

from these time frames. 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: ocular signs (corneal opacity, mydriasis, miosis, nystagmus, discharge); altered equilibrium (unsteadiness, lack of coordination, ataxia); muscular disturbances (tremors, drooping lip, paralysis); altered behavior (anxiety/apprehension, circling, comatose, depression/lethargy, sedation/recumbency); integument changes (alopecia, hair coat condition, pruritus, erythema); gastrointestinal abnormalities (salivation, vomiting, abnormal feces); color of mucous membranes (pale, hyperemic, cyanotic); respiratory signs (dyspnea, tachypnea, coughing, nasal discharge, apnea); renal effects (abnormal urine); and cosmetic effects at the application site (discoloration, matting, spiking, clumping). If any of these were present, additional comments could be made, if needed.

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

3. **Food consumption:** Each animal was given a weighed quantity of food. The amount remaining in the bowl the following day was weighed, and the quantity consumed was recorded. A 100 g quantity was given in the beginning of acclimation, and this amount was gradually increased to 200 g per day due to the growth, and resultant increased food consumption of the puppies. Food was removed from all cages at least 6 hours prior to blood sampling, and the weight of the unconsumed diet in these cases was recorded as not being consumed on the following day.

4. **Hematology and clinical chemistry:** Baseline blood samples were collected on study day 7, and post-treatment blood samples were collected on study days 1 and 7, following a fast of at least 6 hours duration. The report did not include mention of the particular venipuncture site or sites used. Additional samples were collected from some animals on study day 14 and analyzed. According to the protocol, this was to be done if an animal had any parameters outside the reference range in the sample taken on study day 7, but there was no mention of this in the methods section of the study report, and the individual results from these animals, e.g. which parameters needed re-evaluation, were not discussed. 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)* |
| | Platelet count | | Reticulocyte count |
| | Blood clotting measurements | | |
| | (Thromboplastin time)* | | |
| | (Clotting time) | | |
| | (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 (BUN)* |
| X | Phosphorus* | X [a] | Cholesterol |
| X | Potassium* | X | Globulins* |
| X | Sodium* | X | Glucose* |
| | **ENZYMES** | X | Total bilirubin* |
| X | Alkaline phosphatase (ALK)* | X | Direct bilirubin* |
| | Cholinesterase (ChE) | X [a] | Indirect bilirubin |
| X [a] | 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)* | X [a] | Albumin/globulin ratio |
| | Sorbitol dehydrogenase | X [a] | Bicarbonate ($TCO_2$) |
| | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200.
[a]Not included in methods section, but results were reported in the results section and the statistical report.

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. The tabular summary did not include any description of the observations or the time of onset, and no individual animal data were provided. The text mentioned that there was "one situation" involving tremors of the ears, followed by diaphragmatic spasms, and then vomiting, which in turn was followed by depression/lethargy, and

disappearance of the tremors while the animal slept. The author cited a time of occurrence for this group of findings as day 13, but there were no incidences of vomiting or depression/lethargy noted on day 13 in the statistical report.

High incidences of "abnormal feces" were reported in all groups, and the text also mentioned a large incidence of "bloody feces." According to the study author, the abnormal stools were attributable to pre-existing infections with coccidia or giardia (confirmed via fecal examination). Animals in the 3X group had a significantly increased incidence of "abnormal feces" during the afternoon assessments on day 5, when the finding was noted from 2, 8, and 2 animals from the control, 3X, and 5X groups, respectively. The increased incidence in 3X animals on day 5 was not considered treatment-related and may be indicative of more severe infections in these particular animals.

High incidences of "ocular discharge" were also reported in all groups, probably due, in part, to conjunctivitis that was present in an unspecified number of puppies during acclimation and continuing into the main study. Ocular discharge was observed in 4, 7, 2, and 5 animals, prior to the first treatment on day 0 and was noted in 1-7 animals from most groups at most of the examinations. At the afternoon examination on day 2 and the morning observation on day 3, animals treated at 5X had a significantly greater incidence of ocular discharge than controls (0, 3-4, 1, and 5 control, 1X, 3X, and 5X animals affected, respectively).

The study author attributed the high incidence of ocular discharge in some of the animals having "cherry eye" and/or entropion, saying that these are common in beagles, but it is also possible that the conjunctivitis and ocular discharge were manifestations of infectious disease, as an unspecified number of the puppies had upper respiratory signs, including mucopurulent nasal discharge, during acclimation. A breakdown of the incidences of serous ocular discharge/epiphora, conjunctivitis, "cherry eye," and entropion (specific ocular findings mentioned in the text) was not available to the reviewer, but the report did mention the presence of "cherry eye" in a single (unidentified) animal.

In discussion of the "haircoat" observations that were reported as both "abnormal clinical assessments" and "adverse events," the study author mentioned dehydration as a pre-existing condition (presumably continuing into the study interval) that would be included under the "haircoat" category. One of the "haircoat" observations was dandruff, but it is unknown at which treatment level or how many times it was noted.

| TABLE 2: Total incidences of abnormal clinical signs from puppies treated topically with DTE (X-6249-07) [a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Observation** | **Dosage** | | | | | | | |
| | **Control** | **1X** | **3X** | **5X** | **Control** | **1X** | **3X** | **5X** |
| | **Males** | | | | **Females** | | | |
| **Abnormal feces** | 56 | 68 | 116 | 59 | 58 | 57 | 83 | 75 |
| **Ocular discharge** | 35 | 71 | 21 | 69 | 68 | 118 | 67 | 93 |
| **Tremors** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 [b] |
| **Depressed/Lethargic** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 [c] |
| **Vomiting** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 [d] |
| **Pale** | 0 | 0 | 0 | 0 | 0 | 0 | 4 [e] | 0 |
| **Haircoat condition** | 0 | 0 | 0 | 0 | 4 | 1 | 6 | 0 |
| **Pruritus** | 3 | 6 | 3 | 3 | 2 | 3 | 1 | 4 |
| **No feces present** | 56 | 43 | 38 | 50 | 48 | 68 | 46 | 55 |
| **No urine present** | 99 | 94 | 88 | 91 | 104 | 103 | 94 | 101 |

[a]Data taken from text (p. 26) and Tables V (p. 35), 92 and 94 (p. 119), 96 (p. 120), and 104 (p. 129), MRID 47246601.
[b]Observed on day 13.
[c]Observed on day 4 and 13.
[d]Single observations on days 4, 5, and 13.
[e]Single observations on days 0, 4, 6, and 9.

2. **Cosmetic effects:** Matting, spiking, and clumping of the hair were observed in all groups.

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 (X-6249-07) [a] | | | | |
|---|---|---|---|---|
| **Parameter/ Study day or interval** | **Dosage** | | | |
| | **Control** | **1X** | **3X** | **5X** |
| **Males** | | | | |
| **Body Weight (pounds): Day -7** | 3.92±1.13 | 3.97±0.67 | 3.70±0.55 | 3.58±0.49 |
| **Day -1** | 4.35±1.04 | 4.27±0.75 | 4.05±0.62 | 4.13±0.63 |
| **Day 7** | 5.25±1.42 | 5.23±0.65 | 5.05±1.03 | 5.05±0.74 |
| **Day 14** | 6.43±1.45 | 6.37±0.59 | 5.90±1.16 | 6.10±0.87 |
| **BW gain (pounds) [b]: Days -7 to -1** | 0.43 | 0.30 | 0.35 | 0.55 |
| **Days -1 to 7** | 0.90 | 0.96 | 1.00 | 0.92 |
| **Days 7 to 14** | 1.18 | 1.14 | 0.85 | 1.05 |
| **Females** | | | | |
| **Body Weight (pounds): Day -7** | 3.67±0.79 | 3.40±0.47 | 3.80±0.43 | 3.30±0.37 |
| **Day -1** | 3.92±0.65 | 3.82±0.42 | 3.77±0.59 | 3.75±0.61 |
| **Day 7** | 4.53±0.84 | 4.42±0.38 | 4.20±0.74 | 4.30±0.35 |
| **Day 14** | 5.65±1.29 | 5.45±0.75 | 4.82±0.66 | 5.15±0.34 |
| **BW gain (pounds) [b]: Days -7 to -1** | 0.25 | 0.42 | -0.03 | 0.45 |
| **Days -1 to 7** | 0.61 | 0.60 | 0.43 | 0.55 |
| **Days 7 to 14** | 1.12 | 1.03 | 0.62 | 0.85 |

[a] Data taken from p. 69, MRID 47246601. Values are Mean ± Standard Deviation (where available), with n=6 for all groups.

[b] Calculated by reviewer using group mean body weight values; not analyzed statistically

## C. FOOD CONSUMPTION:

Selected mean food consumption data are given in Table 4. Food spillage was common, and when it occurred, no value was recorded for that animal for that day. It was also a common occurrence for the entire provided amount to be eaten. The daily food consumption values showed a lot of variability both within and between groups, and there were no clear treatment-related effects.

There was a statistically significant ($p<0.05$) overall treatment effect for mean daily food consumption; namely, compared to controls, the puppies treated at the 3X level had significantly lower mean daily food consumption averaged over sex and post-treatment day ($p<0.05$). This was not considered treatment-related, as a dose response pattern was not seen.

| TABLE 4: Mean daily food consumption (g) from puppies treated topically with DTE (X-6249-07) [a] | | | | |
|---|---|---|---|---|
| **Study day or interval** | **Dosage** | | | |
| | **Control** | **1X** | **3X** | **5X** |
| **Males** | | | | |
| **Day -6** | 80.7±15.8 | 87.5±17.4 | 92.7±16.3 | 86.1±17.5 |
| **Day -1** | 105.9±50.2 | 137.3±21.2 | 132.2±18.7 | 150.0±0.0 |
| **Day 0** | 111.3±38.5 | 142.3±15.6 | 128.5±29.7 | 150.0±0.0 |
| **Day 1** | 104.5±39.4 | 118.8±35.4 | 99.1±22.9 | 101.6±30.1 |
| **Day 7** | 124.2±61.4 | 113.9±46.8 | 109.2±79.2 | 113.8±67.6 |
| **Day 14** | 138.4±48.1 | 183.6±34.5 | 164.6±29.5 | 187.4±21.7 |
| **Females** | | | | |
| **Day -6** | 75.4±21.4 | 80.3±24.5 | 94.6±10.8 | 88.5±16.1 |
| **Day -1** | 116.5±23.0 | 105.8±36.7 | 103.9±21.7 | 130.4±23.5 |
| **Day 0** | 104.3±27.1 | 88.0±43.9 | 103.0±29.5 | 123.6±21.0 |
| **Day 1** | 88.8±38.6 | 92.6±40.9 | 67.8±29.1 | 103.4±24.3 |
| **Day 7** | 98.8±56.3 | 129.9±43.1 | 83.0±42.1 | 84.5±56.8 |
| **Day 14** | 163.8±51.5 | 160.3±36.0 | 154.9±18.6 | 164.8±21.4 |
| **Combined [b]** | | | | |
| **Days 0-7** | 117.2 | 131.4 (+12) [c] | 111.2 | 125.5 |
| **Days 7-14** | 163.3 | 166.9 | 146.6 (-10) | 165.3 |
| **Days 0-14** | 138.7 | 148.0 | 127.7 | 144.1 |

[a] Data taken from pp. 111-112, MRID 47246601. Values are Mean ± Standard Deviation (where available), with n=3-6 for all groups.
[b] Calculated by reviewer using group mean values.
[c] Numbers in parentheses equal percent different from control; calculated by reviewer.

## D. BLOOD ANALYSES:

1. **Hematology:** Although statistically significant differences or interactions were found for some parameters, none of the statistical findings were considered biologically significant or treatment-related. Where there were sex-by-treatment or sex-by-treatment-by-day interactions, there were no significant differences between treated animals and controls at either time point. In cases where overall treatment effects were found by averaging across treatment days, the mean values for both days fell within the provided reference range and/or the magnitude of the difference from control was of insufficient magnitude to be considered biologically significant.

2. **Clinical chemistry:** Puppies treated at the 3X level had lower calcium than controls on day 7, but this was not considered treatment-related or biologically significant because the mean values for both sexes were well within the provided reference range, the differences from the control values were small in magnitude, and a dose response pattern was not evident. A statistically significant decrease in chloride level

at the 5X treatment level, when the results were averaged over sex and post-treatment day was not considered biologically significant, due to the small magnitude of each day's difference from control and the fact that all mean values (for each sex and the combined sexes) were well within the provided reference range on both days.

## III. DISCUSSION AND CONCLUSIONS

### A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that treatment at 1X, 3X, and 5X the recommended topical dose did not result in any clinically significant "so-called abnormal clinical assessments," adverse events, impaired growth, or effects on hematology or clinical chemistry. The study author also concluded that treatment at all levels, including control, did result in short-term cosmetic effects at the site of administration, which were of little 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 clear adverse effects on body weight, food consumption, hematology, or clinical chemistry. The reviewer agrees that a 5X application of the test material appears to have resulted in a transient increased incidence of ocular discharge.

However, it is the opinion of the reviewer that the impaired health status of the animals complicates the interpretation of the clinical signs data. A treatment-related effect on defecation frequency or quality may have been obscured by the high rate of abnormal feces across all groups, and it is unclear whether the lethargy/depression, vomiting, and tremors exhibited by one or more 5X females were due to treatment or infectious disease. Moreover, although a treatment-related ocular effect was evident, the continued treatment for conjunctivitis may have obscured or lessened its duration or severity.

In addition, the manner in which the clinical signs were recorded and reported was problematic. Recording the presence or absence of *particular* clinical signs is satisfactory only if it does not preclude the mention of other, more serious, signs that are not on the list. For example, it is implied in the text of the report that some animals were dehydrated at the beginning of the study, but this does not appear in the actual data and was, apparently, tabulated as an effect on "haircoat."

**The study is currently classified as unacceptable, although it may be reclassified to marginally accepted, provided data adequately addressing the deficiencies indicated below are provided.**

## C. STUDY DEFICIENCIES:

The following deficiencies were identified in the conduct of this study:

- The exact nature of the control material was not provided or stated.
- Individual animal data were not reported regarding the time of observation of each abnormal sign, its severity, and its subsequent course.
- A tabular summary of the clinical signs data should be provided including a description of the observations and the time of onset. Clinical signs such as ocular discharge, abnormal feces, or abnormal urine should be further characterized.
- The study report should have included mention of which particular puppy or puppies had "cherry eye" and required treatment with topical ophthalmic ointment over the entire study duration. All medications given to the puppies, even during acclimation, should be reported.
- Any discrepancies of inaccuracies in the reporting of the clinical signs should be resolved. For example, the text (p. 26 of MRID 47246601) mentions one "situation" that occurred on day 13, and included tremors of the ears, followed by diaphragmatic spasms, and then vomiting, which in turn was followed by depression/lethargy and disappearance of the tremors while the animal slept. However, according to Table 94 (p. 119) the only incident involving depression/lethargy occurred on day 4 in a 5X female, while tremors only occurred in a 5X female on day 13. In addition, one 5X female is reported (p. 129) to have vomited on day 4, and a 5X female (whether this was the same animal that vomited on day 4 is unknown) is reported to have vomited on day 5, while a control male vomited on day 8. There is no mention of a 5X female which vomited on day 13.