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

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

21 August 2006

MEMORANDUM

Subject: Name of Pesticide Product: PROMERIS SPOT-ON FOR DOGS
EPA Reg. No. /File Symbol: 80490-E
DP Barcode: D327884
Decision No.: 351841
PC Codes: 106201 (Amitraz); 281250 & 281251 (Metaflumizone)

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

Byron T. Backus 8/21/2006
RJW 8/22/2006

To: John Hebert, RM Team 07
Insecticide-Rodenticide Branch
Registration Division (7505P)

Registrant: FORT DODGE ANIMAL HEALTH

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
281250 & 281251 Metaflumizone	14.34%
106201 Amitraz	14.34%
Other Ingredient(s):	71.32%
Total:	100.00%

ACTION REQUESTED: The Risk Manager requests:

"Please conduct a secondary review of the three dog studies on Amitraz/Metaflumizone prepared by a contractor…"

BACKGROUND:

TRB has previously reviewed a 6-pack of acute toxicity studies and two (adult dog and puppy) companion animal studies on this product. In the previous reviews of the companion animal safety studies, TRB expressed concerns regarding indications that there is a less-than-5X margin of safety between the use exposure level and that at which symptoms of toxicity occur.

In order to at least partially address the concerns, the registrant has had several additional dog studies conducted, including the following: "Evaluation of the toxicity in Chihuahua breed dogs of an insecticidal spot on for dogs containing R-28153, 150 mg and amitraz, 150 mg/mL" (in MRID 46672102); "Safety evaluation study of oral exposure from auto- or allogrooming R-28153/amitraz spot-on formulation in dogs" (in MRID 46672103); and "Safety evaluation study of repeated treatments with a topically applied spot-on formulation of R-28153 and amitraz in dogs" (MRID 46672104). As TRB's resources are limited, and the studies were somewhat beyond the usual types of studies (including routine companion animal safety studies conducted according to 870.7200) reviewed by TRB, these studies were routed to HED for primary (contract) reviews, but, at the request of HED, it was agreed that TRB would conduct the secondary reviews.

COMMENTS AND RECOMMENDATIONS:

Overall, the findings from these studies do not indicate that an adequate margin of safety exists between the companion animal (dog) use exposure and that at which symptoms of toxicity occur. This is particularly evident in the Chihuahua study (MRID 46672102), in which possible indications of toxicity occurred at 1X.

The following is the executive summary for MRID 46672102 [Evaluation of the toxicity in Chihuahua breed dogs of an insecticidal spot on for dogs containing R-28153, 150 mg and amitraz, 150 mg/mL]:

In a 21-day companion animal safety study (MRID 46672102), 0.67 mL of 15% R-28153 (metaflumizone)/15% amitraz Spot-on for Dogs (Lot No. 0481704) was applied topically to the dorsal surface between the shoulder blades of five males and one female Chihuahua dog on days 0 and 14. The doses administered ranged from 49 mg/kg to 183 mg/kg body weight each of R-28153 and Amitraz. Six additional dogs (five males and one female) received no test article and served as untreated controls.

All animals survived until the termination of the study. There were no treatment-related effects in any of the dogs on body weight, hematology and clinical chemistry parameters or overall general health. Clinical signs observed immediately after treatment included: hyperactivity (4/6 treated dogs) and pruritus (5/6 treated dogs) with pruritus occurring for up to 7 hours. Lacrimation occurred in 1/6 of treated dogs 24 hours after the first application and in a different treated dog immediately after the 2nd application. The only female dog in the treatment group vomited one time on the first day post-treatment with both treatments suggesting a treatment-related effect. A small swelling was also observed at the area of application in 3/6 treated dogs at days 2, 7 and 11 post-application but was gone by day 21. None of these clinical signs was observed in the untreated control dogs.

This 21-day toxicity study in the dog is **UNACCEPTABLE/NONGUIDELINE** and does not satisfy the guideline requirement for a companion animal safety study (OPPTS 870.7200). Only one dose level was tested and the number of animals tested (5 males and 1 female) was inadequate. Because the administration of 0.67 mL (the recommended labeled dose) 15% R-28153/15% amitraz Spot-on for Dogs topically to Chihuahuas produced clinical signs, a margin of safety was not established.

The following is the executive summary for MRID 46672103 [Safety evaluation study of oral exposure from auto- or allogrooming R-28153/amitraz spot-on formulation in dogs]:

In a ten-day non-guideline companion animal safety study (MRID 46672103), four 5-6 month-old beagle dogs/sex were administered a single oral dose of 0.14 mL/5.0-9.9 kg, which is 0.1 times the proposed commercial dermal dose of a R-28153/amitraz spot-on formulation (Lot #0481704; 15% w/v of each active ingredient). The dogs were observed for nine additional days post-dosing. The placebo control (0.8% bacteriostatic sodium chloride for injection) was administered at the same volume. All animals were observed at least twice a day for morbidity and mortality. Detailed clinical observations were conducted daily. On the treatment day (Day 1), animals were examined prior to treatment and at 5 to 15 minutes, 30 to 45 minutes and 1, 2, 3 and 10 hours post-treatment. Heart rate and body temperature were recorded on Day 1 at 2 and 10 hours post-dosing. Body weight, body weight gain and food consumption were recorded periodically pre- and post-dosing. Hematology and clinical chemistry parameters were measured on Days -1, 2 and 8.

All dogs survived to study termination. The taste of the formulation caused immediate effects in all of the treated dogs upon dosing and included: head shaking, spitting, licking and/or salivation. Other clinical signs observed post-dosing were attributed to amitraz toxicity. Decreased activity was reported in 2/4 males and 3/4 females beginning 1-2 hours post-dosing and continuing for several hours with normal activity by 10 hours post-dosing. Also observed post-dosing in 1/4 of the males and 1/4 of the females was pale gums, skin cold to the touch, and slow capillary refill time. Another female had some ataxia post-dosing. These were seen starting at 1-2 hours post-dosing with most subsiding by 6 hours post-dosing. Body temperature was decreased post-dosing in males and females with the effect lasting up to 10 hours post-dosing in females. A decrease in mean heart rate was observed post-dosing in both the treated males and females with females being more affected. This was first observed at 2 hours post-dosing and rates did not become comparable to controls until 10 hours post-dosing in males and Day 2 in females. All other effects subsided by Day 2 post-dosing.

There was no treatment-related effect on body weight or food consumption. Significant differences in hematology parameters were found in treated groups only when the data for both sexes were combined; these were not considered treatment-related. Several clinical chemistry parameters were affected by treatment only when the data for both sexes were combined so the toxicological significance of the findings is questionable. The only clinical chemistry difference which could be treatment-related is the increase in blood urea nitrogen (BUN) on Day 2 in treated females; however, this was comparable to controls by Day 8.

Amitraz is approved by the U.S. Food and Drug Administration (FDA) as a prescription veterinary drug for the treatment of demodectic mange in dogs as a 19.9% (w/w) formulation under the trade name Mitaban. Comparison of clinical signs observed in acute oral studies with Mitaban (cited in the package insert for the product) and the metaflumizone/amitraz formulation can be made. In the metaflumizone/amitraz study, at a mean mg/kg dose of approximately 3 mg/kg in males and 4 mg/kg in females, decreased activity, pale gums, skin cold to the touch,

ataxia, slow capillary refill time and decreased body temperature were observed. Some of these signs persisted until 10 hours post-dosing. In the Mitaban single oral dose study at a comparable dose (4 mg/kg), the only clinical sign was decreased rectal temperature. Some of the signs observed at 20 mg/kg, the mid-dose treatment in the Mitaban study, were also reported with the metaflumizone/amitraz combination, including decreased activity and ataxia.

Toxicological effects were observed in some of the dogs. **In conclusion, oral administration of 0.14 ml of the metaflumizone/amitraz combination (15% w/v of each ingredient) to Beagle dogs caused some toxicological effects such as pale gums, slow capillary refill time, skin cold to the touch and decreased heart rate.**

This companion animal safety in the dog is **Acceptable (Non-guideline)** and **satisfies** the intent for which it was conducted as a measure of toxicity when the topical product is inadvertently ingested.

The following is the executive summary for MRID 46672104 [Safety evaluation study of repeated treatments with a topically applied spot-on formulation of R-28153 and amitraz in dogs]:

In a companion animal safety study (MRID 46672104), an R-28153/amitraz spot-on formulation (Lot #0481704; 15% w/v of each active ingredient) was topically applied at 14-day intervals over a 14-week period to four/sex/group beagle puppies (approximately 10 weeks old) at 0, 1, 3 or 5 X the label dose. The labeled dose is 0.7 mL for ≤5.0 kg, 1.4 mL for 5.1-10.0 kg and 3.4 mL for 10.1-25.0 kg dogs. The placebo control was administered at the same volume used to treat the 5X dose group. The product or control formulation was applied between the shoulder blades on the dorsal midline extending cranially and caudally. All animals were observed at least twice a day for morbidity and mortality. Clinical observations were conducted twice daily prior to treatment and at least once daily after treatment initiation. On each treatment day, animals were examined prior to treatment and 5 to 15 minutes, and 1, 2 and 10 hours post-treatment. Heart rate and body temperature were recorded during the 10-hour post-treatment observations. Body weight, body weight gain and food consumption were recorded periodically pre- and post-dosing. Hematology, clinical chemistry and urinalysis parameters were measured once pre-treatment, the day after each dose and 7 days after treatments 1, 3, 5 and 7. Complete necropsy examinations were performed and organ weights were recorded for two animals per sex per group. Microscopic examination of tissues was conducted on two animals per sex from the control and 5X groups. The kidneys were examined in all groups.

One male at 3X (number 101) was euthanized *in extremis* on Day 3. The animal had soft/watery feces on Day -1, prior to dosing on Day 1 and again during the 1 and 2 hour post-dosing observations. By Day 2, pale gums at 10 hours post-dosing and decreased activity and watery feces were observed. On Day 3, the animal was ataxic and not eating; therefore, euthanasia was performed. No definitive cause of the clinical signs was established. Soft and/or watery or mucoid feces were observed in control and treated males and females pre- and post-dosing. Lacrimation and salivation were observed in both control and treated animals of both sexes post-dosing and the incidence of the signs was not increased in treated animals.

Body weight, body weight gain and food consumption were not affected by treatment. Blood urea nitrogen was increased on Days 58 and 86 when the data on both sexes were pooled, but there was no dose response on either day. Glucose was increased at the 5X dose on Day 36 and at the 3X and 5X doses on Day 58 when the data for both sexes were pooled. This could be

treatment-related since it is a known effect of amitraz but without statistical significance in individual sexes, it is questionable.

No treatment-related findings were observed on macroscopic or microscopic examination of the tissues at necropsy. Mean absolute liver weight was slightly increased in treated males (102-117% of control value) and females (105-123% of control value). Mean relative liver weights were increased in males at the 5X dose (113% of control value) and all treated females (111-121% of control value). Because the increases were not accompanied by any lesions at histopathology and only two animals/sex/group were necropsied, these changes are not considered treatment-related.

The margin of safety indicated in this study for this spot-on formulation of metaflumizone and amitraz in dogs is 1X because the clinical signs of toxicity which led to euthanasia of the 3X dose animal may be treatment-related. However, the study is considered unacceptable due to deficiencies discussed below.

This companion animal safety in the dog (puppy) is **Unacceptable (Guideline)** and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in dogs (puppies). Study deficiencies included, but are not limited to, the following: the number of animals (four/sex/group) was less than the required six/sex/group; and 2) there are questions about whether the animals were healthy prior to treatment.

DATA EVALUATION RECORD

METAFLUMIZONE AND AMITRAZ

STUDY TYPE: COMPANION ANIMAL SAFETY STUDY
MRID 46672102

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No.125-2006

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Disclaimer

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

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Byron T. Backus, Ph.D. **Signature:** Byron T. Backus
Technical Review Branch, Registration Division (7505P) Date: 8/22/2006

EPA Secondary Reviewer: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) Date: 8/21/06

TXR#: 0053824

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- dogs [non-guideline]

PC CODES: 281250, 281251 and 106201 **DP BARCODE:** D322975

TEST MATERIAL (PURITY): R-28153 (15% w/v) and amitraz (15% w/v)

SYNONYMS: Canine Flea/Tick Spot-On

CITATION: Delport, P.C. 2005. Evaluation of the toxicity in Chihuahua breed dogs of an insecticidal spot on for dogs containing R-28153, 150 mg and amitraz, 150 mg/mL. ClinVet International (Pty) Ltd, Bloemfontein, Republic of South Africa. Study No. 0817-C-SA-02-04 (Sponsor), CV/04/235 (ClinVet). June 13, 2005. MRID 46672102. Unpublished.

SPONSOR: Fort Dodge Animal Health, Princeton, N.J.

EXECUTIVE SUMMARY: In a 21-day companion animal safety study (MRID 46672102), 0.67 mL of 15% R-28153 (metaflumizone)/15% amitraz Spot-on for Dogs (Lot No. 0481704) was applied topically to the dorsal surface between the shoulder blades of five males and one female Chihuahua dog on days 0 and 14. The doses administered ranged from 49 mg/kg to 183 mg/kg body weight each of R-28153 and Amitraz. Six additional dogs (five males and one female) received no test article and served as untreated controls.

All animals survived until the termination of the study. There were no treatment-related effects in any of the dogs on body weight, hematology and clinical chemistry parameters or overall general health. Clinical signs observed immediately after treatment included: hyperactivity (4/6 treated dogs) and pruritus (5/6 treated dogs) with pruritus occurring for up to 7 hours. Lacrimation occurred in 1/6 of treated dogs 24 hours after the first application and in a different treated dog immediately after the 2nd application. The only female dog in the treatment group vomited one

time on the first day post-treatment with both treatments suggesting a treatment-related effect. A small swelling was also observed at the area of application in 3/6 treated dogs at days 2, 7 and 11 post-application but was gone by day 21. None of these clinical signs was observed in the untreated control dogs.

This 21-day toxicity study in the dog is **UNACCEPTABLE/NONGUIDELINE** and does not satisfy the guideline requirement for a companion animal safety study (OPPTS 870.7200). Only one dose level was tested and the number of animals tested (5 males and 1 female) was inadequate. Because the administration of 0.67 mL (the recommended labeled dose) 15% R-28153/15% amitraz Spot-on for Dogs topically to Chihuahuas produced clinical signs, a margin of safety was not established.

COMPLIANCE: Signed and dated Quality Assurance and No Data Confidentiality statements were provided. A signed statement stating the study was not conducted with GLP as set forth in 40 CFR 160 but was conducted in accordance with OECD principles of GLP was included.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** R-28153 (metaflumizone)/amitraz Spot-on for Dogs

Description:	Clear, yellow liquid
Lot/Batch #:	Lot No. 0481704
Purity:	15.2% R-28153 and 15.6% Amitraz.
Compound Stability:	Not supplied
CAS #:	R-28153: 139968-49-3 Amitraz: 33089-61-1
Structure:	No available

2. **Vehicle and/or positive control:** R-28153 and Amitraz were in a mixture including an unknown proprietary solvent of < 40%. Control dogs were not treated.

3. Test animals:

Species:	Dog	
Strain:	Chihuahua	
Age/weight at study initiation:	Body weights were between 550 and 2200 grams and age was 8 weeks and older	
Source:	Loaned from local owners with owner consent	
Housing:	Indoor individual stainless-steel cages	
Diet:	Eukanuba puppy and junior small breed food (Reg V15463, act 36/107), *ad libitum*	
Water:	Potable water, *ad libitum*	
Environmental conditions:	**Temperature:**	20 °C ± 4 °C
	Humidity:	30 to 70%
	Air changes:	12-14 times/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	14 days	

B. STUDY DESIGN:

1. **In life dates** : Start: March 1, 2005; End: April 5, 2005

2. **Animal assignment**: The twelve dogs were ranked and allocated in each study group on Day -1 by body weight and gender. Body weight in males and females was ranked from highest to lowest. Two animals from each gender from top to bottom were chosen to form a block. The first animal from block 1 was assigned to Group 1; the second to Group 2. Then this was reversed and the first animal from block 2 was assigned to Group 2 and the second to Group 1. This continued until all animals were assigned. Groups 1 and 2 were designated Study Groups A or B by random draw with Group A and B being the control and treated groups, respectively. See Table 1.

TABLE 1. Study design

Test Group	Dose (mL)	# Male	# Female
Group A (control)	0	5	1
Group B	0.67 on Day 0 and Day +14	5	1

3. **Dose selection rationale**: The dose used is the recommended label dose level. This dose was determined according to a predetermined dose calculation table. Chihuahua dogs were stated to be chosen based on a possible increased sensitivity to the product; however, no rationale

for this was provided.

4. **Preparation and treatment**: The dogs were administered the formulation according to label directions once on Day 0 and once on Day +14. The hair of the dogs was not clipped or shaven. Dogs were placed standing with all four legs on the table, and the correct dose was administered to the skin in the area between the shoulder blades. No bandage was applied.

5. **Statistics**: The statistical analysis was based on the change from baseline in the hematology and clinical chemistry parameters. Post-exposure values were compared to baseline in an intra-group comparison by means of ANOVA with an animal and observation time as effects. The post-exposure values of the study group were compared to the controls by means of a Co-ANOVA with a treatment effect, and the baseline values as covariate. Significance was considered at $p<0.10$.

C. **METHODS:**

1. **Observations**:

   1a. **Cageside observations**: During the study, animals were observed daily for general signs of health. Dogs were also observed continuously during working hours on Days 0, +1, +14 and +15 which were the days of dosing and the first day post-dosing after each treatment.

   Specific health observations on Days +1, +2, +15 and +16 were conducted for signs of central nervous system affliction (seizures, ataxia, coma and disorientation), general irritation, gastro-intestinal and respiratory signs, polyuria and generalized discomfort.

   1b. **Clinical examinations:** Clinical examinations were conducted on all animals on Days -1, +4, +14 (prior to second application of test material), +17 and +21.

2. **Body weight**: Animals were weighed prior to initiation of the study (Days -14 and -1), and one day during the study (Day +16).

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

4. **Ophthalmoscopic examination**: Ophthalmoscopic examination was not conducted.

5. **Hematology and clinical chemistry**: Blood was collected in pediatric

blood collection tubes for clinical chemistry and hematology on control and treated animals on Days -1, +1, +7, +15 and +21. Information on the site of the blood draw and whether the dogs were fasted was not included in the study report. 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*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for companion animals safety evaluation based on Guideline 870.7200

b. Clinical chemistry

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

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

6. **Sacrifice and pathology**: Dogs were not sacrificed and were returned to their owners at the termination of the study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: Clinical signs observed and the time of observation are in Table 2. The only clinical signs observed in the control group were spots of alopecia in 2/6 dogs which resolved without medication, and diarrhea in 2/6 dogs which was treated with enteritis suspension one time each. In the treated dogs, clinical signs observed immediately post-treatment included pruritus and hyperactivity. Hyperactivity, described as running around the cage and scratching of bedding, was observed in 4/6 of the treated dogs within 2-5 minutes of application and lasted for less than one minute. Pruritus was observed in 5/6 of the treated dogs and was observed for up to 7 hours post-exposure. Lacrimation, described as not severe, was observed in 1/6 dogs on the first day post-treatment after the first application and immediately post-treatment in a different dog after the second application. The only treated female dog vomited one time on the first day post-dosing on both occasions of treatment indicating a possible treatment-related effect. Another sign observed was the presence of a subcutaneous swelling at the site of application that measured approximately either 7 x 7 x 7 mm or 4 x 4 x 4 mm. This was observed in 3/6 of the treated dogs and was observed on the 2nd, 7th and 11th day after application. While the report states that the swellings could be post-injection swellings from a subcutaneous Rabies vaccine and prophylactic imidocarb injection given Day -15, subcutaneous swellings were not observed in any of the control dogs.

TABLE 2. Clinical signs observed in Chihuahua dogs treated with Spot-on

Time of observed sign (post-dosing)	Affected dog ID no. (gender)					
	F3 07T (M)	FF F2T (M)	AF 37T (F)	AC 6BT (M)	FD DOT (M)	8E CET (M)
0-1 hr	Hyperactive[a]	General and local pruritus	Localized pruritus	Localized pruritus	NS[b]	NS
1-4 hrs	General and localized pruritus	NS	General and localized pruritus	General pruritus	General pruritus	NS
4-7 hrs	General pruritus; flaking of test item	Flaking of test item	General pruritus; spiking/ flaking of test item[c]	General pruritus; flaking of test item	Flaking of test item; sneezing	Flaking of test item
7-8 hrs	NS	NS	NS	NS	NS	NS
24-32 hrs (day +1)	General pruritus	NS	Vomiting (1X)	Spiking[c]; lacrimation	NS	NS
48-56 hrs (day +2)	SQ swelling[d] 7x7x7 mm	NS	General pruritus	NS	NS	NS
day +7	SQ swelling 7x7x7 mm	SQ swelling 7x7x7 mm	SQ swelling (day +11) 7x7x7 mm	NS	NS	NS
immediate (2-5 min, day +14)	Hyperactive	Hyperactive	Hyperactive	Hyperactive	Lacrimation[e]	NS
2-4 hrs (day +14)	SQ swelling 4x4x4 mm	Localized pruritus	Localized pruritus	NS	NS	NS
day +15	SQ swelling 4x4x4 mm	Spiking	Vomiting (1X)	Spiking[c]	Spiking[c]	NS
day +16	NS	NS	NS	NS	NS	NS

Data obtained from p. 15-16, MRID 46672102
[a] Hyperactivity started 2-5 minutes post-treatment and lasted for less than 1 minute
[b] NS = no clinical signs observed
[c] The terms "spiking of the test item" or "spiking" were not explained in the study report.
[d] SQ swelling = subcutaneous swelling
[e] lacrimation started after 15 minutes and lasted approximately 1 hour

2. **Mortality**: No animals died in the control or treated groups.

B. **BODY WEIGHT AND WEIGHT GAIN**: There were no treatment-related differences in body weight or body weight gain in any of the dogs. Only one treated dog (FD DOT) did not gain weight during the study but did maintain the same weight from Day -1 to +16. One dog in the control

group (BD 05T) also lost a small amount of weight from Day -1 to Day +16. See Table 3.

TABLE 3. Individual body weight (g) in Chihuahua dogs				
Dog ID no. (sex)	**Group (dose)**	**Day -14**	**Day -1**	**Day + 16**
BD 05T (M)	A (untreated)	2450	2200	2150
FF C9T (M)	A (untreated)	1550	1550	1650
E3 37T (M)	A (untreated)	1050	1250	1300
9D E6T (M)	A (untreated)	1000	800	950
8E F9T (M)	A (untreated)	550	550	650
B2 63T (F)	A (untreated)	1450	1550	1600
FD D0T (M)	B (0.67 mL)	2300	2050	2050
8E CET (M)	B (0.67 mL)	1900	1900	2300
F3 07T (M)	B (0.67 mL)	1250	1250	1650
FF F2T (M)	B (0.67 mL)	1000	1050	1300
AC 6BT (M)	B (0.67 mL)	500	550	750
AF 37T (F)	B (0.67 mL)	1250	1500	1550

[a] Data obtained from page 17, MRID 46672102.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption/efficiency**: Neither food consumption or efficiency were reported in the study.

D. OPHTHALMOSCOPIC EXAMINATION: Ophthalmoscopic examinations were not conducted.

E. BLOOD ANALYSES:

1. **Hematology**: Hematology results taken on the first day post-dosing showed some statistically significant ($p < 0.10$) differences in some of the measured parameters. However, the differences were minor and inconsistent among the treated dogs making them toxicologically insignificant. Mean white blood cell (WBC) count was increased significantly in the treated dogs when compared to controls on Day +1; however the values were still within the reference ranges provided. At all other time points, treated and control values were comparable.

2. **Clinical chemistry**: Clinical chemistry results taken on the first day post-dosing showed some statistically significant ($p < 0.10$) differences in some of the measured parameters. However, the differences were minor and inconsistent among the treated dogs making them toxicologically insignificant. At all other time points, treated and control values were comparable.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigator stated that the dose administered to Chihuahua dogs was well tolerated and offered the following conclusions in regard to the observed clinical signs. All of the dogs were given subcutaneous injections of a Rabies vaccine and prophylactic imidocarb 15 days prior to the start of the study in the dorsal neck area. The small subcutaneous swellings observed in 3 treated animals resembled injection reactions and the investigator concluded that was the likely cause of these swellings. The investigator also stated that both the pruritus and the hyperactivity were due to the application of a liquid to the skin. They supported this by stating the reactions were transient with no local skin reactions observed. The vomiting observed in the treated female may have been treatment-related because the alpha2 agonists found in Amitraz can cause emesis in some dogs; however, the study reports it was transient and not severe.

The investigator also concluded that the statistically significant ($p < .10$) differences in some of the hematology and clinical chemistry parameters were not physiologically relevant and were only present at the Day +1 timepoint.

The investigator concluded that administration of 0.67 ml to Chihuahua dogs with a body weight of 0.55 to 2.05 kg was well tolerated and safe for use in this breed at the labeled dose.

B. **REVIEWER COMMENTS:** The reviewer does not agree that the clinical signs observed in Chihuahua dogs administered the recommended dose of a metaflumizone/amitraz combination product showed a margin of safety at the 1X dose. The reviewer agrees that there were no treatment-related effects in any of the dogs on body weight, hematology and clinical chemistry parameters or overall general health. The pruritus that the investigator associated with the liquid on the skin and stated was transient, was still observed in some dogs for up to 7 hours post-treatment. The reviewer does not agree that the subcutaneous swellings were associated with post-injection swellings as this was not observed in any control animals and was only observed

post-treatment in the treated animals. The vomiting in the only female treated occurred with both applications and suggests a treatment-related effect; although a higher number of females would have to be tested to confirm this finding.

This 21-day toxicity study in the dog is **UNACCEPTABLE/NONGUIDELINE** and does not satisfy the guideline requirement for a companion animal safety study (OPPTS 870.7200). Only one dose level was tested and the number of animals tested (5 males and 1 female) was inadequate. Because the administration of 0.67 mL (the recommended labeled dose) topically to Chihuahuas produced clinical signs, a margin of safety was not established.

C. **STUDY DEFICIENCIES:** The study is deficient in the following:

1. No explanation of the concern for the increased sensitivity in the Chihuahua dog was given.

2. The study was not conducted according to guideline recommendations as only 1X of the recommended dose was administered. A true margin of safety can not be determined as there were clinical signs observed in the treated dogs at this dose. However, due to the use of personally owned dogs, conducting the study under guideline requirements would not be feasible.

3. The study was also deficient in that there was only one female in each of the groups, and the treated female vomited after each treatment. Testing of additional females could establish if this was a treatment-related finding. Also, the total numbers of dogs/sex were less than the recommended 6/sex/dose.

4. The study did not measure food consumption in any of the animals.

DATA FOR ENTRY INTO ISIS

Companion Animal Safety Study - dogs (870.7200)

PC codes	MRID	Study	Species	Duration	Route	Admin	Dose range	Doses	NOAEL	LOAEL	Target organ	Comments
281250 281251 106201	46672102	non-guideline companion animal safety	dog, Chihua hau	21days	derm al	dermal	0, 0.67 mL	0, 1X label dose	Not established	0.67 mL	not established	n/a

DATA EVALUATION RECORD

METAFLUMIZONE AND AMITRAZ SPOT-ON FORMULATION

STUDY TYPE: COMPANION ANIMAL SAFETY STUDY - DOGS - (NON-GUIDELINE)
MRID 46672103

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 125-2006

Primary Reviewer:
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Signature: Dana F. Glass
Date: JAN 0 9 2006

Signature: Robert H. Ross
Date: JAN 0 9 2006

Signature: L. A. Wilson
Date: JAN 0 9 2006

Disclaimer

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

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

EPA Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Technical Review Branch, Registration Division (7505P) Date: 8/21/2006

EPA Work Assignment Manager: P.V. Shah, Ph.D. Signature: P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) Date: 8/21/06

TXR#: 0053824

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/ Dogs [Non-guideline]

PC CODES: 281250, 281251 (metaflumizone); 106201 (amitraz)

DP BARCODE: D322975 **SUBMISSION NO.:** NA

TEST MATERIAL (PURITY): Metaflumizone (15% w/v); amitraz (15% w/v)

SYNONYM: ProMeris for Dogs

CITATION: Lindahl, R.G. (2005) Safety evaluation study of oral exposure from auto-or allogrooming R-28153/amitraz spot-on formulation in dogs. MPI Research, Inc., Mattawan, MI. Study number 817-014, April 29, 2005. MRID 46672103. Unpublished.

SPONSOR: Fort Dodge Animal Health, P.O. Box 5366, Princeton, NJ 08543-5366

EXECUTIVE SUMMARY: In a ten-day non-guideline companion animal safety study (MRID 46672103), four 5-6 month-old beagle dogs/sex were administered a single oral dose of 0.14 mL/5.0-9.9 kg, which is 0.1 times the proposed commercial dermal dose of a R-28153/amitraz spot-on formulation (Lot #0481704; 15% w/v of each active ingredient). The dogs were observed for nine additional days post-dosing. The placebo control (0.8% bacteriostatic sodium chloride for injection) was administered at the same volume. All animals were observed at least twice a day for morbidity and mortality. Detailed clinical observations were conducted daily. On the treatment day (Day 1), animals were examined prior to treatment and at 5 to 15 minutes, 30 to 45 minutes and 1, 2, 3 and 10 hours post-treatment. Heart rate and body temperature were recorded on Day 1 at 2 and 10 hours post-dosing. Body weight, body weight gain and food consumption were recorded periodically pre- and post-dosing. Hematology and clinical chemistry parameters were measured on Days -1, 2 and 8.
All dogs survived to study termination. The taste of the formulation

caused immediate effects in all of the treated dogs upon dosing and included: head shaking, spitting, licking and/or salivation. Other clinical signs observed post-dosing were attributed to amitraz toxicity. Decreased activity was reported in 2/4 males and 3/4 females beginning 1-2 hours post-dosing and continuing for several hours with normal activity by 10 hours post-dosing. Also observed post-dosing in 1/4 of the males and 1/4 of the females was pale gums, skin cold to the touch, and slow capillary refill time. Another female had some ataxia post-dosing. These were seen starting at 1-2 hours post-dosing with most subsiding by 6 hours post-dosing. Body temperature was decreased post-dosing in males and females with the effect lasting up to 10 hours post-dosing in females. A decrease in mean heart rate was observed post-dosing in both the treated males and females with females being more affected. This was first observed at 2 hours post-dosing and rates did not become comparable to controls until 10 hours post-dosing in males and Day 2 in females. All other effects subsided by Day 2 post-dosing.

There was no treatment-related effect on body weight or food consumption. Significant differences in hematology parameters were found in treated groups only when the data for both sexes were combined; these were not considered treatment-related. Several clinical chemistry parameters were affected by treatment only when the data for both sexes were combined so the toxicological significance of the findings is questionable. The only clinical chemistry difference which could be treatment-related is the increase in blood urea nitrogen (BUN) on Day 2 in treated females; however, this was comparable to controls by Day 8.

Amitraz is approved by the U.S. Food and Drug Administration (FDA) as a prescription veterinary drug for the treatment of demodectic mange in dogs as a 19.9% (w/w) formulation under the trade name Mitaban. Comparison of clinical signs observed in acute oral studies with Mitaban (cited in the package insert for the product) and the metaflumizone/amitraz formulation can be made. In the metaflumizone/amitraz study, at a mean mg/kg dose of approximately 3 mg/kg in males and 4 mg/kg in females, decreased activity, pale gums, skin cold to the touch, ataxia, slow capillary refill time and decreased body temperature were observed. Some of these signs persisted until 10 hours post-dosing. In the Mitaban single oral dose study at a comparable dose (4 mg/kg), the only clinical sign was decreased rectal temperature. Some of the signs observed at 20 mg/kg, the mid-dose treatment in the Mitaban study, were also reported with the metaflumizone/amitraz combination, including decreased activity and ataxia.

Toxicological effects were observed in some of the dogs. **In conclusion, oral administration of 0.14 ml of the metaflumizone/amitraz combination (15% w/v of each ingredient) to Beagle dogs caused**

some toxicological effects such as pale gums, slow capillary refill time, skin cold to the touch and decreased heart rate.

This companion animal safety in the dog is **Acceptable (Non-guideline)** and **satisfies** the intent for which it was conducted as a measure of toxicity when the topical product is inadvertently ingested.

COMPLIANCE: The study was not conducted in strict compliance with Good Laboratory Practice regulations. Signed and dated Quality Assurance and Data Confidentiality Statements were present.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: R-28153 (metaflumizone)/amitraz formulation

Description:	Liquid in amber glass bottle
Lot #:	0481704
Purity:	R-28153 (15% w/v); amitraz (15% w/v)
Compound Stability:	Expiration date: March 2005
CAS #of TGAI:	Not provided

2. Placebo control: Bacteriostatic 0.9% Sodium Chloride for Injection, USP (Lot-#16-502-DK)

3. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Age at dosing, males: 23-26 weeks; females: 23-24 weeks; weight at randomization, males: 6.40-7.74 kg; females: 5.16-5.87 kg	
Source:	Covance Research Products, Kalamazoo, MI	
Housing:	Pair-housed in stainless steel cages until Day -4, individually after that	
Diet:	Lab Diet Certified Canine Diet #5007 *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	70-77°F
	Humidity:	32-68%
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Two weeks	

B. STUDY DESIGN:

1. **In life dates:** Start: January 5, 2005; End: January 13, 2005

2. **Animal assignment**: Within each gender, the animals were ranked by weight, assigned to eight blocks of two and then randomly assigned to treatment groups within each block (Table 1). The assignments were known only to the study director, the test material control department, the dosing technician and the department manager.

TABLE 1. Animal assignment[a]

Group	Males	Females	Dose Volume (ml/5.0-9.9 kg)	R-28153/Amitraz (mg/5.0-9.9 kg)
Control	4	4	0.14	0
R-28513/Amitraz	4	4	0.14	21/21

[a] From Page 12 of MRID 46672103.

A single dose of 0.14 mL of the test substance or placebo control was administered orally on Day 1 (there was no Day 0). The oral route is a potential route for inadvertent exposure to the proposed topical spot-on formulation. The dose (10% of the total topical application) is that amount which might be consumed due to licking.

C. **METHODS:**

1. **Observations:**

1a. **Cageside observations**: All animals were observed at least daily for morbidity, mortality, injury and the availability of food and water.

1b. **Clinical examinations**: Clinical observations were conducted twice daily, at least 4 hours apart beginning on Day -4 through Day -1 to establish a baseline prior to treatment. Beginning on Day 1 (there was no Day 0), animals were observed at least once daily until study termination. On the treatment day, animals were examined prior to treatment, at 5 to 15 minutes, 30 to 45 minutes and 1, 2, 3 and 10 hours post-treatment. If an adverse reaction or behavior was observed, the clinical observations continued at hourly intervals until 10 hours post-dosing or until the reaction or behavior was resolved. Heart rate and body temperature were recorded on Day 1 at 2 and 10 hours post-dosing.

1c. **Physical and neurological examinations**: A veterinarian conducted a complete physical and neurological examination (including heart rate and body temperature) on all animals on Day -1, approximately 4 hours post-dosing on Day 1 and on Days 2 and 8.

2. **Body weight:** Animals were weighed and the weight recorded on Days -14, -7, -1 and 8. The values obtained on Day -1 were used as a baseline measurement.

3. **Food consumption:** Food consumption was measured on Days -4, -3, -2, daily beginning on Day 1 and through study termination, but not on Day -1.

4. **Hematology and clinical chemistry:** Blood was collected from the jugular vein of fasted animals on Days -1, 2 and 8. The CHECKED (X) parameters were examined.

a. **Hematology:**

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

Recommended for companion animal safety studies based on Guideline OPPTS 870.7200

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes suggested)*	X	Total bilirubin*
		X	Direct bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G Ratio
X	Alanine aminotransferase (also SGPT)*	X	Amylase
X	Aspartate aminotransferase (also SGOT)*	X	Lipase
	Sorbitol dehydrogenase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for companion animal safety studies based on Guideline OPPTS 870.7200

5. **Study termination**: The animals were transferred to a stock colony at study termination.

6. **Statistics:** Statistical analyses were conducted for each parameter, as described below.

   Body weight and food consumption: Body weight values from Days -1 and 8 were used for the analyses. The three pre-test measurements of food consumption were averaged to provide one pre-treatment value. Body weight and food consumption were analyzed for the post-treatment time point using the PROC MIXED procedure in SAS with pretreatment as the covariate and main effects of treatment, sex and the interaction by sex.

   Clinical pathology parameters: The pretreatment measurement was considered covariant in the analysis. For count data, such as leukocyte count, natural log-transformation was used. Hematology, coagulation and clinical chemistry parameters were analyzed using the PROC MIXED procedure in SAS with pretreatment measurements as corresponding covariates and treatment, sex, and treatment by sex as fixed main effects and interactions.

   Heart rate and body temperature: A repeated measures analysis was performed for heart rate and body temperature using the PROC MIXED

procedure in SAS with pretreatment measurements at Day -1 as the covariate and treatment, sex, treatment by sex, time, treatment by time, sex by time and treatment by sex by time as fixed effects and interactions. Time was the repeated fixed effect.

II. RESULTS

A. OBSERVATIONS

1 **Exposure levels**: The mean predose weight of treated males was 7.13 kg (range: 6.58-7.74 kg); the mean mg/kg dose was 2.96 (2.71-3.19 mg/kg). The mean predose weight of treated females was 5.58 kg (5.16-5.87); the mean mg/kg dose was 3.78 (3.58-4.07 mg/kg).

2. **Mortality**: All animals survived to study termination

3. **Clinical Signs of Toxicity**: Data are presented in Table 2. At the time of dosing, observations of head shaking, spitting, licking, and/or salivation were noted in treated animals but not in the control group. Decreased activity was observed in two males and three females in the treated group beginning one to two hours post-dosing and continuing for several more hours. Normal activity returned by 10 hours post-dosing. One of these males also had pale gums and skin cold to the touch at 2, 3, 5 and 6 hours post-dosing. One female with decreased activity also had ataxia at 1, 2 and 3 hours post-dosing; another had pale gums and slow capillary refill time at 1, 2 and 3 hours post-dosing and skin cold to touch at 5, 6, 7, 8, 9 and 10 hours post-dosing. None of these observations was reported in control animals.

   During the physical and neurological examinations, body temperature was decreased in treated males at the 2 and 4-hour post-dosing intervals and returned to normal by 10 hours post-dosing. In treated females, body temperature was decreased at the 2, 4 and 10 hours post-dosing. On Day 2, body temperature was comparable to the control group in females (Table 2). A decrease in mean heart rate was observed post-dosing in both the treated males and females with females being more affected. This was first observed at 2 hours post-dosing and rates did not become comparable to controls until 10 hours post-dosing in males and Day 2 in females.

TABLE 2: Incidence (Number Affected) of Clinical Findings on Day 1[a]

Finding	Males (n = 4)		Females (n = 4)	
	Control	Treated	Control	Treated
Activity decreased				
1 hr post-dose	0	0	0	2
Unscheduled	NA	2	0	1
2 hr post-dose	0	2	0	3
3 hr post-dose	0	2	0	3
5 hr post-dose	0	2	0	2
6 hr post-dose	0	2	0	1
7 hr post-dose	0	2	0	0
8 hr post-dose	0	2	0	0
9 hr post-dose	0	1	0	0
Discolored gums				
1 hr post-dose	0	0	0	1
Unscheduled	0	1	0	1
2 hr post-dose	0	1	0	1
3 hr post-dose	0	1	0	1
5 hr post-dose	0	1	0	0
6 hr post-dose	0	1	0	0
Skin cold to touch				
2 hr post-dose	0	1	0	0
3 hr post-dose	0	1	0	0
5 hr post-dose	0	1	0	1
6 hr post-dose	0	1	0	1
7 hr post-dose	0	0	0	1
8 hr post-dose	0	0	0	1
9 hr post-dose	0	0	0	1
10 hr post-dose	0	0	0	1
Ataxia				
1 hr post-dose	0	0	0	1
Unscheduled	0	0	0	0
2 hr post-dose	0	0	0	1
3 hr post-dose	0	0	0	1
Slow capillary refill time				
1 hr post-dose	0	0	0	1
Unscheduled	0	0	0	1
2 hr post-dose	0	0	0	1
3 hr post-dose	0	0	0	1
Mean body temperature (°C) ± SD				
Baseline	38.25 ± 0.31	38.65 ± 0.49	38.68 ± 0.21	38.30 ± 0.28
2 hr post-dose	38.15 ± 0.13	37.80 ± 0.39*	38.48 ± 0.28	37.50 ± 0.08*
4 hr post-dose	38.20 ± 0.47	37.63 ± 0.15*	38.38 ± 0.39	37.33 ± 0.29*
10 hr post-dose	38.70 ± 0.36	38.63 ± 0.36	38.58 ± 0.47	37.95 ± 0.34*
Mean heart rate (beats/15 seconds ± SD)				
Baseline	28.3 ± 6.95	29.8 ± 7.27	27.8 ± 4.57	27.8 ± 6.80
2 hr post-dose	23.5 ± 3.42	21.7 ± 4.04	23.3 ± 2.50	19.0 ± 2.45
4 hr post-dose	25.5 ± 7.85	24.3 ± 2.22	24.8 ± 3.77	19.8 ± 4.27
10 hr post-dose	26.0 ± 2.58	25.8 ± 2.87	23.3 ± 4.27	19.8 ± 1.50
Day 2	24.0 ± 5.48	23.3 ± 2.50	23.3 ± 3.10	21.5 ± 2.65

[a] Data from pages 33-36, 55-58, and 64-65, MRID 46672103.
NA = not applicable
* Significant at 5% level

B. BODY WEIGHT AND FOOD CONSUMPTION: There was no evidence of a treatment-related effect on body weight or food consumption during the study.

C. BLOOD ANALYSES:

1. **Hematology**: Several hematology parameters were significantly affected but only when the data from both sexes were pooled. Leukocytes and monocytes were significantly increased and mean corpuscular volume (MCV) decreased on Day 2. On Day 8, lymphocytes were increased and eosinophils were decreased. None of these changes are considered treatment-related since they did not occur when the data for the separate sexes were analyzed.

2. **Clinical chemistry**: Multiple clinical chemistry parameters were affected but the toxicological significance of the findings is questionable. The magnitude of the changes was small and some differences were significant only when the data for both sexes were pooled. Sodium was increased in males and decreased in females on Day 8. BUN was increased in females but only on Day 2. Phosphorus and alkaline phosphatase activity were significantly increased, while ALT activity, creatinine and glucose were significantly decreased on Day 2 when the data for both sexes were pooled. On Day 8, total bilirubin was increased and creatine kinase and creatinine were decreased when the sexes were pooled.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author attributed the following to treatment with the R-28153/amitraz formulation: decreased activity, skin cold to touch, pale gums and decreased body temperature on Day 1 post-dosing. The report concluded that the spot-on formulation, administered orally at 0.1X the proposed commercial topical dose, has no lasting deleterious effects.

B. REVIEWER COMMENTS: All dogs survived to study termination. Immediate effects observed in the treated group at the time of dosing included: head shaking, spitting, licking and/or salivation and were due to the taste of the spot-on formulation. Other signs observed later on the day of dosing in the treated group are attributed to the toxicity of amitraz. Decreased activity was reported in two males and three females beginning 1-2 hours post-dosing and continuing for several hours; normal activity was observed by 10 hours post-dosing. Some of these animals also had pale gums, skin cold to the touch, ataxia and slow capillary refill time. Body temperature was decreased post-dosing in treated males and females; the effect was still observed at 10 hours

post-dosing in females. This finding correlates with the skin cold to touch observation in treated females that was still evident at 10 hours post-dosing. A decrease in mean heart rate was observed post-dosing in both the treated males and females with females being more affected. This was first observed at 2 hours post-dosing and rates did not become comparable to controls until 10 hours post-dosing in males and Day 2 in females.

There was no treatment-related effect on body weight or food consumption. Significant differences in hematology parameters were reported in treated groups only when the data for both sexes were combined and are not considered treatment-related. Several clinical chemistry parameters were affected by treatment only when the data for both sexes were combined so the toxicological significance of the findings is questionable. The only change possibly treatment-related was a transient increase in BUN on Day 2 in treated females. This effect was reported in oral studies with amitraz, as discussed below.

Amitraz is approved by the U.S. Food and Drug Administration (FDA) for the treatment of demodectic mange in dogs as a 19.9% (w/w) formulation under the trade name Mitaban. Acute and subchronic oral studies conducted in non-diseased beagles are cited in the package insert for the product.[1] In an oral toxicity study, death occurred in one of two dogs given a single oral dose of 100 mg/kg. Clinical signs included central nervous system depression, ataxia, hypothermia, bradycardia, muscular weakness, vomition, uncontrolled vocal spasm and micturation. Hemoconcentration and transient elevations in blood glucose, BUN, potassium and alkaline phosphatase activity were reported. Dogs given a single oral dose of 20 mg/kg had similar, though less pronounced, clinical signs and were normal at three days post-treatment. Hemoconcentration and increased BUN were noted, along with transient increases in glucose and alkaline phosphatase activity. Dogs given a single oral dose of 4 mg/kg had decreased rectal temperature within three hours but were normal by 24 hours post-treatment.

In a subchronic study, dogs were administered an oral dose of 0, 0.25, 1 or 4 mg/kg once daily for 90 days. There were no deaths. At 3 hours post-treatment and for the initial three days of the 90 day experiment,

[1] http://vetmeddirect.com/sbsite.php?&item=012upjmit10.6

dogs treated with 4 mg/kg exhibited CNS depression and ataxia; the effects remained for 3 to 6 hours and the dogs were normal within 24 hours post-treatment. Vomition occurred in two dogs on the initial two days of the study. Thereafter (days 4 through 90) the dogs appeared to be subdued for approximately 6 hours after dosing. Dogs treated with 1 mg/kg/day exhibited signs of depression (without ataxia) for 4-6 hours; subsequently the depression became less marked and of shorter duration. At 3 hours after dosing, dogs treated with 1 or 4 mg/kg consistently had subnormal rectal temperatures and pulse rates; both parameters returned to normal within 24 hours post-treatment. At 0.25 mg/kg/day, the dogs appeared normal throughout the experiment. Hyperglycemia consistently occurred in dogs treated with 1 and 4 mg/kg/day and rarely occurred in dogs at the 0.25 mg/kg level; this response peaked within 6 hours post-treatment and serum glucose values returned to normal within 24 hours after treatment.

In MRID 46672103, the mean mg/kg dose of the metaflumizone/amitraz formulation [15% (w/v) of each] was 2.96 and 3.78 mg/kg for males and females, respectively. At this dose, decreased activity, pale gums, skin cold to the touch, ataxia, slow capillary refill time, decreased body temperature and decreased heart rate were observed. Some of these signs persisted until 10 hours post-dosing. In the Mitaban [19% (w/w) amitraz] single oral dose study at 4 mg/kg, the dose comparable to the oral dose in MRID 46672103, the only clinical sign was decreased rectal temperature. Some of the signs observed at 20 mg/kg with Mitaban were also reported at 2.96-3.78 mg/kg in MRID 46672103, including decreased activity (comparable to CNS depression) and ataxia. Clinical pathology parameters could have been altered but not detected because the testing was not done close enough to the dosing. In the Mitaban studies, hyperglycemia was maximal at 6 hours post-treatment and returned to normal by 24 hours. In MRID 46672103, clinical pathology testing was conducted 24 hours after dosing so alterations could have been missed.

In conclusion, oral administration of 0.14 ml of the metaflumizone/amitraz combination (15% w/v of each) to Beagle dogs caused some toxicological effects such as pale gums, slow capillary refill time, skin cold to the touch and a decreased mean body temperature and heart rate.

This companion animal safety in the dog is **Acceptable (Non-guideline)** and **satisfies** the intent for which it was conducted as a measure of toxicity when the topical product is inadvertently ingested.

C. **STUDY DEFICIENCIES:** The conducting laboratory's reference values for the clinical pathology parameters should have been included

with the study report

DATA FOR ENTRY INTO ISIS

Companion Animal Safety Study - cats (870.7200)

PC codes	MRID	Study	Species	Duration	Route	Admin	Dose range	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
281250 281251 106201	46672103	non-guideline companion animal safety	dog	8 days	oral	oral	0.1X label dose	0, 0.1X label dose	not established	0.1X	clinical signs	Toxicity

DATA EVALUATION RECORD

METAFLUMIZONE AND AMITRAZ SPOT-ON FORMULATION

STUDY TYPE: COMPANION ANIMAL SAFETY - DOGS (PUPPIES) - (OPPTS 870.7200)

MRID 46672104

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 125-2006

Primary Reviewer: Virginia A. Dobozy, VMD, MPH	Signature: Date:	Robert H. Ross JAN 0 9 2006
Secondary Reviewers: Dana F. Glass, D.V.M.	Signature: Date:	Dana F. Glass JAN 0 9 2006
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross JAN 0 9 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L.A. Wilson JAN 0 9 2006

Disclaimer

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

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

EPA Reviewer: Byron T. Backus, Ph.D. **Signature:** Byron T. Backus
Technical Review Branch, Registration Division (7505P) **Date** 8/21/06

EPA Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date** 8/21/06

TXR#: 0053824

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety/ Dogs (Puppies) [OPPTS 870.7200]

PC CODES: 281250, 281251 (metaflumizone); 106201 (amitraz)

DP BARCODE: D322975 **SUBMISSION NO.:** NA

TEST MATERIAL (PURITY): Metaflumizone (15% w/v); amitraz (15% w/v)

SYNONYM: ProMeris for Dogs

CITATION: Lindahl, R.G. (2005) Safety evaluation study of repeated treatments with a topically applied spot-on formulation of R-28153 and amitraz in dogs. MPI Research, Inc., Mattawan, MI. Study number 817-011, April 27, 2005. MRID 46672104. Unpublished.

SPONSOR: Fort Dodge Animal Health, P.O. Box 5366, Princeton, NJ 08543-5366

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46672104), an R-28153/amitraz spot-on formulation (Lot #0481704; 15% w/v of each active ingredient) was topically applied at 14-day intervals over a 14-week period to four/sex/group beagle puppies (approximately 10 weeks old) at 0, 1, 3 or 5 X the label dose. The labeled dose is 0.7 mL for ≤5.0 kg, 1.4 mL for 5.1-10.0 kg and 3.4 mL for 10.1-25.0 kg dogs. The placebo control was administered at the same volume used to treat the 5X dose group. The product or control formulation was applied between the shoulder blades on the dorsal midline extending cranially and caudally. All animals were observed at least twice a day for morbidity and mortality. Clinical observations were conducted twice daily prior to treatment and at least once daily after treatment initiation. On each treatment day, animals were examined prior to treatment and 5 to 15 minutes, and 1, 2 and 10 hours post-treatment. Heart rate and body temperature were recorded during the 10-hour post-treatment observations. Body weight, body

weight gain and food consumption were recorded periodically pre- and post-dosing. Hematology, clinical chemistry and urinalysis parameters were measured once pre-treatment, the day after each dose and 7 days after treatments 1, 3, 5 and 7. Complete necropsy examinations were performed and organ weights were recorded for two animals per sex per group. Microscopic examination of tissues was conducted on two animals per sex from the control and 5X groups. The kidneys were examined in all groups.

One male at 3X (number 101) was euthanized *in extremis* on Day 3. The animal had soft/watery feces on Day -1, prior to dosing on Day 1 and again during the 1 and 2 hour post-dosing observations. By Day 2, pale gums at 10 hours post-dosing and decreased activity and watery feces were observed. On Day 3, the animal was ataxic and not eating; therefore, euthanasia was performed. No definitive cause of the clinical signs was established. Soft and/or watery or mucoid feces were observed in control and treated males and females pre- and post-dosing. Lacrimation and salivation were observed in both control and treated animals of both sexes post-dosing and the incidence of the signs was not increased in treated animals.

Body weight, body weight gain and food consumption were not affected by treatment. Blood urea nitrogen was increased on Days 58 and 86 when the data on both sexes were pooled, but there was no dose response on either day. Glucose was increased at the 5X dose on Day 36 and at the 3X and 5X doses on Day 58 when the data for both sexes were pooled. This could be treatment-related since it is a known effect of amitraz but without statistical significance in individual sexes, it is questionable.

No treatment-related findings were observed on macroscopic or microscopic examination of the tissues at necropsy. Mean absolute liver weight was slightly increased in treated males (102-117% of control value) and females (105-123% of control value). Mean relative liver weights were increased in males at the 5X dose (113% of control value) and all treated females (111-121% of control value). Because the increases were not accompanied by any lesions at histopathology and only two animals/sex/group were necropsied, these changes are not considered treatment-related.

The margin of safety for this spot-on formulation of metaflumizone and amitraz in dogs is 1X because the clinical signs of toxicity which led to euthanasia of the 3X dose animal may be treatment-related. However, the study is considered unacceptable due to deficiencies discussed below.

This companion animal safety in the dog (puppy) is **Unacceptable (Guideline)** and **does not satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in dogs (puppies). Study deficiencies included, but are not limited to, the following: the number of animals (four/sex/group) was less than the required six/sex/group; and 2) there are questions about whether the animals were healthy prior to treatment. See **Study Deficiencies** for details.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: R-28153 (metaflumizone)/amitraz formulation

Description:	Clear to yellow liquid
Lot #:	0481704
Purity:	R-28153 (15% w/v); amitraz (15% w/v)
Compound Stability:	Expiration date: December 31, 2004
CAS #of TGAI:	Not provided

2. Placebo control: Formulation with <0.15% w/w of active ingredients (Lot #0381701)

3. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	73 to 79 days of age; males: weight at randomization: 1.76-4.02 kg, females: 1.8-3.23 kg	
Source:	Covance Research Products, Kalamazoo, MI	
Housing:	Pair-housed until Day -5, individually after that	
Diet:	Lab Diet Certified Canine Diet #5007 *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	68 - 81°F
	Humidity:	30-60%
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Two weeks	

B. STUDY DESIGN:

1. **In life dates:** Start: July 14, 2004; End: October 18, 2004

2. **Animal assignment**: Separated by gender, the animals were ranked by weight, assigned to eight blocks of four and then randomly assigned to treatment groups within each block (Table 1).

TABLE 1. Animal assignment[a]

Group[b]	Dose volume			Number of applications	R-28153/Amitraz spot-on		
	mL/ ≤5.0 kg	mL/ 5.1-10.0 kg	mL/ 10.1-25.0 kg		mg/ ≤5.0 kg	mg/ 5.1-10.0 kg	mg/ 10.1-25.0 kg
Control	3.4	6.7	16.7	5	0	0	0
1X	0.7	1.4	3.4	1	105	210	510
3X	2.1	4.0	10.0	3	315	600	1500
5X	3.4	6.7	16.7	5	510	1005	2505

[a] From Page 13 of MRID 46672104.
[b] Each group consisted of four males and four females.

On Day -1, two control males and one female at 5X were replaced due to eye abnormalities observed during the physical examinations. One female at 3X was replaced due to a physical abnormality; the nature of the condition was not provided. Physical and neurological examinations were not conducted on the replacement animals on Day -1.

The test substance is proposed for packaging in volumes of 0.67 mL, 1.3 mL and 3.33 mL to treat dogs in weight categories ≤5.0 kg, 5.1-10.0 kg and 10.1-25.0 kg, respectively. To achieve multiples of the target dose, the volume for each weight band was multiplied by 1X, 3X and 5X.

For the application, a section of the hair between the shoulder blades on the dorsal midline extending cranially and caudally was separated. The test substance or placebo control was applied using a syringe containing the appropriate amount of the material. Care was taken to reduce run-off of the liquid. The dose volume for each animal was based on the body weight recorded on the day of dosing. The puppies were treated with seven topical applications, administered at 14-day intervals, over a 14-week period. Study personnel responsible for data collection did not have knowledge of the grouping of the dogs during the in-life portion of the study.

C. METHODS:

1. Observations:

1a. Cageside observations: All animals were observed at least twice a day for morbidity, mortality, injury and the availability of food and water.

1b. Clinical examinations: Clinical observations were conducted twice daily, at least 4 hours apart beginning on Day -4 through Day -1 to establish a baseline prior to treatment. Beginning on Day 1, animals were observed at least once daily until study termination. On each treatment day, animals were examined prior to treatment and 5 to 15 minutes, and 1, 2 and 10 hours post-treatment. Heart rate and body temperature were recorded during the 10-hour post-treatment observations.

1c. Physical and neurological examinations: A veterinarian conducted a complete physical and neurological examination (including heart rate and body temperature) on all animals on Days -13 and -6, the day prior to each of the seven treatment applications and beginning approximately 4 hours, 24 hours and 7 days post-dosing for each of the seven treatments.

2. **Body weight:** Animals were weighed and the weight recorded on the day of arrival, three times a week during the acclimation, prior to randomization (Day -5), three times a week during the first two weeks and then weekly for the remainder of the study.

3. **Food consumption:** Food consumption was measured daily and recorded weekly beginning the last four days of the acclimation period and continuing throughout the study.

4. **Hematology and clinical chemistry:** Blood was collected from fasted (food only) animals prior to each dose, the day following each dose (approximately 24 hours post-dose) and approximately 7 days post-dose for treatments 1, 3, 5 and 7. The CHECKED (X) parameters were examined.

a. Hematology:

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

* Recommended for companion animal safety studies based on Guideline 870.7200

b. Clinical chemistry:

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin*
x	Chloride*	x	Creatinine*
	Magnesium	x	Urea nitrogen*
x	Phosphorus*	x	Total Cholesterol*
x	Potassium*	x	Globulins*
x	Sodium*	x	Glucose*
	ENZYMES	x	Total bilirubin*
	(more than 2 hepatic enzymes suggested)*	x	Direct bilirubin*
x	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
x	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	x	A/G Ratio
x	Alanine aminotransferase (also SGPT)*	x	Amylase
x	Aspartate aminotransferase (also SGOT)*	x	Lipase
	Sorbitol dehydrogenase		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for companion animal safety studies based on Guideline 870.7200

5. Urinalysis: Urine samples were collected once prior to each dose, the day following each dose and 7 days post-dose for treatments 1, 3, 5 and 7. Urine collections were conducted for up to an 8-hour period during the normal work day. The CHECKED (X) parameters were examined.

	Appearance		Glucose
x	Volume		Ketones
x	Specific gravity/osmolality		Bilirubin
x	pH		Blood/blood cells
	Sediment (microscopic)		Nitrate
	Protein		Urobilinogen

6. **Sacrifice and pathology**: A complete necropsy was performed on a 3X male (number 101) that was euthanized *in extremis*. Two males and two females from each group were sacrificed and necropsied at the conclusion of the study. The animals included numbers 108 (control female) and 118 (1X male); both had sialoceles on clinical observation, and 132 (5X female) had chronic audible breathing and a cough. The other thirteen animals were selected randomly to achieve two/sex/group. The abdominal, thoracic and cranial cavities were examined for abnormalities and the organs removed, examined and where required, placed in neutral buffered formalin, except for the eyes which were fixed in Davidson's fixative. The lungs were infused via the trachea with formalin. Bone marrow evaluations were done on animals at the scheduled necropsies.

   The following organs were weighed and recorded at the scheduled necropsies and appropriate organ weight ratios were calculated: brain, adrenal glands, epididymides, heart, kidneys, liver, lung, pancreas, pituitary gland, prostate gland, mandibular salivary gland, spleen, testes, thymus gland and thyroid/parathyroid gland. Paired organs were weighed together. The thyroid/parathyroid and pituitary gland were weighed following fixation. The organs of the animal which was euthanized *in extremis* were not weighed.

   Tissues from the control and the 5X dose treated groups were examined microscopically using hematoxylin and eosin stain. Only tissues that were abnormal on macroscopic examination, including the kidneys, were microscopically examined in the 1X and 3X dose groups. Findings were graded using a four-step system. The pathologist and necropsy technicians were blinded to the treatment.

7. Statistics: Complex statistical analyses were conducted for each parameter, as described below.

   Body weight change: Any differences in body weight at Weeks 1 through 13 were analyzed using the PROC MIXED procedure in SAS with treatment, sex and treatment by sex as fixed effects. First, the treatment by sex interaction was tested at the 5% significance level. If found significant, then the treated groups' LSMEANS were compared with LSMEAN of the control group for each sex by the 2-sided Student's t-test at the 10% level. If treatment by sex interaction and treatment effect were found not significant for a parameter, no further analysis was conducted.

   Body weight and food consumption: A repeated measures analysis was performed for body weight and food consumption using the PROC

MIXED procedure in SAS. The covariate was the pretreatment measurements at Week -1 (Day -1 for body weight), and the fixed effects and interactions included: treatment, sex, treatment by sex, week, treatment by week, sex by week and treatment by sex by week. Week was the repeated fixed effect. Structure of the covariance matrix was investigated using three assumptions: 1) compound symmetry (CS); 2) autoregressive first order [AR(1)]; and 3) heterogeneous autoregressive first order [ARH(1)]. The assumption giving the minimum Akaike's Information Criterion (AIC) value for a parameter was selected for the final analysis with the selected covariance-matrix structure. Treatment by sex and treatment by sex by week were tested at the 5% level. Sex by week and treatment by week were tested at the 10% level.

Clinical pathology parameters: Pretreatment measurement was considered covariant in the analyses. For count data, such as leukocyte count, log-transformation was used. Hematology, coagulation and clinical chemistry parameters were analyzed by time using the PROC MIXED procedure with pretreatment measurements as corresponding covariates and treatment, sex, and treatment by sex as fixed main effects and interactions. First, treatment by sex interaction was tested at the 5% significance level. If significant for a parameter, then treated groups' LSMEANS were compared to the control group LSMEANs for each sex by the 2-sided Student's t-test at the 10% level. If treatment by sex interaction was not significant and treatment effect was significant at the 10% level, treated groups' LSMEANS were compared with LSMEAN of the control group by the 2-sided Student's t-test at the 10% level. If treatment by sex interaction and treatment effect were not significant for a parameter, no further analysis was done.

Heart rate and body temperature: A repeated measures analysis was performed for heart rate and body temperature using the PROC MIXED procedure in SAS with pretreatment measurements at Day -6 as the covariate and treatment, sex, treatment by sex, time, treatment by time, sex by time and treatment by sex by time as fixed effects and interactions. Time was the repeated fixed effect. The analyses used were similar to those described under body weight and food consumption.

II. RESULTS

A. OBSERVATIONS

1. **Exposure levels**: The mean and range of the topical mg/kg doses for the treated groups are presented in Table 2.

TABLE 2. Mean (range) (mg/kg) of topical doses to treated animals[a]

	Dose group (n = 4)		
Dose	1X	3X	5X
Males			
Dose 1	34.80 (28.46-41.02)	105.66 (90.0-119.32)	170.16 (142.06-195.04)
Dose 2	26.94 (22.01-31.44)	76.59 (68.78-90.52)[b]	137.10 (114.61-165.05)
Dose 3	31.24 (23.18-39.33)	94.91 (73.09-105.82)[b]	158.86 (123.49-194.39)
Dose 4	35.40 (30.43-41.67)	97.70 (87.08-113.85)[b]	130.96 (105.15-159.27)
Dose 5	31.10 (26.52-36.27)	84.37 (75.47-99.83)[b]	161.26 (134.26-192.16)
Dose 6	27.72 (23.73-32.11)	74.43 (66.96-87.98)[b]	140.50 (118.24-163.41)
Dose 7	26.07 (21.88-30.0)	70.29 (63.56-83.22)[b]	133.26 (108.65-159.78)
Females			
Dose 1	39.32 (36.08-42.34)	111.29 (86.3-130.17)	183.06 (147.8-247.57)
Dose 2	32.3 (29.83-35.35)	93.07 (78.16-104.3)	148.38 (121.14-197.67)
Dose 3	26.09 (24.48-27.34)	75.96 (65.49-85.37)	123.29 (102.82-153.61)
Dose 4	31.78 (21.52-41.92)	75.95 (63.77-102.44)	146.65 (119.44-180.76)
Dose 5	38.45 (37.04-41.10)	92.29 (64.95-108.11)	131.78 (102.62-163.41)
Dose 6	33.35 (32.01-36.21)	95.05 (77.62-111.32)	141.80 (113.33-173.28)
Dose 7	31.17 (29.96-33.39)	89.14 (71.68-102.56)	133.62 (113.59-160.54)

[a] Calculated by the reviewer from data on pages 908-921, MRID 46672104.
[b] Three animals in group.

2. **Mortality**: One male at the 3X dose (number 101) was euthanized *in extremis* on Day 3 after receiving one dose. The animal had soft/watery feces on Day -1, prior to dosing on Day 1 and again during the 1 and 2 hour post-dosing observations. By 10 hours post-dosing, the dog had pale gums and by Day 2, decreased activity and watery feces were observed. On Day 3, the animal was ataxic and not eating; therefore, euthanasia was performed. Clinical pathology values during the pretreatment period were normal, but on Day 2, glucose, albumin, sodium, chloride, calcium and phosphorus were decreased. The only macroscopic observation was a small

endocardial cyst in the right ventricle. On microscopic examination of the liver, the following were observed: diffuse, mild hepatocellular vacuolation; minimal single cell necrosis; and minimal Kupffer cell hypertrophy/hyperplasia. Mild lymphoid depletion was noted in the spleen, thymus, lymph nodes and bone marrow. Coccidia were present in the small intestine with no evidence of enteritis. The endocardial cyst noted macroscopically was a endocardial hematocyst on microscopic examination. No definitive cause of death was established. The dose for this animal was 115.81 mg/kg, and the range of doses for the remaining males in the 3X dose group was 90.0-119.32 mg/kg.

3. **Clinical signs of toxicity**: Soft and/or watery or mucoid feces were observed in control and treated males and females pre- and post-dosing. Lacrimation and salivation were also observed in control and treated animals of both sexes post-dosing. A treatment-related increase in clinical signs did not occur. One female at the 5X dose (number 132) had a persistent cough throughout the study. Decreased activity, nasal discharge, soft/mucoid feces, pale gums, lacrimation of both eyes and injected sclera were also observed periodically during the study. This animal was treated with two antibiotics, Clavamox from Days 43-53 and Baytril from Days 57-70, but the coughing persisted until the end of the study. A control female (number 108) and a male at the 1X dose (number 118) had sublingual mucoceles. Mucoceles are formed when saliva leaks from a damaged salivary duct or gland and then accumulates in the surrounding tissues. The cause of salivary mucoceles is rarely identified, although trauma such as from choke collars, bite wounds, or chewing on foreign materials is generally considered to be the most likely initiating event.[2] On Day 58, animal #108 was anesthesized with nitrous oxide and isoflurane and drainage of the mucocele was attempted. No fluid was obtained so the animal was treated with Clavamox for 14 days. The size of the mass remained the same but appeared uncomplicated so no further treatment was provided. Animal number 120, a male at the 5X dose, was treated with triple antibiotic eye ointment and a saline rinse on Days 72-83. No reason for the treatment was provided. The animal was observed to have lacrimation on Days 43-45 of the study.

During the physical and neurological examinations at 10 hours post-dosing, male number 101 (3X dose group) was noted to have a depressed righting reflex and absent panniculus reflex (twitching of

[2]http://www.acvs.org/AnimalOwners/HealthConditions/SmallAnimalTopics/SalivaryMucocele/

the skin in response to a pinch on either side of the spinal column) on Day 1 (4 hours post-dosing) and on Day 2. As discussed above, this animal was euthanized *in extremis* on Day 3. Body temperature was significantly decreased in sex pooled samples from all treated groups at several intervals during the study. The changes were usually noted on treatment days following dosing or on the day after treatment. The 5X dose group was most frequently affected. Summary data are presented in Table 3.

TABLE 3: Summary of significant ($p<0.1$) body temperature data[a]

Parameter	Sex	Day	Dose level increase (+)/ decrease (-)	Treated group (mean temp °C ± SD)	Control (mean temp °C ± SD)
Body temperature	Pooled	1 (10 hr post-dosing)	5X (+)	38.66 ± 0.39	38.36 ± 0.17
	Pooled	8	3X (-)	38.11 ± 0.17	38.29 ± 0.39
	Pooled	15 (4 hr post-dosing)	5X (-)	38.21 ± 0.24	38.58 ± 0.27
	Pooled	57 (10 hr post-dosing)	5X (-)	38.24 ± 0.19	38.56 ± 0.12
	Pooled	72	5X (-)	38.38 ± 0.31	38.60 ± 0.39
	Pooled	86	1X (-)	38.74 ± 0.16	39.15 ± 0.43
	Pooled	86	3X (-)	38.61 ± 0.36	
	Pooled	86	5X (-)	38.33 ± 0.32	

[a] Data from page 27, MRID 46672104

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weight and body weight gain data are presented in Table 4. There was no evidence of a treatment-related effect on body weight or body weight gain during the study.

TABLE 4: Mean body weight and body weight gain[a]

	Dose group							
	Males				Females			
	0	1X	3X	5X	0	1X	3X	5X
Body weight (kg ± SD)								
Day -1	2.9 ± 1.0	3.1 ± 0.5	3.0 ± 0.4	3.1± 0.5	2.6 ± 0.4	2.7 ± 0.2	2.9 ± 0.5	2.9 ± 0.6
Day 7	3.1± 1.1	3.5 ± 0.6	3.7 ± 0.6	3.5 ± 0.6	3.0 ± 0.5	3.0 ± 0.3	3.1 ± 0.5	3.2 ± 0.6
Day 21	3.9 ± 1.3	4.6 ± 0.7	4.8 ± 0.7	4.4 ± 0.7	3.7 ± 0.6	3.7 ± 0.1	3.9 ± 0.5	4.0 ± 0.7
Day 42	5.2 ± 1.2	6.0 ± 0.9	6.2 ± 0.8	5.6 ± 1.0	4.8 ± 0.8	4.9 ± 0.2	5.0 ± 0.6	5.0 ± 0.9
Day 96	8.7 ± 1.4	9.0 ± 1.3	9.7 ± 1.3	8.7 ± 1.7	7.3 ± 0.8	7.5 ± 0.4	7.6 ± 1.0	7.4 ± 1.6
Body weight gain (kg)[b]								
Day -1 to Day 7	0.2	0.4	0.7	0.4	0.4	0.3	0.2	0.3
Day 7-21	0.8	1.1	1.1	0.9	0.7	0.7	0.8	0.8
Day 21-42	1.3	1.4	1.4	1.2	1.1	1.2	1.1	1.0
Day 42-96	3.5	3.0	3.5	3.1	2.5	2.6	2.6	2.4
Day -1 to Day 96	5.8	5.9	6.7	5.6	4.7	4.8	4.7	4.5

[a] Data from pages 171-176, MRID 46672104, with values rounded off by the reviewer.
[b] Calculated by the reviewer from rounded off values in Table 4.

C. FOOD CONSUMPTION: Mean food consumption data are presented in Table 5. There was no evidence of a treatment-related effect.

TABLE 5: Mean food consumption[a] (g/animal/day ± SD)				
	Dose group			
	0	1X	3X	5X
Males				
Week -1	144.9 ± 59.0	176.9 ± 29.3	164.2 ± 65.2	194.1 ± 31.3
Week 1	142.3 ± 43.2	180.9 ± 36.6	200.9 ± 40.2	167.9 ± 36.5
Week 3	202.4 ± 51.1	224.3 ± 46.7	253.8 ± 31.0	239.6 ± 32.3
Week 6	225.1± 60.5	248.3 ± 50.9	259.6 ± 40.6	279.3 ± 82.2
Week 14	284.0 ± 39.6	280.7 ± 49.8	301.3 ± 39.2	280.9 ± 109.7
Females				
Week -1	156.0 ± 28.7	148.0 ± 15.8	149.9 ± 7.5	139.2 ± 32.9
Week 1	159.7 ± 40.7	138.5 ± 11.3	139.3 ± 25.1	176.6 ± 34.4
Week 3	230.0 ± 11.3	192.3 ± 40.8	210.3 ± 11.5	209.0 ± 24.7
Week 6	232.5 ± 85.1	281.2 ± 94.0	232.8 ± 44.7	237.7 ± 113.4
Week 14	264.7 ± 47.8	293.4 ± 144.8	255.2 ± 52.0	203.0 ± 62.7

[a] Data from pages 206-211, MRID 46672104.

D. BLOOD ANALYSES:

1. **Hematology**: Throughout the study, slight differences were observed in the male and female 5X dose groups when compared to controls in leukocytes, neutrophils, monocytes and fibrinogen but they were sporadic, in varied individuals and did not show a dose-response. Female 132 at 5X had markedly increased values for these parameters; however, it was most likely due to the respiratory illness in this animal. Some hematology parameters [red blood cells (RBC's), hemoglobin (HGB) and hematocrit (HCT)] were increased in all male treated groups on Day 36, but there was no dose response. HGB was significantly decreased in the 5X females on Day 86. HCT was significantly decreased in females at the 3X and 5X doses on Day 86. These changes are not considered treatment-related since they were sporadic and inconsistent.

2. **Clinical Chemistry**: Increases in blood urea nitrogen (BUN) occurred on Days 58 and 86 in all treated groups when sex-pooled data were analyzed. However, there was not a dose-response or corresponding changes observed in creatinine, urinalysis or histopathological lesions in the kidneys; therefore, the increase appeared to be from a non-renal cause. Glucose levels also increased in the males and females at the 5X dose following some of the treatments but the mean pre-treatment values were increased in this group as well and all values were within the expected range of values. Data are in Table 6.

TABLE 6: Summary of significant (p<0.1) clinical chemistry data[a]					
Parameter	**Sex**	**Day**	**Dose level increase (+)/ decrease (-)**	**Treated group (± SD)**	**Control (± SD)**
Urea nitrogen (mg/dL)	Pooled	58	1X (+)	15.3 ± 3.5	11.6 ± 1.9
	Pooled	58	3X (+)	18.1 ± 3.4	
	Pooled	58	5X (+)	15.9 ± 3.4	
	Pooled	86	1X (+)	16.4 ± 3.2	13.3 ± 2.8
	Pooled	86	3X (+)	19.3 ± 5.0	
	Pooled	86	5X (+)	17.6 ± 3.3	
Glucose (mg/dL)	Pooled	30	5X (+)	129.3 ± 26.9	108.0 ± 9.0
	Pooled	58	3X (+)	111.4 ± 15.3	96.4 ± 7.4
	Pooled	58	5X (+)	122.5 ± 15.8	

[a] Data from page 29, MRID 46672104

3. **Urinalysis:** No treatment-related findings were reported.

E. POSTMORTEM RESULTS:

1. Macroscopic observations: The only macroscopic lesions were a small endocardial cyst in the right ventricle of animal 101 (from the 3X dose group, euthanized on Day 3) and a small ectopic focus of splenic tissue on the urinary bladder serosa of a placebo control animal.

2. Organ weight: Data are presented in Table 7. Mean absolute liver weight was slightly increased in treated males (102-117% of control value) and females (105-123% of control value). Mean relative liver weight was increased in males at 5X the label dose (113% of control value) and in all treated females (111-121% of control value).

TABLE 7: Mean absolute (g± SD) and relative to body weight (%± SD)) liver weight data[a]				
Parameter	Dose group (n = 2)			
	Control	1X	3X	5X
Males				
Terminal body wt (kg ± SD)	8.52 ± 2.55	8.72 ± 1.42	9.18 ± 1.78	8.70 ± 2.28
Liver, absolute	224.54 ± 56.25	229.43 ± 15.20 (102)[b]	242.60 ± 37.49 (108)	262.53 ± 73.46 (117)
Liver, relative	2.66 ± 0.13	2.65 ± 0.26	2.65 ± 0.11	3.01 ± 0.06 (113)
Females				
Terminal body wt (kg ± SD)	7.54 ± 0.40	7.14 ± 0.62	7.80 ± 1.65	7.33 ± 2.54
Liver, absolute	190.47 ± 11.63	200.16 ± 10.11 (105)	233.42 ± 57.50 (123)	218.09 ± 38.7 (115)
Liver, relative	2.54 ± 0.29	2.82 ± 0.39 (111)	3.0 ± 0.11 (118)	3.07 ± 0.54 (121)

[a] Data from pages 877- 883 MRID 46672104.
[b] Number in parentheses is percent of control, calculated by reviewer.

3. **Microscopic observations:** The male in the 3X dose group (number 101) that was euthanized *in extremis* on Day 3 had mild hepatocellular vacuolation, minimal single cell necrosis, minimal Kupffer cell hypertrophy/hyperplasia and minimal subacute inflammation. Mild lymphoid depletion was present in the spleen and thymus. Coccidia were present in the small intestine but there was no evidence of enteritis. The endocardial cyst noted macroscopically was a endocardial hematocyst on microscopic examination.

   The female in the 5X dose group (number 132) with respiratory signs had chronic, active bronchial, bronchiolar and alveolar inflammation. In the other animals sacrificed at study termination, minimal tubular mineralization was reported in control and treated animals.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study report author concluded that multiple administrations of the R-28513/amitraz spot-on formulation, administered to mimic a full season of treatment in dogs beginning at 10 weeks of age at 1X, 3X and 5X the proposed commercial dose resulted in no substantial deleterious effects.

B. REVIEWER COMMENTS: The euthanasia of one male at the 3X dose *in extremis* and the inability to diagnose the cause of its illness are a concern. There was minimal evidence that the animal was not healthy prior to treatment. It had soft/watery feces on Day -1 and prior to dosing on Day 1. Clinical pathology values during the pretreatment period were normal. The health of the animal deteriorated by Day 2 and it was euthanized on Day 3. In a single dose study in eight-week old puppies with this R-28153/amitraz product (MRID 46401004), a female at the 3X dose was euthanized *in extremis* on Day 9 and a male at the 1X dose was found dead on Day 9. No cause of death could be determined for either animal. These deaths could be the result of including unhealthy animals in the studies; however, it cannot be ruled out that these animals were especially sensitive to the product.
There was no evidence that clinical signs of toxicity were increased in treated animals. Soft and/or watery or mucoid feces were observed in control and treated males and females pre- and post-dosing. Lacrimation and salivation were observed in control and treated animals of both sexes post-dosing. Amitraz is approved by the U.S. Food and Drug Administration (FDA) under the trade name Mitaban for the treatment of demodectic mange in dogs. In one dermal toxicity study submitted to FDA for approval of the product, non-diseased adult beagles were administered a single dose of either 250 (recommended dose), 1250 or 2500 ppm. The following were observed: transient sedation at all doses; decreased rectal temperature 4 hours post-treatment at 1250 and 2500 ppm; and increased glucose at 4 hours post-treatment at 250 ppm in females and at 1250 and 2500 ppm in both sexes.[3] Glucose and rectal temperatures returned to normal by 24 hours. In another study, groups of healthy beagles were topically treated with either 250, 750 or 1250 ppm of the active ingredient at 14-day intervals for 12 weeks. Glucose values were increased at 750 ppm 4 hours post-treatment after three of six treatments and after five of six treatments at 1250 ppm. In MRID 46672104, body temperatures were decreased in treated animals but only when data for both sexes were pooled.

Body weight, body weight gain and food consumption were not affected by treatment. Increases in WBC, fibrinogen, neutrophils and monocytes at the 5X dose when data for both sexes were pooled are most likely due to the large increases in these parameters in female #132, the animal with a respiratory illness. BUN was increased on Days 58 and 86 when pooled data were evaluated, but there was no dose response on either day. Glucose was increased at the 5X dose on Day 36

[3] http://vetmeddirect.com/sbsite.php?&item=012upjmit10.6

and at the 3X and 5X dose on Day 58 when data for both sexes were pooled. This could be treatment-related since it is a known effect of amitraz but without statistical significance in individual sexes, it is questionable. As discussed above, increased glucose levels at four hours post-treatment were observed in the Mitaban studies but the levels returned to normal by 24 hours. In MRID 46672104, clinical pathology testing was done 24 hours post-treatment; therefore, the timing was probably not adequate for detecting an increase.

No treatment-related findings were observed on macroscopic or microscopic examination of the tissues at necropsy. The increases in mean absolute and relative liver weights in treated groups were not accompanied by any histopathology findings and considering necropsy and organ weight data were evaluated from only two animals/sex/group, these changes are not considered treatment-related.

It is concluded that the clinical signs of toxicity which led to euthanasia of the one 3X dose animal may be treatment-related and therefore, the margin of safety for this spot-on formulation of metaflumizone and amitraz in dogs is 1X. However, the study is considered unacceptable due to deficiencies discussed below.

C. STUDY DEFICIENCIES:

1. The ingredients in the control group were not adequately identified. In the Certificate of Analysis for Lot #0381701 (page 905), the specifications for R-28513 and amitraz are given as less than 0.15% w/v and water content as not more than 0.1% w/w. The main ingredient is identified as "Density (M-3234)". The study report should have identified this chemical(s).

2. The study did not comply with the Companion Animal Safety Guideline (OPPTS 870.7200) in the following:

   a. Four animals/sex/group were used instead of the required six/sex/group.
   b. Observations should have been conducted hourly after dosing for at least four hours. They were conducted prior to treatment and 5-15 minutes, 1, 2 and 10 hours post-treatment.
   c. The Guideline states that animals should be healthy when entered into the study. The health of the puppies in this study is questioned. Four animals had to be replaced immediately prior to treatment; two were noted to be thin and two had increased activity on physical examination. Soft and/or watery or mucoid feces were observed in control and treated groups prior to dosing. Several animals required treatment during the study, including antibiotics for animal number 132 (5X female) that had a persistent cough throughout the study. Two animals (control female and 1X male) had sialoceles, one of which was treated with antibiotics. Another animal (5X male) was treated with an antibiotic eye ointment. Therapeutic treatments during a study could complicate the interpretation of findings in these animals.

3. Reference values for the clinical pathology parameters should have been provided.

4. Parameters were considered statistically significant at $p<.10$ which is not consistent with the accepted value of $p<0.05$.

DATA FOR ENTRY INTO ISIS

Companion Animal Safety Study - dogs (870.7200)

PC codes	MRID	Study	Species	Duration	Route	Admin	Dose range	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
281250 281251 106201	46672104	companion animal safety	dog (puppy)	14 weeks	topical	topical	1X-5X label dose	0, 1X, 3X, 5X label dose	1X	3X	clinical signs	Toxicity