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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

April 15, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	ADVANTAGE IGR 5
	EPA Reg. No. /File Symbol:	11556-RLN
	DP Barcode:	DP 372322
	Decision No.:	424201
	Action Code:	R310
	PC Codes:	129099 (Imidacloprid: 9.1%)
		129032 (Pyriproxyfen: 0.46%)

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

Byron T. Backus
04-15-2010
M Hasler

To: Kable Davis/Venus Eagle, RM 01
Insecticide-Rodenticide Branch
Registration Division (7505P)

Registrant: BAYER HEALTHCARE LLC

FORMULATION FROM LABEL:

Active Ingredient(s):	By wt.
129099 Imidacloprid	9.10%
129032 Pyriproxyfen	0.46%
Other Ingredient(s):	90.44%
TOTAL	100.00%

ACTION REQUESTED: The Risk Manager requests:

"...Please review the attached companion animal data for a new spot-on for cats and kittens. The cover letter details the regulatory history of this product. In addition to the cover letter, I also included copies of the proposed label, proposed csf and previous companion animal and protocol reviews..."

BACKGROUND:

The material received includes a companion animal safety study (in MRID 47924801) titled: "Evaluation of the General Safety of Imidacloprid + Pyriproxyfen Spot-On in 8-Week-Old Kittens"), a CSF, a proposed label for this product (Advantage® IGR 5), and a cover letter from the registrant dated November 30, 2009. The proposed product would be packaged in single-use tubes which would provide an application of 0.23 mL.

COMMENTS AND RECOMMENDATIONS:

1. The registrant is citing a previously reviewed cat companion animal safety study in MRID 45097001 to support this product's use on adult cats. A comparison of the CSF (dated November 20, 2009) for 11556-RLN with the analysis of the test material used in the study in MRID 45097001 (available from Documentum as MRID 45007001.CA.tif) indicates they are toxicologically similar. The study involved a 5X dose level of 2.0 mL (1X = 0.4 mL) for cats weighing less than 9 lbs and a 5X dose level of 4.0 mL (1X = 0.8 mL) for cats weighing >9 lbs. The test material (containing 9.1% Imidacloprid and 0.9% Pyriproxyfen) was applied on study days 0, 7, 14 and 21. On Day -1 the mean weight of the Group A (test material) females was 6.00 (S.D. = 0.56) lb, with a range from 5.23 to 6.92 lbs; the mean weight of the Group A males was 9.34 (S.D. = 1.16) lb, with a range from 8.27 to 10.9 lbs. All of the females weighed less than 9 lbs, and each was treated with 2.0 mL (mean amount: 0.3333 mL/lb) Two males weighed more than 9 lb, and were treated with 4.0 mL; the remaining 4 weighed less than 9 lbs and were each treated with 2.0 mL. The mean dosage for males on a body weight basis was 0.278 mL/lb. The mean 1X treatment for females was (0.3333 mL/lb)/5 = 0.06666 mL/lb and for males was (0.278 mL/lb)/5 = 0.0556 mL/lb. A dosage of 0.23 mL would be supported for adult female cats $\geq$ 3.45 lbs and for adult male cats $\geq$ 4.13 lbs, and labeling (for adult cats) should be revised accordingly.

2. The name "Advantage" is being used by Bayer for both dog and cat products. One of the recommendations made as a result of the recent Agency adverse incident data analysis for pet spot-ons is the requirement for different brand names for dog and cat products.

3. The study (on 8-week-old kittens) in MRID 47924801 was reviewed in TRB, and was then secondarily reviewed in HED. This study has been classified as acceptable and can be used to support the proposed use of Advantage® IGR 5 in 8-week-old and older kittens, with an application rate of 0.23 mL and retreatment no more frequently than at 14 days.

4. The study in MRID 47924801 was conducted on test material consistent with that in the basic formulation CSF (dated November 20, 2010) for this product. The material received by this reviewer did not include any alternate formulation CSFs.

5. The following is the executive summary from the DER for MRID 47924801:

In a companion animal safety study (MRID 47924801), 5 groups, each containing 6 males and 6 females, of domestic shorthair kittens (54-57 days old on Day 0; Day -1 body weights: males: 0.691-1.012 kg; females: 0.555-0.935 kg; source: Liberty Research, Inc., Waverly, NY), were topically treated (on Day 0) with (Group 1): mineral oil at a total dose of 1.15 mL; (Group 2): 3X

vehicle substance at a total dose of 0.63 mL; (Group 3): 5X vehicle substance at a total dose of 1.05 mL; (Group 4): 3X test substance at a total dose of 0.69 mL; and (Group 5): 5X dose test substance at a total dose of 1.15 mL. For each group, the total dose was split into three sub-applications which were administered at approximately 60-minute intervals. The application site was the skin on the dorsal midline from the base of the skull to the interscapular region. The dosing was repeated on Day 14.

The groups and test materials they received (with amounts applied) are shown in the table below:

Group	Test Material Applied	Volume of each application	Cumulative amount applied on Day 0; also on Day 14
1	Mineral oil	1st app = 0.35 mL; 2nd & 3rd = 0.4 mL	1.15 mL
2	Vehicle of proposed formulation (no active ingredients) at 3X	3 applications @ 0.21 mL	0.63 mL
3	Vehicle of proposed formulation (no active ingredients) at 5X	3 applications @ 0.35 mL	1.05 mL
4	Proposed formulation (with active ingredients) at 3X	3 applications @ 0.23 mL	0.69 mL
5	Proposed formulation (with active ingredients) at 5x	1st app = 0.35 mL; 2nd & 3rd = 0.4 mL	1.15 mL

The animals were observed twice daily (morning and afternoon) except on days of treatment and on the first day following each dosage. On treatment days (0 and 14) kittens were observed pre-treatment, and at 1, 2, 3 and 4 hours (± 15 minutes) following completion of the third and final sub-application. On days 1 and 15, kittens were observed at approximately 8:00 am, 11:00 am, and 3:00 pm (± 30 minutes). Body weights were determined on days -7, -3, -1, 1, 15 and 28. Physical examinations were conducted by a veterinarian on days -7, -3, 1, 15 and 28.

Blood for hematology and clinical chemistry was collected from all the kittens on study days -7, 1 (approximately 21 hours post-treatment), 15 (approximately 24 hours post-treatment), and at termination on day 28. To avoid overstressing the kittens, only 1 to 3 mL of blood was collected at each time, consequently, coagulation times were not determined.

All animals survived to the end of the study.

All kittens showed hair coat effects at 1-4 hours post-dose, mostly on days 0 and 14, but in some cases these effects were noted on subsequent days, primarily in Group 1 (all 12 kittens on day 15, and in 5 on day 16), with a few occurrences in Group 2 (two kittens on day 15 and one on day 16), Group 3 (two kittens on day 15 and one on day 16) and one in Group 5 (day 15 only). One Group 3 female had hair coat effects on day 21.

One Group 5 male (08KPK1) was lethargic on day 15 (the day following the second dosage). This was the only reported occurrence of lethargy in the study; also it was the only kitten in this group with coat effects on day 15; this animal had shown diarrhea on day 14 pre-dose and on day 15. The overall mean food consumption of this animal (49.4 g/day) from week 1 to 4 was lower than values of the other males (range: 52.9 to 69.0 g/day) in this group, and was particularly low (36.6 g/day) during week 2 (presumably from day 7 through 13, a period that did not include an application of the test material). According to the report summary this kitten demonstrated intermittent anorexia (days 11 and 15) resulting in mild weight loss and transient dehydration

and lethargy, with immediate improvements in food consumption and general condition noted following supplementation of the diet with moist food.

All kittens gained weight from Day -1 to Day 28. Mean body weight gains of Group 5 (5X test material) males and females were noticeably lower than those of the other groups in the period from Day -1 to Day 20 (which included applications on Days 0 and 14). Group 5 males had a weight gain that was 87% of that for Group 1 males, and Group 5 females had a value that was 92.6% that of Group 1 females. Group 4 (3X test material) males and females had values slightly greater than those of their Group 1 counterparts.

It is concluded that the margin of safety in kittens administered topical application of the product formulation is at least 3X. Possible effects observed at 5X included lethargy in one male kitten following the second set of applications, and decreased body weight gains in both males and females in the period from day -1 to day 20. As noted in the current 870.7200 Guidelines: "Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)."

This companion animal safety study in male and female domestic shorthair kittens is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in 54-57 day (8 week) kittens.

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

Signature: Byron T. Backus
Date: 04/15/2010

EPA Secondary Reviewer: Ayaad Assaad, D.V.M., Ph.D.
Toxicology and Epidemiology Branch, HED (7509PY)

Signature: A. E. Assaad
Date: 4/15/2010

Template Version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- kittens – OPPTS 870.7200

PC CODES: 129099- Imidacloprid, 129032- Pyriproxyfen, **DP BARCODE:** 372322

TEST MATERIAL (PURITY): M880 Insecticide (Bayer Imidacloprid/Pyriproxyfen/Dog/Cat SpotOn), Formula No. BB-06-139; Lot No. BB-06-139-M880-06-05-60; described as a clear amber liquid with a specific gravity of 1.095 g/mL (see p. 18 of MRID 47924801) containing 9.1% Imidacloprid and 0.46% Pyriproxyfen.

TRADE NAME: Advantage® IGR 5

CITATION: Madsen, T. (2009) Evaluation of the General Safety of M880. Bayer Animal Health Study No.: 152.141; In-Life Testing Facility Study No. S07648; Bayer Animal Health Report No.: 33714. Sinclair Research Center, Inc., 562 State Road DD, Auxvasse, MO 65231, 9 October 2009. MRID 47924801. Unpublished. 193 p.

SPONSOR: Bayer HealthCare LLC / Animal Health Division

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47924801), 5 groups, each containing 6 males and 6 females, of domestic shorthair kittens (54-57 days old on Day 0; Day -1 body weights: males: 0.691-1.012 kg; females: 0.555-0.935 kg; source: Liberty Research, Inc., Waverly, NY), were topically treated (on Day 0) with (Group 1): mineral oil at a total dose of 1.15 mL; (Group 2): 3X vehicle substance at a total dose of 0.63 mL; (Group 3): 5X vehicle substance at a total dose of 1.05 mL; (Group 4): 3X test substance at a total dose of 0.69 mL; and (Group 5): 5X dose test substance at a total dose of 1.15 mL. For each group, the total dose was split into three sub-applications which were administered at approximately 60-minute intervals. The application site was the skin on the dorsal midline from the base of the skull to the interscapular region. The dosing was repeated on Day 14.

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All kittens gained weight from Day -1 to Day 28. Mean body weight gains of Group 5 (5X test material) males and females were noticeably lower than those of the other groups in the period from Day -1 to Day 20 (which included applications on Days 0 and 14). Group 5 males had a

weight gain that was 87% of that for Group 1 males, and Group 5 females had a value that was 92.6% that of Group 1 females. Group 4 (3X test material) males and females had values slightly greater than those of their Group 1 counterparts.

It is concluded that the margin of safety in kittens administered topical application of the product formulation is at least 3X. Possible effects observed at 5X included lethargy in one male kitten following the second set of applications, and decreased body weight gains in both males and females in the period from day -1 to day 20. As noted in the current 870.7200 Guidelines: "Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)."

This companion animal safety study in male and female domestic shorthair kittens is **Acceptable/Guideline** and **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in 54-57 day (8 week) kittens.

COMPLIANCE: Signed and dated GLP Compliance, Quality Assurance and [No] Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials:

1a. Control/reference substance (Group 1)

Description:	Mineral oil, light, from Fisher Chemical, a clear colorless viscous liquid
Lot no.:	084662
Purity:	
Storage:	"Controlled room temperature"
Compound Stability:	
CAS #:	8042-47-5

1b. Vehicle Control; M880 Insecticide Placebo (Group 2 at 3X; Group 3 at 5X))

Description:	Clear amber liquid
Lot no.:	08-05-30
Purity:	LOD (<0.018%) Pyriproxyfen; LOD (<0.01%) Imidacloprid
Storage:	"Controlled room temperature"
Compound Stability:	
CAS #:	Not reported

1c. M880 Insecticide (Bayer Imidacloprid/Pyriproxyfen/Dog/Cat Spot On)

Description:	Clear amber liquid
Lot no.:	011902-09
Purity:	0.46% w/w Pyriproxifen; 9.1% Imidacloprid
Storage:	At room temperature
Compound Stability:	Expiration date: January 19, 2011
CAS #:	95737-68-1 (Pyriproxyfen); 138261-41-3 (Imidacloprid)

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

3. **Test animals:**

Species:	Cat
Strain:	Domestic shorthair
Age/weight	Day 0: 54-57 days old; males: 0.691-1.012 kg; females: 0.555-0.935 kg
Source:	Liberty Research, Inc., Waverly, NY
Housing:	Individually housed in 3 ft x 3 ft stainless steel pens
Diet:	Purina® Kitten Chow or equivalent, 150 g/kitten/day; animals that exhibited inappetance (= consumption ≤ 25 g/kitten/day) were offered 20 to 75 g of moist food (Purina® Friskies, Mariner's Catch) per day.
Water:	Well water, sourced from an on-site deep well, *ad libitum*
Environmental conditions:	
Temperature:	67-87° F
Humidity:	≤ 15-86%
Air changes:	"appropriate hourly air exchanges."
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	One week

B. STUDY DESIGN:

1. **In life dates:** Start: February 2, 2009; End: March 9, 2009. Day 0 for Replicate A was February 2 and for Replicate B was February 9.

2. **Animal assignment:** Sixty kittens were assigned to the study. Because of difficulty in obtaining kittens within the narrow age range, the study was conducted in two replicates, with 3/sex/group in each replicate. Unique randomization tables were generated for each replicate. On day -1 of each replicate, kittens meeting the inclusion criteria were separated by gender and ranked by day -1 body weights in descending order. A pre-generated table was then used to assign ranked kittens to one of the five treatment groups. The table consisted of pre-generated random numbers in sets of 5, by which the five heaviest kittens of one sex were each assigned to one of the five treatment groups, with the smallest number assigned to Group 1, the next smallest to Group 4, the next to Group 5, then to Group 3 and finally Group 2. This allocation process was continued for the remaining 5 sets of kittens. A littermate review was then conducted to ensure that the two 5X treatment groups (Groups 3 and 5) did not contain more than one male and one female from the same litter.

Table 1: Study design			
Group	**Test Material Applied**	**Volume of each application**	**Cumulative amount applied on Day 0; also on Day 14**
1	Mineral oil	1st application = 0.35 mL; 2nd & 3rd = 0.4 mL	1.15 mL
2	Vehicle of proposed formulation (no active ingredients) at 3X	3 applications @ 0.21 mL	0.63 mL
3	Vehicle of proposed formulation (no active ingredients) at 5X	3 applications @ 0.35 mL	1.05 mL
4	Proposed formulation (with active ingredients) at 3X	3 applications @ 0.23 mL	0.69 mL
5	Proposed formulation (with active ingredients) at 5x	1st app = 0.35 mL; 2nd & 3rd = 0.4 mL	1.15 mL

3. **Dose selection rationale**: According to a cover letter dated November 30, 2009 from the registrant the proposed product Advantage IGR 5 is "especially designed for small cats and kittens in a smaller single-use tube (0.23 mL)." This is consistent with the cumulative 3X dosage of 0.69 mL indicated above, as well as the cumulative 5X dosage of 1.15 mL.

4. **Application**: The test and control substances were topically applied using a calibrated pipette, with each dose split into 3 sub-applications at approximately 60-minute intervals. Application was directly to the skin on the dorsal midline from the base of the skull to the interscapular (between the shoulder blades) region.

5. **Statistics**: From p. 21 of MRID 47924801: "...The experimental unit was defined as the individual animal. Descriptive statistics (mean and standard deviation) were analyzed for all variables for all treatment groups... Statistical analyses were performed to further evaluate body weight, food consumption, and liver values (ALT, AST, ALP and GGT) for a potential treatment effect (using an alpha of 0.05). A repeated measures analysis of covariance including the classification terms 'treatment,' 'time,' and 'sex'; the two-way interactions 'treatment by time,' 'sex by time,' and 'treatment by sex'; the three-way interaction 'treatment by time

C. METHODS:

1. Observations:

a. **Observations**: The animals were observed twice daily (morning and afternoon) except on days of treatment and on the first day following each dosage. On treatment days (0 and 14) kittens were observed pretreatment, and at 1, 2, 3 and 4 hours (± 15 minutes) following completion of the third and final sub-application. On days 1 and 15, kittens were observed at approximately 8:00 am, 11:00 am, and 3:00 pm (± 30 minutes).

b. **Veterinary examinations**: Physical examinations were conducted by a veterinarian on days -7 -3, +1, +15 and +28. The examinations included but were not limited to heart rate, auscultation of the heart and lungs, mucous membranes, eyes, ears and genital organs.

2. **Body weight**: Animals were weighed on Days -7, -3, -1, +6, +13, +20 and +28.

3. **Food consumption:** Food consumption was "assessed" once daily between days -7 through termination (day 28). In addition to dry food (150 g offered/day), any kitten that exhibited inappetance and/or abnormal feces (loose stools or diarrhea) was offered moist food. The amounts of moist food offered and consumed were recorded in the raw data.

4. **Hematology and clinical chemistry**: Blood was collected for hematology and clinical chemistry assessments on unfasted kittens on the following days: - 7, +1 (approximately 21 hours post-treatment), 15 (approximately 24 hours post-treatment), 19 (Group 5 kitten 08KPK1 only) and at termination on day 28. To avoid putting additional stress on the kittens, only 1 to 3 mL of blood/kitten was collected at each time, consequently, coagulation times were not determined. All whole blood and serum specimens were shipped with frozen ice packs. Hematology and chemistry analyses were conducted by Antech Diagnostics, Morrisville, NC 27560. 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 (HBD)
	(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	Blood urea nitrogen*
X	Phosphorus *		Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase (CK)		Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
X	Gamma glutamyl transpeptidase (GGT)		TCO_2 Bicarbonate
	Amylase		
	Sorbitol dehydrogenase		

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

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

6. **Sacrifice and pathology**: The study did not have a scheduled necropsy, and all kittens survived.

II. RESULTS

A. **DOSES ADMINISTERED ON A BODYWEIGHT BASIS**: The only kittens treated with the active ingredients were in Groups 4 (3X the proposed dosage treatment) and 5 (5X the proposed dosage treatment). The cumulative doses in these groups (0.69 mL at 3X, 1.15 mL at 5X) on days 0 and 14 remained the same. As all kittens gained weight between day -1 and 13, the dosages of active ingredients on a body weight basis decreased.

Table 2. Dosages for actives on a mg/kg basis				
Group 4 (3X)	**Day 0 (mg/kg)**		**Day 14 (mg/kg)**	
	Pyriproxyfen	Imidacloprid	Pyriproxyfen	Imidacloprid
Minimum	3.999	75.725	2.770	52.452
Maximum	5.575	105.564	4.741	89.778
Mean	4.609	87.279	3.462	65.549
Group 5 (5X)	**Day 0 (mg/kg)**		**Day 14 (mg/kg)**	
	Pyriproxyfen	Imidacloprid	Pyriproxyfen	Imidacloprid
Minimum	5.848	110.744	4.177	79.092
Maximum	9.655	182.827	7.417	140.443
Mean	7.350	139.184	5.526	104.645

B. **OBSERVATIONS:**

1. **Cosmetic effects**: All kittens showed cosmetic hair coat effects (greasy, matted, and/or spike hair at dose site) at 1-4 hours post-dose on days 0 and 14, and in some cases these effects were noted on subsequent days, particularly in Group 1 (all 12 kittens on day 15, and 5 on day 16), with a few occurrences in Group 2 (two kittens on day 15 and one on day 16), Group 3 (two kittens on day 15 and one on day 16), and one in Group 5 (day 15 only). One Group 3 female had hair coat effects on day 21.

2. **Clinical signs of toxicity**: Loose stools and/or diarrhea occurred sporadically throughout the study in all groups. There seemed to be an increased incidence on days 14, 15 and 16 relative to days 12 and 13, but a closer examination of the data shows that days 5 and 6 also had reduced incidences. Since days 0 and 14 were Mondays, days 5-6 and 12-13 were weekends, and possibly the kittens were not observed as closely on those days as at other times; refer to Table 3, below:

Table 3. Occurrences of loose stools and diarrhea by group and sex on days 0 through 21.

Group & Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1M	1L		2L	1L	1L			1L 1d	1L 1D	1L 1d	2D	2D			1L 1d	1D	1L 1d				1D	1D
1F					1D	1D	1D	1D		1D	1L 1D	1L 1d	1D		1L 1d	1D	1L	2D			1D	1D
2M	1L	1D	1D	1L	1L			1D							1L	1D	1L	1L 1d			1L	1L 1D
2F	1L		2D	2D	1L			2L 1d		1L 1D	1L 1d 1D			1D	1L 1D			1D	1D		1D	
3M	1L	1L 1d		1L	1L 1D			1L				1L			1L 1D	1L 1D		1D	1D		1D	
3F			1L				1D		1L	1L 1D	1D	1D					1L 1d					
4M		1L		1D											1L	2L 1d	1L 1d	2D	1D			
4F		2L	1L	2L	1D			1L 1D	2L 1D	3L	2D	1L 2D	2D		2L	2L	1L	1L		1D	1D	1L 2D
5M				1D	1L 1d		1D	1D	1L	1L 1D	2D	1L 1d 1D			1D	2D	1D					
5F		1L 1d	1L 1D	2L 1D	1L 2D	1D	1D	2L 1d	1L 1d	1L 1D	1L 1D	1D		1D	2L	1L 1D	3L	2D	2D	1D	1D	3L
Total	4	7	9	12	12	2	4	14	9	14	14	13	3	2	15	14	13	11	5	2	7	10

Number = number of kittens with one or more occurrences on that day; L = loose stools; d = diarrhea with occurrence of loose stools for that kitten on same day; D = diarrhea without occurrence of loose stools.

On study day 15, kitten 08KPK1 (a male in Group 5) was observed to be lethargic. This kitten also showed abnormal feces (loose stools and/or diarrhea on days 11, 14 and 15) and had also shown intermittent anorexia (days 11 and 15) and a 3 g decrease in body weight (from 939 to 936 g) between days 6 and 13.

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

B. **BODY WEIGHT AND WEIGHT GAIN**: Body weight data are presented in Table 3. All individual kittens gained weight from Day -1 to Day 28, although some individual kittens lost weight during some of the study intervals (Group 1 female 08QNM3 lost 33 g between days 13 and 20; Group 4 male 08QNM1 lost 45 g between days 13 and 20; Group 5 male 08KPK1 lost 3 g between days 13 and 20, and Group 5 female 08JNG3 lost 62 g between days 20 and 28). Mean body weight gains of Group 5 males and females were noticeably lower than those of the other groups in the period from Day -1 to Day 20 (which included applications on Days 0 and 14), and this was particularly pronounced in Group 5 females as their mean weight gains from Day -1 to 6 and from Day 13-20 (the test material was applied at 5X on Days 0 and 14) were considerably lower than the means from other females of the other groups.

Group & Sex	Table 4. Mean body weights (kg)					
	Day -7	Day -1	Day 6	Day 13	Day 20	Day 28
1 M	0.712	0.841	1.010	1.166	1.348	1.581
2 M	0.734	0.851	1.016	1.176	1.375	1.588
3 M	0.727	0.845	1.003	1.157	1.310	1.518
4 M	0.696	0.825	1.002	1.134	1.336	1.563
5 M	0.750	0.863	1.020	1.151	1.304	1.524
1 F	0.627	0.739	0.883	0.990	1.142	1.317
2 F	0.643	0.758	0.913	1.035	1.163	1.358
3 F	0.629	0.742	0.897	1.022	1.201	1.353
4 F	0.619	0.731	0.861	0.963	1.148	1.299
5 F	0.664	0.772	0.876	1.039	1.145	1.263

Group & Sex	Table 5. Mean body weight gains (kg)						
	Day -7 to Day -1	Day -1 to Day 28	Day -1 to Day 20	Day -1 to Day 6	Day 6 to Day 13	Day 13 to Day 20	Day 20 to Day 28
1 M	0.129	0.740	0.507	0.169	0.156	0.182	0.233
2 M	0.117	0.737	0.524	0.165	0.160	0.199	0.213
3 M	0.118	0.673	0.465	0.158	0.154	0.153	0.208
4 M	0.129	0.738	0.511	0.177	0.132	0.202	0.227
5 M	0.113	0.661	0.441	0.157	0.131	0.153	0.220
1 F	0.112	0.578	0.403	0.144	0.107	0.152	0.175
2 F	0.115	0.600	0.405	0.155	0.122	0.128	0.195
3 F	0.113	0.611	0.459	0.155	0.125	0.179	0.152
4 F	0.112	0.568	0.417	0.130	0.102	0.185	0.151
5 F	0.108	0.491	0.373	0.104	0.163	0.106	0.118

C. **FOOD CONSUMPTION:** No treatment-related effects were reported or are evident from group or individual data (refer to Table 5.3, pages 182-184 of MRID 47924801). From p. 19: "Food consumption was assessed once daily between days -7 through termination (day 28)." According to the text on p. 23: "Although slight decreases in food consumption were recorded for several animals in all treatment groups on the day of treatment and/or the initial 2-3 days post-treatment, food consumption gradually increased, as expected for growing kittens, over the course of this study."

From p. 19: "In addition to dry food, any kitten that exhibited inappetance and/or abnormal feces (i.e., loose or diarrhea) was offered moist food." The following is from p. 24:

[a]Moist Food Offered During the Exposure Period				
Animal ID (Sex)	Group	Treatment	Timepoint(s) (when moist food offered)	Amount of Moist Food Offered per Timepoint (g)
08KPV3 (M)	1	0X (Control/Reference)	Days 10, 11	20, 20
08QNM3 (F)	1	0X (Control/Reference)	Days 17, 18	20, 20
08KPG5 (F)	1	0X (Control/Reference)	Days 17, 18	20, 20
08QNP3 (F)	2	3X (Vehicle Substance)	Days 10, 11, 17	20, 20, 20
08KPK4 (F)	2	3X (Vehicle Substance)	Days 17, 18	20, 20
08KPV4 (F)	3	5X (Vehicle Substance)	Days 10, 11	20, 20
08QN2 (M)	3	5X (Vehicle Substance)	Days 17, 18	20, 20
08KPW6 (F)	3	5X (Vehicle Substance)	Day 25	20
08QNP4 (F)	4	3X (Test Substance)	Days 10, 11, 23, 24	20, 20, 20, 20
08QNM1 (M)	4	3X (Test Substance)	Days 17, 18	20, 20
08KPY2 (F)	4	3X (Test Substance)	Day 22	20
08KPI8 (F)	4	3X (Test Substance)	Days 22, 24	20, 20
08KPZ1 (M)	5	5X (Test Substance)	Days 10, 11	20, 20
08KPW2 (M)	5	5X (Test Substance)	Days 10, 11	20, 20
08KPW8 (F)	5	5X (Test Substance)	Days 10, 11, 23, 24, 25	20, 20, 20, 20, 20
08KPK1 (M)	5	5X (Test Substance)	Days 15, 16, 17, 18	75, 75, 20, 20
08QNR4 (F)	5	5X (Test Substance)	Day 22	20
08KQA7 (F)	5	5X (Test Substance)	Days 22, 23, 24, 25	20, 20, 20, 20
08JNG3 (F)	5	5X (Test Substance)	Days 24, 25	20, 20

[a]Table from p. 24 of MRID 47924801.

According to the protocol (see page 40 of MRID 47924801): "Kittens exhibiting inappetance may be offered moist food" with no mention of loose stool and/or diarrhea. It appears that the decision to provide moist food to kittens with diarrhea and/or loose stools may have been made about day 10. Seven kittens were given moist food on day 10, although 13 showed loose stool and/or diarrhea on that date.

Since individual food consumption values (in g/kitten/day) are reported in MRID 47924801 on a weekly (rather than daily) basis, TRB requested and received individual daily food consumption data.

From the individual food consumption data, there is no indication that exposure to the test material (or the control reference or vehicle substance) on day 0 resulted in a decrease in food consumption. One control reference (Group 1) male kitten (08KPF3) is reported to have consumed 114 g on Day 0. One Group 5 female (08KPI6) consumed only 20 g on Day 1, but this animal had consumed only an average of 32 g/day from Day -7 to -1. On Day 14 (second treatment) Group 5 male consumed only 6 grams, and then only 20 grams on Day 15; this kitten was then offered (in addition to the usual ration) 75 g of moist food on Days 16 and 17, and consumed 84 and 81 g of food on those days, respectively.

D. CLINICAL PATHOLOGY ANALYSES:

1. **Hematology**: No treatment-related changes were observed in any of the parameters. On Day 15 (refer to p. 25 and p.p. 114-115 of MRID 47924801), group 5 kitten 08KPK1 had an increased percentage (82.5) of neutrophils, an increased percentage (37.72) of absolute neutrophils, with elevated percentages of basophils and absolute basophils (2.2 and 1.2, respectively). This is suggestive of a response to a bacterial infection.

2. **Clinical Chemistry**: No treatment-related changes were observed in any of the parameters. On Day 15 (refer to p. 26 and p. 141-142 of MRID 47924801, group 5 male 08KPK1 had a high BUN (105 mg/dL), normal creatinine, low sodium and low chloride, elevated potassium, elevated total protein and slightly elevated glucose. From p. 26: "The observed hematology and serum chemistry changes were likely secondary to dehydration. Decreased sodium and chloride values may also be secondary to hyperproteinemia..." An elevated BUN >60 mg/dL with a normal creatinine level suggests a moderate-to-severe degree of acute renal failure. This kitten had loose stool and diarrhea on days 11, 14 and 15. Blood was taken from this kitten on day 19 and hematology and clinical chemistry parameters were measured (see p. 149); by day 19 the BUN (36 mg/dL), sodium, chloride and potassium levels were within normal reference ranges.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that no treatment-related clinical signs or effects on the variables measured were observed in kittens 8 weeks of age and older and/or up to 5 pounds treated topically, biweekly for two consecutive treatments with 0, 3, or 5 times the label dose of the imidacloprid + pyriproxyfen spot-on.

B. **REVIEWER COMMENTS:** All animals survived to the end of the study. There were no indications of dose-related signs at the 3x dose level. Possible indications of systemic toxicity at the 5x dose level included the lethargy seen in male 08KPK1 (while this kitten was reported to have had pre- and post-dose diarrhea on day 14, as well as diarrhea on day 15, and had clinical chemistry results from day 15 that suggested electrolyte loss and dehydration – consistent with the diarrhea - the possibility that treatment and/or exposure to the test material on day 14 exacerbated its condition cannot be discounted). In addition, mean body weight gains in Group 5 males and females in the period from day -1 to 20 were lower than the corresponding values from other groups, and this was particularly pronounced in Group 5 females for weeks the test material was applied (Days -1 to 6 and 13-20).

It is concluded that the margin of safety in kittens administered topical application of M880 Insecticide (9.1% imidacloprid and 0.46% pyriproxyfen) was at least 3X. According to the OPPTS 870.7200 Companion Animal Safety Test Guidelines the targeted adequate margin of safety is 5X, but consideration can be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life threatening signs). In this case, the possible (but ambiguous) signs of toxicity observed at 5X were limited to lethargy in one kitten following treatment on Day 14, and lower weight gains in the test group (particularly females, which showed reduced weight gains for the weeks in which they were treated with the test material).

This study is acceptable and can be used to support the proposed use of M880 Insecticide (9.1% imidacloprid and 0.46% pyriproxyfen) on kittens 8 weeks old and older at a dosage rate of 0.23 mL/application, with retreatment no more often than once every 14 days.

1. **DP BARCODE:** 372322
2. **PC CODES:** 129099 (Imidacloprid); 129032 (Pyriproxyfen)
3. **CURRENT DATE:** April 15, 2010
4. **TEST MATERIALS:** Controls (Group 1): Mineral Oil; Vehicle Controls (Groups 2 & 3): Test material without active ingredients; M880 Insecticide (Groups 4 & 5): Bayer Imidacloprid/Pyriproxyfen Dog/Cat Spot On containing 9.1% Imidacloprid and 0.46% Pyriproxyfen.

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion Animal Safety Study/Kittens Sinclair Research, Auxvasse, MO 65231 Bayer Animal Health Study No. 152.141 / October 9, 2009.	47924801	Five groups (each 6M & 6F) of 8 week old kittens were treated on Days 0 & 14. Group 1 was treated with a total of 1.15 mL mineral oil; Group 2 with a total of 0.63 mL formulation vehicle; Group 3 with 1.05 mL formulation vehicle; Group 4 with 3X (=0.69 mL) proposed formulation; Group 5 with 5X (=1.15 mL) proposed formulation. Possible (but ambiguous) signs of toxicity at 5X were lethargy in one kitten following day 14 application, and reduced mean weight gains, particularly in females on weeks of treatment.	N/A	A

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