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

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

March 31, 2008

MEMORANDUM

Name of Pesticide Product:	PROMERIS SPOT ON FOR DOGS
EPA Reg. No. /File Symbol:	80490-2
DP Barcode:	D348052
Decision No.:	386841
Action Code:	R340
PC Codes:	281250 & 281251 (Metaflumizone: 14.34%) 106201 (Amitraz: 14.34%)

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

Byron T. Backus 7-31-2008
M. Cole

To: Julie Chao/John Hebert, RM 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 review the attached reproduction studies, submitted in support of 80490-2, a combination product for dogs containing both metaflumizone and amitraz. A copy of the cover letter is included, along with copies of the proposed labelling and CSF..."

BACKGROUND:

The material received for review includes two dog (beagle studies), one titled: "Effects of Repeated 3x Treatments with Topically Applied Metaflumizone / Amitraz on the Seminal Quality in Male Beagles" (MRID 47303301; in 5 volumes), and the other titled "A Reproductive Study of Repeated 3X Treatments with a Topically Applied Spot-On Formulation of Metaflumizone / Amitraz in Female Beagle Dogs" (in MRID 47295301; in 3 volumes), and a CSF. The two submitted studies are being used as data to support a proposed label statement (also received) that: *Promeris*™ *for dogs* can be used in breeding males, as well as breeding, pregnant and lactating bitches."

COMMENTS AND RECOMMENDATIONS:

1. An Agency contractor, Oak Ridge National Laboratory, conducted the primary review of the two companion animal safety studies which were included in this submission. TRB performed the secondary review and made changes as necessary.

2. It should be noted the protocols for these non-guideline studies were not submitted to the Agency for review or comment prior to commencement of the studies. The Agency preference would have been for a maximum 5X dosage, rather than the 3X that was used in these studies, and there would have been additional comments regarding the scheduling of the doses and the adequacy of the study to support the proposed label claims on breeding, pregnancy and nursing female dogs.

3. The following is from the DER executive summary from the study on male beagles in MRID 47303301:

This companion animal safety study in adult male beagle dogs is **Acceptable/Non-Guideline;** it **does not satisfy** the normal guideline requirements for a companion animal safety study (OPPTS 870.7200) in the dog. The study adequately supports the claim that Promeris™ can be used on breeding male dogs. Study deficiencies observed (but not affecting the study results) include treatment with expired product, an incomplete study report, testing with only one dose and lack of laboratory references for semen parameters.

4. The following is from the DER executive summary from the study on female beagles in MRID 47295301:

This companion animal safety study in adult female beagles is **Unacceptable/Non-Guideline; it does not satisfy** the normal guideline requirements for a companion animal safety study (OPPTS 870.7200). The study does not support the proposed label claim that Promeris™ can be used on breeding female dogs. There was an application at approximately 4 weeks before the dogs were bred; the next application was one day after the second mating. TRB's concern is the reduced mean litter size in the 3X treated group (4.7 puppies vs 6.9 puppies in the control group) as it may have been due to a reduced number of implantations caused by exposure to the test substance at one day after the second mating. In addition, it is noted that the test material was not applied immediately before breeding or during the breeding session. Study deficiencies

affecting the study results included the lack of exact dates when applications to individual dogs were made and whether the dosages were 4.0 or 10 mL, lack of information on prior pregnancies and their results, absence of reporting as to the individual female ages or food consumption, insufficient data on the pups that were euthanized or died during the study, the use of only one dose and incomplete data to evaluate litter size.

DATA EVALUATION RECORD

AMITRAZ AND METAFLUMIZONE
COMPANION ANIMAL SAFETY STUDY- MALE DOGS – (NON-GUIDELINE)
MRID 47303301

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 1-11

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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.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: 7-31-2008

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- male dogs [Non-Guideline]

PC CODES: 106201 (amitraz); 281250 and 281251 (metaflumizone) **DP BARCODE:** 348052

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

TRADE NAME: ProMeris for Dogs

CITATION: McKeown, D. (2006) Effects of repeated 3x treatments with topically applied metaflumizone/amitraz on the seminal quality in male beagles. International Bio-Institute Corp., Fergus, Ontario, Canada. Test Facility Study No. IOSD-0405, October 24, 2006. MRID 47303301. Unpublished.

SPONSOR: Fort Dodge Animal Health, Princeton, N.J., 08543

EXECUTIVE SUMMARY: In a non-guideline companion animal safety study (MRID 47303301), groups of 8 male beagle dogs/group were administered topical applications of either the vehicle control (Lot # 0381701) or a metaflumizone-amitraz combination (both 15% w/v; Lot No. 0381702; ProMeris for dogs) at 3x the recommended dose every 30 days beginning on day -30 (cohort 1) or day -23 (cohort 2) and then on days 0, 30 and 60. The animals were examined for mortality and moribundity, clinical signs, evidence of irritation at the application site and body weight. Semen was collected on pretreatment days -47, and -33 (cohort 1) or -32 and -26 (cohort 2). Semen was collected from all dogs on days 7, 35, 49, 63, 77 and 91.

The dogs ranged from 2 to 6 years old, and weighed from 6.31 to 20.82 kg at first treatment, and from 6.31 to 22.43 kg immediately prior to any of the 4 treatments. Dogs weighing up to and including 9.92 kg were treated with 4.0 mL of vehicle control or test material, while dogs weighing 10.07 kg or more were treated with 10 mL of vehicle control or test material. Label directions specify application of 0.045 fluid oz. (1.33 mL) for dogs weighing from 11 to 22 lbs (5-10 kg), and an application of 0.113 fluid oz. (3.34 mL) for dogs weighing from 22 to 55 lbs (10-25 kg), so the 4.0 mL applications to dogs weighing from 6.31 to <10.0 kg and the 10.0 mL applications to dogs >10.0 kg and up to 22.43 kg were consistent with 3X application rates.

All dogs survived until the end of the study. No treatment-related clinical signs of toxicity or body weight effects were observed. Some control and treated dogs reacted to the application of the material (out of a total of 64 applications there were 8 incidences – 6 following 10.0 mL and 2 following 4.0 mL dosages - involving rolling around, scratching back and/or one or both ears) but there was no evidence of skin irritation.

Statistically significant changes in semen parameters were observed but were not biologically

significant as some variations between dogs are normal and differences between means for controls and treated dogs in the study were generally within 10%. Over all treatment days, the treated animals had a statistically significant lower mean total sperm per ejaculate (437.1 x 10^6 vs. 473.9 x10^6 in the control group). The total normal sperm per ejaculate was statistically lower in the treated group (355.8 x 10^6 vs. 393.0 x 10^6 for the control group; however, the report states that the lower limit for normal sperm counts in medium-sized dogs is 200 x 10^6/ejaculate). On day 49, the treated animals had a statistically higher percent of abnormal tails (2.8% vs. 1.5% in the control group). On day 77, the treated animals had a statistically higher percent of distal droplets (4.3% vs. 2.3% in the control group). From http://www.vet.purdue.edu/vcs/Peter/514fall99.htm turnover time for the dog is 70 days, while the period between first application of the test material and the last semen specimen in this study was 121 days. It is concluded then that if any significant effects had occurred impacting on spermatogenesis and/or transit time they would be evident in the results.

The study demonstrated that the amitraz-metaflumizone combination (ProMeris for Dogs) did not produce any biologically significant changes in semen parameters when administered to beagle dogs at 3x the recommended dose every 30 days for four treatments. The study is classified as acceptable, but did have numerous reporting deficiencies.

This companion animal safety study in adult male beagle dogs is **Acceptable/Non-Guideline;** it **does not satisfy** the normal guideline requirements for a companion animal safety study (OPPTS 870.7200) in the dog. However, the study adequately supports the claim that Promeris™ can be used on breeding male dogs. Study deficiencies observed (but not affecting the study results) includes treatment with expired product, an incomplete study report, only testing with a single dose and lack of laboratory references for semen parameters.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The study was not conducted in compliance with U.S. GLP as set forth in 40 CFR 160, but was conducted "in accordance within principles of Good Clinical Practices as outlined in VICH GL9."

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	Metaflumizone and Amitraz combination
Description:	Not provided
Lot #:	0381702
Purity:	15% w/v for both active ingredients
Storage:	Room temperature
Compound Stability:	The product tested expired on August 5, 2005. Therefore, the test material used in the study was over the expiration date by approximately 1 week and 5 weeks for the third and fourth treatments, respectively.
CAS #:	Not provided

2. **Vehicle and/or positive control:** The control is described as an investigational veterinary product vehicle with no active ingredients.

3. **Test animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Males: approximately 2-6 years; approximately 6-20 kg at first treatment	
Source:	Marshall BioResources (no address provided) and International Bio-Institute Corp. (Fergus, Ontario, Canada)	
Housing:	Individually in cages	
Diet:	Not provided	
Water:	Not provided	
Environmental conditions:	**Temperature:**	17-28^0 C
	Humidity:	25-75%
	Air changes:	14/hour
	Photoperiod:	Not provided
Acclimation period:	Seven days	

B. STUDY DESIGN:

1. **In life dates:** Start: June 16, 2005; End: October 15, 2005

2. **Animal assignment:** A total of 16 intact male beagles were entered into the study. For logistical reasons, the dogs were split into two cohorts of 8 dogs each. Within a cohort, dogs were ranked by decreasing weight into four blocks of 2 dogs per block. Dogs within a block were then allocated to either the control or treated group. The dose of the test material was three times the recommended commercial dose based on the weight categories below. The volume of vehicle applied to the control dogs was similar to the volume applied to the treated group.

Dog wgt. (kg)	Dose Vol. (mL/dog)
<5.0	2.0
5.0 – 9.9	4.0
10.0 – 24.9	10.0
25.0 – 39.9	16.0
40.0 – 49.9	20.0

3. **Dose selection rationale**: The dose was three times the label recommended dose.

4. **Preparation and treatment**: The hair at the application site on the dog's upper back between the shoulder blades was parted in a longitudinal line until the skin was visible. The end of a 10 mL syringe filled with the appropriate dose of either test material or vehicle was placed on the skin and a small amount was applied in a longitudinal line against the grain of the hair in a caudal to cranial direction. In the same area of the dogs back, a new line of hair was parted parallel to the first and more of the total dose volume was applied directly to the skin. This was repeated until the full volume was administered with minimal runoff into the hair coat. Treatments were administered approximately every 30 days, on study days -30 (cohort 1) or -23 (cohort 2), then on days 0, 30 and 60 to animals in both cohorts. The study was blinded to all personnel, except for the persons involved with administering the test material and vehicle.

5. Statistics: Semen quality data were analyzed by Repeated Measures Analysis using the SAS PROC MIXED Procedure with a model that considered the average pre-treatment measurement for each parameter as the covariate along with the fixed effects of treatment, day and the interaction treatment-by-day and the random effect of block. Day was the repeated measure.

In order to perform a repeated measures analysis, structure of the covariance matrix was investigated using three assumptions: 1) compound symmetry; 2) autoregressive first order; and 3) heterogeneous autoregressive first order. The assumption, which gave the minimum value of the Akaike's Information Criterion for a parameter, was selected as the best assumption for the final analysis. The covariate was tested at the 5% level of significance. As the covariate only has one degree of freedom, it was retained in the analysis even if found to be non-significant. The treatment effect and interaction were tested at the 10% level of significance. If the treatment-by-day interaction was found to be significant at the 10% level, a by-day analysis for that parameter was performed with a model that considered treatment as a fixed effect and block as a random effect.

Body weight data for each day were analyzed separately by an Analysis of Variance (ANOVA) with a model that considered treatment as a fixed effect and block as a random effect. Treatment was tested with the residual error at the 10% level of significance.

Least Square Means of each treatment group were computed and compared for all semen parameters and body weights. Since there were only two treatment groups, the ANOVA F-test was used to determine if the difference in the treated group, relative to the control group was significant at the 10% level.

C. METHODS:

1. Observations

a. General health observations: General health observations were conducted on each animal twice daily. The application site was examined for evidence of swelling, pain, erythema and heat at 1, 3, 6 and 24 hours post-treatment for each treatment day throughout the study.

b. Veterinary examinations: Physical examinations were conducted on days -53, -30, -23, -1, 29, 35, 60 and 91.

2. Body weight: Animals were weighed on days -53, -30, -23, -1, 29, 35, 60 and 91.

3. Food consumption: Food consumption was not measured.

4. Hematology and clinical chemistry: Blood was collected for hematology and clinical chemistry assessments on control and treated animals at trial commencement. The MARKED (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 corpuscle. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscle. volume (MCV)*
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

*Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. Clinical chemistry

	ELECTROLYTES		**OTHER**
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Sodium/Potassium ratio	X	Urea nitrogen*
X	Phosphorus *	X	Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, e.g., *)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase	X	Albumin/Globulin ratio
X	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*	X	BUN/Creatinine ratio
X	Gamma glutamyl transferase (GGT)		TCO_2 Bicarbonate
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		

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

5. Urinalysis: Urine was collected by catheterization at trial commencement. The CHECKED (X) parameters were examined.

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

6. **Fecal Examination:** A fecal analysis of fresh stool from each dog included a wet mount with saline and sucrose for *Coccidia, Giardia* and *Trichomonas* and fecal flotation for metazoan ova, cysts and oocysts.

7. **Heartworm testing:** Blood was collected for the presence of microfilariae using the Knott's technique during the baseline period. Blood was also examined for the presence of heartworm antigen.

8. **Semen collection and evaluation:**

a. Examination and sample collection: Treatment began on day -30 for cohort 1 and day -23 for cohort 2. Semen was collected on days -47 and -33 pre-treatment. Because some dogs had poor libido or had poor sperm morphology, two additional semen evaluations were conducted on days -32 and -26 for cohort 2. An estrogenized spayed female dog was used as a teaser dog. Semen was also collected from each dog on days 7, 35, 49, 63, 77 and 91. Tubes were exchanged when each of the second and third fractions of semen were noted in the tube or when the dog showed characteristic changes in behavior during collection or when more than 4-5 mL of the first fraction had been collected or if discolored semen was noted in the tubes. The external genitalia were examined at the same time and included visual inspection and palpation of the scrotum and palpation of scrotal contents (epididymides, spermatic cords and testes).

b. Motility and morphology assessment: Immediately following semen collection, motility was evaluated by examining at least 10 high power fields and evaluating how many of every ten sperm were moving at all ("total" as percent) and, of the ones moving, what percent were going in a straight line in a forward direction ("progressive" as percent). These parameters resulted in a number reflecting the total progressive motility of the semen. The speed of the movement was assessed simultaneously and scored as fast, moderate or slow. The presence of other solids in the second fraction was determined and scored as none to 4+. Solids in the second fraction were classified as bacteria, epithelial cells, white blood cells and other. Other included debris, crystals and the like. Following the motility assessment, a slide was prepared for sperm morphology evaluation using a drop of eosin/nigrosin stain and a drop of semen. One hundred sperm were counted and categorized as follows: normal morphology, head, midpiece or tail defects, proximal or distal cytoplasmic droplets as well as whether the heads had no tails, called "loose heads". The morphology assessments were conducted at the Theriogenology Laboratory at the University of Guelph's Ontario Veterinary College.

c. Semen analysis: After preparing the morphology slide, a sample of the second fraction was evaluated for sperm concentration using an Improved Nebauer Counting Chamber. The volume of the second fraction was multiplied by the concentration to yield the total sperm in the ejaculate. The color of the ejaculate and the pH of the second and third fractions were recorded. If the prostatic fluid (third fraction) had an appearance other than clear, a slide for cytological assessment was prepared and stained with Wright's/Giemsa.

d. Indices: From the semen quality data, total progressive motility (T_PMOT), total progressively motile sperm per ejaculate (T_PMSPE), total sperm per ejaculate (T_SPE) and total normal sperm per ejaculate (T_NSPE) were calculated as follows:

$$T\_PMOT = \frac{\%\ Motility \times \%\ Progressive\ Motility}{100}$$

$$T\_PMSPE = \frac{Sperm\ Conc. \times Vol.of\ Sperm\ Rich\ Fraction \times \%\ Motility \times \%\ Progressive\ Motility}{10{,}000}$$

$$T\_SPE = Volume\ of\ Sperm\ Rich\ Fraction \times Sperm\ Conc.$$

$$T\_NSPE = \frac{Volume\ of\ Sperm\ Rich\ Fraction \times Sperm\ Conc. \times \%\ Normal\ Sperm}{100}$$

9. Sacrifice and pathology: No animal died or had to be euthanized during the study. The 870.7200 Guidelines state that routine sacrifice or necropsy is not required for surviving animals.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity: One control dog was diagnosed with acute hemorrhagic colitis one hour post-treatment; the condition resolved by three hours post-treatment. The condition was attributed to the dog's super activity and nervousness. A treated dog had clear watery vomit at the one hour observation after the fourth treatment. A week later, the same dog had posterior weakness and reduced sensation and motor function to both hind legs. The dog recovered in approximately one week without treatment. Two treated dogs had loose stool on one occasion each. No treatment-related clinical signs were observed. None of these effects were considered treatment-related.

2. Application site examination: Three treated and three control dogs in both cohorts reacted to the application of the material on their back at the first treatment. One of the control dogs reacted on three more occasions (days 0, 30 and 60). Another treated dog reacted on day 60; this dog had bilateral hair loss on the flank and abdomen. None of the affected animals had evidence of skin irritation and did not require treatment.

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

B. BODY WEIGHT AND WEIGHT GAIN:

Body weight data are presented in Table 1. No treatment-related changes in mean body weight were observed.

Table 1: Mean body weight (kg) [a]		
Day	**Control group**	**Treated group**
Pretreatment	10.51	10.23
-1	10.96	10.70
29	11.42	11.09
35	11.48	11.15
60	11.45	11.12
91	10.86	10.43

[a] Extracted from Table IX, page 276, MRID 47303301.

C. FOOD CONSUMPTION: Food consumption was not measured.

D. CLINICAL PATHOLOGY ANALYSES: The study report stated that all results for hematology, fecal, urine and serum chemistry testing were normal, but only individual animal data were presented and no analyses were conducted.

E. SEMEN ANALYSES

1. Pre-treatment semen analyses: The covariate values were statistically significant for pH of sperm-rich fraction, percent normal sperm, percent abnormal head, percent loose head, pH of third fraction, total progressively motile sperm per ejaculate, total sperm per ejaculate and total normal sperm per ejaculate.

2. Sperm concentration and total sperm count: The mean sperm concentration was 227.4 x 10^6 and 278.1 x 10^6 in the treated and control groups, respectively. There was no significant difference between the Least Square means of the two groups when using pre-treatment values as a covariate. The total number of sperm per ejaculate was calculated by multiplying the sperm concentration by the volume of the sperm-rich fraction. The mean total sperm count per ejaculate was 437.1 x 10^6 and 473.9 x 10^6 in the treated and control groups, respectively. The ANOVA indicated that over all treatment days, the treated animals had a statistically significantly lower total sperm per ejaculate compared to animals in the control group, but this was not considered to be biologically significant (Table 2).

3. Sperm motility: No statistically significant difference in percentage total motility or in the percentage progressive motility was observed. The total progressively motile sperm per ejaculate was calculated by multiplying the percentage total progressive motility with the total sperm count per ejaculate. The mean value was 364.1 and 334.3 in the control and treated groups, respectively.

4. Sperm morphology: No significant difference in the percentage of morphologically normal sperm between the treated and control groups was observed. The total normal sperm per ejaculate was calculated by multiplying the percentage of normal sperm times the sperm per ejaculate. The total normal sperm/ejaculate was statistically significantly lower in the treated

group (355.8 x 10^6) as compared to the control group (393.0 x 10^6), but this was not considered to be biologically significant (Table 2).

No significant difference in the percentage of abnormal midpieces between the treated and control groups was observed. A statistically significant treatment-by-day interaction for percent of abnormal tails and percent of distal droplets was reported. Therefore, a day-by-day analysis was performed for these parameters. This analysis indicated that on day 49, the treated animals had a statistically higher percent of abnormal tails compared to the controls. The day-to-day analyses also indicated that on day 77, the treated animals had a statistically higher percent of distal droplets when compared to the control group, but these were not considered to be biologically significant (Table 2).

5. Semen pH: No significant difference in pH of the sperm-rich fraction or the prostatic fraction between the treated and control animals was observed.

6. Semen volume: Separation of the first and second ejaculate fractions into tubes was not always possible. Therefore, the volume of the sperm-rich fraction reflected the combined volume of the first and second fractions in some instances. There was no significant difference between the treated and control groups in the first fraction and sperm-rich volumes.

Table 2: Mean values for selected semen parameters[a]

Parameter	Control group	Treated group
PH2		
Day 7	6.3	6.1
Day 35	6.2	6.1
Day 49	6.3	6.1
Day 63	6.3	6.1
Day 77	6.3	6.3
Day 91	6.3	6.3
Mean	6.3	6.1
PNSPRM		
Day 7	82.3	80.3
Day 35	80.6	79.4
Day 49	86.0	82.1
Day 63	82.5	80.8
Day 77	83.1	75.0
Day 91	83.4	83.9
Mean	83.0	80.3
PABHD		
Day 7	3.0	4.1
Day 35	3.1	4.6
Day 49	3.5	5.1
Day 63	4.0	5.8
Day 77	3.1	6.4
Day 91	1.8	3.8
Mean	3.1	5.0

PLHD		
Day 7	6.0	5.3
Day 35	4.6	5.3
Day 49	3.8	3.8
Day 63	4.3	4.1
Day 77	4.1	4.3
Day 91	3.8	4.9
Mean	4.4	4.6

PTAIL		
Day 7	1.5	1.6
Day 35	1.3	1.1
Day 49	1.5	2.8*
Day 63	1.4	0.5
Day 77	1.4	1.0
Day 91	1.4	0.3
Mean	1.4	1.2
PDISTD		
Day 7	1.6	1.1
Day 35	4.8	2.9
Day 49	1.9	0.6
Day 63	2.4	2.8
Day 77	2.3	4.3*
Day 91	3.0	1.0
Mean	2.6	2.1
PH3		
Day 7	6.3	6.3
Day 35	6.4	6.3
Day 49	6.7	6.3
Day 63	6.5	6.4
Day 77	6.4	6.4
Day 91	6.5	6.4
Mean	6.5	6.3
T_PMSPE		
Day 7	275.7	312.8
Day 35	293.4	299.3
Day 49	316.1	237.8
Day 63	455.9	353.1
Day 77	403.9	419.9
Day 91	439.8	378.4
Mean	364.1	334.3
T_SPE		
Day 7	377.2	412.2
Day 35	374.7	392.1
Day 49	407.5	314.1
Day 63	584.7	449.1
Day 77	543.8	561.3
Day 91	555.6	488.4
Mean	473.9	437.1*

T_NSPE		
Day 7	307.7	335.1
Day 35	300.7	316.7
Day 49	351.4	259.8
Day 63	480.5	374.5
Day 77	453.4	425.4
Day 91	464.1	418.4
Mean	393.0	355.8*

[a] Extracted from Tables 1, 2 and 6, pages 256-273, MRID 47303301.
PH2 = pH of sperm-rich fraction; PNSPRM = percent normal sperm (%); PABHD = percent abnormal head (%); PLHD = percent loose head (%); PDISTD = percent distal droplet (%); T_PMSPE = total progressively motile sperm/ejaculate (in millions); T_SPE = total sperm/ejaculate (mL); T_NSPE = total normal sperm/ejaculate (mL)
* statistically significant compared to controls, $p<0.10$.

7. **External genitalia examination:** Some differences in the size of the testes and epididymides between the right and left sides were observed in both control and treated dogs, but there was no evidence of inflammation, such as swelling and pain.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that, although the treated group had a statistically significant lower total sperm count (and hence lower total progressively motile and total normal sperm) compared to the control group, these parameters increased over the course of the study in both groups. Furthermore, the differences both before and after treatments were not biologically significant as they were well within the normal variation seen in individual dogs. Therefore, the treatment appeared to have no biologically measurable effect on the dogs' semen parameters over three months of treatment.

B. REVIEWER COMMENTS: There was no evidence of systemic toxicity in male beagle dogs when the amitraz and metaflumizone combination (ProMeris for Dogs) was applied at 3x the recommended dose every 30 days for four treatments. All dogs survived until the end of the study. No treatment-related clinical signs of toxicity or body weight effects were observed. Both control and treated dogs reacted to the application of the material on their back, but there was no evidence of skin irritation.

Statistically significant changes in semen parameters were observed but were not biologically significant as some variations between dogs are normal and differences between means for controls and treated dogs in the study were generally within 10%. Over all treatment days, the treated animals had a statistically significant lower mean total sperm per ejaculate (437.1×10^6 vs. 473.9×10^6 in the control group). The total normal sperm per ejaculate was statistically lower in the treated group (355.8×10^6 vs. 393.0×10^6 for the control group; however, the report states that the lower limit for normal sperm counts in medium-sized dogs is 200×10^6/ejaculate). From http://www.vet.purdue.edu/vcs/Peter/514fall99.htm turnover time for the dog is 70 days, while the period between first application of the test material and the last semen specimen in this study was 121 days. It is concluded then that if any significant effects had occurred impacting on spermatogenesis and/or transit time they would be evident in the results.

The study report argues that the control dogs were heavier and thus had slightly more sperm per ejaculate in the pre-treatment analyses. This affected all the "total" parameters including total sperm per ejaculate, total progressively motile sperm and total normal sperm in each ejaculate. The study report further argues that statistical differences found after the pre-treatment differences were accounted for were not biologically significant and cites normal values for male beagle dogs. The percent of abnormal tails for day 49 was higher in the treated group (2.8% vs. 1.5% in the control group). The study report states that, when the percentage of sperm is evaluated, the testing laboratory expects values to be repeatable within 10% as regards percent normal. Normal dogs are considered to have less than 10% of any one morphological effect and percent normal >75%. Thus, it was argued one cannot see a significant biological difference between 2% and 3% of abnormal tails. A similar argument was made for the increase in the percent of distal droplets in treated dogs on day 77. The 2% difference between the treated (4.3%) and control (2.3%) groups would not be biologically significant. The statistically significant difference in total sperm counts between the control (393.0) and treated (355.8) groups were within the testing laboratory's 10% limit and would not be biologically meaningful. Because the total sperm counts were affected, the parameters total progressively motile sperm and total normal sperm were also changed.

Although this reviewer agrees that the findings were not biologically significant, more information should have been provided to help support the findings. The study author should have included the following to further explain the difference in semen parameters between the control and treated groups:

1) Data on the pre-treatment semen evaluations were not tabulated and presented in the study report or the statistician's report to establish that the values were higher in the control group. Even when the pre-treatment values in the control group were accounted for in the statistical analyses, significant differences were still found for several parameters.

2) The theriogenologist's report cited normal values for dog semen parameters, but no references were provided. The normal values should be for beagles and from the supplier of the animals.

3) The theriogenologist's report did not provide citations for the endnotes used to establish the lack of biological significance in the findings.

The study demonstrated that the amitraz-metaflumizone combination (ProMeris for Dogs) did not produce any biologically significant changes in semen parameters when administered to beagle dogs at 3x the recommended dose every 30 days for four treatments. The study is classified as acceptable, but did have a number of reporting deficiencies.

C. STUDY DEFICIENCIES:

1. According to the stability data, the product tested expired on August 5, 2005. The third and fourth treatments were on August 15, 2005 and September 14, 2005.

2. The study report was incomplete in the following:

a) The control was identified only as an investigational veterinary product vehicle with no active ingredients. It should be clarified if this is the ProMeris for Dogs product without the metaflumizone and amitraz.

b) Some data, including clinical pathology testing and pre-treatment semen analyses, were not provided.

c) The Table of Contents was not complete in that page numbers were not supplied for the Appendices.

3. The interval between treatments was not consistent for the two cohorts. Treatment 1 was administered on study day -30 for cohort 1 and on day -23 for cohort 2. The next treatment was on day 0 for both cohorts.

4. Having only one dose level made it more difficult to determine if some changes observed were related to treatment as the reviewer could not determine if a dose response had occurred.

DATA EVALUATION RECORD

AMITRAZ AND METAFLUMIZONE (R-28153)
COMPANION ANIMAL SAFETY STUDY- FEMALE DOGS (NON-GUIDELINE)
MRID 47295301

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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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:** 3-31-2008

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- reproductive study in female dogs; [Non-guideline]

PC CODES: 106201; 281250; 281251 **DP BARCODE:** 348052

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

SYNONYMS: Metaflumizone (R-28153)/Amitraz Spot-On; Promeris Spot On for Dogs

CITATION: Lloyd, Z. (2006) A reproductive study of repeated 3X treatments with a topically applied spot-on formulation of Metaflumizone/Amitraz in female beagle dogs. MPI Research, Inc., Mattawan, MI. Study No. 0817-C-US-16-05 (Sponsor) and 817-015 (Test Facility). October 31, 2006. MRID 47295301. Unpublished.

SPONSOR: Fort Dodge Animal Health, PO Box 5366, Princeton, N.J., 08543-5366

EXECUTIVE SUMMARY: In a non-guideline companion animal safety reproductive study (MRID 47295301), eight adult female beagle dogs/group were administered a spot-on formulation of a placebo control of metaflumizone (R-28153) and amitraz (both 0% w/v; Lot No. 0381701) or metaflumizone (R-28153) and amitraz (both 15% w/v; Lot No. 0381702) at 3x the recommended dose every 30 days for a total of four doses; doses were given as a single dose approximately 4 weeks (± 3 days) prior to anticipated mating, one day after the second mating, Day 28 of gestation and Lactation Day (LD) 5. Sixteen untreated males were utilized for breeding purposes only. The response to treatment in the adult females was evaluated by monitoring clinical observations, body weight, clinical pathology (hematology and clinical chemistry) and reproductive parameters (mating, parturition, and lactation). Pup growth and development were measured by calculating a stillborn index, whelping index, pup survival, and weaning index. Pups were also monitored for clinical signs, body weight, hematology and clinical chemistry parameters and by performing gross examination at necropsy on any descendents or sacrifices.

No deaths or treatment-related clinical signs of toxicity were observed in the adult beagles. Body weight, changes in hematology and clinical chemistry parameters measured were not affected by treatment. There were no treatment-related effects on fertility, gestation length, delivery or lactation. Litter size in the 3x females was statistically significantly lower than the control females, with 3/7 of the treated females having litters with ≤ 4 pups. Three pups (1 control and 2 treated) were found dead on day 0 but only one 3x pup was confirmed to be stillborn with hemorrhage in the abdominal cavity. The other two had normal observations at necropsy (control pup) or moderate perforations in the skin and thoracic cavity (3x pup). Four more control pups and 1 treated pup died during the 42 days of observation with either inconclusive causes of death

(control pups) or normal findings (treated pup) at necropsy. The stillborn index, whelping index, survival rate and weaning index were similar between treated and control dogs. There were more male pups in the control group and more females in the 3x group but this was not considered to be treatment-related. A statistically significant increase in body weight of male pups in the 3x group was observed when compared to the controls but this was not an adverse effect. No treatment-related differences were observed in the hematology or clinical chemistry parameters measured in the pups.

There was a statistically significant (p = 0.0444) reduction in mean litter size in the 3x group (4.7 pups) as compared to controls (6.9 pups). This was associated with two 3x litters which each contained only 2 puppies; a third 3x litter contained only 4 puppies. Litter sizes in the placebo control group ranged from 5 to 8 puppies, as did 4/7 litters in the 3x group.

This companion animal safety study in adult female beagles is **Unacceptable/Non-Guideline; it does not satisfy** the normal guideline requirements for a companion animal safety study (OPPTS 870.7200). The study does not support the proposed label claim that Promeris™ can be used on breeding female dogs. There was an application at approximately 4 weeks before the dogs were bred; the next application was one day after the second mating. TRB's concern is that the reduced mean litter size in the 3X treated group (4.7 puppies vs 6.9 puppies in the control group) may have been due to reduced number of implantations caused by exposure to the test substance at one day after the second mating. In addition, it is noted that the test material was not applied immediately before breeding or during the breeding session. Study deficiencies observed but not affecting the study results included the lack of exact dates when applications to individual dogs were made and whether the dosages were 4.0 or 10 mL, lack of information on prior pregnancies and their results, absence of reporting as to the individual female ages or food consumption, insufficient data on the pups that were euthanized or died during the study, the use of only one dose and incomplete data to evaluate litter size.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Metaflumizone (R-28153)/Amitraz Spot-On

Description:	Liquid in an amber glass vial
Batch #:	Lot No. 0381702
Purity:	15% w/v R-28153/15% Amitraz
Compound Stability:	May 26, 2006
CAS #:	Metaflumizone: 139968-49-3; Amitraz: 33089-61-1

2. **Vehicle and/or positive control:** A placebo control of Metaflumizone (R-28153) (0%w/v) / Amitraz (0% w/v) (Lot No. 0381701) was used as a control.

3. **Test animals:**

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	Adult (no specific or individual ages provided); Males: 9.40 -14.81 kg and Females: 8.96 – 15.01 kg
Source:	Covance Research Products (CRP, Kalamazoo, MI)
Housing:	Individually in cages while at CRP during the premating and early gestation. Females housed in the male's cage during mating. Then, females were transferred to MPI Research during GD 6-13 and were housed individually in runs and provided a whelping box upon time for parturition.
Diet:	Certified Canine Diet #5007, PMI Nutrition International, Inc., *ad libitum* when housed at CRP and Eukanuba premium performance canine diet (IAMS Corp.), *ad libitum*, at MPI Research.
Water:	Potable water, *ad libitum*
Environmental conditions:	**Temperature:** 69-79^0 F **Humidity:** 35-68% **Air changes:** Not provided **Photoperiod:** 12 hours/day
Acclimation period:	~ 2 weeks

B. STUDY DESIGN:

1. **In life dates:** Start: June 9, 2005; End: November 23, 2005

2. **Animal assignment:** Twenty male and twenty female proven fertile adult beagle dogs were chosen for the study. Males had to have sired and females had to have delivered at least 2 uneventful litters, each with a minimum of 4 pups. Females considered suitable for the study were weighed and randomized, by sex, into treatment groups using a standard, by weight, block randomization. Males considered suitable were weighed and randomized into a non-treatment-specific group using a simple, by weight, randomization procedure. All females acceptable for randomization were sorted in ascending order by body weight and separated into blocks of two animals each. Each animal in the block was randomly assigned either number 1 or 2 which corresponded to the group number assignment in the study. After randomization, the group designation of all dogs was changed to Group 4 within the Provantis™ computer system to blind the original two groups. Records identified animals by groups, not treatment. Following completion of data collection, the study groups were decoded and data presented by group and treatment. See Table 1.

Table 1. Study design[a]

Test Group	Treatment	Number of males	Number of females
Group 1	Placebo control	NA	8
Group 2	Test material (3x)	NA	8
Group 3	Untreated	16	NA

[a] Data from p. 13 in MRID 47295301

3. Dose selection rationale: The dose level was chosen by the Sponsor to be 3x the proposed commercial dose (1x) in canines; the sponsor stated that testing a test substance at 3x the proposed rate in sexually mature females is an appropriate safety margin.

4. Preparation and treatment: The placebo and test material were administered once every 30 days for a total of 4 doses. They were applied to females once at approximately 4 weeks prior to anticipated mating, one day after the second tie, day 28 of gestation and day 5 of lactation. Doses were administered using disposable syringes at volumes of 4.0 or 10.0 mL/kg based on animal body weight. Doses were applied to the skin of the dorsal midline at one site between the shoulder blades.

5. Statistics: A statistical report was prepared by a study biostatistician. Parental body weights were used in the repeated measures analysis of covariance (RMANCOVA) with the average of the last two pre-dose body weights collected closest to dosing used as the covariate. The analysis was run using three different covariance types, autoregressive (AR(1)), heterogeneous autoregressive (ARH (1)) and Compound Symmetry (CS) with that having the smallest Akaike criterion used for the repeated measure analysis. Clinical pathology endpoints were analyzed using an analysis of covariance (ANCOVA) with the pretest value used as the covariate. Gestation length and litter size were analyzed with an analysis of variance (ANOVA), however, the analysis for litter size was run 3 different ways, once with all pups pooled, once with male pups only and once with female pups only. The live pup ratio, stillborn index, and pup sex ratio were computed for each litter. The pup survival, and weaning index were computed three different ways, with sexes pooled, only male pups and only female pups. After these endpoints were calculated, they were transformed with an arcsine-square-root transformation and analyzed with an ANOVA. Pup body weight on day 1 was analyzed three ways (sexes pooled, males only and females only) by ANCOVA using the litter size as the covariate. Pup body weights collected for the remainder of the study were analyzed by RMANCOVA, also using litter size as the covariate. The same three types were run as described above (AR (1); ARH (1) and CS). Clinical pathology endpoints for pups were analyzed using an ANOVA. All hypotheses were tested at an alpha of 0.10, 0.05 and 0.01.

C. METHODS:

1. Observations

a. General health observations: All dogs were observed cage-side at least twice daily (6 hours apart), seven days/week for morbidity, mortality, injury and availability of food and water.

b. Clinical assessments: Detailed clinical observations of the female dogs were conducted immediately pre- and post-dosing and at 1, 3, 6 and 24 hours after treatment. On non-treatment days, all females were observed for pharmacotoxic signs twice daily (6 hours apart). Observations included: abnormalities of the site of application, details of the nervous system (unsteadiness, ataxia, tremors, decreased activity, salivation, abnormal behavior, panting, restless and aggression), ocular effects (nystagmus, blindness, chemosis, congestion and discharge), effects on the integument (alopecia, condition of haircoat, pruritus and erythema), gastrointestinal effects (stool consistency, diarrhea, and vomiting) and cardiovascular effects

(heart rate, rhythm and color of mucous membranes). In addition, all dogs were given a complete physical/neurological examination by a veterinarian once prior to randomization, and on the females and pups between LD 40 and 42.

2. Body weight: Body weights were measured for adult males and females prior to randomization, and weekly thereafter for adult females. The first gestation weight was recorded on the day of mating and the first lactation body weight on LD1.

3. Food consumption: Food consumption was not recorded.

4. Hematology & Clinical Chemistry: Blood was collected from the jugular vein for hematology and clinical chemistry assessments on all adult dogs prior to randomization, on females at the end of lactation and on puppies 3 days prior to vaccinations given on LD 25-33. Adults were fasted (food only) overnight and pups were removed from their mother for 6 hours. The MARKED (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 corpuscle. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscle. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Sodium/Potassium ratio	X	Urea nitrogen*
X	Phosphorus *	X	Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, e.g., *)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase	X	Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)		Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
X	Gamma glutamyl transferase (GGT)		TCO_2 Bicarbonate
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		

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

5. Reproductive Parameters:

a. Breeding procedures: Females were checked for estrus visually three times weekly until proestrus was observed. Then they were put into a cage with a male for breeding, seven days after proestrus was observed. The first female coming into heat was mated with the first numbered male and continued in that manner until all females had been mated. Two days after mating, females were paired for mating a second time with the same male. Mating was observed by a visual confirmation of a "tie". The day of observation of the first mating was designated Gestation Day (GD) 0.

b. Delivery and Lactation: Around 10 days prior to expected parturition, a whelping box was placed in with the females and they were monitored twice daily. After delivery was complete, pups were sequentially identified by sex and the litters were allowed to remain with their mother for 6 weeks. Gestation time and any abnormalities during gestation or parturition were recorded. Any females not delivering were radiographed or underwent ultrasound to determine if there were any retained fetuses or to confirm they were not pregnant. If they were not pregnant, the females were removed from the study.

c. Litter evaluations: The day whelping was observed was considered Lactation Day (LD) 0. Pups were examined on LD 1 and the following data recorded: litter size, number and sex of dead and live born, and gross visceral or skeletal abnormalities. Litters were examined twice daily and detailed clinical examinations were performed on each pup on postnatal days 1, 4, 7, 14, 21, 28, 35 and 42. Individual pup weights were also obtained on these days.

d. Pup Indices: The following indices were determined for the pups: stillborn index, whelping index, pup sex ratio, pup survival, and weaning index.

$$\text{Stillborn index} = \frac{\text{\# of stillborn pups per litter}}{\text{total \# of pups delivered per litter}} \times 100$$

$$\text{Whelping index} = \frac{\text{\# of live pups delivered}}{\text{\# of pups delivered}} \times 100$$

$$\text{Pup sex ratio} = \frac{\text{\# of males per litter at day 0 or 1}}{\text{total \# of pups delivered per litter}} \times 100$$

$$\text{Pup survival} = \frac{\text{total \# of pups surviving per litter at specified time}}{\text{\# of live pups per litter at specific day}} \times 100$$

$$\text{Weaning index} = \frac{\text{number of pups weaned per litter}}{\text{number of pups whelped per litter}} \times 100$$

6. Termination of study: All males were returned to the CRP stock colony after the females were mated. After the pups were 42 days old, they and the females were returned to the stock colony of MPI Research. Any pups born dead, found dead or euthanized underwent a complete necropsy examination to determine the cause of death and tissues were saved in 10% neutral buffered formalin.

II. RESULTS FOR ADULT DOGS

A. Observations:

1. Clinical signs of toxicity: No treatment-related clinical signs were observed. The most common finding in both the control and treated females was the presence of interdigital cysts which resulted from the caging environment and was not treatment-related. Lacrimation and salivation were observed around the time of dosing but were observed in both the control and treated dogs.

2. Mortality: No adult animals died.

B. Body Weight and Weight Gain: Select mean body weight data are presented in Table 2. No treatment-related changes in mean pre-mating body weight were observed in the adult male or female dogs. A statistically significant ($p<0.05$) finding of increased body weight in treated females was identified in gestation week 2 but the amount increased, (<10% when compared to controls), was not toxicologically significant.

Table 2. Mean body weight (kg± SD) of adult female dogs treated with metaflumizone (R-28153) and amitraz[a]		
Week of Study	**Controls**	**Treated (3x)**
Premating body wt.		
Week 1	11.21 ± 1.75	11.60 ± 1.55
Week 3	11.22 ± 1.68	11.84 ± 1.63
Week 6	11.05 ± NA[b]	9.32 ± NA[b]
Gestation body wt.		
Week 1	11.22 ± 1.86	11.46 ± 1.44
Week 2	10.98 ± 1.75	11.66* ± 1.59
Week 3	11.16 ± 1.75	11.07 ± 1.75
Week 6	10.74 ± 1.71	11.32 ± 1.77
Week 9	12.95 ± 1.88	13.13 ± 1.41
Lactation body wt.		
Week 1	10.74 ± 1.66	11.21 ± 1.48
Week 3	10.84 ± 1.31	11.33 ± 1.17
Week 6	11.58 ± 1.49	11.86 ± 1.52

[a] Extracted from Tables 3-5, pp. 158-173 in MRID 47295301

[b] Value is from one animal only.

* Statistically significant compared to controls, $p < 0.05$

C. Food Consumption: Food consumption was not measured in the study.

D. Blood Analyses:

1. Hematology: Male beagles exhibited no abnormalities in the hematology parameters measured prior to randomization. Treated females had no significant changes in hematology parameters measured when compared to controls taken prior to randomization or at the end of lactation.

2. Clinical Chemistry: Selected clinical chemistry parameters are included in Table 3. No significant findings were identified in the male dogs. Treated females had a significant decrease in alanine aminotransferase (ALT) when compared to controls at the end of lactation that was not considered toxicologically significant as physiologically this does not present a problem. Other hepatic enzyme activities measured were not affected. These included alkaline phosphatase (AP), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and total bilirubin.

Table 3. Mean clinical chemistry parameters of adult female dogs treated with metaflumizone (R-28153) and amitraz[a]		
Parameter	**Controls**	**Treated (3x)**
ALT (U/L)		
pre-treatment	31.5 ± 4.93	31.1 ± 6.62
lactation	42.0 ± 16.12	30.1* ± 6.20
AST (U/L)		
pre-treatment	20.6 ± 3.16	21.4 ± 2.62
lactation	38.4 ± 22.59	27.6 ± 10.90
AP (U/L)		
pre-treatment	28.3 ± 6.52	35.0 ± 14.03
lactation	58.1 ± 20.71	59.8 ± 33.75
GGT (U/L)		
pre-treatment	4.1 ± 0.99	4.0 ± 0.53
lactation	4.1 ± 0.64	4.6 ± 2.33
Total bilirubin (mg/dL)		
pre-treatment	0.25 ± 0.076	0.26 ± 0.052
lactation	0.21 ± 0.035	0.21 ± 0.035

[a] Extracted from Table 9, pp. 246-281 in MRID 47295301.
* Statistically significant from controls, $p < 0.1$

E. Reproductive Parameters:

1. Fertility: Male sperm were evaluated prior to breeding and no abnormalities were observed in motility, morphology and volume. Female fertility indices were similar between the control and treated females, both were 87.5%. One female out of eight in both the control and 3x females did not produce a litter.

2. Gestation length: Gestation length for the control group was 64.43 days and for the 3x treated group, 63.71 days; therefore, there was no difference observed.

3. Litter size: Litter size data are provided in Table 4. Litter size was analyzed by males only, females only and then pooled. Statistical significance was observed in the males and pooled data and showed a decrease in litter size in these two groups when compared to the control group. The differences were the result of two litters from the 3x females only having 1-2 pups, thus lowering the mean of the treated groups.

Table 4. Mean litter size from adult female dogs treated with metaflumizone (R-28153) and amitraz[a]		
Litter size	**Controls**	**Treated (3x)**
Males only	4.3 ± 1.80	1.1* ± 1.07
Females only	2.6 ± 0.98	3.6 ± 1.62
Males/Females pooled	6.9 ± 1.21	4.7** ± 2.21

[a] Data obtained from Table 13, p. 296 in MRID 47295301.
*Statistically significant from controls, $p < 0.01$ or ** $p < 0.05$

III. RESULTS FOR PUPS

1. Clinical signs of toxicity: No treatment-related clinical signs were observed in any of the pups. Clinical signs were observed in pups from both the control and treated females and included: lacrimation, scabs in the abdominal area and discolored (red) abdominal areas.

2. Mortality: One placebo control pup and two treated 3x pups were found dead on day 0. A lung floatation test was only performed on one of the 3x pups and indicated that the pup was stillborn; on gross examination, there was free hemorrhage in the abdominal cavity. The control pup found dead on day 0 had no findings on gross examination and the 3x pup had multiple perforations in the thoracic cavity and skin. There was no statistical significance in the stillborn index or whelping index between the control and treated group. Besides the three found dead on day 0, four control pups (2 male and 2 females) and 1 female 3x pup either died or were euthanized during the 42 days of lactation. Gross examination of the pups either showed normal findings (treated pup) or inconclusive causes of death (control pups- red fluid in abdomen, discolored myocardium). These deaths did not appear to be treatment-related as most were in control pups and the treated pup that died had normal findings on examination. The pathology tables were provided on the pups that died, but otherwise information in the study report text was very limited.

3. Pup sex ratio: Pup sex ratio data are provided in Table 5. The sex ratio of the pups was computed on day 0 and day 1 for each litter. There was no difference between day 0 and day 1 but for both days, there was a significant decrease in the number of male pups in the 3x treated groups when compared to the controls.

Table 5. Pup sex ratio from adult female dogs treated with metaflumizone (R-28153) and amitraz[a]

Pup sex ratio[b] (%)	Controls	Treated (3x)
Day 0	57.79 ± 22.33	22.98* ± 19.86
Day 1	55.41 ± 22.11	22.98* ± 19.86

[a] Data obtained from Table 17, p. 309 in MRID 47295301.

[b] Pup sex ratio is (# of male pups per litter/total # of pups delivered per litter) x 100

*Statistically significant from controls, $p < 0.05$

4. Pup survival: Survival for the pups was calculated on days 4, 7, 14, 21, 28, 35 and 42 by males, females and the pooling of the sexes. No statistical differences or effect of treatment were observed.

5. Weaning index: The weaning index for the pups was calculated by males, females and the pooling of the sexes. No statistical differences or effects of treatment were observed.

B. BODY WEIGHT AND WEIGHT GAIN: Select mean body weight data are presented in Table 6. Statistically significant increases in body weight were observed in the male pups from treated females consistently throughout the study, when compared to pups from controls. Female pups in the treated group also had increased, although not statistically significant, body weight when compared to controls. Weight gain in the 3x male and female pups combined was also increased compared to the controls. Individual body weight data indicated, however, that a few

of the 3x pups were much larger than the rest, thus increasing the overall mean body weight on Day 1 of the study.

Table 6. Mean body weight and wt. gain of pups from dams treated with metaflumizone (R-28153) and amitraz[a]		
Day of Study	**Controls**	**Treated (3x)**
Body wt. (g± SD)		
	Males	
Day 1	269.57 ± 35.35	336.93** ± 40.45
Day 7	494.86 ± 71.56	664.25** ± 73.76
Day 21	1098.23 ± 195.16	1324.67 ± 237.86
Day 35	1823.84 ± 201.66	2185.03** ± 242.57
Day 42	2293.91 ± 209.17	2685.08** ± 217.75
	Females	
Day 1	249.94 ± 33.82	309.51 ± 68.72
Day 7	463.32 ± 63.34	577.99 ± 92.17
Day 21	1020.36 ± 154.32	1219.73 ± 169.40
Day 35	1669.42 ± 151.44	1940.26* ± 186.80
Day 42	2070.03 ± 155.79	2316.79 ± 209.44
	Pooled	
Day 1	260.32 ± 31.66	322.89 ± 64.11
Day 7	480.46 ± 62.79	610.13 ± 85.33
Day 21	1055.83 ± 153.07	1256.02 ± 198.92
Day 35	1751.51 ± 147.78	2016.65 ± 215.95
Day 42	2196.67 ± 137.66	2434.93 ± 216.39
Body wt. gain (g)[b]		
	Males	
wt. gain (day 1-7)	225.29	327.32
wt. gain (day 7-21)	603.37	660.42
wt gain (day 21-35)	725.61	860.36
Total wt gain (1-42)	2024.34	2348.15
	Females	
wt. gain (day 1-7)	213.38	268.48
wt. gain (day 7-21)	557.04	641.74
wt gain (day 21-35)	649.06	720.53
Total wt gain (1-42)	1820.09	2007.28
	Pooled	
wt. gain (day 1-7)	220.14	287.24
wt. gain (day 7-21)	575.37	645.89
wt gain (day 21-35)	695.68	760.63
Total wt gain (1-42)	1936.35	2112.04

[a] Data obtained from Table 21, p. 336-348 in MRID 47295301.
[b] Body wt. gain calculated by reviewer.
*Statistically significant from controls, $p < 0.10$ or ** $p < 0.05$

C. <u>FOOD CONSUMPTION:</u> Food consumption was not reported.

D. <u>BLOOD ANALYSES</u>:

1. <u>Hematology</u>: Selected hematology parameters are included in Table 7. Statistical analysis of hematology parameters from pooled samples indicated some significant differences; however,

none of the differences noted between the control and treated pups were toxicologically significant as they were minor, were still within normal ranges and did not have any accompanying side effects. Erythrocytes ($p<0.10$) were increased and MCV ($p<0.01$) and MCH ($p<0.05$) were decreased in the 3x pups when compared to the controls. The other red blood cell (RBC) index, MCHC, the platelet count, hematocrit and hemoglobin were also not affected. Eosinophils were increased in the 3x pups.

Table 7. Hematology parameters (±SD) for pups from dams treated with metaflumizone (R-28153) and amitraz[a]

Parameter	Controls	Treated (3x)
Males		
Erythrocytes ($10^6/\mu L$)	3.88 ± 0.30	4.01 ± 0.36
MCV (fL)	78.31 ± 2.80	74.94 ± 3.77
MCH (pg)	22.93 ± 0.95	22.43 ± 1.44
Eosinophils ($10^3/\mu L$)	0.12 ± 0.05	0.30 ± 0.13
Females		
Erythrocytes ($10^6/\mu L$)	4.00 ± 0.49	4.14 ± 0.34
MCV (fL)	79.47 ± 3.31	76.87 ± 3.40
MCH (pg)	22.87 ± 0.84	22.31 ± 0.10
Eosinophils ($10^3/\mu L$)	0.15 ± 0.09	0.23 ± 0.19
Pooled		
Erythrocytes ($10^6/\mu L$)	3.93 ± 0.38	4.10* ± 0.34
MCV (fL)	78.74 ± 3.01	76.36*** ± 3.54
MCH (pg)	22.91 ± 0.90	22.34** ± 1.11
Eosinophils ($10^3/\mu L$)	0.13 ± 0.07	0.25*** ± 0.18

[a] Data obtained from Table 22, pp. 351-382 in MRID 47295301
*Statistically significant from controls, $p< 0.10$, ** $p< 0.05$ or *** $p<0.01$

2. <u>Clinical Chemistry</u>: Selected clinical chemistry parameters are included in Table 8. Statistical analysis of clinical chemistry parameters from pooled samples indicated some significant differences; however, none of the differences noted between the control and treated pups were toxicologically significant as they were minor, were still within normal ranges and did not have any accompanying side effects. Sodium ($p< 0.01$), potassium ($p<0.05$), chloride ($p<0.01$), alanine aminotransferase (ALT) ($p<0.01$) and glucose ($p<0.10$) were all decreased in the pooled samples in the 3x pups when compared to controls. Calcium ($p<0.01$) was increased.

Table 8. Clinical chemistry parameters (±SD) for pups from dams treated with metaflumizone (R-28153) and amitraz[a]		
Parameter	**Controls**	**Treated (3x)**
Males		
Sodium (mEq/L)	144.2 ± 1.74	142.3 ± 1.58
Potassium (mEq/L)	5.20 ± 0.42	5.03 ± 0.39
Chloride (mEq/L)	107.8 ± 1.65	106.5 ± 2.51
Calcium (mg/dL)	11.31 ± 0.56	11.9 ± 0.45
ALT (U/L)	16.3 ± 4.06	12.6 ± 4.50
Glucose (mg/dL)	135.2 ± 8.94	131.9 ± 15.62
Females		
Sodium (mEq/L)	145.0 ± 2.25	143.0 ± 1.31
Potassium (mEq/L)	5.09 ± 0.31	4.90 ± 0.37
Chloride (mEq/L)	108.9 ± 2.63	107 ± 1.45
Calcium (mg/dL)	11.23 ± 0.65	11.63 ± 0.47
ALT (U/L)	13.9 ± 2.64	12.2 ± 3.31
Glucose (mg/dL)	136.1 ± 17.18	129.3 ± 11.72
Pooled		
Sodium (mEq/L)	144.5 ± 1.96	142.8*** ± 1.40
Potassium (mEq/L)	5.16 ± 0.38	4.94** ± 0.38
Chloride (mEq/L)	108.2 ± 2.10	106.9*** ± 1.76
Calcium (mg/dL)	11.28 ± 0.59	11.7*** ± 0.48
ALT (U/L)	15.4 ± 3.75	12.3*** ± 3.58
Glucose (mg/dL)	135.5 ± 12.40	130.0* ± 12.64

[a] Data obtained from Table 23, pp. 387-420 in MRID 47295301

*Statistically significant from controls, $p< 0.10$, ** $p< 0.05$ or *** $p<0.01$

IV. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: The study author concluded that the topically applied spot-on formulation of metaflumizone (R-28153) and amitraz at 3x the proposed commercial dose to female dogs was well tolerated and had no effect in the female dogs, breeding behavior, reproductive capacity, or clinical pathology of adults. No treatment-related findings were observed in any of the pups or in most of the parameters measured. The study author stated there were significant differences in the live pup sex ratio between the controls and 3x pups as there were more males in controls and more females in the treated group but considered this to be non-treatment-related. Litter size was also decreased in the 3x group compared to the controls but the study author contributed this to two litters in the 3x group having only 1-2 pups in them. The study author also stated that the typical average litter for this age and breed of dog from the vendor suggested that this difference was not related to treatment. The investigator stated that the typical average litter size at Covance Research Products (CRP) was approximately 5.9.

B. <u>REVIEWER COMMENTS</u>: The reviewer agrees that few or no effects were observed in the adult beagle dogs when treated with 3x of the recommended dose of metafluizone (R-28153) and amitraz spot-on formulation. There were no clinical signs, body weight loss, neurological findings or any differences of significance in the hematology and clinical chemistry parameters

measured. The reviewer also agrees that most reproductive parameters did not appear to be affected with treatment. There were some increases in body weight and body weight gain in the treated pups but this was not considered toxicologically significant as it is not an adverse effect. The primary concern of this reviewer was with the reduced mean litter size in the 3x group. Two of the seven females in the 3x group produced only two pups/litter and 1/7 had a litter of 4 pups with all seven control females having at least 5 pups/litter. The investigator stated that this was not treatment related because the typical average litter size at CRP is approximately 5.9; however, this does not explain the low litter size in three of the 3x females and data from CRP to support this were not included. The study report stated that females accepted into the study were chosen based on producing two previous uneventful litters, thus proving their fertility and that these litters each had a minimum of 4 pups each thus adding support that 1-2 pups was smaller. Because the possibility exists that treatment may be associated with a decrease in litter size, a more thorough developmental/reproductive study should be considered to look at more reproductive details (# implantation sites, # of implantations, # of corpora lutea, # of resorptions). One concern is that the reduced mean litter size in the 3X treated group may have been due to a reduced number of implantations caused by exposure to the test substance at one day after the second mating. In addition, it is noted that the test material was not applied immediately before breeding or during the breeding session, so that the proposed claim for use on breeding females is inappropriate.

The report also failed to mention pup deaths that occurred during the 42 days of lactation. The report stated only that the survival rates were not statistically significantly different and that the postmortem evaluations did not indicate any treatment effects. The findings from the gross examination at necropsy should have been included in the study report. The reviewer had to look at individual necropsy tables to identify the pups sacrificed or euthanized during the study and the cause of their death.

C. STUDY DEFICIENCIES:

1. Having only one dose level made it more difficult to determine if some changes observed were related to treatment as the reviewer could not determine if a dose response had occurred.

2. The exact dates (and whether dosages were 4.0 or 10 mL) when applications were made to individual females were not reported.

3. Historical data on CRP breeding success and number of litters produced, as well as the previous pregnancy data on these animals (as well as their ages at the time of this study) were not provided.

4. Data were not included in the study report text on the incidence of pups that either died or were euthanized, *in extremis* during the lactation period and the postmortem findings on them. The report mentioned only that there were no treatment-related findings on those pups.

5. Although not a guideline study, food consumption measurements would have been helpful. Mean body weights of treated females as well as their pups were consistently increased compared to the controls, and the food consumption would have provided more data.

1. **DP BARCODE:** D348052
2. **PC CODES:** 281250 & 281251 (Metaflumizone); 106201 (Amitraz)
3. **CURRENT DATE:** March 13, 2008
4. **TEST MATERIAL:** Metaflumizone (R-28153)/amitraz formulation containing 15% w/v metaflumizone and 15% w/v amitraz. Described as a liquid in an amber glass vial.

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety (nonguideline) adult male dog (beagle) International Bio-Institute Corp., Fergus, Ontario, Canada.. Test Facility Study No. IOSD-0405, Oct. 24, 2006.	47303301	Testing involved two groups each with 8 intact male adult beagles weighing 6.31-20.82 kg at first treatment. One group was administered vehicle control; the other a metaflumizone-amitraz combination at 3x the recommended dose (3x dose = 4.0 mL for dogs weighing less than 10.0 kg, and 10.0 mL for dogs >10 kg). Dogs were treated on day -30 (cohort 1) or -23 (cohort 2), and then on days 0, 30 & 60. Semen was collected pretreatment on days -47 and -33 (cohort 1), or -32 and -26 (cohort 2), and then from all dogs on days 7, 35, 49, 63, 77 and 91. All dogs survived to the end of the study. No treatment-related clinical signs of toxicity or body weight effects were seen. Some control and treated dogs reacted to application by rolling around, scratching back and/or one or both ears, but there was no skin irritation. The study demonstrated that the amitraz-metaflumizone combination (ProMeris for dogs) did not product any biologically significant changes in semen parameters when administered to male beagles at 3x the recommended dose every 30 days for 4 treatments; the study is classified as acceptable but did have some reporting deficiencies. The study adequately demonstrates that ProMeris can be used on breeding male dogs.	N/A	A

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

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety (nonguideline) adult female dog (beagle) MPI Research, Inc., Mattawan, MI. Test Facility Study No. 817-015, Oct. 31, 2006.	47295301	Testing involved two groups each with 8 female adult beagles weighing 8.96-15.01 kg. One group was administered vehicle control; the other a metaflumizone-amitraz combination at 3x the recommended dose (3x dose = 4.0 mL for dogs weighing less than 10.0 kg, and 10.0 mL for dogs >10 kg). Dogs were treated at 4 weeks (± 3 days) prior to anticipated mating; one day after second mating, Day 28 of gestation and Lactation day 5. No deaths or treatment-related clinical signs of toxicity were observed in the adult female beagles. Body weight, changes in hematology and clinical chemistry parameters were not affected by treatment. Mean lkitter size in the 3x females was statistically significantly lower than the controls (4.7 vs. 6.9). One concern is that the reduced mean litter size may have been due to a reduced number of implantations caused by exposure to the test material at one day after the second mating. In addition, it is noted that the test material was not applied immediately before breeding or during the breeding session. Study deficiencies observed but not affecting the study results included the lack of exact dates (and whether the dosages were 4.0 or 10 mL) when applications to individual dogs were made, lack of information on prior pregnancies and their results, absence of reporting as to the individual female ages or food consumption, insufficient data on the pups that were euthanized or died during the study, the use of only one dose and incomplete data to evaluate litter size.	N/A	U

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