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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

OFFICE OF PESTICIDE PROGRAMS
REGISTRATION DIVISION (7505P)

January 27, 2012

MEMORANDUM: Companion Animal Safety Studies for 11556-RLL (FLUMETHRIN COLLAR)

Subject:	Name of Pesticide Product:	PNR1427 INSECTICIDE
	EPA Reg. No. /File Symbol:	11556-RLL
	DP Barcode:	DP 385560 + DP 396978
	Decision No.:	440307
	Action Code:	R110.0
	PC Codes:	129099 (Imidacloprid)
		036007 (Flumethrin)

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

Byron T. Backus
Jan. 27, 2012
M Ashi

To: BeWanda Alexander/Richard Gebken RM 10
Insecticide Branch
Registration Division (7505P)

Registrant: BAYER HEALTHCARE LLC

FORMULATION FROM LABEL:

Active Ingredient(s):		by wt.
129099 Imidacloprid		10.0%
036007 Flumethrin		4.5%
Other Ingredient(s):		85.5%
	TOTAL	100.0%

ACTION REQUESTED: The Risk Manager requests:

For DP 385560: "Please review attached companion animal safety studies submitted in support of a new end use product containing two active ingredients: one currently registered (imidacloprid) and a new active ingredient (flumethrin).

For DP 396978: "Please review attached requested companion animal safety studies on adult cats and adult dogs per teleconference on October 20, 2011. Bayer has recalculated the exposure of the target

[companion] animals to the levels of the two active ingredients released from the collars worn by the pets during the study.

BACKGROUND:

The material for review in DP 385560 consists of eight companion animal safety studies for collars (containing 10% imidacloprid and 4.5% flumethrin), as follows: MRID 48240108 (61-day adult cat study); 048240109 (61-day adult dog study); 48240110 (180-day beagle puppy study starting when puppies were 7 weeks old); 48240111 (180-day kitten study starting when kittens were ~10 weeks old); 48240112 (safety of collar + reflectors in puppies); 48240113 (safety of collar + reflectors in kittens); 48240114 (safety of collar ± reflectors in adult cats); and 48240115 (safety of collars ± reflectors in adult dogs).

The material for review in DP 396978 consists of two final report amendments relating to the rate of release of the actives from the collar: MRID 48674701 (Final Report Amendment 1 to the study in MRID 48240109) and 48674702 (Final Report Amendment 2 to the study in MRID 48240108). These two reports were reviewed by TRB and the reviews were incorporated into the DERs for MRIDs 48240109 and 48240108, respectively.

COMMENTS AND RECOMMENDATIONS:

1. A contractor (Summitec Corporation) did the primary reviews on the eight companion animal safety studies and produced a DER for each study; TRB secondarily reviewed the studies and DERs, making revisions where appropriate. The two reports in MRIDs 48674701 and 4867402 were reviewed by TRB, and the reviews were incorporated into the final DERs for MRIDs 48240109 and 48240108, respectively.

2. The studies in MRIDs 48240108 (with the report amendment in 48674702), 48240109 (with the report amendment in 48674701), 48240110 and 48240111 have all been classified as acceptable guideline studies. These studies support the proposed use of this collar in adult dogs and puppies of seven weeks of age and older, and in adult cats and kittens of 10 weeks of age and older.

3. The studies in MRIDs 48240112, 48240113, 48240114 and 48240115 have all been classified as acceptable non-guideline studies. These studies demonstrate that there is no indication of a decrease in safety (or increase in toxicological hazard to the companion animals) associated with the wearing of collars with reflectors.

4. Refer to the attached DERs for the executive summaries and additional comments.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427 INSECTICIDE COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY - CATS (OPPTS 870.7200)

MRID 48240108

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

Prepared by
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Task Order No. 3-C-04

Primary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H.	Signature:	Virginia A. Dobozy, AE
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	Date:	SEP 27 2011

Disclaimer

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

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

Signature: Byron T. Backus
Date: Jan. 27, 2012

EPA Secondary Reviewer: Masih Hashim, Ph.D., D.V.M
Technical Review Branch, Registration Division (7505P)

Signature: M Hashim
Date: 1-31-12

Template version 02/06

DATA EVALUATION RECORD

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

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **DP BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)]

TRADE NAME: Not provided

CITATIONS: Madsen, T.J. (2010) Safety of PNR 1427 in adult cats. Sinclair Research Center, Auxvasse, MO. In-Life Testing Facility Study No. S10065, April 1, 2010. MRID 48240108. Unpublished.

Madsen, T.J. (2010) Final report amendment 1 to SRC study S10065. Sinclair Research Center, Auxvasse, MO. In-Life Testing Facility Study No. S10065, June 2, 2010. MRID 48240108. Unpublished.

Chopade, H. (2011) Final Report Amendment 2 to Bayer Report 33800 (MRID 48240108) - Safety of PNR 1427 in Adult Cats. Project Number: 33968, S10065, 152/152. November 22, 2011. MRID 48674702. Unpublished.

SPONSOR: Bayer HealthCare LLC/Animal Health Division, Shawnee Mission, KS

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 48240108), the safety of PNR 1427 insecticide collars containing imidacloprid (10% w/w) and flumethrin (4.5% w/w) was tested in adult domestic short hair cats (9.5 to 9.7 months of age). One group of three male and three female cats served as negative control. Another group of three male and three female cats served as a placebo control and wore five end-use collars minus the active ingredients for 61 days continuously. A third group (1x) of three male and three female cats wore one end-use collar for 61 days continuously. In a fourth group (5x) of six male and six female cats, five end-use collars were applied on day 0 and then replaced with five new collars on days 14, 28 and 42 and removed on day 61. In addition to measuring the required parameters, the end-use collars worn by selected cats in the 1x and 5x groups were analyzed post-removal to determine the exposures of cats to the active ingredients.

All animals survived to the end of the study. Clinical observations reported included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge in all treatment groups; however, no raw or summarized data were included in the final report. No effects on body weight, body weight gain, food consumption or clinical pathology parameters were observed. Statistically

significant findings for hematology and clinical chemistry parameters were not considered treatment-related since they were either isolated, inconsistent or not associated with clinical signs. Based on the chemical analyses of worn collars and additional information reported in MRID 48674702, cats in the 5x group were exposed to 5.2x the dose of imidacloprid and 4.0 x the dose of flumethrin received by cats in the 1x group.

It is concluded that the margin of safety in adult cats exposed to PNR 1427 insecticide [imidacloprid (10% w/w) + flumethrin (4.5% w/w)] collar for 61 days is 4x based on the chemical analyses of the worn collars which yielded 5.2x the recommended dose of imidacloprid and 4.0x the recommended dose of flumethrin. The mean collar weight (after trimming) applied to the 1x cats was 11.17 g equivalent to 2.82 g/kg body weight.

This companion animal safety study in male and female adult cats is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the cat. Minor study deficiencies are listed under Section III.C. Deficiencies of this review.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] (Lot # KP05KTJ). Each collar measured approximately 8 x 4.5 mm (width x height), 35 cm (length) and 12.5 g (weight).

2. **Placebo control:** End-product collar minus the active ingredients (Lot # KP05U04). Each collar measured approximately 8 x 4.5 mm (width x height), 35 cm (length) and 12.5 g (weight).

3. **Test animals:**

Species:	Feline
Strain:	Domestic short hair
Age/weight	On Day 0 - 9.5 to 9.7 months old On Day -1 – 2.779 to 5.310 kg body weight
Source:	SRC's open feline colony (originally sourced from Liberty Research Inc., Waverly, NY)
Housing:	Individually housed in stainless steel pens
Diet:	Purina® Cat Chow – 200 grams/day; cats exhibiting inappetence (<25 grams of dry food per day) were offered moist food (Purina® Friskies, approximately 25 grams per day).
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	68 - 82° F
Humidity:	15 – 96%
Air changes:	"Appropriate hourly air exchanges"
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Fourteen days

B. STUDY DESIGN:

1. **In life dates:** Start: September 30, 2009; End: November 30, 2009

2. **Animal assignment:** There were four groups in the study, each containing either 6 cats (3 males and 3 females) or 12 cats (6 males and 6 females), as shown in Table 1. Prior to randomization on day -1, the cats were blocked by gender and ranked within each block by descending order of body weight. Each gender block was divided into three subgroups. Each subgroup contained five cats of the same gender and similar body weight (heavy, intermediate, light). A unique random number was assigned to each cat within each subgroup. The final assignments were based on a pre-designed relationship between subgroup, unique random number and treatment detailed in Table 3 of the study report. Although not stated in the final study report, the animal information table on page 135 of MRID 48240108 indicates that there were three replicates for Groups I, II and II and six replicates for Group IV. The study was not blinded.

Table 1: Animal Assignment

Group	Treatment	Number of Cats	
		Male	Female
I (0x; negative control)	No collar	3	3
II (5x; placebo control)	5x Vehicle-only collar	3	3
III (1x end-use product)	1x Imidacloprid + Flumethrin collar	3	3
IV (5x end-use product)	5x Imidacloprid + Flumethrin collar	6	6

3. **Dose selection rationale**: No dose rationale was provided for the percentage of active ingredients in the end-use product.

4. **Preparation and treatment**: On day 0, five vehicle collars were applied to each cat in Group II, one end-use collar was applied to each cat in Group III and five end-use collars were applied to each cat in Group IV. The multiple collars in Groups II and IV were affixed around the neck in a 3 x 2 bi-layer arrangement. A single layer of three collars was in contact with the cat's neck. A second tier of two collars encircled and directly contacted the foundation layer. The multiple collars were banded together with nylon cable (zip) ties. Surplus collar length, in excess of 2 cm, was trimmed and removed. On days 14, 28 and 42, the five collars worn by each cat in Group IV were removed and replaced with five new end-use collars. The collars worn by Group II cats remained in place until day 61. At each observation period, the collars were adjusted if removed by the cat, loosened, damaged or missing ties. After the removal of the collars from all cats on day 61, the final collar weight was determined and each removed collar was stored.

5. **Statistics**: The individual animal was defined as the experimental unit. Descriptive statistics (mean and standard deviation) were calculated for all numeric variables, including food consumption (average daily consumption for each weekly interval), body weight, hematology, coagulation and clinical chemistry parameters. All continuous numeric measurements were plotted

across time for individual animals and group averages. All continuous numeric measurements were analyzed with a repeated measures analysis of covariance. When significant effects were observed (treatment x time or overall treatment effects), pair-wise group comparisons (versus Group I alone and Group I and II combined) were conducted between all groups (except 1x group). An alpha level of 0.05 was used to define significant effects.

C. METHODS:

1. Observations:

a. **General health observations**: The animals were observed twice daily for general health from days -14 to 61.

b. **Veterinary examinations**: A physical examination was performed by a veterinarian on days -14, -1, 13, 30, 47 and 61.

c. **Local observations**: The hair and skin of all cats, both underneath and adjacent to the collars, was inspected for signs of dermal irritation and hair loss at least once daily on days -7, -1, 0 (pre- and post-application), 2, 3, 7, 21, 35, 49, 56, 61 and 62 through 68. In addition, local observations were conducted on all cats in Group IV on treatment days 14, 28 and 42 (pre- and post-application).

2. **Body weight:** Animals were weighed on days -14, -1, 13, 30, 44 and 61.

3. **Food consumption:** On days -7 through 61, food consumption was measured once daily for each cat. The cats were offered 200 grams of dry food per day. Cats exhibiting inappetence (<25 grams of dry food per day) were offered moist food.

4. **Hematology and clinical chemistry:** On days -13 and -2, blood was collected from all cats for hematology and clinical chemistry testing. On days 15, 33, 47 and 61, blood was collected from all cats in Groups I, II and IV. Cats were fasted overnight prior to the blood collection. 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	Heinz bodies
X	(Activated partial 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	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase (CPK)		Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Amylase		
	Sorbitol dehydrogenase		

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

5. **Urinalysis:** Urinalysis was not conducted. It is not required by the OPPTS 870.7200 guideline.

6. **Sacrifice and pathology:** The study did not have a scheduled necropsy. It is not required by the OPPTS 870.7200 guideline.

7. **Chemical Analysis of Collars:** To determine the 1x and 5x exposure rates, the collars worn by selected cats in Group IV (5x end-use product) were periodically analyzed for residual percentages (weight/weight) of imidacloprid and flumethrin. On days 14, 28, 42 and 61, one collar from each gender in Group IV, demonstrating the greatest weight loss between each application and removal event (i.e., days 0-14, 14-28, 28-42 and 42-61), was selected for chemical analysis. For Group III (1x end-use product), each individual collar from all cats was removed on day 61, packaged and shipped to Ecto Development Corporation, Excelsior Springs, MO, for analysis.

II. RESULTS

A. CHEMICAL ANALYSIS OF COLLARS: The concentrations of imidacloprid at 9.88% (w/w) and flumethrin at 4.67% (w/w) were used to calculate the average initial amounts (mg) of each active

ingredient in the collars applied to Groups III and IV, as shown in Table 2.

Table 2: Summary of initial amounts of imidacloprid and flumethrin in collars for adult cats[a]

Group	Treatment days	Average Imidacloprid (mg)	Average Imidacloprid (%)	Average Flumethrin (mg)	Average Flumethrin (%)
III	0 to 61	1103.40	9.88	521.55	4.67
IV	0 to 14	5618.51	9.88	2655.71	4.67
	14 to 28	5652.15	9.88	2671.61	4.67
	28 to 42	5832.86	9.88	2757.03	4.67
	42 to 61	5861.31	9.88	2770.48	4.67

[a] Extracted from Table 12, page 10, MRID 48674702.
Group III = 1x the end-use product; Group IV = 5x the end-use product

The mean collar weight applied to the 1x (group III) cats can be calculated by (Average flumethrin in mg)/(Average flumethrin %) = (521.55 mg)/(0.0467) = 11168 mg = 11.17 g.

The average amounts (mg) of imidacloprid and flumethrin remaining in the selected end-use product collars at each exposure event for Groups III and IV are presented in Table 3.

Table 3: Summary of imidacloprid and flumethrin amounts remaining in collars removed from adult cats[a]

Group	Treatment days	Average Imidacloprid (mg)	Average Imidacloprid (%)	Average Flumethrin (mg)	Average Flumethrin (%)
III	0 to 61	755.58	7.44	422.83	4.17
IV	0 to 14	5246.11	9.34	2648.91	4.72
	14 to 28	5291.65	9.35	2681.60	4.74
	28 to 42	5246.21	8.99	2594.54	4.45
	42 to 61	5282.25	9.02	2601.80	4.45

[a] Extracted from Table 13, page 35, MRID 48240108 and Table 13, page 11, MRID 48674702
Group III = 1x the end-use product; Group IV = 5x the end-use product

B. **ACTUAL DOSES ADMINISTERED**: The average imidacloprid and flumethrin exposures in cats, based on the analysis of the used collars, are presented in Table 4. Cats in Groups III (1x) and IV (5x) were on average exposed to 89.93 + 25.15 and 467.30 + 100.76 imidacloprid + flumethrin/kg of body weight, respectively. Therefore, the cats in Group IV were subjected to 5.2x the imidacloprid and 4.0x the flumethrin exposures received by cats in Group III.

Table 4: Release (Exposure) rates of imidacloprid and flumethrin in adult cats[a]

Group	Treatment days	Average Imidacloprid (mg)	Average Imidacloprid (mg/kg bw)	Average Flumethrin (mg)	Average Flumethrin (mg/kg bw)[b]
III	0 to 61	347.82	89.93	98.72	25.15
IV	0 to 14	331.08	81.74	37.44	9.36
	14 to 28	329.46	82.68	20.06	6.15
	28 to 42	586.65	160.84	162.49	43.88
	42 to 61	593.29	142.04	175.41	41.38
	0 to 61 cumulative	1840.48	467.30	395.39	100.76

[a] Extracted from Table 14, page 11, MRID 48674702
[b] The average body weights for each event were: Group III (0-61 days) = 4.033 kg, Group IV (0-14 days) = 3.998 kg, (14-28 days) = 4.090 kg, (28-42 days) = 3.379 kg and (42-61 days) = 4.249 kg
Group III = 1x the end-use product; Group IV = 5x the end-use product

C. OBSERVATIONS:

1. **Cat removal of collars**: From p. 24 of MRID 48240108 five cats in Group IV (5x) were found not wearing their collar set on 11 occasions. Each incident represented no more than an 8-19 hour void in continuous collar exposure.

2. **Clinical signs of toxicity**: Clinical observations reported in the final study report included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge. No raw or summarized data on clinical observations were included with the study report.

3. **Application site examination**: Abnormal local findings were detected in seven different cats over the course of the study. The findings in Groups I (0x), II (5x vehicle) and IV (5x end-use product) consisted of scabs, hair loss, thinning hair around the throat and abrasion on the throat (1 cat). The findings in three cats in Group IV were considered to be mechanical irritations induced by multiple collars. After the collars were removed on day 61, these cats were retained for post-study recovery/observation. During this period, each cat wore one end-use product collar. Hair re-growth was detected in all three cats.

4. **Mortality**: All cats survived to the end of the study.

D. BODY WEIGHT AND WEIGHT GAIN: Body weight and body weight gain data are presented in Table 5. No treatment-related effects on body weight and body weight gain were observed.

Table 5: Group body weight (kg) and body weight gain (kg)[a]

	Groups			
	I	II	III	IV
Day	**Males**			
-1	4.44±0.43	4.53±0.43	4.68±0.57	4.52±0.39
13	4.63±0.43	4.72±0.48	4.86±0.61	4.73±0.47
30	4.66±0.37	4.63±0.43	4.83±0.55	4.74±0.52
44	4.80±0.40	4.68±0.41	4.92±0.55	4.81±0.52
61	4.84±0.29	4.62±0.28	4.93±0.63	4.78±0.57
Weight gain (days -1 to 61)[b]	0.4	0.09	0.25	0.26
	Females			
-1	3.16±0.41	3.22±0.31	3.23±0.31	3.21±0.31
13	3.30±0.55	3.35±0.36	3.34±0.20	3.31±0.31
30	3.21±0.57	3.33±0.34	3.29±0.26	3.28±0.38
44	3.23±0.62	3.35±0.31	3.27±0.31	3.35±0.44
61	3.16±0.72	3.30±0.26	3.29±0.37	3.25±0.45
Weight gain (days -1 to 61)[b]	0	0.08	0.06	0.04

[a] Extracted from data table on page 140, MRID 48240108.
[b] Calculated by the reviewer.
Group I = Control with no collars; Group II = 5 placebo collars; Group III = 1x the end-use product; Group IV = 5x the end-use product

E. FOOD CONSUMPTION: Except for one incident, food consumption among all cats was consistent and within acceptable limits. On day 0, a female cat in Group IV (5x) consumed only 16 grams of dry food. On day 1, moist food (≈25 grams) was offered to this animal and normal food consumption was observed throughout the remainder of the study. Food consumption was decreased in all cats on days -3, 14, 32, 46 and 60, presumably due to overnight fasting before blood was collected.

F. CLINICAL PATHOLOGY ANALYSES:

1. Hematology: None of the following hematology findings is considered treatment-related since they were either not consistent, present pre-treatment or not associated with clinical signs.

The main effect of treatment was significant ($p=0.0343$) for the absolute basophil count. There were no significant pair-wise comparisons between Group I vs. Group IV, Group II vs. Group IV and Groups I and II vs. Group IV.

For MCV, the treatment x day interaction was significant ($p=0.0346$) but none of the pair-wise comparisons evaluated for each post-treatment sampling day (days 15, 33, 47 and 61) were significant.

The treatment x sex interactions were significant for absolute neutrophil ($p=0.0431$) and differential neutrophil count ($p=0.0045$). Absolute neutrophil counts in males were significantly lower ($p\leq0.05$) in Group IV vs. Group II and in Group IV vs. combined controls (Groups I and II). Throughout the exposure period, mean neutrophil counts in all male treatment groups were consistently greater than the upper limit of the reference range for the testing laboratory. The magnitude of the increases was substantially greater in Groups I and II as compared to Group IV. Differential neutrophil counts in female cats were significantly higher ($p\leq0.05$) in Group IV, as compared to Group I, whereas the percentage of neutrophils in male cats was significantly lower (≤0.05) in Group IV, as compared to Group I, II and the combined controls.

For the differential lymphocyte count, both the treatment x day ($p=0.046$) and the treatment x sex ($p=0.0109$) interactions were significant. None of the pair-wise control comparisons for each individual post-treatment sampling day (days 15, 33, 47 and 61) were significant. However, the percentage of lymphocytes in male cats was significantly increased ($p\leq0.05$) in Group IV, as compared to Group II and the combined controls.

Heinz bodies were detected in three cats in Group I, two cats in Group II and four cats in Group IV. Multiple occurrences were reported in some cats, and they were observed on day -2 prior to collar exposure in three cats (one from Group II, one from III, and one from IV). Heinz bodies are commonly seen in cat RBCs and are not considered pathological unless associated with anemia.

During the exposure period, ten cats from all groups, had platelet counts, conducted using automated equipment, below the lower limit of the laboratory reference range. A clinical pathologist conducted a quantitative assessment of the platelet numbers (i.e., increased, adequate, decreased) in a stained blood smear whenever automated platelet counts were below the reference range. The evaluation of the blood smears confirmed platelet clumping, with adequate numbers, in all cats with low automated platelet counts.

On day -13, prothrombin times for most cats were 1.0 to 38.0 secs. greater than the upper limit of the normal range (10 to 25 secs.). On day -2, prothrombin times generally were greater than 120 secs. (normal range = 6 to 11 secs.) and activated partial thromboplastin times were 2.0 to 88.0 secs. greater than the upper limit of the normal range. On days 15, 33, 47 and 61, coagulation results were generally normal. The changes pre-treatment are most likely due to a laboratory error since they occurred in most animals and were not found during the exposure period.

2. **Clinical Chemistry:** None of the following clinical chemistry findings is considered treatment-related since they were either isolated changes or not associated with clinical signs.

   Chloride was significantly increased ($p \leq 0.05$) in Group IV, as compared to Group I and the combined controls. However, all treatment group means and all individual animal values remained within the normal reference range.

   The three-way interaction between treatment, sex and day was significant for aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine phosphokinase (CPK). The treatment group means and most individual values for AST remained within the normal reference range, except for three results in two cats, one in Group III and the other in Group IV. All treatment group means and all individual animal values for BUN were within the normal reference range.

   Elevations of CPK were observed in all treatment groups: Group I – eight results in 6 of 6 cats, Group II – six results in 5 of 6 cats, Group III – one result in 1 of 6 cats and Group IV – 19 results in 8 of 12 cats. Except for one cat in Group IV, all CPK elevations were either transient in duration or only mildly or moderately increased. The cat in Group IV had markedly elevated CPK levels, including values of 6016 and 17061 U/L (reference range: 56-529 U/L)on days -13 and -2, respectively. The values remained elevated (4302-6126 U/L) during the exposure period.

   For phosphorus, the treatment x day interaction was significant ($p=0.0341$) but none of the pair-wise control comparisons for each individual post-treatment day (days 15, 33, 47 and 61) were significant.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that no adverse treatment-related findings were observed in male or female adult cats treated continuously for 61 days, either with zero, one or five PNR 1427 collars or with five placebo collars. Mild thinning of hair, presumed to be induced by mechanical irritation associated with multiple collars, was observed in the throat region of three Group IV (5x end-use product) cats. However, these local changes normalized within 7 days following replacement of the 5-collar set with a single (1) end-use product collar.

B. **REVIEWER COMMENTS:** A proposed study protocol (MRID 47776601) was reviewed by the Registration Division, OPP, in a Memorandum dated August 26, 2009 (Decision No. 415122). The Agency agreed that three males and three females in the negative and placebo control groups and the 1x group, as opposed to six males and six females per group required by the OPPTS 870.7200 Guideline, were sufficient. The Agency also agreed to the elimination of the 3x group,

which is provided for in the OPPTS 870.200 Guideline as long as there is no evidence of toxicity at 5x the recommended dose.

There were numerous amendments to the protocol after the Agency review. The most significant change was in the placement of the collars in Groups III (5x the placebo control) and IV (5x the end-use product). The initial protocol stated that the collars for these groups would be placed side-by-side in a single layer with all five collars touching the animal's neck. In protocol amendment number 1 (page 89 of the final report, MRID 48240108), the placement of the collars was changed to a 3 x 2 bilayer arrangement. The amendment states that this arrangement was approved by Dr. Byron Backus (the EPA reviewer) in a teleconference on September 2, 2009.

All animals survived to the end of the study. Clinical observations reported included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge in all treatment groups; however, no raw or summarized data were included in the final report. No effects on body weight, body weight gain, food consumption or clinical pathology parameters were observed. Statistically significant findings for hematology and clinical chemistry parameters were not considered treatment-related since they were either isolated, inconsistent or not associated with clinical signs. Based on chemical analyses of the worn collars and information reported in MRID 48674702, cats in the 5x group were exposed to 5.2x the recommended dose of imidacloprid and 4.0x the recommended dose of flumethrin.

It is concluded that the margin of safety in adult cats exposed to PNR 1427 insecticide [imidacloprid (10% w/w) + flumethrin (4.5% w/w)] collar for 61 days is 1x based on the chemical analyses of the worn collars which yielded 5.2x the recommended dose of imidacloprid and 4.0x the recommended dose of flumethrin. The mean collar weight (after trimming) applied to the 1x cats was 11.17 g equivalent to 2.82 g/kg body weight.

This companion animal safety study in male and female adult cats is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the cat.

C. MINOR STUDY DEFICIENCIES:

1. On study days 14, 28, 42 and 61, a set of collars from each gender in Group IV (5x the end-use product) demonstrating the greatest weight loss between each application and removal event (i.e., days 0-14, 14-28, 28-42 and 42-61) were selected for chemical analysis. By selecting the collars with the greatest weight loss, the analyses were biased to provide the highest mg/kg body weight exposure of the active ingredients.

2. No raw data or summary tables were provided for the clinical observations results.

3. The study protocol states that cats would be excluded from the study due to several conditions, including distinctly abnormal hematology/coagulation/clinical chemistry results. However, some animals had markedly abnormal clinical pathology values pretreatment and were still included in the study. For example, 4 cats in Group I, one cat in Group III and 4 cats in Group IV had elevated CPK values on days -13 and -2. One cat in Group IV had a value of >30 times the upper value of the reference range on day -2. The study author speculated that the increased CPK values could be due to increased muscular activity occurring during study

procedures associated with non-cooperation, resistance, struggle and muscular exertion. The reason for the abnormal clinical pathology values should have been explored before the animals were included in the study.

The deficiencies indicated above are considered to be minor, and do not affect the acceptable classification of this study.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL)
[PNR 1427, IMIDACLOPRID (10%, W/W) + FLUMETHRIN (4.5%, W/W) COLLAR]

OPPTS 870.7200
STUDY TYPE: COMPANION ANIMAL SAFETY STUDY- ADULT DOGS
MRID 48240109

Prepared for
Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
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Task Order No. 3-C-04

Primary Reviewer: Donna L. Fefee, D.V.M.	Signature:	Donna L. Fefee, AE
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	Date:	SEP 27 2011
Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	SEP 27 2011
Quality Assurance: Jennifer Goldberg, B.S.	Signature:	Jennifer Goldberg
	Date:	SEP 27 2011

Disclaimer

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

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

Signature: Byron T. Backus
Date: Jan. 27, 2012

EPA Secondary Reviewer: Masih Hashim, Ph.D., D.V.M
Technical Review Branch, Registration Division (7505P)

Signature: M Hashim
Date: 1-31-12

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Adult Dogs; OPPTS 870.7200

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **DP BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (9.96% Imidacloprid and 4.35% Flumethrin; Lot No. KP05KF6)

SYNONYMS: M915 Insecticide Animal Collar (large collar)

CITATIONS: Madsen, T. (2010) Safety of PNR 1427 in adult dogs. Sinclair Research Center, LLC (SRC), Auxvasse, Missouri. Study Number S10064, April 23, 2010. MRID 48240109. Unpublished.

Chopade, H. (2011) Final Report Amendment 1 to Bayer Report 33805 (MRID 48240109) - Safety of PNR 1427 in Adult Dogs. Sinclair Research Center, Inc. Project Number: 33967, S10064, 152/151. Final Report Amendment 1: November 22, 2011. MRID 48674701. Unpublished.

SPONSOR: Bayer HealthCare LLC, Animal Health Division, 12809 Shawnee Mission Parkway, Shawnee Mission, Kansas.

EXECUTIVE SUMMARY: In a 61-day companion animal safety study (MRID 48240109), groups of adult male and female beagle dogs were treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars (9.96% Imidacloprid and 4.35% Flumethrin; Lot No. KP05KF6) at 1X (one collar; applied to 3 animals/sex) or 5X (five collars; applied to six animals/sex) the intended label use rate for dogs weighing greater than 8 kg. Two additional groups of three males and three females were untreated or treated with five "Placebo Collars." The multiple collars applied to the placebo controls and 5X animals were affixed around the neck in a "3 x 2" bi-layer arrangement such that a single layer of three collars was in contact with the dog's neck, while a second tier of two collars encircled and directly contacted the foundation layer, and the multiple collars were banded together with nylon cable (zip) ties. Initial treatment was on day 0, and the 5X animals were re-treated on days 14, 28 and 42.

There were no treatment-related effects on mortality, systemic clinical signs, body weight or body weight gain, food consumption, hematology, clinical chemistry, or coagulation parameters. Reversible, treatment-related abnormal local findings such as erythema and hair loss or thinning of the hair on the throat, back of the neck, or side of the neck were noted on two placebo control, two 1X, and five 5X dogs at one or more observations during the treatment interval.

Based on the information in MRID 48674701, the dogs in the 1X and 5X groups were respectively exposed to 83.42 mg/kg bw and 907.88 mg/kg bw of imidacloprid and 19.09 mg/kg bw and 77.50 mg/kg bw of flumethrin, i.e. the average imidacloprid exposure of the 5X animals was 10.88X that of the dogs in the 1X group, and the average flumethrin exposure of the 5X was 4.05X that of the dogs in the 1X group.

The margin of safety in 8.67 to 13.44-kg adult beagle dogs treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars for 61 days is 4.05X the recommended dose of one collar per dog. The mean collar weight for the 1X dogs was 37.93 grams or 3.22 grams/kg b.w.

It is concluded that this companion animal safety study in dogs is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in adult dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar

Description:	Gray solid collar, 66 cm (length) x 14 mm (width) x 5 mm (thickness), weighing 45 g
Lot #:	KP05KF6
Purity:	9.96% Imidacloprid and 4.35% Flumethrin
Compound Stability:	Shown via analysis to be stable for the study duration.
CAS #:	138261-41-3 (Imidacloprid) and 69770-42-2 (Flumethrin)

2. Vehicle control: Placebo Collar [End-product collar, minus active ingredients]

Description:	Gray solid collar, 66 cm (length) x 14 mm (width) x 5 mm (thickness), weighing 45 g
Lot #:	KP05TZ7
Purity:	0.0% Imidacloprid and 0.0% Flumethrin
Compound Stability:	Not applicable
CAS #:	Not Provided

3. Positive control: No positive control was used.

4. **Test animals:**

Species:	Dog	
Breed:	Beagle	
Age/weight at study initiation:	10.3-11.2 months old on Day 0/ Males: 11.11-13.44 kg; Females: 8.67-11.25 kg (Day -1)	
Source:	Ridglan Farms, Mount Horeb, Wisconsin	
Housing:	Individually; During acclimation and through Day 8: pens (~3.5 ft x 6.5 ft) with chain-link fence and frame walls and front gate; Beginning Day 8 or 9: stainless-steel cages (~3 ft x 5 ft) with solid walls and a front gate that had vertical bars.	
Diet:	Purina® Dog Chow, 300 g/day through Day 20, 400 g/day beginning on Day 21; any dog that consumed <25 g of dry food/day was offered ~25 g/day of supplemental moist food (Purina Pro Plan) until the expected dry food consumption was observed.	
Water:	*Ad libitum* water from an on-site deep well	
Environmental conditions:	**Temperature:**	63-77° F.
	Humidity:	15% (the lowest measurable level) to 100%
	Air changes:	Not reported, but stated to be appropriate
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Two weeks.	

B. STUDY DESIGN:

1. **In life dates:** Start: October 7, 2009; End: January 11, 2010.

2. **Animal assignment:** The study design is given in Table 1. The animals were assigned to groups according to sex and body weight on day -1, using a stratified block randomization procedure. The animals on study were assigned into six replicates: the first three replicates (1-3) included one animal/sex/group from all groups, and the last three replicates (4-6) included one animal/sex from Group IV. However, all replicates were treated and observed contemporaneously. The study was not blinded.

TABLE 1: Study design [a]

Test Group	Treatment	Treatment Day(s)	Number assigned	
			Males	Females
I. Negative control	No collar	--	3	3
II. Placebo control	Five Placebo Collars	Day 0, only	3	3
III. 1X	One End-use Collar	Day 0, only	3	3
IV. 5X	Five End-use Collars	Days 0, 14, 28, and 42	6	6

[a] Data taken from Table 2, p. 13, MRID 48240109.

3. **Dose selection rationale:** The study author stated that the study design, including dose selection, was based on OPPTS 870.7200, on recommendations provided to the sponsor by the EPA during two study design teleconferences (August 8, 2008, and March 9, 2009), and on the EPA's formal comments on a draft protocol review (issued on August 31, 2009).

According to the provided Application for Pesticide Registration and proposed product labeling, a "large size" collar, 70 cm in length and weighing 45 g, is intended for use on dogs that weigh

greater than 8 kg, with an intended period of use of up to 8 months per collar. Usage of five end-use collars as the highest dose level tested is in accordance with OPPTS 870.7200. Absence of a 3X group (and 1X group) is acceptable under OPPTS 870.7200 in the case of a limit test, provided no evidence of toxicity is seen at the 5X dose. Usage of just three males and three females in the negative and placebo control groups, as opposed to the six males and six females per group recommended under OPPTS 870.7200, was approved by a representative of Registration Division, OPP, in a Memorandum dated August 31, 2009 (Decision No. 415125). To maximize exposure, the 5X animals had their collars replaced with new collars three times, at retreatment intervals of 14 days.

4. **Treatment:** On day 0, five placebo collars were applied to each placebo control dog, one end-use collar was applied to each 1X dog, and five end-use collars were applied to each 5X dog. A two-finger space was maintained between the collar(s) and the neck. The multiple collars applied to the placebo controls and 5X animals were affixed around the neck in a "3 x 2" bi-layer arrangement such that a single layer of three collars was in contact with the dog's neck, while a second tier of two collars encircled and directly contacted the foundation layer, and the multiple collars were banded together with nylon cable (zip) ties. Surplus collar length, in excess of 2 cm, was trimmed and saved. On days 14, 28 and 42, the five collars worn by each 5X animal were removed, and five new end-use collars were applied to each animal in an identical manner as was done on Day 0. The collars worn by the placebo controls and 1X dogs were intended to remain in place until day 61, the conclusion of the exposure period, and were not replaced unless necessary, i.e. if the collars were chewed or otherwise damaged.

5. **Recovery period:** Any animal with abnormal local changes to the skin and/or hair presumed to be mechanically induced by the presence of the collar(s) was retained for a recovery period of up to five weeks' duration. During the first four weeks (Days 61-89), each dog wore a single new collar, either a placebo collar or an end-use collar, as appropriate. Any dog whose abnormal local findings remained present through day 89 was maintained in recovery for an additional week (through day 96) without wearing any collar, until the local abnormalities normalized.

6. **Statistics:**

The individual animal was the experimental unit. Body weight, food consumption, and the hematology, clinical pathology parameters were analyzed using a repeated measures analysis of covariance (RMANCOVA). The RMANCOVA used the following as fixed effects: a covariate (the pretreatment baseline), treatment, time, and sex, the two-way interactions "treatment by time," "treatment by sex," "sex by time," and the three-way interaction "treatment by time by sex."

Four covariance structures were compared for each variable using the Akaike Information Criterion (AIC): compound symmetry; heterogeneous compound symmetry; first-order autoregressive; and heterogeneous first-order autoregressive. Results from the model with the lowest AIC value were used. Regardless of the result of the "sex by time" interaction, the analysis continued. If the "treatment by sex by time" interaction was significant ($p \leq 0.05$), the statistical analysis of the variable was deemed inconclusive, and no further statistical analysis was conducted. If the "treatment by sex" or "treatment by time" interaction was significant ($p \leq 0.05$), within sex or within time treatment effects were evaluated as follows: negative controls vs. 1X (if applicable) and 5X, placebo controls vs. 1X (if applicable) and 5X, and combined negative and

placebo controls vs. 1X (if applicable) and 5X. If none of the interactions were significant, the treatment main effect was evaluated. If the treatment main effect was not significant ($p>0.05$), the results were deemed not significant, and no further statistical analysis was conducted. If the treatment effect was significant ($p\leq0.05$), comparisons were made at a 5% significance level as follows: negative controls vs. 1X (if applicable) and 5X, placebo controls vs. 1X (if applicable) and 5X, and combined negative and placebo controls vs. 1X (if applicable) and 5X.

The daily food consumption data were analyzed over four intervals (weeks 1, 3, 5, and 7), using the average intake during the three days prior as the covariate.

Profile plots were provided for all of the analyzed endpoints and included all data collected during acclimation and the treatment interval (Days 0-61). For each endpoint, the individual data and the average for the combined sexes were presented separately for each treatment group. For clinical pathology endpoints, the lower and upper limits of the reference range were included, and the body weight plots and "dose event" plots for food consumption included a plot of the baseline value.

C. METHODS:

1. Observations:

a. General health observations: During acclimation and the treatment interval, the animals were observed twice per day, morning and afternoon. On treatment days, i.e. on day 0 for the placebo controls and 1X animals and on days 0, 14, 28, and 42 for the 5X animals, the treated animals were also observed pre-treatment and 1, 2, 3, and 4 hours (± 15 minutes) post-dosing.

b. Clinical assessments: All animals received physical examinations on days -14, -1, 13, 30, 47, and 61.

c. Local observations: The hair and skin underneath and adjacent to the collars were inspected for signs of dermal irritation and hair loss at least once daily on days -7, -1, 0 (pre- and post-application), 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 61, and daily during days 62 through 96 for any animals retained for recovery. Additional pre- and post-treatment local observations were conducted on all 5X dogs on days 14, 28, and 42.

2. Body weight: The animals were weighed on days -14, -1, 13, 30, 44, and 61.

3. Food consumption: Food consumption was measured and recorded daily.

4. Clinical pathology: Blood for hematology, clinical chemistry and coagulation evaluation was collected on days -13 and -2 (from all animals) and on days 15, 33, 47, and 61 (from the negative controls, placebo controls, and 5X animals). Food was withheld overnight prior to collections. Blood was collected from the jugular, cephalic, and/or saphenous vein. The sampling order used was not reported. The CHECKED (X) parameters were examined.

a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count* (absolute and percentage)
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		Morphology (if indicated)
X	(Thromboplastin time)*		Heinz body formation
	(Clotting time)		
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 (BUN)*
X	Phosphorus*		Cholesterol
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)		Indirect bilirubin
X	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		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Amylase		

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

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

7. **Test article investigations:**

a. **Collar weights:** The placebo and end-use collars were weighed prior to application, and, following application, the trimmed surplus collar lengths were maintained in individual labeled Ziploc® bags for the later determination of the final weights of the applied collars. When collars were removed on days 14, 28, and 42 (for 5X animals, only), on day 61 (for placebo controls and 1X and 5X animals), or at other intervals during the study, as required by damage to the collar,

each collar or set of collars was placed in a tared, pre-labeled Ziploc® bag, and the final collar weight was recorded.

b. **Chemical analysis of collars:** On study days 14, 28, 42, and 61, two sets of collars (one from a 5X male and one from a 5X female) were selected for chemical analysis. Selection was based on demonstration of the greatest weight loss between application and removal among the sets that had not been chewed or otherwise damaged. All of the collars used on the 1X animals, including chewed/damaged collars that had required replacement, were submitted for chemical analysis. The analytical testing was done at Ecto Development Corporation (Excelsior Springs, Missouri).

II. RESULTS

A. Results of the collar weight measurements and chemical analyses of the collars are given in Tables 2 and 3. The original percentage of flumethrin was initially reported as 4.35% (MRID 48240109). From p. 10 of MRID 48674701: "The results of the first contemporaneous analysis of the end-product large collars (Lot No. KP05KF6) performed on 17-Nov-09 showed the concentrations of imidacloprid at 9.93% (w/w) and flumethrin at 4.61% (w/w). The results of the second contemporaneous analysis performed on 08-Jan-10 showed the concentrations of imidacloprid at 9.99% (w/w) and flumethrin at 4.70% (w/w). Therefore, the average of these two analyses for the two active ingredients [9.96% imidacloprid (w/w) and 4.65% flumethrin (w/w)] were considered much closer to the actual concentrations in the large collars, and therefore these values were used to calculate the average initial amounts (mg) of individual active ingredients present in the collars applied to the test animals in Groups III (1x) and IV (5x)..."

Table 2: Summary of initial amounts of imidacloprid and flumethrin in collars [a]

Group	Treatment days	Average Initial Collar Weight (g)	Average Active Ingredient (% w/w)		Average Active Ingredient (mg/animal)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 61	37.929	9.96	4.65	3777.76	1742.18
IV [c] [5X]	0 to 14	196.320	9.96	4.65	19553.42	8539.90
	14 to 28	184.861	9.96	4.65	18412.16	8041.45
	28 to 42	195.540	9.96	4.65	19475.73	8505.97
	42 to 61	194.968	9.96	4.65	19418.76	8481.09

[a] Data taken from amended Table 14, p. 10 of MRID 48674701.

[b] Calculations for this group were based on 3 animals.

[c] All calculations for this group were based on 2 animals per interval.

Table 3: Summary of amounts of imidacloprid and flumethrin remaining in removed collars [a]						
Group	Treatment days	Average Final Collar Weight (g)	Average Active Ingredient (% w/w)		Average Active Ingredient (mg/animal)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 61	35.140	8.00	4.38	2805.97	1538.78
IV [c] [5X]	0 to 14	193.865	9.03	4.76	17506.01	9218.03
	14 to 28	182.555	8.72	4.61	15911.01	8407.71
	28 to 42	192.911	8.76	4.48	16897.55	8632.55
	42 to 61	192.931	8.66	4.60	16708.73	8875.13

[a] Data taken from Table 15, p. 34, and pp. 128-129, MRID 48240109, and p. 10 of MRID 48674701.

[b] Calculations for this group were based on 3 animals.

[c] All calculations for this group were based on 2 animals per interval.

B. <u>ACTUAL DOSES ADMINISTERED</u>: During the 61-day exposure interval, there were a total of 28 incidences when an animal's collar or collars was or were observed to be removed, chewed, and/or otherwise damaged. Each incident represented an interval of no more than 6-18 hours. In the placebo control, 1X, and 5X groups, there were 7, 5, and 17 occurrences, involving 4, 3, and 6 animals, respectively. The average exposure rates to the active ingredients expressed as mg/kg bw are given in Table 4. Based on these results, compared to the 1X group, the 5X group received 10.88X the imidacloprid exposure and 4.05X the flumethrin exposure.

Table 4: Summary of release (exposure) rates of imidacloprid and flumethrin [a]					
Group	Treatment days	Average Active Ingredient (mg/animal)		Average Active Ingredient (mg/kg bw)	
		Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 61 Cumulative	971.79	224.93	83.42	19.09
IV [d] [5X]	0 to 14	2047.41	0 [c]	192.43	0 [c]
	14 to 28	2501.15	188.32	224.89	17.50
	28 to 42	2578.18	460.04	237.15	42.35
	42 to 61	2710.03	190.86	253.41	17.66
	Cumulative	9836.77	893.22	907.88	77.50

[a] Data taken from amended Table 16, p. 11 of MRID 48674701.

[b] Calculations for this group were based on 3 animals.

[c] The amount of flumethrin in the collars did not decrease during this exposure event, based on the initial average concentration. Therefore 0 was reported.

[d] All calculations for this group were based on 2 animals per interval.

C. <u>OBSERVATIONS</u>:

1. **<u>Clinical signs of toxicity</u>:** The study report did not provide the individual animal data for the time of observation of each abnormal sign and its subsequent course, and no tabular summary was provided either. The study author stated that abnormal clinical signs included mild ocular discharge, emesis, abnormal feces (loose stool or diarrhea), and interdigital cysts. According to the study author, the ocular discharge and episodes of abnormal feces were recorded across all treatment groups and throughout the study, without any pattern indicating a relationship to treatment, and the vomiting was much less frequent and was not observed on the day of or the day following application of a new collar.

2. **Local effects:** Treatment-related abnormal local findings such as erythema and hair loss or thinning of the hair on the throat, back of the neck, or side of the neck were noted on two placebo control, two 1X, and five 5X dogs at one or more observations during the treatment interval (days 0-61). Eight animals (two placebo control, one 1X, and five 5X dogs) were retained for recovery, due to the presence of local effects on day 61. Unequivocal hair regrowth (and/or presumably resolution of erythema) was noted on one placebo control and all 5X animals, on or before day 89, while they were wearing a single placebo or end-use collar. The remaining placebo control and 1X animal showed unequivocal hair regrowth on day 96, after a week without wearing any collar.

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

D. BODY WEIGHT AND WEIGHT GAIN: Body weight data are given in Table 5. There were no treatment-related effects on body weight or body weight gain.

TABLE 5: Body weight data from adult beagles [a]

Parameter/ Study day or interval		Treatment			
		Negative Control	Placebo Control	1X	5X
Males					
Absolute body weight (kg):	**Day -1**	12.54±0.70	12.14±0.89	12.42±0.62	12.39±0.85
	Day 13	12.51±0.70	12.18±0.87	12.33±0.74	12.32±0.95
	Day 30	12.66±0.49	12.16±0.56	11.91±0.89	12.36±0.71
	Day 44	12.97±0.42	12.13±0.59	11.89±1.04	12.38±0.84
	Day 61	13.14±0.43	12.08±0.61	12.08±0.71	12.50±0.77
Body weight change (kg) [b]:	**Days -1 to 13**	-0.03	0.04	-0.09	-0.07
	Days 13 to 30	0.15	-0.02	-0.42	0.04
	Days 30 to 44	0.31	-0.03	-0.02	0.02
	Days 44 to 61	0.17	-0.05	0.19	0.12
	Day -1 to 61	0.60	-0.06	-0.34	0.11
Females					
Absolute body weight (kg):	**Day -1**	10.13±0.65	10.08±0.79	10.33±0.86	9.92±0.90
	Day 13	10.13±0.50	9.87±0.71	9.98±0.98	9.57±0.90
	Day 30	10.16±0.57	9.88±1.10	9.77±1.03	9.77±0.98
	Day 44	10.12±0.36	9.84±1.12	9.89±0.97	9.77±0.94
	Day 61	10.18±0.67	9.73±0.84	9.86±0.91	9.84±0.97
Body weight change (kg) [b]:	**Days -1 to 13**	0.00	-0.21	-0.35	-0.35
	Days 13 to 30	0.03	0.01	-0.21	0.20
	Days 30 to 44	-0.04	-0.04	0.12	0.00
	Days 44 to 61	0.06	-0.11	-0.03	0.07
	Day -1 to 61	0.05	-0.35	-0.47	-0.08

[a] Data taken from p.151, MRID 48240109. Values are Mean ± Standard Deviation, with n=3 for negative control, placebo control, and 1X groups, and n=6 for the 5X group.

[b] Calculated by reviewer using group mean absolute body weight values.

E. **FOOD CONSUMPTION:** Prior to day 21 when the maximum offered amount of food was increased to 400 grams, all of the negative control males and some of the other animals of both sexes consumed the entire 300-g ration on most or all days. Throughout the study there were twelve incidences of inappetence (defined as consumption of less than 25 g of food), all of which were a single day in duration, preceded and followed by normal food consumption. Inappetence of one 5X female that consumed 0 grams on day 0, may have been treatment-related or simply due to the stress of the unaccustomed handling and other activities related to treatment. The remaining incidences were not treatment-related: six incidences occurred on the day the animals were moved from pens to cages; one placebo control and two 5X animals had inappetence on the day of or the day preceding one of the blood collections; and one placebo control and one 1X animal consumed just one gram of food on day 40 or day 23, respectively.

The manner in which the numerical data were presented made it difficult to assess food consumption. Mean weekly food consumption values were not subjected to statistical analysis, and daily food consumption data were only analyzed (separately) over weeks 1, 3, 5, and 7, i.e. the four "dose events" commencing when new collars were applied. No statistically significant differences were found during those four weeks; however, the profile plots did show decreases (usually below baseline) in the food consumption of all groups on days 14, 33, and 47, which were the days food was removed prior to blood collection, along with decreases in the mean food consumption of most groups, including negative controls, on treatment days, i.e. days 0, 14, 28, and 42.

Mean weekly food consumption of the 5X males was 11-22% less than that of the negative controls throughout the study. This difference may be treatment-related but is not considered adverse because there was no significant corresponding effect on body weight gain or absolute body weight. However, it must be noted that the usefulness of the food consumption data is somewhat limited because the animals were not always offered more food than they were likely to eat. This decreased the likelihood that the food consumption and body weight data would detect and accurately reflect an adverse effect.

F. **BLOOD ANALYSES:**

1. **Hematology:** Statistically significant hematological findings included a treatment main effect for platelets, a "treatment by sex by day" interaction for absolute lymphocyte count, and increased absolute and relative eosinophils on day 33 in 5X animals, relative to negative and/or placebo controls. None of the statistical effects were considered biologically or toxicologically significant. For the lymphocyte and eosinophil counts, all of the group mean and individual values fell within the provided reference ranges. For the platelet counts, the 5X animals had greater platelet counts for the study overall, compared to placebo controls, but not compared to negative controls or the combined controls, the "treatment by day" interaction was not significant, and all of mean values of the 5X group remained within the reference range.

2. **Coagulation parameters:** On day 33, one placebo control female and two 5X females had APTT values that were recorded as >240 seconds, along with increased PT values (32.2-93.3 seconds). These differences are most likely due to sample mishandling or a technical problem in the testing laboratory. Although not mentioned in the study report or indicated in the summary tables, the APTT values for these animals were excluded from the group means for this day; however, the PT values were not excluded. One negative control female and one 5x female had prolonged

prothrombin times on days -2, 15, 33, and 47 (29.1-102.7 seconds vs. a reference range of 6-12 seconds) with much smaller increases seen on day 61 (14.5-16.9 seconds). The increases in these two animals are not considered treatment-related because the alterations began prior to the commencement of treatment.

Statistical analysis revealed a significant treatment main effect for APTT, which was significantly increased in 5X animals for the study overall, as compared to placebo controls and the combined controls, but not as compared to the negative controls alone. This difference is not considered biologically significant because, with the exception of the above-mentioned APTT values, none of the 5X individual or group mean APTT values fell above the provided reference range (10-25 seconds).

3. **Clinical chemistry:** Statistically significant "treatment by day" interactions were found for serum calcium, glucose, potassium, and total protein concentrations, and for all four parameters statistically significant differences were found between the 5X animals and negative and/or placebo controls on one or more treatment days. However, none of these differences were considered treatment-related because all mean and individual values of the 5X animals fell well within the provided reference ranges.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** According to the study author, no adverse treatment-related findings were observed in male or female adult dogs treated continuously for 61 days either with zero, one, or five PNR 1427 collars or with five placebo collars. The study author stated that mild thinning of the hair under or adjacent to the collars, which was presumed to be related to mechanical irritation from the collars, was noted on dogs in the treated groups (two placebo controls, two 1X animals, and five 5X animals). These changes began to reverse in one placebo control and all 5X animals within 28 days of wearing a single placebo or end-use collar, and the remaining placebo control and 1X animal showed unequivocal hair regrowth after an additional week spent without wearing any collar. The study author concluded that continuous treatment of adult dogs at 5X the recommended label dose for 61 consecutive days was determined to be safe.

B. **REVIEWER COMMENTS:** The reviewer is in agreement with the study author that there was no indication of systemic toxicity. Reversible local effects under or adjacent to the collars most likely were related to mechanical irritation.

Based on the information in MRID 48674701, the dogs in the 1X and 5X groups were respectively exposed to 83.42 mg/kg bw and 907.88 mg/kg bw of imidacloprid and 19.09 mg/kg bw and 77.50 mg/kg bw of flumethrin, i.e. the average imidacloprid exposure of the 5X animals was 10.88X that of the dogs in the 1X group, and the average flumethrin exposure of the 5X was 4.05X that of the dogs in the 1X group.

The margin of safety in 8.67 to 13.44-kg adult beagle dogs treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars for 61 days is 4.05X the recommended dose of one collar per dog. The mean collar weight for the 1X dogs was 37.93 grams or 3.22 grams/kg b.w.

It is concluded that this companion animal safety study in dogs is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in adult dogs.

C. STUDY DEFICIENCIES:

Deficiencies included the following:

- The study report did not provide the individual animal data for the time of observation of each abnormal sign and its subsequent course, and no tabular summary was provided either.
- There was no comparison of the placebo control group to the negative controls.
- Mean daily food consumption should have been analyzed for the entire study rather than just the 5 "treatment periods."
- The data should have been analyzed separately by sex, regardless of whether the "treatment by sex" interaction was significant.

The deficiencies indicated above are considered to be minor, and do not affect the acceptable classification of this study.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427, IMIDACLOPRID (10%, W/W) + FLUMETHRIN (4.5%, W/W) COLLAR]

OPPTS 870.7200
STUDY TYPE: COMPANION ANIMAL SAFETY STUDY- PUPPIES
MRID 48240110

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Task Order No. 3-C-04

Primary Reviewer: Donna L. Fefee, D.V.M.	Signature:	Donna L Fefee, AE
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Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	SEP 27 2011
Quality Assurance: Jennifer Goldberg, B.S.	Signature:	Jennifer Goldberg
	Date:	SEP 27 2011

Disclaimer

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

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

Signature: Byron T. Backus
Date: Jan. 27, 2012

EPA Secondary Reviewer: Masih Hashim, Ph.D., D.V.M
Technical Review Branch, Registration Division (7505P)

Signature: M Hashim 1/31/12
Date:

Template version 02/06

DATA EVALUATION RECORD

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

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **DP BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (Small collars: Lot No. KP05KTJ, 10.18% imidacloprid and 4.47% flumethrin; Large collars: Lot No. KP05KF6, 9.96% Imidacloprid and 4.35% Flumethrin)

SYNONYMS: M915 Insecticide Animal Collar (large collar and small collar)

CITATIONS: Madsen, T. (2010) Safety of PNR1427 in puppies. Sinclair Research Center, LLC (SRC), Auxvasse, Missouri. Study Number S10062, June 16, 2010. MRID 48240110. Unpublished.

SPONSOR: Bayer HealthCare LLC, Animal Health Division, 12809 Shawnee Mission Parkway, Shawnee Mission, Kansas.

EXECUTIVE SUMMARY: In a 180-day companion animal safety study (MRID 48240110), groups of six male and six female 7-week-old beagle puppies were treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars (9.96% Imidacloprid and 4.35% Flumethrin; Lot No. KP05KF6) at 1X (one collar), 3X (three collars), or 5X (five collars) the intended label use rate. Two additional groups of three males and three females were untreated or were treated with five "Placebo Collars." The multiple collars applied to the placebo controls and 5X animals were affixed around the neck in a "3 x 2" bi-layer arrangement such that a single layer of three collars was in contact with the dog's neck, while a second tier of two collars encircled and directly contacted the foundation layer, and the multiple collars were banded together with nylon cable (zip) ties. Initial treatment was on day 0, and the animals were re-treated (existing collars replaced with new collars) on days 29, 90, 125, and 148.

There were no treatment-related effects on mortality, absolute body weight, food consumption, hematology or clinical chemistry. The 5X females had a transient decrease in body weight gain (25% less than controls during days -1 through 17). Reversible abnormal local findings such as erythema, hair loss or thinning of the hair, bruising, abrasions, scabbing, or a part in the hair were noted on two negative controls, five placebo controls, nine 1X animals, eight 3X animals, and ten 5X animals at one or more observations during the treatment interval. Mildly increased creatine kinase activity in 3X and 5X animals (349, 940, 1074 U/L for negative controls, 3X, and 5X, respectively) is of undetermined significance.

Based on determination of the collar weight losses and chemical analyses of the worn collars, the imidacloprid exposure of puppies in the 3X and 5X groups was 3.02X and 4.89X that of the puppies

in the 1X group, and the flumethrin exposure of puppies in the 3X and 5X groups was 2.46X and 1.45X that of the puppies in the 1X group.

Based on transient decreased body weight gain, it is concluded that the margin of safety in 7-week-old 1.53- to 2.96-kg beagle puppies treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars is 3X the recommended dose (of one collar per puppy). The average collar dosage rate for 1X animals on Day 0 was 10.156 g, equivalent to 5.096 g/kg.

This companion animal safety study in puppies is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in juvenile dogs. However it must be noted that, although the puppies in the 5X group were exposed to 4.89X the recommended dose of imidacloprid, the chemical analyses of the worn collars indicates they were exposed to only 1.45X the recommended dose of flumethrin.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:

a. PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (small collar)

Description:	Gray solid collar, measuring approximately 35 cm (length) x 8 mm (width) x 4.5 mm (thickness), weighing 12.5 g;
Lot #:	KP05KTJ
Purity:	10.18% Imidacloprid and 4.47% Flumethrin;
Compound Stability:	Shown via analysis to be stable for the study duration.
CAS #:	138261-41-3 (Imidacloprid) and 69770-42-2 (Flumethrin)

b. PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (large collar)

Description:	Gray solid collar, measuring approximately 66 cm (length) x 14 mm (width) x 5 mm (thickness), weighing 45 g
Lot #:	KP05KF6
Purity:	9.96% Imidacloprid and 4.35% Flumethrin
Compound Stability:	Shown via analysis to be stable for the study duration.
CAS #:	138261-41-3 (Imidacloprid) and 69770-42-2 (Flumethrin)

2. Vehicle control:

a. Placebo Collar [End-product collar, minus active ingredients] (small collar)

Description:	Gray solid collar, measuring approximately 35 cm (length) x 8 mm (width) x 4.5 mm (thickness), weighing 12.5 g
Lot #:	KP05U04
Purity:	0.0% Imidacloprid and 0.0% Flumethrin

b. Placebo Collar [End-product collar, minus active ingredients] (large collar)

Description:	Gray solid collar, measuring approximately 66 cm (length) x 14 mm (width) x 5 mm (thickness), weighing 45 g
Lot #:	KP05TZ7
Purity:	0.0% Imidacloprid and 0.0% Flumethrin

3. Positive control: No positive control was used.

3. Test animals:

Species:	Dog
Breed:	Beagle
Age/weight at study initiation:	48-50 days old on day 0/ Males: 1.80-2.96 kg; Females: 1.53-2.45 kg (day -1)
Source:	Ridglan Farms, Mount Horeb, Wisconsin
Housing:	Individually, in stainless-steel cages (~3 ft x 5 ft) with solid walls and a front gate that had vertical bars.

Diet:	Purina® Puppy Chow, 300 g/day through Day 20, 200 g/day on days -4 to 7, 300 g/day on days 8-34, 400 g/day on days 35-104, and 300 g/day on days 105-191; any puppy that consumed <25 g of dry food/day was offered ~25 g/day of supplemental moist food until the expected dry food consumption was observed.	
Water:	*Ad libitum* water from an on-site deep well	
Environmental conditions:	**Temperature:**	64.1-84.7° F.
	Humidity:	15% (the lowest measurable level) to 98.4%
	Air changes:	Not reported; stated to be appropriate
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Two weeks.	

B. STUDY DESIGN:

1. **In life dates:** Start: September 23, 2009; End: April 2, 2010.

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups according to sex and body weight on day -1, using a stratified block randomization procedure. The animals on study were assigned into six replicates: the first three replicates (1-3) included one animal/sex/group from all groups, and the last three replicates (4-6) included one animal/sex from Groups III, IV, and IV. However, all replicates were treated and observed contemporaneously. The study was not blinded.

TABLE 1: Study design [a]

Test Group	Treatment	Number assigned	
		Males	Females
I. Negative control	No collar	3	3
II. Placebo control	Five Placebo Collars	3	3
III. 1X	One End-use Collar	6	6
IV. 3X	Three End-use Collars	6	6
V. 5X	Five End-use Collars	6	6

[a] Data taken from Table 2, p. 14, MRID 48240110.

3. **Dose selection rationale:** The study author stated that the study design, including dose selection and treatment frequency was based on OPPTS 870.7200, on recommendations provided to the sponsor by the EPA during two study design teleconferences (August 8, 2008, and March 9, 2009), and on the EPA's formal comments on a draft protocol review (issued on August 31, 2009).

4. **Treatment:** On day 0, five placebo collars were applied to each placebo control dog, and one, three, or five end-use collars were applied to each 1X, 3X, or 5X puppy, as appropriate. A two-finger space was maintained between the collar(s) and the neck. The study report stated that the multiple collars applied to the placebo controls and 5X animals were affixed around the neck in a "3 x 2" bi-layer arrangement such that a single layer of three collars was in contact with the puppy's neck, while a second tier of two collars encircled and directly contacted the foundation layer, and the multiple collars were banded together with nylon cable (zip) ties. It is assumed that the multiple collars applied to the 3X animals were affixed around the neck in a single layer, with the multiple collars banded together with nylon cable (zip) ties. Surplus collar length, in excess of 2 cm, was trimmed and saved. On days 29, 90, 125, and 148, the placebo or end-use collar(s) worn by each animal were removed and replaced with new collar(s), applied in an identical

manner as was done on Day 0. On day 180 (the conclusion of the treatment interval), the collars were terminally removed, except as described below under "recovery period." On days 0, 29, and 90, the puppies were treated according to their most recent body weight measurements: puppies weighing up to 8 kg were treated with small placebo or end-use collars, as appropriate, and puppies weighing greater than 8 kg were treated with large placebo or end-use collars, as appropriate. On days 125 and 148, all puppies were treated with large placebo or end-use collars (as appropriate) because any puppy that still weighed less than 8 kg was expected to exceed 8 kg by day 148 or day 180. Outside of the scheduled treatment days, collars were replaced with new collars if damage was noted.

5. **Recovery period:** Any animal with abnormal local changes to the skin and/or hair underneath or adjacent to the collar(s) was retained for a recovery period of up to eleven days' duration. During recovery, each puppy wore a single new collar, either a placebo collar or an end-use collar, as appropriate, until the local abnormalities normalized, e.g. until unequivocal signs of hair regrowth were seen.

6. **Statistics:**

The individual animal was the experimental unit. Body weight, food consumption, and the hematology, clinical pathology parameters were analyzed using a repeated measures analysis of covariance (RMANCOVA). The RMANCOVA used the following as fixed effects: a covariate (the pretreatment baseline), treatment, time, and sex, the two-way interactions "treatment by time," "treatment by sex," "sex by time," and the three-way interaction "treatment by time by sex."

Two covariance structures were compared for each variable using the Akaike Information Criterion (AIC): compound symmetry and heterogeneous compound symmetry. Results from the model with the lowest AIC value were used. If the "treatment by sex by time" interaction was significant ($p \leq 0.05$), the statistical analysis of the variable was deemed inconclusive, and no further statistical analysis was conducted. If the "treatment by sex" interaction, "treatment by time" interaction, or overall treatment effect was significant ($p \leq 0.05$), pair-wise comparisons were made between negative controls vs. 1X, 3X, and 5X, placebo controls vs. 1X, 3X, and 5X, and combined negative and placebo controls vs. 1X, 3X, and 5X. If the "treatment by sex" interaction was not significant, comparisons were made using the combined sexes, otherwise the two sexes were evaluated separately.

The daily food consumption data were only analyzed over five 7-day intervals that commenced on the days of the collar applications (on days 0, 29, 90, and 148), using the average intake during the three days prior to application as the baseline covariate.

Profile plots were provided for all of the analyzed endpoints and included all data collected during acclimation and the treatment interval (Days 0-180). For each endpoint, the individual data and the average for the combined sexes were presented separately for each treatment group. For clinical pathology endpoints, the lower and upper limits of the reference range were included, and the "dose event" plots for food consumption included a plot of the baseline value.

The reviewer has the following issues with the statistical analysis:

- There was no comparison of the placebo control group to the negative controls.

- Mean daily food consumption should have been analyzed for the entire study rather than just the 5 "treatment periods."
- The data should have been analyzed separately by sex, regardless of whether the "treatment by sex" interaction was significant.
- The study report should have presented the summary data (means and standard deviations) for the combined control groups (separately by sex and with the sexes combined).
- It would have been helpful to have profile plots showing all of the group averages on the same plot.

C. METHODS:

1. Observations:

a. General health observations: During acclimation and the treatment interval, the animals were observed twice per day, morning and afternoon. On treatment days, i.e. on days 0, 29, 90, 125, and 148, the treated and placebo control animals were also observed pre-treatment and 1, 2, 3, and 4 hours (± 15 minutes) post-dosing. During the recovery interval (days 181-191), the animals were observed once daily.

b. Clinical assessments: All animals received physical examinations on days -14, -1, 15, 29, 61, 90, 120, 148, and 180.

c. Local observations: The hair and skin underneath and adjacent to the collars were inspected for signs of dermal irritation and hair loss at least once daily on days -7, -4, -1, 0, 1, 2, 3, 4, 7, 14, 21, 29, 35, 42, 49, 56, 63, 70, 77. 84, 90, 98, 105, 112, 119, 125, 133, 140, 148, 154, 161, 168, 175, 180, and daily during days 181-191 for any animals retained for recovery. Additional pre- and post-treatment local observations were conducted on all treated and placebo control puppies on days 0, 29, 90, 125, and 148.

2. Body weight: The animals were weighed on days -14, -11, -7, -1, 2, 5, 9, 13, 17, 21, 24, 28, 31, 35, 42, 49, 56, 63, 70, 77, 84, 89, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, and 180.

3. Food consumption: Food consumption was measured and recorded daily on days -4 through 180.

4. Clinical pathology: Blood for hematology and clinical chemistry evaluation was collected on days -14, -1, 15, 33, 61, 96, 120, 152, and 180. At less than 6 months of age, the puppies were not fasted, but food was withheld overnight prior to the collections on days 120, 152, and 180. Blood was collected from the jugular, cephalic, and/or saphenous vein. The sampling order used was not reported. The CHECKED (X) parameters were examined. A representative of Registration Division, OPP, authorized omitting the evaluation of coagulation parameters required under OPPTS 870.7200, in a Memorandum dated August 28, 2009 (Decision No. 415123). Coagulation data were generated for this collar in an adult dog study (MRID 48240109).

a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count* (absolute and percentage)
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		Morphology (if indicated)
	(Thromboplastin time)*	X	Heinz body formation
	(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*		Cholesterol
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)		Indirect bilirubin
X	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		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Amylase		

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

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

7. **Test article investigations:**

a. **Collar weights:** The placebo and end-use collars were weighed prior to application, and, following application, the trimmed surplus collar lengths were maintained in individual labeled Ziploc® bags for the later determination of the final weights of the applied collars. When collars were removed on days 29, 90, 125, 148, and 180 or at other intervals during the study, as required by damage to the collar, each collar or set of collars was placed in a tared, pre-labeled Ziploc® bag, and the final collar weight was recorded.

b. **Chemical analysis of collars:** On study days 29, 90, 125, 148, and 180, one set of collars/sex/group from each of the 3X and 5X groups were selected for chemical analysis. Selection was based on demonstration of the greatest weight loss between application and removal among the sets that had not been chewed or otherwise damaged. All of the collars used on the 1X animals, including chewed/damaged collars that had required replacement, were submitted for chemical analysis. The analytical testing was done at Ecto Development Corporation (Excelsior Springs, Missouri).

II. RESULTS

A. Results of the collar weight measurements and chemical analyses of the collars are given in Tables 2 and 3.

Table 2: Summary of initial amounts of imidacloprid and flumethrin in collars for puppies [a]

Group	Treatment days	Average Initial Collar Weight (g)	Average Active Ingredient (% w/w)		Average Active Ingredient (mg/animal)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 29	11.102	9.88	4.66	1096.87	517.35
	29 to 90	11.314	9.88	4.66	1117.85	527.24
	90 to 125	22.906	9.90	4.64	2270.88	1059.61
	125-148	37.028	9.93	4.61	3676.88	1706.99
	148 to 180	37.834	9.93	4.61	3756.95	1744.16
IV [c] [3X]	0 to 29	33.932	9.88	4.66	3352.43	1581.21
	29 to 90	33.693	9.88	4.66	3328.82	1570.07
	90 to 125	74.826	9.91	4.64	7420.85	3458.85
	125-148	114.607	9.93	4.61	11380.48	5283.38
	148 to 180	115.599	9.93	4.61	11478.93	5329.09
V [c] [5X]	0 to 29	58.887	9.88	4.66	5818.04	2744.13
	29 to 90	61.638	9.88	4.66	6089.79	2872.31
	90 to 125	198.417	9.93	4.61	19702.81	9147.02
	125-148	202.247	9.93	4.61	20083.08	9323.56
	148 to 180	206.876	9.93	4.61	20542.74	9536.96

[a] Data taken from Table 27, pp. 47-48, MRID 48240110.

[b] All calculations for this group were based on 12 animals per interval, except days 148-180, which used 9 animals.

[c] All calculations for this group were based on 2 animals per interval.

Table 3: Summary of amounts of imidacloprid and flumethrin remaining in collars removed from puppies [a]						
Group	Treatment days	Average Final Collar Weight (g)	Average Active Ingredient (% w/w)		Average Active Ingredient (mg/animal)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 29	10.156	8.94	4.58	877.73	465.61
	29 to 90	9.792	8.01	4.42	784.01	432.51
	90 to 125	21.483	7.63	4.52	1633.17	985.46
	125-148	35.772	8.69	4.63	3110.56	1655.28
	148 to 180	36.262	8.49	4.56	3078.48	1652.35
IV [c] [3X]	0 to 29	31.540	8.30	4.51	2615.40	1422.45
	29 to 90	29.505	8.00	4.40	2360.20	1296.95
	90 to 125	71.281	8.17	4.87	5903.83	3500.54
	125-148	111.347	8.55	4.99	9519.09	5549.80
	148 to 180	110.771	8.45	4.60	9349.34	5092.37
V [c] [5X]	0 to 29	56.670	7.86	4.77	4452.60	2703.36
	29 to 90	56.126	7.34	4.51	4119.25	2531.39
	90 to 125	192.206	8.32	4.92	15991.21	9456.67
	125-148	197.954	8.66	5.02	17134.17	9938.99
	148 to 180	201.109	8.60	4.68	17298.15	9410.71

[a] Data taken from Table 28, page 48, MRID 48240110.

[b] All calculations for this group were based on 12 animals per interval, except days 148-180, which used 9 animals.

[c] All calculations for this group were based on 2 animals per interval.

B. <u>ACTUAL DOSES ADMINISTERED</u>: The average exposure rates to the active ingredients expressed as mg/kg bw are given in Table 4. Based on these results, compared to the 1X group, the 3X group received 3.02X the imidacloprid exposure and 2.46X the flumethrin exposure, and the 5X group received 4.89X the imidacloprid exposure and 1.45X the flumethrin exposure.

Table 4: Summary of release (exposure) rates of imidacloprid and flumethrin [a]					
Group	Treatment days	Average Active Ingredient (mg/animal)		Average Active Ingredient (mg/kg bw)	
		Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
III [b] [1X]	0 to 29	219.14	51.74	76.98	18.69
	29 to 90	333.84	94.74	58.45	16.56
	90 to 125	637.71	74.15	70.21	8.71
	125-148	566.32	56.80	57.98	5.99
	148 to 180	678.47	91.81	65.15	8.63
	Cumulative	**2435.48**	**369.24**	**328.77**	**58.58**
IV [c] [3X]	0 to 29	737.03	158.76	270.46	61.10
	29 to 90	968.62	273.12	180.16	50.65
	90 to 125	1517.02	78.81	156.49	10.98
	125-148	1861.39	0 [d]	192.38	0 [d]
	148 to 180	2129.59	236.72	192.21	21.49
	Cumulative	**7213.64**	**747.40**	**991.70**	**144.22**
V [c] [5X]	0 to 29	1365.43	40.78	375.46	11.57
	29 to 90	1970.54	340.92	344.90	63.35
	90 to 125	3711.60	0 [d]	363.10	0 [d]
	125-148	2948.91	0 [d]	258,79	0 [d]
	148 to 180	3244.59	126.25	264.76	9.91
	Cumulative	**13241.06**	**507.95**	**1607.02**	**84.83**

[a] Data taken from Table 29, page 48, MRID 48240110.

[b] All calculations for this group were based on 12 animals per interval, except days 148-180, which used 9 animals.

[c] All calculations for this group were based on 2 animals per interval.

[d] Flumethrin amounts (mg) remaining in the collars exceeded the amounts initially present.

C. OBSERVATIONS:

1. **Removal of collars by puppies**: During the 180-day exposure interval, there were a total of five incidences (involving three animals) in the 1X group and one incidence in the 3X group when an animal's collar or collars was or were observed to be removed, chewed, and/or otherwise damaged. Each incident represented an interval of no more than 6-18 hours.

2. **Clinical signs of toxicity:** The study report did not provide the individual animal data for the time of observation of each abnormal sign and its subsequent course, and no tabular summary was provided either. The study author stated that one female negative control suffered a mild seizure on day 83, was treated with intravenous diazepam, and did not exhibit any additional seizures thereafter. The study author also stated that mild ocular discharge, emesis, and abnormal feces (loose stool or diarrhea) were recorded across all treatment groups, without any pattern indicating a relationship to treatment. Five puppies (one negative control, and 1, 2, and 1 puppy from each of the 1X, 3X, and 5X groups) were noted to have unilateral persistent prolapsed gland of the third eyelid.

3. **Local effects:** Treatment-related abnormal local findings such as erythema, hair loss or thinning of the hair, bruising, abrasions, scabbing, or a part in the hair were noted on two negative controls, five placebo controls, nine 1X animals, eight 3X animals, and ten 5X animals at one or more observations during the treatment interval (days 0-180). Five animals (two placebo control, one 3X, and two 5X puppies) were retained for recovery, due to the presence of local effects on day 180. Unequivocal hair regrowth and/or resolution of erythema was noted on all of these animals on or before day 191, while they were wearing a single placebo or end-use collar.

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

D. BODY WEIGHT AND WEIGHT GAIN: Selected body weight data are given in Table 5. Statistical analysis did not detect any significant treatment interactions or a significant treatment main effect on absolute body weight. Body weight gain of the 5X females was 25% less than that of negative controls during days -1 to 17.

TABLE 5: Body weight data from beagle puppies [a]					
Parameter/ Study day or interval	Treatment				
	Negative Control	Placebo Control	1X	3X	5X
Males					
Absolute body weight: Day -1	2.428±0.448	2.226±0.355	2.150±0.285	2.308±0.320	2.409±0.357
Day 17	3.627±0.730	3.421±0.789	3.203±0.375	3.519±0.533	3.596±0.530
Day 31	4.873±0.865	4.687±0.889	4.171±0.502	4.625±0.667	4.671±0.664
Day 63	7.650±1.435	7.708±1.217	6.576±0.575	7.228±0.921	7.307±1.037
Day 89	9.620±1.685	9.849±1.532	8.257±0.744	9.025±0.905	8.918±1.352
Day 126	11.397±2.011	11.860±1.631	10.126±0.920	10.919±0.948	11.224±1.712
Day 154	12.291±1.996	12.901±1.641	11.062±0.936	11.987±0.846	12.227±1.832
Day 180	12.690±2.182	13.543±1.792	11.523±1.041	12.648±0.965	12.744±1.993
Body weight change [b]:					
Days -1 to 17	1.199	1.195	1.053	1.211	1.187
Days 17 to 31	1.246	1.266	0.968	1.106	1.075
Days 31 to 63	2.777	3.021	2.405	2.603	2.636
Days 63 to 89	1.97	2.141	1.681	1.797	1.992
Days 89 to 126	1.777	2.011	1.869	1.894	1.925
Days 126 to 154	0.894	1.041	0.936	1.068	1.003
Days 154 to 180	0.399	0.642	0.461	0.661	0.517
Day -1 to 180	10.262	11.317	9.373	10.34	10.335
Females					
Absolute body weight: Day -1	1.921±0.155	2.001±0.397	1.836±0.216	1.846±0.197	1.908±0.299
Day 17	2.945±0.152	3.106±0.793	2.765±0.443	2.724±0.353	2.678±0.464
Day 31	3.841±0.182	4.127±1.056	3.697±0.574	3.587±0.445	3.604±0.517
Day 63	5.911±0.072	6.443±1.760	5.898±0.951	5.575±0.579	5.595±0.769
Day 89	7.429±0.447	8.080±2.222	7.451±1.318	6.958±0.683	7.011±1.044
Day 126	8.875±0.608	9.521±2.680	9.081±1.752	8.206±0.900	8.465±1.384
Day 154	9.612±0.705	10.372±2.856	9.870±1.911	8.893±0.848	9.272±1.768
Day 180	10.052±0.714	10.740±2.950	10.037±2.000	9.173±0.935	9.588±1.969
Body weight change [b]:					
Days -1 to 17	1.024	1.105	0.929	0.878	0.770 (-25) [c]
Days 17 to 31	0.896	1.021	0.932	0.863	0.926
Days 31 to 63	2.07	2.316	2.201	1.988	1.991
Days 63 to 89	1.518	1.637	1.553	1.383	1.416
Days 89 to 126	1.446	1.441	1.63	1.248	1.454
Days 126 to 154	0.737	0.851	0.789	0.687	0.807
Days 154 to 180	0.44	0.368	0.167	0.28	0.316
Day -1 to 180	8.131	8.739	8.201	7.327	7.680

[a] Data taken from pp. 216-225, MRID 48240110. Values are Mean ± Standard Deviation, with n=3 for negative control, placebo control, and 1X groups, and n=6 for the 5X group.

[b] Calculated by reviewer using group mean absolute body weight values.

[c] Numbers in parentheses equal percent different from control; calculated by reviewer.

E. **FOOD CONSUMPTION:** Throughout the study there were four incidences of inappetence (defined as consumption of less than 25 g of food). One placebo control female consumed 22 and 27 grams on days 1 and 2 and had normal food consumption thereafter. The remaining incidences (in two 1X animals and 1 3X animal) were a single day in duration, preceded and followed by normal food consumption.

Mean weekly food consumption values were not subjected to statistical analysis. As a correlate to the decreased body weight gain in 5X females, the mean food consumption of the 5X females was marginally decreased during weeks 2 and 3 (16% and 10% less than negative controls, respectively).

A treatment by day interaction was detected for "period 3," i.e. days 90-97. In addition to differences seen only in the lower dose groups on day 91, on day 93 the food consumption of the 5X group was increased 28% compared to the negative controls and was also increased relative to placebo controls and combined controls. Food consumption of the 3X group was increased compared to the placebo controls, and the food consumption of the 1X group was increased compared to the placebo controls and combined controls. For the 1X and 3X groups, respectively, mean food consumption for the combined sexes on day 93 was 15% and 9% greater than that of negative controls. These differences are not considered toxicologically significant, due to short duration.

The profile plot for the day 148 "dose event" did show decreases (usually below baseline) in the food consumption of all groups on day 151, which was one of the days on which food was removed prior to blood collection.

F. **BLOOD ANALYSES:**

1. **Hematology:** Statistically significant treatment main effects, treatment interactions, and/or differences from negative and/or placebo controls at one or more time points were found for most of the hematology parameters, including the following: relative eosinophils, leukocyte count, erythrocyte count, hematocrit, hemoglobin, MCV, MCHC, absolute and relative neutrophil counts, relative lymphocytes, and platelets. However, none of the statistical findings were considered biologically significant or treatment-related due to the absence of a dose response or because the mean values fell within the provided reference ranges and/or within two standard deviations of the negative control mean.

2. **Clinical chemistry:** Statistically significant treatment main effects, "treatment by day" or "treatment by sex" interactions, and/or differences from negative and/or placebo controls at one or more time points were found for most of the evaluated parameters, including ALT, ALP, AST, and CPK activities and serum calcium, phosphorus, chloride, sodium, potassium, and glucose concentrations. In general, the differences were present without a dose-related trend or were of small magnitude with the mean values and all or most individual values falling within the provided reference range or within two standard deviations of the negative control mean in cases where the negative control mean was outside the provided reference range. The exception was CPK activity on day 120, when 3X and 5X animals had significantly increased mean CPK activity relative to negative controls, placebo controls, and the combined controls (for the combined sexes, 349±248, 940±857, and 1074±927 for negative controls, 3X, and 5X, respectively), with 5 and 6 individual values from the 3X and 5X groups, respectively, that fell outside of the provided

reference range (59-895 U/L). The significance of this apparently treatment-related finding is unclear. Elevated CK activity of muscle origin could result from scratching, but if this were the case, it would not make sense for the increases to only be noted at one of the seven measuring intervals.

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: According to the study author, no adverse treatment-related findings were observed in male or female puppies treated continuously for 180 days either with zero, one, three, or five PNR 1427 collars or with five placebo collars. The study author stated that mild thinning of the hair in the throat region under or adjacent to the collars was presumed to be related to mechanical irritation from wearing multiple collars. The study author concluded that continuous treatment of puppies at 1X, 3X, and 5X the recommended label dose for 180 consecutive days was well tolerated and determined to be safe.

B. <u>REVIEWER COMMENTS</u>: In disagreement with the study author, toxicity was evident at 5X as a transient decrease in body weight gain in females following the initial treatment on day 0. Elevated CK activity on day 120 in 3x and 5x animals is of undetermined significance.

Based on determination of the collar weight losses and chemical analyses of the worn collars, the imidacloprid exposure of puppies in the 3X and 5X groups was 3.02X and 4.89X that of the puppies in the 1X group, and the flumethrin exposure of puppies in the 3X and 5X groups was 2.46X and 1.45X that of the puppies in the 1X group. During two of the retreatment intervals, the Flumethrin amounts (mg) remaining in the sets of collars exceeded the amounts initially present. This may be due to a problem with the analytical method, a manufacturing problem, or a problem with the study design. It is possible that the 3 x 2 bi-layer arrangement of the collars and/or the use of zip ties to band the collars together inhibited the release of flumethrin from the collars.

Based on transient decreased body weight gain, it is concluded that the margin of safety in 7-week-old 1.53- to 2.96-kg beagle puppies treated with PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars is 3X the recommended dose (of one collar per puppy). The average collar dosage rate for 1X animals on Day 0 was 10.156 g, equivalent to 5.096 g/kg.

C. <u>STUDY DEFICIENCIES</u>: The following deficiencies were identified:

- The level of flumethrin exposure for the 5X group measured only 1.45X that of the animals dosed according to the label recommendation of one collar per dog.
- The study report did not provide the individual animal data for the time of observation of each abnormal sign and its subsequent course, and no tabular summary was provided, either.
- On study days 29, 90, 125, 148 and 180, one set of collars/sex/group from each of the 3X and 5X groups was selected for chemical analysis. Selection was based on demonstration of the greatest weight loss between application and removal among the sets that had not been chewed or otherwise damaged. By selecting the collars with the greatest weight loss, the analyses were biased to provide the highest mg/kg body weight exposure of the active ingredients.
- There was no comparison of the placebo control group to the negative controls.
- Mean daily food consumption should have been analyzed for the entire study rather than just the 5 "treatment periods."

- The data should have been analyzed separately by sex, regardless of whether the "treatment by sex" interaction was significant.
- Due to weight gain (from a weight less than 8 kg to a weight exceeding 8 kg) between the collar applications on days 90 and 125, at least ten, and possibly eleven animals (4-5 1X, 3 3X, and 3 5X animals) were underdosed for 1-5 weeks of the study.

The deficiencies indicated above are considered to be minor, and do not affect the acceptable classification of this study.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427 INSECTICIDE COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY – KITTENS (OPPTS 870.7200)

MRID 48240111

Prepared for
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Disclaimer

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

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Template version 02/06

DATA EVALUATION RECORD

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

PC CODE: 129099 (Imidacloprid), 036007 (Flumethrin) **BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)]

TRADE NAME: Not provided

CITATIONS: Madsen, T.J. (2010) Safety of PNR 1427 in kittens. Sinclair Research Center, Auxvasse, MO. In-Life Testing Facility Study No. S10063, June 16, 2010. MRID 48240111. Unpublished.

SPONSOR: Bayer HealthCare LLC/Animal Health Division, Shawnee Mission, KS

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 48240111), the safety of PNR 1427 insecticide collars containing imidacloprid (10% w/w) and flumethrin (4.5% w/w) was tested in domestic short hair kittens (68 to 71 days old at initiation of treatment). One group of three male and three female kittens served as negative control. Another group of three male and three female kittens served as a placebo control and wore five end-use collars minus the active ingredients for 180 days continuously. In a third group (1x) of six male and six female kittens, one end-use collar was applied on day 0 and then replaced with new collars on days 29, 90 and 149 and removed on day 180. In a fourth group (3x) of six male and six female kittens, three end-use collars were applied on day 0 and then replaced with three new collars on days 29, 90 and 149 and removed on day 180. In a fifth group (5x) of six male and six females kittens, five end-use collars were applied on day 0 and then replaced with five new collars on days 29, 90 and 180 and removed on day 180. In addition to measuring the required parameters, the end-use collars worn by selected kittens in the 1x, 3x and 5x groups were analyzed post-removal to determine the exposures of the kittens to the active ingredients.

All animals survived to the end of the study. Clinical observations reported included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge in all treatment groups; however, no raw or summarized data were included in the final report. No effects on body weight, food consumption or clinical pathology parameters were observed. Statistically significant findings for hematology and clinical chemistry parameters were not considered treatment-related since they were either isolated, inconsistent or not associated with clinical signs. Body weight gain over the course of the study (days -1 to 180) was decreased 17% in the 5x group females, as compared to the

placebo control group. Based on chemical analyses of the worn collars, kittens in the 3x and 5x groups were exposed to 3.59x and 3.13x the imidacloprid exposure, respectively, received by kittens that wore one end-use collar. The kittens in the 3x and 5x groups were exposed to 5.09x and 5.57x the flumethrin exposure, respectively, received by kittens that wore one end-use collar.

It is concluded that the margin of safety in kittens exposed to PNR 1427 [imidacloprid (10% w/w) + flumethrin (4.5% w/w)] insecticide collar for 180 days is 3x the recommended dose based on decreased body weight gain over the course of the study (days -1 to 180) in females at 5x the recommended dose. In addition, the exposure from the 5x treatment was 5.57x the recommended dose of flumethrin but only 3.13x the recommended dose of imidacloprid, based on the chemical analyses of the worn collars. The average collar dosage rate for 1X kittens on Day 0 was 9.387 g, equivalent to 4.15 g/kg body weight.

This companion animal safety study in male and female kittens is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in the kitten. However it must be noted that, although the kittens in the 5x group were exposed to 5.57x the recommended dose of flumethrin, they were exposed to only 3.13x the recommended dose of imidacloprid, based on the chemical analyses of the worn collars. Additional study deficiencies are listed under III.C. Deficiencies in this review.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] (Lot # KP05KTJ). Each collar measured approximately 8 x 4.5 mm (width x height), 35 cm (length) and 12.5 g (weight).

2. **Placebo control:** End-product collar minus the active ingredients (Lot # KP05UGW). Each collar measured approximately 8 x 4.5 mm (width x height), 35 cm (length) and 12.5 g (weight).

3. **Test animals:**

Species:	Feline
Strain:	Domestic short hair
Age/weight	On Day 0 – 68 to 71 days old On Day -1 – 0.774 to 1.466 kg body weight
Source:	Liberty Research Inc., Waverly, NY
Housing:	Group housed on days -14 through -5; individually housed in stainless steel pens on days -5 through 180
Diet:	Purina® Kitten Chow – 100 grams/day on days -4 through 6; 150 grams/day on days 7 through 180; kittens eating <25 grams per day were offered moist food (Purina® Friskies, Mariner's Catch, ≈25 grams/day)
Water:	Tap water, *ad libitum*

Environmental conditions:	
Temperature:	50.5 – 85.7° F
Humidity:	15 – 89.71%
Air changes:	"Appropriate hourly air exchanges"
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Fourteen days

B. STUDY DESIGN:

1. **In life dates:** Start: October 7, 2009; End: April 5, 2010

2. **Animal assignment:** There were five groups in the study, each containing either 6 cats (3 males and 3 females) or 12 cats (6 males and 6 females), as shown in Table 1. The kittens were blocked by gender and ranked within each block by descending order of body weight. Each gender block was divided into three subgroups. Each subgroup contained eight kittens of the same gender and similar body weight (heavy, intermediate, light). A unique random number was assigned to each kitten within each subgroup. The final assignments were based on a pre-designed relationship between subgroup, unique random number and treatment which is detailed in Table 3 of the study report. Although not stated in the final study report, the animal information table on page 173-174 of MRID 48240111 indicates that there were three replicates for Groups I and II and six replicates for Groups III, IV and V. The study was not blinded.

Table 1: Animal Assignment

Group	Treatment	Number of Cats	
		Male	Female
I (0x; negative control)	No collar	3	3
II (5x; placebo control)	5x Vehicle-only collar	3	3
III (1x end-use product)	1x Imidacloprid + Flumethrin collar	6	6
IV (3x end-use product)	3x Imidacloprid + Flumethrin collar	6	6
V (5x end-use product)	5x Imidacloprid + Flumethrin collar	6	6

3. **Dose selection rationale:** No dose rationale was provided for the percentage of active ingredients in the end-use product.

4. **Preparation and treatment:** On day 0, five vehicle collars were applied to each kitten in Group II, one end-use collar was applied to each kitten in Group III, three end-use collars to each kitten in Group IV and five end-use collars to each kitten in Group V. The multiple collars in Groups II and IV were affixed around the neck in a 3 x 2 bi-layer arrangement. A single layer of three collars was in contact with the cat's neck. A second tier of two collars encircled and directly contacted the foundation layer. The multiple collars were banded together with nylon cable (zip) ties. Surplus collar length, in excess of 2 cm, was trimmed and removed. On days 29, 90 and 148, the end-use collars worn by kittens in Groups III, IV and V were removed and replaced with new end-use

collars. On day 180, the collars were removed from all groups. At each observation period, the collars were adjusted if removed by the cat, loosened, damaged or missing ties. After the removal of the collars, the final collar weight was determined and each removed collar was stored.

5. **Statistics:** The individual animal was defined as the experimental unit. Descriptive statistics (mean and standard deviation) were calculated for all numeric variables, including food consumption, body weight, hematology and clinical chemistry parameters. All continuous numeric measurements were plotted across time for individual animals and group averages. All continuous numeric measurements were analyzed with a repeated measures analysis of covariance, with the most appropriate baseline value(s) as the covariate and the best fitted covariance structure. When significant effects were observed (treatment x sex, treatment x time or overall treatment effects), pair-wise group comparisons with Group I versus III, IV and V, Group II versus III, IV and V and the combined control (Groups I + II) versus III, IV and V were made. If the treatment x sex interaction was not significant, analyses were performed using the combined sexes, otherwise analyses were performed for males and females, respectively. An alpha level of 0.05 was used to define significant effects.

   For food consumption, time intervals surrounding each collar application (days 0, 29, 90 and 148) were statistically analyzed. The average of three days prior to initial application (day 0) or removal (days 0, 20, 90 and 148) were used as the baseline covariate. The following four time intervals were analyzed for daily food consumption: days -3 to 6, 26 to 35, 87 to 96 and 145 to 154.

C. METHODS:

1. Observations:

d. **General health observations:** The animals were observed twice daily for general health, except on days of treatment (application of new set of collars). On treatment days, observations were performed at pre-treatment and 1, 2, 3 and 4 hours (±15 minutes) post-dose.

e. **Veterinary examinations:** A physical examination was performed by a veterinarian on days -14, -1, 15, 29, 61, 90, 120, 148 and 180.

f. **Local observations:** The hair and skin of all kittens, both underneath and adjacent to the collars, was inspected for signs of dermal irritation and hair loss at least once daily on days -7, -1, 0 (pre- and post-application), 1, 2, 3, 4, 7, 14, 21, 29 (pre- and post-application), 35, 42, 49, 56, 63, 70, 77, 84, 90 (pre- and post-application), 98. 105, 112, 119, 126, 133, 140, 148 (pre- and post-application), 154, 161, 168, 175 and 180.

2. **Body weight:** Animals were weighed on days -14, -11, -7, -4, -1, 2, 5, 9, 13, 17, 21, 24, 28, 31, 35, 42, 49, 56, 63, 70, 77, 84, 89, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, and 180.

3. **Food consumption:** On days -4 through 180, food consumption was measured once daily for each kitten.

4. **Hematology and clinical chemistry:** On days -14, -1, 15, 33, 61, 96, 120, 152 and 180, blood was collected from all kittens for hematology and clinical chemistry testing. During the initial 2.5

months, growing kittens were not fasted overnight prior to blood collection (days -14, -1, 15, 33 and 61). Once kittens became 5 months of age, all animals were fasted overnight prior to blood collection (days 96, 120, 152 and 180). 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	Heinz bodies
	(Activated partial thromboplastin time)		
	(Fibrinogen)		
	(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	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase (CPK)		Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
X	Amylase		
	Sorbitol dehydrogenase		

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

5. **Urinalysis:** Urinalysis was not conducted. It is not required by the OPPTS 870.7200 guideline.

6. **Sacrifice and pathology:** The study did not have a scheduled necropsy. It is not required by the OPPTS 870.7200 guideline.

7. **Chemical Analysis of Collars:** To determine the 1x, 3x and 5x exposure rates, the collars worn by selected kittens in Group III, IV and V were periodically analyzed for residual percentages (weight/weight) of imidacloprid and flumethrin. On days 29, 90, 148 and 180, a collar from each animal in Group III (1x end-use product) plus any previously worn collar(s) that had been chewed/damaged were packaged separately for chemical analysis. On days 29, 90, 148 and 180, a collar set from each gender in Groups IV and V demonstrating the greatest weight loss between

each application and removal event (i.e., days 0-29, 29-90, 90-148 and 148-180) and that had not been chewed/damaged, was selected for chemical analysis. The collars were shipped to Ecto Development Corporation, Excelsior Springs, MO, for analysis.

II. RESULTS

A. CHEMICAL ANALYSIS OF COLLARS: The pre-treatment analysis of the end-use product showed concentrations of imidacloprid at 10.18% (w/w) and flumethrin at 4.47% (w/w). The mid-treatment analysis of the end-use collar showed concentrations of 9.88% (w/w) imidacloprid and 4.66% (w/w) flumethrin. The post-treatment analysis of the end-use collar showed concentrations of imidacloprid at 10.2% (w/w) and 4.66% (w/w) flumethrin. All the collars were from the same lot number (KP05KTJ). The mid-treatment concentrations were used to calculate the average initial amounts (mg) of each active ingredient in the collars applied to Groups III, IV and V as shown in Table 2.

Table 2: Summary of initial amounts of imidacloprid and flumethrin in collars for kittens[a]

Group	Treatment days	Average Imidacloprid (mg)/kitten	Average Imidacloprid (%, w/w)	Average Flumethrin (mg)/kitten	Average Flumethrin (%, w/w)
III[b]	0 to 29	927.44	9.88	437.43	4.66
	29 to 90	1013.24	9.88	477.90	4.66
	90 to 148	1011.51	9.88	477.09	4.66
	148 to 180	1095.86	9.88	516.87	4.66
IV[c]	0 to 29	2715.47	9.88	1280.78	4.66
	29 to 90	2992.70	9.88	1411.54	4.66
	90 to 148	3145.25	9.88	1483.49	4.66
	148 to 180	3302.14	9.88	1557.49	4.66
V[c]	0 to 29	5045.81	9.88	2379.91	4.66
	29 to 90	5351.45	9.88	2524.07	4.66
	90 to 148	5570.49	9.88	2627.38	4.66
	148 to 180	5757.72	9.88	2715.68	4.66

[a] Extracted from Table 21, page 44, MRID 48240111.
[b] All calculations for this group were based on 12 animals per interval, except days 29-90 which used 11 animals.
[c] All calculations for this group were based on 2 animals per interval.
Group III – 1x the end-use product; Group IV – 3x the end-use product; Group V – 5x the end-use product

The average amounts (mg) of imidacloprid and flumethrin remaining in the selected end-use product collars at each exposure event for Groups III, IV and V are presented in Table 3.

Table 3: Summary of imidacloprid and flumethrin amounts remaining in collars removed from kittens[a]					
Group	**Treatment days**	**Average Imidacloprid (mg)/kitten**	**Average Imidacloprid (%, w/w)**	**Average Flumethrin (mg)/kitten**	**Average Flumethrin (%, w/w)**
III[b]	0 to 29	744.62	8.28	424.75	4.72
	29 to 90	742.45	7.87	462.60	4.89
	90 to 148	801.20	8.42	439.71	4.62
	148 to 180	865.81	8.29	500.00	4.76
IV[c]	0 to 29	2009.02	7.61	1159.47	4.39
	29 to 90	2117.06	7.45	1326.57	4.67
	90 to 148	2329.87	7.73	1396.89	4.64
	148 to 180	2681.74	8.29	1570.42	4.85
V[c]	0 to 29	4477.14	8.92	2250.72	4.49
	29 to 90	4332.48	8.29	2463.17	4.71
	90 to 148	4936.33	9.01	2518.50	4.60
	148 to 180	5083.00	8.89	2604.88	4.55

[a] Extracted from Table 22, page 45, MRID 48240111.
[b] All calculations for this group were based on 12 animals per interval, except days 29-90 which used 11 animals.
[c] All calculations for this group were based on 2 animals per interval.
Group III – 1x the end-use product; Group IV – 3x the end-use product; Group V – 5x the end-use product

B. <u>ACTUAL DOSES ADMINISTERED</u>: The average imidacloprid and flumethrin exposures in kittens, based on the analysis of the used collars, are presented in Table 4. The release rates for imidacloprid and flumethrin in kittens wearing 1x (Group II), 3x (Group III) and 5x (Group IV) end-use collars worn 180 days were on average exposed to 369.11mg + 31.16 mg, 1325.60 mg + 158.71 mg and 1155.84 mg + 173.43 mg of imidacloprid + flumethrin of body weight, respectively. Therefore, the kittens in Groups IV (3x) and V (5x) were subjected to 3.59x and 3.13x the imidacloprid exposure, respectively, received by the kittens in Group III (1x). Based on the analysis for flumethrin, the kittens in Groups IV and V were subjected to 5.09x and 5.57x the flumethrin exposure, respectively, by kittens in Group III (1x). Based on the combined imidacloprid + flumethrin exposure (mg/kg), the kittens in Groups IV and V received 3.71x and 3.32x the chemical exposure received by kittens in Group III, respectively.

Table 4: Release (Exposure) rates of imidacloprid and flumethrin in kittens[a]					
Group	**Treatment days**	**Average Imidacloprid (mg)/kitten**	**Average Imidacloprid (mg/kg bw)**	**Average Flumethrin (mg)/kitten**	**Average Flumethrin (mg/kg bw)[b]**
III[b]	0 to 29	182.81	123.42	12.69	8.51
	29 to 90	270.79	115.56	15.30	6.44
	90 to 148	210.31	65.80	41.01	11.69
	148 to 180	230.04	64.34	16.87	4.52
	0 to 180 cumulative	**893.96**	**369.11**	**85.87**	**31.16**
IV[c]	0 to 29	706.45	494.57	121.31	84.73
	29 to 90	875.64	397.98	84.97	40.67
	90 to 148	815.38	250.67	86.60	31.10
	148 to 180	620.40	182.38	10.02	2.21
	0 to 180 cumulative	**3017.87**	**1325.60**	**302.89**	**158.71**
V[c]	0 to 29	568.67	362.95	129.19	82.12
	29 to 90	1018.98	399.65	60.90	23.87
	90 to 148	634.16	169.54	108.88	27.10
	148 to 180	674.72	223.71	110.81	40.34
	0 to 180 cumulative	**2896.54**	**1155.84**	**404.77**	**173.43**

[a] Extracted from Table 23, page 46, MRID 48240111.

[b] All calculations for this group were based on 12 animals per interval, except days 29-90 which used 11 animals.

[c] All calculations for this group were based on 2 animals per interval.

The average body weights for each event were: Group III (0-180 days) = 10.806 kg, Group IV (0-180 days) = 11.019 kg, Group V (0 to 180 days) = 11.065.

Group III – 1x the end-use product; Group IV – 3x the end-use product; Group V – 5x the end-use product.

C. OBSERVATIONS:

1. **Clinical signs of toxicity:** Multiple kittens in Groups II, III, IV and V were found to not being wearing their collar on one or more occasions. Each incident represented no more than an 8-19 hour void in continuous collar exposure. One cat had acute respiratory distress after blood collection on day 33. This animal received supportive oxygen therapy for approximately 15 minutes until the episode resolved. Clinical observations noted in the final study report included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge. No raw or summarized data on clinical observations were included with the study report.

2. **Application site examination:** Abnormal local findings were detected in 13 different kittens over the course of the study. The findings (hair thinning) in 11 kittens were present in all dose groups and were presumed to be mechanical irritations induced by the presence of multiple collars. The local findings had resolved by day 180.

3. **Mortality:** One male in the placebo control (Group II) died after blood collection on day 33. A necropsy revealed that the cause of death was idiopathic hypertrophic cardiomyopathy and was not associated with treatment.

D. **BODY WEIGHT AND WEIGHT GAIN:** Selected body weight and body weight gain data are presented in Table 5. No treatment-related effects on body weight were observed. Body weight gain over the course of the study (days -1 to 180) in females was decreased 7% and 17% in the 3x and 5x groups, respectively, as compared to the placebo control group (II).

Table 5: Group body weight (kg) and body weight gain (kg)[a]

Day	Groups				
	I	II	III	IV	V
	Males				
-1	1.18±0.06	.22±0.14	1.19±0.05	1.20±0.14	1.17±0.08
2	1.27±0.07	.31±0.15	1.30±0.06	1.29±0.15	1.25±0.09
89	3.36±0.23	3.62[b]	3.44±0.34	3.42±0.19	3.36±0.37
140	4.16±0.32	4.14[b]	4.36±0.58	4.31±0.36	4.22±0.57
180	4.28±0.34	4.23[b]	4.62±0.73	4.45±0.44	4.43±0.61
Weight gain (days -1 to 180)[c]	3.10	3.01	3.43	3.25	3.26
	Females				
-1	1.00±0.08	.04±0.07	1.07±0.08	0.99±0.11	1.05±0.09
2	1.08±0.07	.09±0.11	1.16±0.08	1.06±0.10	1.14±0.10
89	2.52±0.12	.47±0.33	2.48±0.16	2.41±0.25	2.33±0.33
140	2.80±0.23	.75±0.50	2.77±0.20	2.68±0.27	2.58±0.43
180	2.72±0.19	.81±0.50	2.81±0.33	2.64±0.29	2.52±0.36
Weight gain (days -1 to 180)[c]	1.72	1.77	1.74	1.65 (↓7%)[d]	.47 (↓17%)[d]

[a] Extracted from pages 184-193, MRID 48240111.
[b] Weight based on only two animals.
[c] Calculated by the reviewer.
[d] Percentage decrease as compared to the placebo control group (II).

E. **FOOD CONSUMPTION:** Mean food consumption was occasionally increased or decreased in the treated groups but the effect was not consistent or dose-related.

F. **CLINICAL PATHOLOGY ANALYSES:**

1. **Hematology:** None of the following hematology findings is considered treatment-related since they were either not consistent, present pre-treatment or not associated with clinical signs. In addition, the kittens were 68 to 71 days of age at the start of the study. The clinical pathology laboratory used in the study did not have age-appropriate reference values for kittens.

   Statistical analysis of the hematological results revealed: 1) significant treatment x sex and significant treatment x day interactions for MCH; and 2) significant treatment x day interactions for % eosinophils, hemoglobin, lymphocyte count, % lymphocytes and monocyte count. In female kittens, mean MCH was significantly lower ($p \leq 0.05$) in Group III (1x) as compared to controls

(Group II and combined Groups I & II) while in male kittens, mean MCH was significantly higher ($p \leq 0.05$) in Group V (5x) versus the controls (Group I, II and combined Groups I & II). On day 120, mean MCH was significantly lower ($p \leq 0.05$) in Group III (1x) versus the controls (Groups I, II and combined I & II) while on day 152, MCH was significantly higher ($p \leq 0.05$) in Group V (5x) versus the controls (Group I and combined Groups I & II).

On days 96, 120 and 180, the mean % eosinophils was significantly greater in Group III (1x) as compared to controls (Groups II and combined Groups I & II), whereas on day 180, the % eosinophils was significantly lower in Group IV (3x) as compared to controls (Group I).

On day 120, the mean hemoglobin value was significantly lower in Group III (1x) as compared to controls (Groups I, II and combined I & II), whereas on day 180, mean hemoglobin was significantly lower in Group IV (3x) as compared to controls (Groups I and combined I & II).

On day 33, the mean lymphocyte count was significantly lower in Group IV (3x) as compared to controls (Groups I and combined I & II), whereas on day 180, the mean lymphocyte count was significantly higher in Group IV (3x) as compared to controls (Groups II and I & II).

On days 33 and 61, significant interactions between treatment (Groups III and IV) and control (Groups I and II) occurred for % lymphocytes.

On day 96, mean absolute monocyte counts were significantly lower in Groups III (1x) and IV (3x) as compared to controls (Groups I and combined I & II), whereas on day 120, mean absolute monocyte counts were significantly greater in Group V (5x) as compared to controls (Groups I, II and combined I & II).

Ten kittens from four different groups had platelet counts measured by automated equipment that were below the lower limit of the laboratory reference range. The evaluation of blood smears from these kittens confirmed the presence of platelet clumping and adequate numbers in all except for four isolated incidences.

2. **Clinical Chemistry:** None of the following clinical chemistry findings is considered treatment-related since they were isolated changes and not associated with clinical signs.

   Statistical analysis of the clinical chemistry indices revealed: 1) a significant treatment x sex x day interaction for amylase; 2) a significant treatment x sex interaction for globulin; and 3) a significant treatment x day interaction for chloride.

   The three-way interaction between treatment, sex and day was significant ($p = 0.054$) for amylase. Additional statistical analyses were not possible. No signs of abdominal pain or gastrointestinal distress usually associated with increased amylase were observed.

   The treatment x sex interaction was significant ($p = 0.0209$) for globulin. When all control pairwise comparisons within each gender were evaluated, no values were statistically significant.

   The treatment by day interaction was significant ($p=0.0027$) for chloride. Eleven significant ($p \leq 0.05$) interactions, involving different treatment (Groups III, IV and V) and control (Groups I,

II and combined I & II) combinations were noted on days 33, 61, 120, 152 and 180. All significant treatment means were within the normal reference range.

The mathematical model used for analysis of CPK results could not be applied. The majority of CPK results were within the normal reference range. The values that were outside the reference range were usually seen in two smaller control kittens and one Group V (5x) kitten. Review of the individual animal data shows isolated increases in CPK in multiple animals from all groups. On day 61, the most animals were affected with 3, 4, 4 and 5 kittens in groups II, III, IV and V, respectively, having elevated CPK levels. Some of the isolated increases throughout the study were so high that this reviewer questions whether lab errors occurred.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that no adverse treatment-related findings or clinical effects were observed in male or female kittens treated continuously, for 180 days, either with zero, one, three or five PNR 1427 collars or with five placebo collars.

B. REVIEWER COMMENTS: A proposed study protocol (MRID 47776801) was reviewed by the Registration Division, OPP, in a Memorandum dated August 28, 2009 (Decision No. 415124). The Agency agreed that three males and three females in the negative and placebo control groups, as opposed to six males and six females per group recommended by the OPPTS 870.7200 Guideline, were sufficient.

Based on chemical analyses of the worn collars, kittens in the 3x and 5x groups were exposed to 3.59x and 3.13x the imidacloprid exposure, respectively, received by kittens that wore one end-use collar. The kittens in the 3x and 5x groups were exposed to 5.09x and 5.57x the flumethrin exposure, respectively, received by kittens that wore one end-use collar. The comparatively low exposures of the 5x group may be due to a problem with the analytical method, a manufacturing problem, or a problem with the study design.

All animals survived to the end of the study. Clinical observations reported included mild signs of abnormal feces (loose stool and/or diarrhea), emesis and ocular discharge in all treatment groups; however, no raw or summarized data were included in the final report. No effects on absolute body weight, food consumption or clinical pathology parameters were observed. Statistically significant findings for hematology and clinical chemistry parameters were not considered treatment-related since they were either isolated, inconsistent or not associated with clinical signs. Body weight gain over the course of the study (days -1 to 180) was decreased 17% in the 5x group females, as compared to the placebo control group. These are considered treatment-related, although similar changes were not seen in the kittens of the 3x group, who may have received very similar exposures.

It is concluded that the margin of safety in kittens exposed to PNR 1427 [imidacloprid (10% w/w) + flumethrin (4.5% w/w)] insecticide collar for 180 days is 3x the recommended dose based on decreased body weight gain over the course of the study (days -1 to 180) in females at 5x the recommended dose. In addition, the exposure from the 5x treatment was 5.57x the recommended dose of flumethrin but only 3.13x the recommended dose of imidacloprid, based on the chemical analyses of the worn collars. The average collar dosage rate for 1X kittens on Day 0 was 9.387 g, equivalent to 4.15 g/kg body weight.

C. STUDY DEFICIENCIES:

1. Kittens in the 5x group were exposed to 5.57x the recommended dose of flumethrin but only 3.13x the recommended dose of imidacloprid, based on the chemical analyses of the worn collars. This reviewer questions whether the placement of collars in the 3 x 2 bilayer arrangement contributed to decreased exposure to imidacloprid (discussed in item 3 below).

2. The proposed protocol reviewed by the Agency indicated that collars would be applied side by side in a single layer to Groups II (5x placebo control) and V (5x end-use collar) so that all collars would touch the animal's neck. In the completed study, the placement of the collars was changed to a 3 x 2 bilayer arrangement. This reviewer questions whether the placement of collars in the 3 x 2 bilayer arrangement contributed to decreased exposure to imidacloprid.

3. On study days 29, 90, 148 and 180, a set of collars from each gender in Groups IV (3x) and V (5x) demonstrating the greatest weight loss between each application and removal event (i.e., days 0-29, 29-90, 90-148 and 148-180) were selected for chemical analysis. By selecting the collars with the greatest weight loss, the analyses were biased to provide the highest mg/kg body weight exposure of the active ingredients.

4. No raw data or summary tables were provided for the clinical observations results.

The deficiencies indicated above are considered to be minor, and do not affect the acceptable classification of this study.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL)
[PNR 1427, IMIDACLOPRID (10%, W/W) + FLUMETHRIN (4.5%, W/W) COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY STUDY- PUPPIES;
NON-GUIDELINE
MRID 48240112

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Task Order No. 3-C-04

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Disclaimer

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

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

Signature: Byron T. Backus
Date: Jan. 27, 2012

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Technical Review Branch, Registration Division (7505P)

Signature: M Hashim
Date: 1-31-12

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Puppies; Non-guideline.

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **DP BARCODE:** 385560

TEST MATERIAL (PURITY): PNR1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (10.18% Imidacloprid and 4.47% Flumethrin; Lot No. KP05KTJ)

SYNONYMS: M915 Insecticide Animal Collar (small collar)

CITATIONS: Madsen, T. (2010) Safety of PNR1427 with reflectors in puppies. Sinclair Research Center, LLC (SRC), Auxvasse, Missouri. Study Number S10064, August 30, 2010. MRID 48240112. Unpublished.

SPONSOR: Bayer HealthCare LLC, Animal Health Division, 12809 Shawnee Mission Parkway, Shawnee Mission, Kansas.

EXECUTIVE SUMMARY: In a 30-day non-guideline companion animal safety study (MRID 48240112), two groups of three 9-week-old beagle puppies were treated with single PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars either without (Group I, one male and two females) or with (Group II, two males and one female) attached polyamide (Grilamid® TR90) reflectors. Animals were treated on day 0 and observed for 30 days, and the weights of the collars applied on day 0 and the final collar weights (on day 30) were recorded.

There were no treatment-related effects on mortality, systemic or local clinical signs, body weight, cumulative body weight gain, or food consumption.

The percentage of collar weight lost from the collars without reflectors ranged from 6.713% to 10.663% and averaged 9.056%. The percentage of collar weight lost from the collars with attached reflectors (excluding the weight of the reflectors) ranged from 7.159% to 9.028% and averaged 7.861%.

It is concluded that, under the conditions used in this study, the attachment of three polyamide (Grilamid® TR90) reflectors to PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars worn by 9-week-old, 3.077- to 4.951-kg beagle puppies for thirty days was not associated with increased wear on the collars and did not result in a decrease in safety (or increase in toxicological hazard). The percentage weight loss from the collars with the reflectors was 13.2% lower than from collars without the reflectors.

This companion animal safety study in dogs is **Acceptable/Non-guideline.** It does not satisfy the guideline requirement for a companion animal safety study (OPPTS 870.7200) in puppies but does provide scientifically valid information.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

a. PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collar (small collar)

Description:	Gray solid collar, measuring approximately 35 cm (length) x 8 mm (width) x 4.5 mm (thickness), weighing 12.5 g;
Lot #:	KP05KTJ
Purity:	10.18% Imidacloprid and 4.47% Flumethrin
Compound Stability:	Shown via analysis to be stable for the study duration.
CAS #:	138261-41-3 (Imidacloprid) and 69770-42-2 (Flumethrin)

b. Reflector

Description:	Polyamide (Grilamid® TR90), manufactured by Ultra Reflex GmbH, Renchen, Germany
Lot #:	051109
Purity and stability:	Not provided

2. Vehicle and/or positive control: none.

3. Test animals:

Species:	Dog	
Breed:	Beagle	
Age/weight at study initiation:	61-65 days old on day 0/ Males: 3.098-4.951 kg (day -1); Females: 3.077-3.270 kg (day -1)	
Source:	Ridglan Farms, Mount Horeb, Wisconsin	
Housing:	Individually, in stainless-steel cages (~3 ft x 5 ft) with solid walls and a front gate that had vertical bars.	
Diet:	Purina® Puppy Chow, 300 g/day.	
Water:	*Ad libitum* water from an on-site deep well	
Environmental conditions:	**Temperature:**	68.1-79.0° F.
	Humidity:	27.0-98.4%
	Air changes:	Not reported; stated to be appropriate
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Two weeks.	

B. STUDY DESIGN:

1. In life dates: Start: May 5, 2010; End: June 4, 2010.

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups according to sex and body weight on day -1, using a stratified block randomization procedure. The animals on study were assigned into two replicates; however, both replicates were treated and observed contemporaneously. The study was not blinded.

TABLE 1: Study design [a]			
Test Group	Treatment	Number assigned	
		Males	Females
I. 1X	One end-use collar	1	2
II. 1X with 3 reflectors	One end-use collar with three attached reflectors	2	1

[a] Data taken from Table 2, p. 11, MRID 48240112.

3. **Dose selection rationale:** The study author stated that the study design, including dose selection, frequency of treatment, treatment duration, and group size was based on OPPTS 870.7200, on recommendations provided to the sponsor by the EPA during a study design teleconference (June 6, 2009), and on email correspondence between the sponsor and EPA (March 26 and 30, 2010).

4. **Treatment:** On day 0, one end-use collar was applied to each puppy in group I and one end-use collar with three attached reflectors was applied to each puppy in group II. The collars were fitted to maintain a two-finger space between the collar and the neck, and surplus collar length, in excess of 2 cm, was trimmed and saved. Three reflectors were attached to the collar of each puppy in group II by tightly pressing the fastener until it clicked into place. The reflectors were spaced as evenly as possible over the non-overlapping portion of the collar.

5. **Statistics:** The individual animal was the experimental unit. Descriptive statistics were presented for body weight, food consumption, and numerical variables related to the collars.

C. METHODS:

1. **Observations:**

a. **General health observations:** During acclimation and the treatment interval, the animals were observed twice per day, morning and afternoon. At each observation, each animal's collar was examined for proper fit and adjusted if necessary.

b. **Clinical assessments:** All animals received physical examinations on day -2.

c. **Local observations:** The hair and skin underneath and adjacent to the collars were inspected for signs of dermal irritation and hair loss at least once daily on day -7 and days -1 through 30.

2. **Body weight:** The animals were weighed on days -14, -1, and 30.

3. **Food consumption:** Food consumption was measured and recorded daily on days -4 through 30.

4. **Clinical pathology:** Clinical pathology was not evaluated.

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

7. **Collar weights:** The collars were weighed prior to application, and, following application, the trimmed surplus collar lengths were maintained in individual labeled Ziploc® bags for the later determination of the final weights of the applied collars. The reflectors were also pre-weighed. When collars were removed on day 30, each collar (with or without reflectors) was placed in a tared, pre-labeled Ziploc® bag, and the final collar weight was recorded.

II. RESULTS

A. **DOSES ADMINISTERED:** The mean administered mg/kg doses of the active ingredients are given in Table 2, and the net weight losses of the collars with and without the reflectors attached are given in Table 3. Based on the average net collar weight losses of 9.056% and 7.861% from the collars worn by Group I and Group II animals, respectively, the percentage of weight lost from the collars with the reflectors was 13.2% lower than from collars without the reflectors.

Table 2: Mean collar "doses" and administered doses of imidacloprid and flumethrin [a]

Group	Mean collar weight (g)	Mean collar "dose" (g/kg bw)	Content active ingredients (mg) [b]		Dose active ingredients (mg/kg bw)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
I	11.616	3.138	1161.6	522.7	313.8	141.2
II	11.570	3.289	1157.0	520.7	328.9	148.0

a Data taken from Table D, page 63, MRID 48240112.

b Based on the nominal concentrations of the active ingredients, i.e. 10% (w/w) imidacloprid and 4.5% (w/w) flumethrin.

Table 3: Individual and mean collar net weight losses during 30-day application intervals on beagle puppies [a]

Group	Animal ID	Collar weight at application [b] (g)	Net weight loss (g)	Net weight loss (%)	Mean (± SD [c]) net weight loss (g)	Mean (± SD [c]) net weight loss (%)
I	1M1	12.287	1.203	9.791	1.052±0.243 [23.1%] [d]	9.056±2.075 [22.9%]
	1F1	11.076	1.181	10.663		
	1F2	11.486	0.771	6.713		
II	2M1	11.675	1.054	9.028	0.910±0.125 [13.7%]	7.861±1.018 [12.9%]
	2M2	11.678	0.836	7.159		
	2F1	11.357	0.840	7.396		

a Data taken or derived from Tables D and E, pp. 63 and 64, respectively, MRID 48240112.

b Prior to attachment of reflectors to collars placed on Group II animals.

c Calculated by reviewer.

d Numbers in brackets equal the coefficient of variation, calculated by reviewer.

B. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no treatment-related clinical signs. The single Group I male had a prolapsed third eyelid on day 6 and vomited on days 9 and 14. One Group I female vomited on days 3 and 19.

2. **Local effects:** No abnormal local observations were observed.

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

C. BODY WEIGHT AND WEIGHT GAIN: Body weight data are given in Table 4. From the available data, no treatment-related effects on body weight or body weight gain were seen.

TABLE 4: Body weight data from beagle puppies [a]

Parameter/ Study day or interval		Treatment	
		Collar (without reflectors)	Collar *with* reflectors
Absolute body weight (kg):	Day -14	3.021±0.826	2.714±0.410
	Day -1	3.702±1.082	3.518±0.585
	Day 30	6.132±1.639	5.871±1.006
Body weight change (kg) [b]:	Days -14 to -1	0.681±0.262	0.804±0.176
	Days -1 to 30	2.430±0.588	2.353±0.445

[a] Data taken or derived from p. 19-20 and Table C, p.63, MRID 48240112. Values are Mean ± Standard Deviation, with n=3 for both groups.

[b] Calculated by reviewer.

D. FOOD CONSUMPTION: There were no occurrences of inappetence, and the animals of both groups had comparable food consumption.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that there were no major adverse treatment-related effects seen in male or female puppies wearing one PNR1427 collar, with or without three attached reflectors, for 30 consecutive days and that the collars were well tolerated and determined to be safe. The study author also concluded that the presence of the reflectors did not cause any increase in the net weight loss from the collars, but actually resulted in an average 13.2% decrease in the net weight loss from the collars during the 30-day exposure period.

B. REVIEWER COMMENTS: The reviewer agrees that no adverse effects were seen. However, it must be noted that the small group size and measuring body weight only at the beginning and end of the 30-day treatment-interval limit the study's sensitivity for detecting a transient effect on body weight. The reviewer also agrees that the percentage of weight lost from the collars with the reflectors was 13.2% lower than the percentage of weight lost from the collars without the reflectors. It is unknown to the reviewer whether a 13.2% lower percentage weight loss would sufficiently decrease exposure to the two active ingredients enough to compromise efficacy. As

the range of percentages lost from the collars without reflectors bracketed the range of percentages lost from the collars with reflectors (6.713%-10.663%, without reflectors, vs. 7.159%-9.028%, with reflectors), it is possible that a study done using a larger sample size would find a smaller difference between the two groups.

It is concluded that, under the conditions used in this study, the attachment of three polyamide (Grilamid® TR90) reflectors to PNR 1427 [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)] Collars worn by 9-week-old, 3.077- to 4.951-kg beagle puppies for thirty days was not associated with increased wear on the collars and did not result in a decrease in safety (or increase in toxicological hazard). The percentage weight loss from the collars with the reflectors was 13.2% lower than from collars without the reflectors.

C. **STUDY DEFICIENCIES:** The stated study objective was to evaluate the general safety of PNR1427 with reflectors in puppies. In addition to comparison of collars with reflectors to those without reflectors rather than testing for an adequate (5X) margin of safety, the study design deviates from OPPTS 870.7200 with respect to the following:

- There were only three animals per group.
- Careful clinical observations were not conducted at hourly intervals on the day of treatment for at least 4 hours after treatment.
- The animals were not weighed on days 7 and 14.
- Clinical pathology evaluation was not done.

It was stated that the study design was based on recommendations provided to the sponsor by the EPA during a study design teleconference (June 6, 2009), and on email correspondence between the sponsor and EPA (March 26 and 30, 2010). No documentation of these communications was provided to the reviewer, but, assuming the study design was fully in accordance with EPA recommendations, the above-listed deviations from OPPTS 870.7200 do not impact the study classification.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427 INSECTICIDE COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY - KITTENS (NON-GUIDELINE)

MRIDs 48240113

Prepared for
Registration Division
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Prepared by
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Task Order No. 3-C-04

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Disclaimer

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

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

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Date: 1-31-12

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Kittens; Non-Guideline

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)]

TRADE NAME: Not provided

CITATIONS: Madsen, T.J. (2010) Safety of PNR 1427 with reflectors in kittens. Sinclair Research Center, LLC, Auxvasse, MO. In-Life Testing Facility No. S10605, August 30, 2010. MRID 48240113. Unpublished.

SPONSOR: Bayer HealthCare LLC/Animal Health Division, Shawnee Mission, KS

EXECUTIVE SUMMARY: In a non-guideline companion animal safety study (MRID 48240113), the influence of reflectors on the net weight loss of PNR 1427 insecticide collars (10% imidacloprid + 4.5% flumethrin) was tested in short hair kittens. One group of one male and two female kittens wore a single end-use collar for 30 days. The other group of two male and one female kittens wore a single end-use collar with three affixed polyamide reflectors. The kittens were observed for systemic and/or local reactions, body weight and food consumption before and after wearing the collar. The weights of the collars pre- and post-treatment were compared.

All animals survived to the end of the study. No systemic or local signs of reactions were observed. Food consumption was comparable between groups and both groups of kittens gained weight over the course of the study.

In Group I, the average collar weight at application was 9.328 g. The average collar dosage based on body weights of kittens in Group I was 8.467g collar/kg body weight, which is equivalent to 846.7 mg imidacloprid + 381.0 mg flumethrin/kg body weight. At the end of the 30-day exposure period, the average weight of the collars removed from kittens in Group I was 8.874 g. The average net loss from the collars in Group I was 0.454 g or 4.874%.

In Group II, the average collar weight (with three reflectors) at application was 10.950g.The average collar dosage calculated based on body weights of kittens in Group II was 7.759g collar/kg body weight, which is equivalent to 775.9 mg imidacloprid + 349.2 mg flumethrin/kg body weight. At the

end of the 30-day exposure period, the average weight of the individual collars removed from kittens in Group II was 10.511 g. The average net loss from the collars was 0.439 g or 4.713%.

Based on the average net collar weight loss of 4.874% from Group I and 4.713% from Group II, the comparative weight loss from the collars with the reflectors was 3.3% lower than from collars without the reflectors.

The weight loss of PNR 1427 collars with three reflectors was decreased by 3.3% after 30 days of wear in adult cats as compared to collars without reflectors, indicating no decrease in safety (or increase in toxicological hazard) associated with the reflectors.

This non-guideline companion animal safety study in male and female kittens is acceptable for its intended purpose as a measure of the effect of reflectors on the loss of active ingredients from PNR 1427 (10% imidacloprid + 4.5% flumethrin) collars.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test item 1:** End-product collar [imidacloprid (10%, w/w) + flumethrin (4.5% w/w)]- small –Lot number KP05KTJ. Dimensions = 8 x 4.5 mm; length = 35 cm; weight = 12.5 g; recommended for use in kittens and cats up to 8 kg body weight

2. **Test item 2:** Test item 1 collar with three polyamide reflectors

3. **Test animals:**

Species:	Feline
Strain:	Domestic short hair
Age/weight	Age: 68 days on day 0 Weight: 1.025 to 1.325 kg on day -1
Source:	Liberty Research, Inc., Waverly, NY
Housing:	Group housed through day -8 of acclimation; individually housed in stainless steel cages from day -8 to study termination
Diet:	Purina® Kitten Chow – 100 g/day
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	70.5° to 79.0° F
Humidity:	23.3% to 100%
Air changes:	"appropriate hourly air exchanges"
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Fourteen days

B. STUDY DESIGN:

1. **In life dates:** Start: May 12, 2010; End: June 12, 2010

2. **Animal assignment:** Kittens were randomized to treatments on day -1. The kittens were blocked by gender and ranked within each block by descending order of body weights. Group I was assigned one male and two female kittens. Group II was assigned two male and one female kittens. A random number was assigned to each animal using Microsoft Excel. The largest male random numbers and the smallest female random numbers were assigned to Group II. The study was not blinded. The treatment groups are presented in Table 1.

Table 1: Animal Assignment

Group	Treatment	Number of Cats	
		Male	Female
I	1x imidacloprid + flumethrin collar	1	2
II	1x imidacloprid + flumethrin collar with three reflectors	2	1

3. **Dose selection rationale:** No dose rationale was provided for the percentage of active ingredients in the end-use product.

4. **Preparation and treatment:** On day 0, one pre-weighed end-use collar was applied to each kitten in Groups I and II. The collars were adjusted around the animal's neck with a space of two fingers between the collar and the neck. Any excess collar length beyond 2 cm was clipped and removed. Three pre-weighed reflectors were affixed to each collar of cats in Group II by tightly pressing the fastener until it clicked. The reflectors were distributed as evenly as possible over the non-overlapping part of the collar. The fit of the collar was checked once daily.

   At the study conclusion on day 30, the collars from Group I and the collars with reflectors from Group II were removed and weighed.

5. **Statistics:** The numerical data were described by descriptive statistical methods, such as mean value and standard deviation.

C. METHODS:

1. Observations:

g. **General health observations:** During the acclimation (days -7 and -1) and exposure periods (days 0 to 30), kittens were observed twice daily.

h. **Veterinary examinations:** On day -2, physical examinations were performed by a veterinarian.

i. **Local observations:** The hair and skin in all kittens, both underneath and adjacent to the collars, was inspected for signs of dermal irritation and hair loss at least once daily on day -7 and days -1 through 30.

2. **Body weight:** Animals were weighed on days -14, - 1 and +30.

3. **Food consumption:** Food consumption was measured once daily for each kitten on days -4 through 30.

4. **Hematology and clinical chemistry:** No clinical pathology testing was conducted.

II. RESULTS

A. **COLLAR WEIGHTS:** The average collar dosage based on body weights of kittens in Group I was 8.467g collar/kg body weight, which is equivalent to 846.7 mg imidacloprid + 381.0 mg flumethrin/kg body weight.

The average collar dosage calculated based on body weights of kittens in Group II was 7.759g collar/kg body weight, which is equivalent to 775.9 mg imidacloprid + 349.2 mg flumethrin/kg body weight.

Table 2: Mean exposure to imidacloprid and flumethrin[a]

Group	Collar dosage (g/kg bw)	Content imidacloprid (mg)	Content flumethrin (mg)	Dose imidacloprid (mg/kg bw)	Dose flumethrin (mg/kg bw)
I	8.467	932.8	419.8	846.7	381.0
II	7.759	931.3	419.1	775.9	349.2

[a] Extracted from Table D, page 65, MRID 48240113.

At the end of the 30-day exposure period, the weight of the collars removed from kittens in Group I ranged between 8.682 and 9.149 g with an average of 8.874 g. The net loss from the collars in Group I ranged between 0.372g and 0.606 g with an average of 0.454 g. This net collar weight loss ranged between 4.038% and 6.525% with an average of 4.874%.

At the end of the 30-day exposure period, the weight of the individual collars removed from kittens in Group II ranged between 10.429 g and 10.672 g with the average of 10.511 g. The net loss ranged between 4.21% and 5.672% with an average of 4.713%.

Based on the average net collar weight loss of 4.874% from Group I and 4.713% from Group II, the comparative weight loss from the collars with the reflectors was 3.3% lower than from collars without the reflectors.

Table 3: Mean collar weights (g) after 30 days of wear[a]

Group	Mean weight of applied collar	Mean collar weight after removal	Mean weight loss	Relative weight loss (%)
I	9.328	8.874	0.454	4.874
II	10.950	10.511[b]	0.439	4.713

[a] Extracted from Tables D & E, pages 65- 66, MRID 48240113
[b] Weight includes the three reflectors

B. **OBSERVATIONS:**

1. **Clinical signs of toxicity:** On days 25 and 29, one Group II male kitten was observed with a collar caught in his mouth. The incident resulted in hair loss and/or erythema around the upper lip of the kitten's mouth which resolved by the day after the observation.

2. **Application site examination:** No local reactions were observed.

3. **Mortality:** All cats survived to the end of the study.

C. **BODY WEIGHT AND WEIGHT GAIN:** No effect on body weight or body weight gain was reported. Kittens in both groups gained weight over the course of the study.

D. **FOOD CONSUMPTION:** Food consumption was comparable in both groups.

E. **CLINICAL PATHOLOGY ANALYSES:** Clinical pathology parameters were not measured.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the three reflectors affixed to each collar did not increase the net weight loss by the collars and therefore did not increase the release of the two active ingredients as compared to the average net weight loss from a single collar without reflectors. Instead, the presence of the reflectors on the collar resulted in 3.3% decrease in the net collar weight loss as compared to the net weight loss from the collars without reflectors during the 30-day exposure period.

B. **REVIEWER COMMENTS:** All animals survived to the end of the study. No systemic or local signs of reactions were observed. Food consumption and weight gain was comparable between the two groups of kittens.

In Group I, the average collar weight at application was 9.328 g. The average collar dosage based on body weights of kittens in Group I was 8.467g collar/kg body weight, which is equivalent to 846.7 mg imidacloprid + 381.0 mg flumethrin/kg body weight. At the end of the 30-day exposure period, the average weight of the collars removed from kittens in Group I was 8.874 g. The average net loss from the collars in Group I was 0.454 g. The average net collar weight loss was 4.874%.

In Group II, the average collar weight (with three reflectors) at application was 10.950g.The average collar dosage calculated based on body weights of kittens in Group II was 7.759g collar/kg body weight, which is equivalent to 775.9 mg imidacloprid + 349.2 mg flumethrin/kg body weight. At the end of the 30-day exposure period, the average weight of the individual collars removed from kittens in Group II was 10.511 g. The average net loss from the collars was 0.439 g. The average net collar 4.713%.

Based on the average net collar weight loss of 4.874% from Group I and 4.713% from Group II, the comparative weight loss from the collars with the reflectors was 3.3% lower than from collars without the reflectors.

The weight loss of PNR 1427 collars with three reflectors was decreased by 3.3% after 30 days of wear in adult cats as compared to collars without reflectors, indicating no decrease in safety (or increase in toxicological hazard) associated with the reflectors.

C. STUDY DEFICIENCIES:

Only three animals per group were included in the study. The Companion Animal Safety Guideline (CAS) (OPPTS 870.7200) requires six animals/sex/group. However, according to the study protocol, the study design was agreed to during teleconferences (June 6, 2009) and email correspondence (March 16 & 30, 2010) between the sponsor and the EPA. In addition, this study is not considered a CAS guideline study.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427 INSECTICIDE COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY - CATS (NON-GUIDELINE)

MRIDs 48240114

Prepared for
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Disclaimer

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

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Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: Jan. 27, 2012

Signature: M Hashim
Date: 1-31-12

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Cats – Non-Guideline

PC CODES: 129099 (Imidacloprid), 036007 (Flumethrin) **BARCODE:** 385560

TEST MATERIAL (PURITY): PNR 1427 Insecticide Collar [Imidacloprid (10% w/w) and Flumethrin (4.5% w/w)]

TRADE NAME: Not provided

CITATIONS: Delport, P.C. (2010) Target animal safety with PNR 1427 collar with and without reflectors when applied once to adult cats. ClinVet International (Pty) Ltd, Bloemfontein, Republic of South Africa. Lab Study No. CV 09/676, May 18, 2010. MRID 48240114. Unpublished.

SPONSOR: Bayer HealthCare LLC/Animal Health Division, Shawnee Mission, KS

EXECUTIVE SUMMARY: In a non-guideline companion animal safety study (MRID 48240114), the influence of reflectors on the net weight loss of PNR 1427 insecticide collars (10% imidacloprid + 4.5% flumethrin) was tested in adult short hair cats. One group of four males and four females wore a single collar for 30 days. The other group of four males and four females wore a collar with three affixed polyamide reflectors. The cats were observed for systemic and/or local reactions and body weight before and after wearing the collar. The weights of the collars pre- and post-treatment were compared.

All animals survived to the end of the study. No systemic or local signs of reactions were observed. The cats maintained their body weight over the course of the study.

For the cats wearing the collars without reflectors, the calculated average weight of the collars before application was 10.149 g and upon removal on day 30, it was 9.748 g. The calculated average net weight loss of the collars was 0.401 g, which is equivalent to 3.951% of the collar weight before application.

For the cats wearing collars with the reflectors, the calculated average weight of the collars was 12.064 g and upon removal on day 30, it was 11.688 g. The calculated average weight loss of the collars was 0.378 g, which is equivalent to 3.622% of the collar weight before affixing the three reflectors and before application.

The weight loss of PNR 1427 collars with three reflectors was decreased by 8.33% after 30 days of wear in adult cats as compared to collars without reflectors.

This non-guideline companion animal safety study in male and female adult cats is acceptable for its intended purpose as a measure of the effect of reflectors on the loss of active ingredients from the PNR 1427 (10% imidacloprid + 4.5% flumethrin) collars.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test item 1:** IMI/FLU 10/4.5 Collar Small (each gram contains 98 mg imidacloprid + 44 mg flumethrin) – Batch #KP05KTJ

2. **Test item 2:** Test item 1 collar with three polyamide (Grilamid® TR90) reflectors

3. **Test animals:**

Species:	Feline
Strain:	Domestic short hair
Age/weight	Age: older than 6 months at study start Weight: 2.04 to 4.96 kg (5 days prior to treatment)
Source:	ClinVet International (Pty) Ltd colony
Housing:	Individually housed in stainless steel cages
Diet:	IAMS Multi cat adult + Eukanuba cat food
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	$20 \pm 4^{\circ}$ C
Humidity:	Not provided
Air changes:	12 to 14/hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Fourteen days

B. STUDY DESIGN:

1. **In life dates:** Start: February 9, 2010; End: March 25, 2010

2. **Animal assignment:** Sixteen cats were allocated at random to two study groups of eight animals each using body weights which were taken five days prior to treatment initiation on day 0. First the animals were separated according to sex and within each gender group ranked according to decreasing order of body weights. The cats were allocated to blocks containing two cats each. The cats from each block were assigned to the study groups by random number draw, either 1 or 2. The study was not blinded. The treatment groups are presented in Table 1.

Table 1: Animal Assignment			
Group	Treatment	Number of Cats	
		Male	Female
A	Single 10% imidacloprid + 4.5% flumethrin collar (test item 1)	4	4
B	Single 10% imidacloprid + 4.5% flumethrin collar with three affixed reflectors (test item 2)	4	4

3. **Dose selection rationale:** No dose rationale was provided for the percentage of active ingredients in the end-use product.

4. **Preparation and treatment:** On day 0, one collar was applied to each cat in Groups A and B. The collars remained in place until study termination (day 30). The animals received a small collar intended for cats ≤ 8 kg. The collars were adjusted around the animal's neck with a space of two fingers between the collar and the neck. Any excess collar length beyond 2 cm was clipped and removed. Three reflectors were affixed to the collar of each cat in Group B by tightly pressing the fastener until it clicked. The reflectors were distributed as evenly as possible over the non-overlapping part of the collar. The fit of the collar was checked once daily.

   Before the application on study day 0, each collar was weighed using a calibrated electronic scale; the weights were determined to three decimal places. After the reflectors were affixed to the collars of Group B cats, the collars with the reflectors were removed, weighed and reapplied.

   At the study conclusion on day 30, the collars from Group A and the collars with reflectors from Group B were removed and weighed.

5. **Statistics:** The numerical data were described by the following descriptive statistical methods: mean value, standard deviation, relative standard deviation and minimum-maximum values.

C. METHODS:

1. Observations:

j. **General health observations:** Daily observations, included but were not limited to habitus, appetite, color of urine, color and consistency of feces, salivation, vomiting, skin lesions and change in general condition.

k. **Specific health observations:** On the day of collar application (day 0), clinical observation was performed prior to application and at approximately 2 hours and 4 hours after the application.

l. **Local observations:** On the day of collar application (day 0), observation of hair coat and skin was performed prior to application and at approximately 2 hours and 4 hours after the application. Thereafter, a daily local tolerance examination was conducted.

2. **Body weight:** Animals were weighed on days -14, - 5 and +30.

3. **Food consumption:** Food consumption was not measured.

4. **Hematology and clinical chemistry:** No clinical pathology testing was conducted.

II. RESULTS

A. **COLLAR WEIGHTS:** The mean levels of exposure to imidacloprid and flumethrin in the collars applied to cats in Groups A and B are presented in Table 2. The cats in Group A received from 2.182 to 4.660 g (mean 3.388 g) collar per kg body weight based on the day -5 weight. Therefore, this dose is equivalent to 213.8 – 456.7 mg (mean 332.1 mg) of imidacloprid per kg body weight and 96.0 – 205.0 mg (mean 149.1 mg) of flumethrin per kg body weight.

The cats in Group B received from 2.306 g to 4.863 g (mean 3.577 g) collar per kg based on the -5 day weight. Therefore, this dose is equivalent to 226.0 - 476.5 mg (mean 350.5 mg) of imidacloprid per kg body weight and 101.5 – 214.0 mg (mean 157.4 mg) flumethrin per kg body weight.

Table 2: Mean (±SD) exposure to imidacloprid and flumethrin based on day -5 weights[a]

Group	Dose collar (g/kg)	Content imidacloprid (g)	Content flumethrin (g)	Dose imidacloprid (mg/kg)	Dose flumethrin (mg/kg)
A	3.388±0.919	0.995±0.050	0.447±0.022	332.062±90.036	149.089±40.424
B	3.577±0.918	1.023±0.062	0.459±0.028	350.517±89.945	157.375±40.384

[a] Extracted from Table 4, page 27, MRID 48240114.

For Group A, the calculated average weight of the collars before application was 10.149 g and upon removal on day 30, it was 9.748 g (Table 3). The calculated average net weight loss of the collars was 0.401 g, which is equivalent to 3.951% of the collar weight before application.

For Group B, the calculated average weight of the collars was 10.435 g. After affixing the three reflectors and before application, the calculated average weight of the collars was 12.064 g and upon removal on day 30, it was 11.686 g. The calculated average weight loss of the collars was 0.378 g, which is equivalent to 3.622% of the collar weight before affixing the three reflectors and before application.

Table 3: Mean collar weights (g) (±SD) after 30 days of wear[a]

Group	Mean weight of applied collar	Mean collar weight after removal	Mean weight loss	Relative weight loss (%)
A	10.149 ± 0.509	9.748 ± 0.520	0.401 ± 0.061	3.951
B	10.435±0.629[b] 12.064 ± 0.631[c]	11.686 ± 0.595	0.378 ± 0.083	3.622

[a] Extracted from Table 5, page 28, MRID 48240114
[b] Weight before affixing the three reflectors.
[c] Weight after affixing the three reflectors.

B. **OBSERVATIONS:**

1. **Clinical signs of toxicity:** The study report states that one cat in Group B vomited food prior to application of the test collar, but no other clinical signs were observed. No clinical observation data were included with the final study report.

2. **Application site examination:** The study report states that no signs were reported during the study period, but no data were included with the final study report.

3. **Mortality:** All cats survived to the end of the study.

C. **BODY WEIGHT AND WEIGHT GAIN:** No treatment-related changes were reported.

D. **FOOD CONSUMPTION:** Food consumption was not measured.

E. **CLINICAL PATHOLOGY ANALYSES:** Clinical pathology parameters were not measured.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the three reflectors affixed on to each collar did not increase the net weight loss by the collars and therefore did not increase the release of the two active ingredients as compared to the collars without the reflectors.

B. **REVIEWER COMMENTS:** All animals survived to the end of the study. No systemic or local signs of reactions were observed. The cats maintained their body weight over the course of the study.

For the cats wearing the collars without reflectors, the calculated average weight of the collars before application was 10.149 g and upon removal on day 30, it was 9.748 g. The calculated average net weight loss of the collars was 0.401 g, which is equivalent to 3.951% of the collar weight before application.

For the cats wearing collars with the reflectors, the calculated average weight of the collars was 12.064 g and upon removal on day 30, it was 11.686 g. The calculated average weight loss of the collars was 0.378 g, which is equivalent to 3.622% of the collar weight before affixing the three reflectors and before application.

The weight loss of PNR 1427 collars with three reflectors was decreased by 8.33% after 30 days of wear in adult cats as compared to collars without reflectors.

C. **STUDY DEFICIENCIES:**

No raw data or summary tables were provided for the clinical observations results.

DATA EVALUATION RECORD

CYCLOPROPANECARBOXYLIC ACID, 3-(4-CHLOROPHENYL) [PNR 1427, IMIDACLOPRID (10%, W/W) + FLUMETHRIN (4.5%, W/W) COLLAR]

STUDY TYPE: COMPANION ANIMAL SAFETY STUDY- ADULT DOGS; NON-GUIDELINE MRID 48240115

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Disclaimer

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

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Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study - Adult Dogs; Non-guideline.

PC CODE: 129099 (Imidacloprid), 036007 (Flumethrin) **DP BARCODE:** 385560

TEST MATERIAL (PURITY): IMI/FLU 10/4,5 Collar (9.9% Imidacloprid and 4.5% Flumethrin; Lot No. KP05KGL)

CITATIONS: Bach, T. (2010) Target animal safety with a 10% imidacloprid + 4.5% flumethrin collar with or without reflector clips applied once to adult dogs. Bayer Animal Health GmbH, Research and Development, Clinical Research and Development - Animal Centre, Monheim, Germany. Study Number 35637, April 27, 2010. MRID 48240115. Unpublished.

SPONSOR: Bayer HealthCare LLC, Animal Health Division, P.O. Box 390, Shawnee Mission, Kansas.

EXECUTIVE SUMMARY: In an 30-day non-guideline companion animal safety study (MRID 48240115), two groups of four male and four female adult beagle dogs were treated with single IMI/FLU 10/4,5 Collars (9.9% Imidacloprid and 4.5% Flumethrin; Lot #KP05KGL), either without (Group 1) or with (Group 2) attached polyamide (Grilamid® TR90) reflectors. Animals were treated on day 0 and observed for 30 days, and the weights of the collars applied on day 0 and the final collar weights (on day 30) were recorded.

There were no treatment-related effects on mortality, systemic clinical signs, body weight, or cumulative body weight gain. Local effects included a 1-cm^2 area with broken hair on the ventral neck of one Group 1 animal on days 25-29, which was noted to be partially hairless on day 30, an approximately 3 cm by 0.5 cm area on the dorsal neck with broken hair, hairlessness, and erythema on the same animal at an unspecified time, and areas of broken hair on the ventral neck area (measuring less than 1 cm^2) on a different Group 1 animal and two Group 2 animals on day 30.

The percentage of collar weight lost from the collars without reflectors ranged from 2.447% to 4.754% and averaged 3.225%. The percentage of collar weight lost from the collars with attached reflectors (excluding the weight of the reflectors) ranged from 2.224% to 4.263% and averaged 3.114%.

It is concluded that, under the conditions used in this study, the attachment of three polyamide (Grilamid® TR90) reflectors to IMI/FLU 10/4,5 Collars worn by adult, 8.7- to 11.7-kg beagle dogs for thirty days does not appreciably decrease safety. The percentage of weight loss from the collars with the reflectors was 3.4% lower than from collars without the reflectors.

This companion animal safety study in dogs is **Acceptable/Non-guideline.** It does not satisfy the guideline requirement for a companion animal safety study (OPPTS 870.7200) in adult dogs but does provide scientifically valid information.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

a. IMI/FLU 10/4,5 Collar Big

Description:	Gray solid collar, weighing 46.4 g;
Batch #:	KP05KGL
Purity:	9.9% Imidacloprid and 4.5% Flumethrin
Compound Stability:	Retest date: December 31, 2010
CAS #:	138261-41-3 (Imidacloprid) and 69770-42-2 (Flumethrin)

b. Collar reflector large ZSB

Description:	Polyamide (Grilamid® TR90) reflector clips, manufactured by Ultra Reflex GmbH, Renchen, Germany
Lot #:	091109
Purity and stability:	Not provided

2. Vehicle and/or positive control: none.

3. Test animals:

Species:	Dog	
Breed:	Beagle	
Age/weight at study initiation:	9-13 months on day 0/ Males: 8.7-10.7 kg (day -4); Females: 9.4-11.7 kg (day -4)	
Source:	Marshall BioResources, North Rose, New York	
Housing:	Individually, in 6-m^2 pens	
Diet:	Commercially available dry dog food (Ssniff Hd Ereich); quantities not reported	
Water:	*Ad libitum*, source not specified	
Environmental conditions:	**Temperature:**	19-21° F.
	Humidity:	30%-65% with brief excursions up to 90% during cleaning
	Air changes:	Not reported
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Two weeks.	

B. STUDY DESIGN:

1. In life dates: Start: November 16, 2009; End: December 16, 2009.

2. **Animal assignment:** Study design is given in Table 1. The animals were assigned to groups according to sex and body weight on day -4, using a stratified block randomization procedure. The study was not blinded.

TABLE 1: Study design [a]			
Test Group	Treatment	Number assigned	
		Males	Females
1	One end-use collar	4	4
2	One end-use collar with three attached reflectors	4	4

[a] Data taken from 12-13, MRID 48240115.

3. **Dose selection rationale:** No dose selection rationale was provided.

4. **Treatment:** On day 0, one end-use collar was applied to each dog in group 1 and one end-use collar with three attached reflectors was applied to each dog in group 2. The collars were fitted to maintain a two-finger space between the collar and the neck, and surplus collar length, in excess of 2 cm, was trimmed and saved. Three reflectors were attached to the collar of each dog in group 2 by tightly pressing the fastener until it clicked into place. The reflectors were spaced as evenly as possible over the non-overlapping portion of the collar.

5. **Statistics:** The individual animal was the experimental unit. Descriptive statistics were presented for body weight and numerical variables related to the collars.

C. METHODS:

1. **Observations:**

a. **General health observations:** During acclimation and the treatment interval, the animals were observed once per day. On day 0, the animals were observed prior to the application and approximately two and four hours after the application of the test item(s). At each observation, each animal's collar was examined for proper fit and adjusted if necessary.

b. **Clinical assessments:** All animals received physical examinations on days -14 and -4.

c. **Local observations:** The hair and skin of the neck region were inspected for signs of dermal irritation and hair loss prior to application and approximately two and four hours after application of the test item(s) on day 0 and daily thereafter. The test items, themselves, were also checked for intactness and proper placement at these times.

2. **Body weight:** The animals were weighed on days -14, -4, and 30.

3. **Food consumption:** Food consumption was not measured.

4. **Clinical pathology:** Clinical pathology was not evaluated.

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

7. **Collar weights:** The collars were weighed prior to application, and, following application, the trimmed surplus collar lengths were maintained in individually labeled, sealed plastic bags for the later determination of the final weights of the applied collars. The reflectors were also pre-weighed. When collars (and collars with attached reflectors) were removed on day 30, each collar (with or without reflectors) was re-weighed.

II. RESULTS

A. **DOSES ADMINISTERED:** The mean administered mg/kg doses of the active ingredients are given in Table 2, and the net weight losses of the collars with and without the reflectors attached are given in Table 3. Based on the average net collar weight losses of 3.225% and 3.114% from the collars worn by Group 1 and Group 2I animals, respectively, the percentage of weight lost from the collars with the reflectors was 3.4% lower than from collars without the reflectors.

Table 2: Mean collar "doses" and administered doses of imidacloprid and flumethrin [a]

Group	Mean collar weight (g)	Mean collar "dose" (g/kg bw)	Content active ingredients (mg) [b]		Dose active ingredients (mg/kg bw)	
			Imidacloprid	Flumethrin	Imidacloprid	Flumethrin
1	35.412	3.483	3505.8	1593.5	344.8	156.7
2	36.337	3.640	3597.3	1635.2	360.3	163.8

[a] Data taken from Table 5, p. 28, MRID 48240115.

[b] Based on the reported concentrations of the active ingredients on the certificate of analysis, i.e. 9.9% (w/w) imidacloprid and 4.5% (w/w) flumethrin.

Table 3: Individual and mean collar net weight losses during 30-day application intervals on adult beagles [a]						
Group	Animal ID	Collar weight at application [b] (g)	Net weight loss (g)	Net weight loss (%)	Mean (± SD [c]) net weight loss (g)	Mean (± SD [c]) net weight loss (%)
1	9535	35.073	0.984	2.806	1.144±0.277	3.225±0.743
	2519	34.260	0.997	2.910		
	7902	34.738	0.850	2.447		
	9352	37.330	1.090	2.920		
	3871	34.136	1.288	3.773		
	5008	36.406	1.248	3.428		
	3089	36.308	1.726	4.754		
	5050	35.043	0.968	2.762		
2	7375	35.526	1.234	3.474	1.132±0.272	3.114±0.738
	4083	37.322	0.830	2.224		
	9395	36.666	1.402	3.824		
	4156	35.266	0.786	2.229		
	5161	37.673	1.156	3.069		
	3276	36.569	1.559	4.263		
	6176	35.743	0.930	2.602		
	6202	35.929	1.161	3.231		

a Data taken or derived from Table 5, p. 28, MRID 48240115.

b Prior to attachment of reflectors to collars placed on Group 2 animals.

c Calculated by reviewer.

B. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs were seen. One Group 1 male had ocular discharge on days -13 through -11 and was found to have a congenital abnormality (atresia of the ventral puncta) as an underlying cause of chronic lacrimation. A different Group 1 male had diarrhea on day 3.

2. **Local effects:** One Group 1 animal had a 1 cm by 1 cm area with broken hair on the ventral neck on days 25-29, which was noted to be partially hairless on day 30. This same animal had an approximately 3 cm by 0.5 cm area on the dorsal neck with broken hair, hairlessness, and erythema (interval not specified). A different Group 1 animal and two Group 2 animals had areas of broken hair on the ventral neck area (measuring less than 1 cm by 1 cm) on day 30.

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

C. **BODY WEIGHT AND WEIGHT GAIN:** Body weight data are given in Table 4. From the available data, no treatment-related effects on body weight or body weight gain were seen. Most individuals lost 0.1 to 0.6 kg during acclimation and/or during the study interval.

TABLE 4: Body weight data from adult beagles [a]		
Parameter/ Study day or interval	**Treatment**	
	Collar (without reflectors)	**Collar *with* reflectors**
Absolute body weight (kg): Day -14	10.41±0.74	10.43±0.94
Day -4	10.20±0.64	10.05±0.97
Day 30	10.16±0.56	9.85±0.88
Body weight change (kg) [b]: Days -14 to -4	-0.21±0.17	-0.38±0.10
Days -4 to 30	-0.04±0.33	-0.20±0.26

[a] Data derived from Table 3, p. 26, MRID 48240115. Values are Mean ± Standard Deviation, with n=8 for both groups.
[b] Calculated by reviewer.

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: The study author concluded that IMI/FLU 10/4,5 Collars, with or without three attached reflectors, were well tolerated when worn by adult dogs for 30 consecutive days, and that the treatment-related local signs, such as thinning of the hair and skin irritation, were due to mechanical rubbing of the collars against the skin rather than toxicity of the active ingredients. The study author also concluded that the presence of the reflectors did not cause any increase in the net weight loss from the collars, and therefore did not result in increased release of the active ingredients from the collars.

B. <u>REVIEWER COMMENTS</u>: The reviewer agrees that no adverse effects were seen. However, it must be noted that measuring body weight only at the beginning and end of the 30-day treatment-interval and omission of food consumption measurements limit the study's sensitivity. The reviewer also agrees that the percentage of weight lost from the collars with the reflectors was lower than the percentage of weight lost from the collars without the reflectors. It is unknown to the reviewer whether a 3.4% lower percentage weight loss would sufficiently decrease exposure to the two active ingredients enough to compromise efficacy.

It is concluded that, under the conditions used in this study, the attachment of three polyamide (Grilamid® TR90) reflectors to IMI/FLU 10/4,5 Collars worn by adult, 8.7- to 11.7-kg beagle dogs for thirty days does not appreciably decrease safety. The percentage of weight loss from the collars with the reflectors was 3.4% lower than from collars without the reflectors.

C. <u>STUDY DEFICIENCIES</u>: In addition to comparison of collars with reflectors to those without reflectors rather than testing for an adequate (5X) margin of safety, the study design deviates from OPPTS 870.7200 with respect to the following:

- No raw data or summary tables were provided for the clinical observation data.
- There were only four animals per sex per group.
- Careful clinical observations were not conducted at hourly intervals on the day of treatment for at least 4 hours after treatment.
- The animals were not weighed on days 7 and 14.
- Clinical pathology evaluation was not done.

However, only the first deviation is considered to be a deficiency. The stated study objective was to evaluate the local tolerance of a collar containing 10% imidacloprid plus 4.5% flumethrin when worn by adult dogs with or without reflectors and to investigate whether the reflector clips influence the net weight loss from the collar during use, as a measure of the amount of active ingredients released. The latter modifications to the OPPTS 870.7200 protocol do not compromise the investigators' ability to reach the stated objective.

1. **DP BARCODES:** 385560, 396978
2. **PC CODES:** 129099 (Imidacloprid); 036007 (Flumethrin)
3. **CURRENT DATE:** January 26, 2012
4. **TEST MATERIAL:** PNR 1427 insecticide collar (10.0% Imidacloprid and 4.5% Flumethrin)

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Study/Adult Cats Sinclair Research Center, MO Study No. S10065/April 1, 2010 (+2 amendments dated June 2, 2010 & November 22, 2011)	48240108 48674702	61-day study: One group of 3M & 3F adult cats served as negative controls; one group of 3M & 3F were in the placebo control group and wore 5 end-use collars minus the active ingredients continuously for 61 days. A third group of 3M & 3F wore one end-use collar continuously for 61 days. In a fourth group of 6M & 6F, five end-use collars were applied on day 0 and were replaced by new collars on days 14, 28 and 42. The end-use collars worn by selected cats in the 1x and 5x groups were analyzed post-removal to determine the exposures of these cats to the active ingredients. No effects were observed. Based on the chemical analyses of worn collars and information reported in MRID 48674702, cats in the 5x group were exposed to 5.2x the recommended dose of imidacloprid and 4.0x the recommended dose of flumethrin. The mean collar weight (after trimming) applied to the 1x cats was 11.17 g equivalent to 2.82 g/kg body weight.	N/A	A (guide-line)
Companion Animal Safety Study/Adult Dogs Sinclair Research Center, MO Study No. S10064/April 23, 2010 (+1 amendment dated November 22, 2011)	48240109 48674701	61-day study: One group of 3M & 3F adult dogs served as negative controls; one group of 3M & 3F were in the placebo control group and wore 5 end-use collars minus the active ingredients continuously for 61 days. A third group of 3M & 3F wore one end-use collar continuously for 61 days. In a fourth group of 6M & 6F, five end-use collars were applied on day 0 and were replaced by new collars on days 14, 28 and 42. No effects were observed except for erythema and/or hair loss on the throat or neck. Based on the chemical analyses of worn collars and information reported in MRID 48674701, dogs in the 5x group were exposed to 10.88x the imidacloprid and 4.05x the flumethrin that dogs in the 1x group were exposed to. The mean collar weight (after trimming) applied to the 1x dogs was 37.93 g or 3.22 g/kg b.w.		A (guide-line)

Companion Animal Safety Study/Puppies (7 weeks old on day 0) Sinclair Research Center, MO Study No. S10062/June 16, 2010	48240110	180-day study: Groups of 6M & 6F beagle puppies (7 weeks old on Day 0) were treated at 1x (1 collar), 3x (3 collars), and 5x (5 collars). Two additional groups of 3M & 3F were untreated or were treated with 5 placebo (no active ingredient) collars. For the 5x group initial collars were placed on the animals on day 0, and the puppies were retreated (existing collars replaced with new collars) on days 29, 90, 125 and 148). There were no treatment-related effects on mortality, absolute body weight, food consumption, hematology or clinical chemistry. The 5x females had a transient decrease in body weight gain (25% less than controls from day -1 to 17). Mildly increased creatinine kinase activity in 3x and 5x animals (349, 940, 1074 U/L for negative controls, 3x and 5x, respectively). There were local findings such as erythema, hair loss or thinning of hair, bruising, abrasions or scabbing that were presumably due to the mechanical trauma of wearing one or more collars. Based on collar weight losses and chemical analyses of worn collars, the imidacloprid exposure of puppies in the 3x and 5x groups was 3.02x and 4.89x that of the 1x group, and the flumethrin exposure of puppies in the 3x and 5x groups was 2.46x and 1.45x that of the dogs in the 1x group. Based on transient decreased body weight gain in 5x females, it is concluded that the margin of safety in 7-week-old 1.53-2.96 kg beagle puppies treated with PNR collars is 3x the recommended dose of one collar per puppy. The average collar dosage rate for 1x animals on day 0 was 10.156 g, equivalent to 5.096 g/kg b.w.		A (guide-line)

Companion Animal Safety Study/Kittens (68-71 days old on day 0) Sinclair Research Center, MO Study No, S10063/June 16, 2010	48240111	180 day study: Groups of 6M & 6F kittens (68-71 days old on day 0) were treated at 1x (1 collar), 3x (3 collars), and 5x (5 collars). Two additional groups of 3M & 3F were untreated or were treated with 5 placebo (no active ingredient) collars. For the 1x, 3x and 5x groups the kittens were retreated (existing collars taken off and replaced with new collars) on days 29, 90 and 149. End-use collars worn by selected kittens in the 1x, 3x and 5x groups were analyzed after removal to determine exposures to the active ingredients. All kittens survived to the end of the study; there were no effects except body weight gain over the course of the study (days -1 to 180) was decreased 17% in the 5x group females as compared to the placebo control group. Based on chemical analyses of the worn collars kittens in the 3x and 5x groups were exposed to 3.59x and 3.13x the imidacloprid that kittens in the 1x group were exposed to. Kittens in the 3x and 5x groups were exposed to 5.09x and 5.59x the flumethrin that kittens in the 1x group were exposed to. It is concluded that the margin of safety in kittens exposed to the PNR 1427 collar is 3x, based on decreased body weight gain over the 180 day study in 5x females. The average collar dosage rate for 1x kittens on day 0 was 9.387 g, equivalent to 4.15 g/kg.		A (guide-line)
Companion Animal Safety Study (non-Guideline)/Puppies Sinclair Research Center, MO Study No. S10064/August 30, 2010	48240112	30-day non-guideline study: 2 groups of 3 9-week-old beagle puppies were treated with single PNR 1427 collars, either without (Group I, 1M & 2F) or with (Group 2 (2M & 1F) attached polyamide reflectors. There were no treatment-related effects on mortality, systemic or local clinical signs, body weight, cumulative weight gain or food consumption. The percentage of collar weight lost from collars without reflectors ranged from 6.713% to 10.663% averaging 9.056%; percentage of collar weight lost from collars with reflectors (excluding weightof reflectors) ranged from 7.159% to 9.028% and averaged 7.861%. Conclusion: attachment of 3 polyamide reflectors was not associated with increased wear or the collars and did not result in an decrease in safety (or increase in toxicological hazard). Percentage weight loss from collars with the reflectors was 13.2% lower than from collars without the reflectors.		A (non-guide-line)

Companion Animal Safety Study (non-Guideline)/Kittens Sinclair Research Center, MO Study No. S10605/August 30, 2010	48240113	30 day study: Group I (1M and 2F) wore a single collar without a reflector; Group II (2M & 1F) wore a single collar with attached 3 polyamide reflectors. No systemic or local reactions were seen Based on the average collar weight loss of 4.874% from Group I and 4.713% from Group II, the comparative weight loss from the collars with reflectors was 3.3% lower than from collars without reflectors.		A (non-Guide-line)
Companion Animal Safety Study (non-Guideline)/Adult Cats ClinVet International Ltd, Bloemfontein, South Africa Study No. CV/09/676 / May 18, 2010	48240114	30 day study: Group I (4M & 4F) wore a single collar without a reflector; Group II (4M & 4F) wore a collar with 3 attached polyamide reflectors. No systemic or local reactions were seen. Based on the average collar weight loss of 3.951% in Group I and 3.622% in Group II, the weight loss of the collars with 3 reflectors was decreased by 8.33%.		A (non-Guide-line)
Companion Animal Safety Study (non-Guideline)/Adult Dogs Bayer Animal Health GmbH Research & Development, Monheim, Germany Study No. 35637/April 27, 2010	48240115	30 day study: Group I (4M & 4F beagles) wore a single collar without reflectors; Group II (4M & 4F) wore a collar with 3 attached polyamide reflectors. No indications of systemic effects. Local effects (seen in 2 Group I and 2 Group II dogs) included partial hairlessness on neck with erythema, and "broken hair on the ventral neck area." Percentage of collar weight loss was 3.225% (range: 2.447-4.754%) in Group I and 3.114% (range: 2.224-4.263%) in Group II. The percentage weight loss from the collars with reflectors was 3.4% lower than from collars without the reflectors.		A (non-Guide-line)

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