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

PESTICIDES
SUBSTANCES

OFFICE OF PREVENTION,
AND TOXIC

Date: 27 May 2005

MEMORANDUM

Subject: EPA File Symbol: 80490-E PROMERIS SPOT-ON FOR DOGS
DP Barcode: D311482
Decision No.: 351841
PC Codes: 106201 (Amitraz); 281250 [and/or 281251?] (Metaflumizone)

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

To: Ann Hanger/John Hebert, RM 07
Insecticide Branch
Registration Division (7505C)

Applicant: FORT DODGE ANIMAL HEALTH

FORMULATION DECLARATION FROM LABEL:

Active Ingredient(s):	% by wt.
Metaflumizone (CAS #139968-49-3)	14.34%
Amitraz (CAS #33089-61-1)	14.34%
Other Ingredients:	71.32%
Total:	100.00%

ACTION REQUESTED:

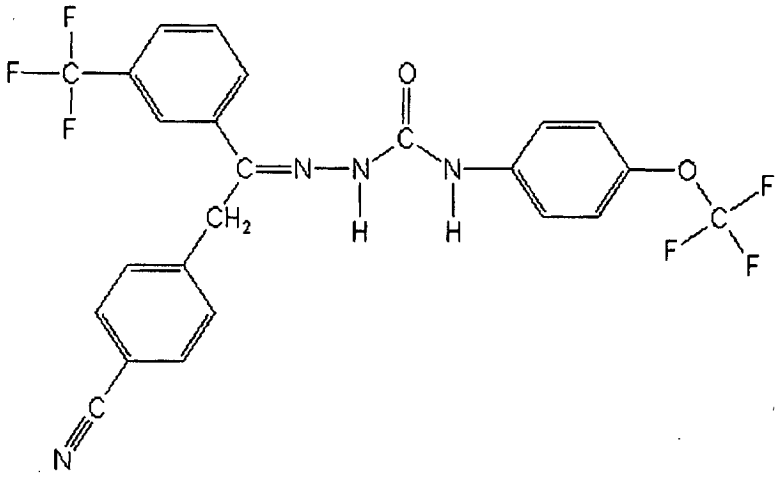
The Risk Manager requests:

> Please review the animal safety data submitted for this new flea and tick end use product for dogs (EPA File Symbol 80490-E). There are two a.i.'s–the pending new a.i. Metaflumizone (also known as as BAS 320 I and currently undergoing registration as file symbol 7969-EEA) and Amitraz (registrant is pursuing a new tech registration as file symbol 80490-R).

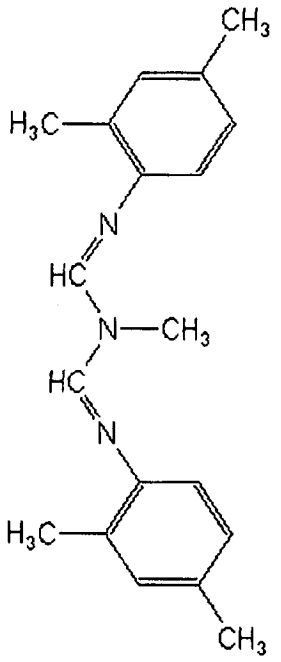
BACKGROUND:

The material received by TRB consists of two companion animal safety studies for this spot-on product. One of these studies (MRID 46401003, in 4 volumes) was conducted on young adult beagles; the other (MRID 46401004, also in 4 volumes) was conducted on eight-week old beagle puppies.

This product contains the new active ingredient, Metaflumizone (also known as BAS 320 I; also known as R-28153; 2-[2-(4-cyanophenyl)-1-[3-(trifluoromethyl)phenyl] ethylidene]-*N*-[4-(trifluoromethoxy)phenyl]hydrazinecarboxamide-1, with the following structure:



The structure of Amitraz is shown below:



COMMENTS AND RECOMMENDATIONS:

1. While the conduct and reporting of the companion animal (adult dog) safety study (OPPTS 870.7200) in MRID 46401003 (OPPTS 870.7200) were adequate, the study does not demonstrate an adequate margin of safety (5X) associated with the proposed use of this formulation, and so does not support registration. This conclusion is based on the following:

   i. A depressed righting reflex was seen in 4/12 of the 5X dogs. In two of these dogs it was observed on Days 1, 2, 8 and 22, indicating the possibility of a permanent effect.

   ii. All dogs (including those at 0X) were very quiet and inactive ("little activity or barking compared to the previous day, even during repeated entries by the technical staff") on the afternoon of Day 1 (applications of test material or placebo were made during the morning). While the report states that this could be a result of increased handling which had occurred earlier (during application of the test substance or placebo), Amitraz can cause sedation. Although dogs in the placebo control group also showed the same or a similar effect, one or more of the inerts in this formulation have pharmacological activity, and these controls were being exposed to essentially a 7X dosage of the formulation without the actives.

   iii. The formulation has tested positive as a dermal sensitizer. Possibly associated with this, total leukocyte counts were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, for combined sexes at the 3X and 5X dose levels 24 hours postdose. Concurrently, neutrophils were increased 1.2-fold at the 3X and 1.5-fold at 5X.

Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges. An elevation in leukocyte counts is normally a response to infection.

iv. The increases in glucose and urea nitrogen levels for all dose groups (1X, 3X and 5X) at 24 hours are consistent with known effects of Amitraz, and indicate pharmacologically significant quantities of this active are absorbed at even the 1X dose level.

v. The proposed label suggests that this would be an over-the-counter product, readily available to dog owners, particularly as the only mention of veterinarians is in the standardized statements under the heading HAZARDS TO DOMESTIC ANIMALS ("Consult a veterinarian before using this product on aged, debilitated, medicated, pregnant or nursing animals. Individual sensitivities while rare may occur after the use of any pesticide product. If signs persist or become more severe, consult a veterinarian immediately...").

vi. The label for Mitaban® (a formulation containing 19.9% Amitraz, approved by the FDA for treating generalized canine demodicosis) includes the following WARNING statement: "Toxicology studies conducted in the dog and other species suggest amitraz may alter the animal's ability to maintain homeostasis. Animals treated with MITABAN (Amitraz) should not be subjected to stress for a period of at least 24 hours posttreatment. Adverse reactions including three fatalities were reported during the clinical studies. In excess of 1100 patients with generalized demodicosis were topically treated with MITABAN." In addition, the Mitaban® label includes the statement: "**Caution:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

vii. The overall exposure level to Amitraz associated with the proposed use of this product seems to be comparable to that associated with the use of Mitaban® . However, there are indications that Amitraz from the Promeris formulation is absorbed more rapidly and/or to a greater extent than Amitraz in the 250 ppm Mitaban-use dilution. Publically available information on Mitaban® includes the following [see myweb.cableone.net/bdturner/**Mitaban**.pdf]: "Blood glucose values were elevated at 4 hours posttreatment in the 250 ppm female group, and in both sexes at the 1250 and 2500 ppm concentrations. ...glucose values returned to normal within 24 hours posttreatment. In another study, groups of healthy beagles were topically treated with either 250 ppm, 750 ppm or 1250 ppm of active drug at 14 day intervals and for 12 weeks. Blood glucose values were elevated at the 750 ppm concentration at 4 hours posttreatment after 3 of 6 treatments, and after 5 of 6 treatments at the 1250 ppm level. In the 750 ppm group, serum glucose values returned to normal at 24 hours posttreatment, however for the 1250 ppm group, at 24 hours and after 3 of 6 treatments the levels remained significantly elevated." In the adult dog Promeris study [MRID 46401003] 1/6 of the 3X females and 5/6 5X females had elevated glucose levels ($\geq$120 mg/dL) at 24 hours, suggesting that Amitraz absorption from 5X Promeris treatment (7.0 mL) is greater than that from exposure to 2500 ppm Amitraz (or a 10X concentration of the use-dilution of Mitaban®), and a 1X (1.4 mL) Promeris treatment of an adult dog would then be associated with greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban® solution.

viii. There is also potential for adverse drug reactions: from http://www.aava.org/pub/iatrogenic.html: "Ivermectin which is in Heartgard heart worm preventative and is sometimes used to treat "mange" and ear mites, causes increases in

monoamine neurotransmitter metabolites which could result in important adverse drug reactions with amitraz (Mitaban topical mange treatment)..." At the very least, if this product is registered it should be available only through licensed veterinarians, as is Mitaban®.

2. The companion animal safety study in MRID 46401004, conducted on 8-week old puppies, also does not demonstrate an adequate (5X) margin of safety for the proposed use. The greatest concern involves the death of 3X female #122, sacrificed *in extremis* on Day 9. This animal had a urea nitrogen of 8 mg/dL pretest, 22 mg/dL at 24 hours postdose, and 33 mg/dL at 8 days postdose, as well as an elevated serum potassium level at 24 hours, consistent with known effects of Amitraz. While no deaths occurred in 5X puppies, female #122 may have been representative of a more sensitive population subgroup. In addition, 1X puppy #125 was found dead on Day 9. While this puppy had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, the possibility that exposure to Amitraz may have contributed to death cannot be ruled out. Increases in blood urea nitrogen (BUN) and a dose-related trend of increasing incidences (Placebo: 1/12; 1X: 2/12; 3X: 3/12; 5X: 5/12) of elevated glucose (>120 mg/dL) at 24 hours postexposure indicate that physiologically significant amounts of Amitraz had been absorbed.

3. Refer to the executive summaries for MRIDs 46401003 and 46401004 for additional comments and conclusions regarding TRB's reviews of these studies.

EPA Primary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Technical Review Branch, Registration Division (7505C) Date 5/27/05
EPA Secondary Reviewer: William Dykstra, Ph.D. Signature: William Dykstra
Health Effects Division (7509C) Date 5/25/05
EPA Tertiary Reviewer: Kit Farwell, DVM, DABT Signature: Kit Farwell
Health Effects Division (7509C) Date 5-26-05

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Dogs OPPTS 870.7200
PC CODES: 106201 (Amitraz), 281250 (Metaflumizone)
DP BARCODE: D311482 **RISK MANAGER:** (EPA): 07 **DECISION NO.:**351841

PRODUCT AND TEST MATERIAL: 15% w/v R-28153/15% w/v Amitraz spot-on Lot No. 0381702, a pale yellow liquid with a specific gravity of 1.047 g/mL containing (from p. 480 of MRID 464010-03) 14.70% R-28153 (Metaflumizone) and 14.65% Amitraz.

CITATION: Lindahl, R.G. (2004) Safety Evaluation Study of a Topically Applied Spot-On Formulation of R-28153 and Amitraz in Adult Dogs. Study No. 817-007; Sponsor Study No. 0817-C-US-03-03. Unpublished study prepared by MPI Research, Inc. 54943 North Main St. Mattawan, MI 49071-9399. Study Completion Date: 19 March 2004. MRID 46401003.

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

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46401003), 4 groups, each containing 12 (6/sex) young adult beagles (source: Covance Research Products; males: 9.99 to 12.02 kg; females: 6.66-9.54 kg) were treated at 0X (7 or 17 mL/kg placebo), 1X (1.4 or 3.4 mL spot-on formulation), 3X (4.2 or 10.2 mL spot-on formulation) and 5X (7 or 17 mL spot-on formulation). Dogs weighing >10.5 kg (no females were in this category) received the higher doses. Each dog was treated in a single application along the spine, starting on the back between the shoulder blades and then continuing caudally, with application using syringes. The placebo was the test formulation without the two actives (so dogs in the 0X group were actually dosed with an approximately 7X application of the "inactive" ingredients, or 1.4X of the "inactives" that the 5X group received).

According to proposed label directions the product would be applied as a spot-on at the following dosage rates: dogs $\leq$ 5 kg: 0.67 mL; 5-10 kg: 1.33 mL; 10-25 kg: 3.33 mL; 25-40 kg: 5.33 mL; and 40-50 kg: 6.66 mL.

All animals were observed at least twice daily. On the day of dosing (Day 1: there was no Day 0) clinical observations were made predose and at 1, 2 and 3 hours postdose. Otherwise, clinical observations were twice daily, approximately 4 hours apart, during both the acclimation period and then from Days 2 through 28, except on days of neurological and physical examinations (Days -1, 1, 2, 8 and 22).

Dogs were individually weighed on Days -7, -1, 1 (just before dosing) and then postdose on Days 8, 15 and 22. Food consumption is reported (g/dog/day) for weeks -1, 1, 2, 3 and 4. Blood samples were taken (following overnight fasts) once pretest (Week -1) and then on Days 2 (about 24 hours postdose), 8 and 22.

All dogs survived and there was no indication of any effects on body weight or food consumption.

Neurologically, two dogs at the 5X dose level had a depressed righting reflex on Days 1 and 2, and an additional two dogs (one male, one female) at this dose level had a depressed righting reflex on Days 1, 2, 8 and 22, not observed before exposure. Although the report states that this was "transitory" and not considered to be clinically significant, no subsequent (after Day 22) observations are reported for these two dogs. Other occurrences of this finding (a control female on Day 8, a 1X female on Day 2, another 1X female on Day 8, a 3X female on Day 22) were sporadic.

In addition, all dogs (including those at 0X) were very quiet and inactive ("little activity or barking compared to the previous day, even during repeated entries by the technical staff") on the afternoon of Day 1 (applications of test material or placebo were made during the morning). While the report states that this could be a result of increased handling which had occurred earlier (during application of the test substance or placebo), Amitraz can cause sedation. From http://www.medi-vet.com/Mitaban.aspx: "The most frequently observed adverse reaction in the [Amitraz] clinical studies was transient sedation, which occurred in approximately 8% of the generalized demodicosis patients. This effect was observed within 2 to 6 hours posttreatment, and usually dissipated within 24 to 72 hours. In approximately 40% of the affected generalized demodicosis patients, the effect dissipated in less than 24 hours." While the report states that this effect was seen in the 0X group dogs, the CSF indicates there are one or more inert ingredients present with potential pharmacological activity.

For clinical observations, few or absent feces were observed in 4/12 dogs in the 5X group on day 2, and purulent discharge was observed in one eye of a 3X male on days 2-5, and from both eyes of a 5X female on days 2-6.

The following clinical chemistry and hematology effects were noted:

Glucose: Significantly increased at 24 hours postdose in the 3X and 5X groups, with a dose relationship [Males: controls: mean 97.7 mg/dL; 1X: mean 113.0 (+15.7%); 3X: mean 131.2 (+34.3%); 5X: mean 144.2 (+47.6%); Females: controls: mean: 98.7; 1X: mean: 106.7 (+8.1%); 3X: 116.2 (+17.7%); 5X: 159.2 (+61.3%)]. P values (combined sexes) were: 1X: 0.1229 [not considered significant]; 3X: 0.0006; 5X: 0.0000. The report (p. 22 of MRID 46401003) states: "Glucose was increased 24 hours postdose at 3X and 5X. This effect was not seen at 1X or at subsequent intervals." However, all 1X dogs at 24 hours had glucose values $\geq$ 100 mg/dL, not seen in any group pretest, nor on Days 8 or 22. Of the four 1X males >10.5 kg treated with 3.4 mL formulation one had an elevated glucose level (125 mg/dL) outside the normal range (80-120 mg/dL, as given in http://www.ahc.umn.edu/rar/RefValues.html) and two showed high normal values (116 and 119 mg/dL). Mitaban treatment of dogs is known to cause a transient hyperglycemia. In an Amitraz 2-year feeding study in beagle dogs (MRIDs 00030493; 00040856) blood samples collected during weeks 40 and 53 showed significant increases in mean blood sugar concentration 3 hours after dosing in the 1 mg/kg/day group, but no effects at lower doses (0.1 and 0.25 mg/kg/day).

Calcium: "In the 24 hour postdose interval a significant treatment effect was found for calcium ($p=0.0005$). Follow-up analyses were done in this interval with sexes combined. This analysis showed significance for...groups 3X and 5X when compared to the Control with $p=0.0305$ and $p<0.0001$, respectively." Also, "at 24 hours, calcium was slightly increased at 3X and 5X (combined sexes: 1.03-fold and 1.07-fold respectively). However, there were suggestions of an effect in males at all dose levels, with the following incidences of calcium >11.6 mg/dL (normal range: 9-11.4 mg/dL) in males at 24 hours: 0X: 1/6; 1X: 4/6; 3X: 2/6; 5X: 5/6.

BUN: At 24 hours postdose there were significant increases in urea nitrogen at 1X, 3X and 5X

(combined sexes: 1.4. 1.4 and 1.6-fold, P values of 0.0053, 0.0006 and 0.0000 respectively), and 1.2-fold on Day 8 at 5X, with decreases on Day 22 at 1X (5%) and 5X (19%). A normal range for BUN in the dog is 7-24 mg/dL; a number of dogs in the 1X, 3X and 5X groups (males: 0X: 0/6, 1X: 0/6: 3X: 1/6; 5X: 3/6; females: 0X: 0/6; 1X: 1/6; 3X: 0/6; 5X: 2/6) had values slightly above 24 mg/dL (up to 27 mg/dL) at 24 hours, and this was the only time during the study that any dogs were outside (or above) this reference range.

Aspartate aminotransferase (AST) was decreased at all dose levels 24 hours postdose (combined mean for both sexes as percentage of control mean: 1X: 87.8%; 3X: 85.0%; 5X: 78.1%). The significance (if any) of this finding is not evident, as there was no concurrent dose-related change in ALT. In any case, all values observed in this study for AST were below 105 U/L, and so were normal for the dog.

Cholesterol: Significantly increased at 5X at 24 hours and at 3X and 5X on day 8. The mean increases (relative to preexposure values) at 24 hours for the 5X group were: males: 14.7%; females: 14.8%; combined: 14.8%; day 8: 3X: males: 7.9%; females: 10.1%; combined: 9.0%; 5X: males: 20.8%; females: 15.1%; combined: 18.2%. However, the range of values (112-300 mg/dL) seen at 24 hours and on Day 8 was within the normal reference range (110-330 mg/dL), and the means for the 1X, 3X and 5X groups on Day 22 were not significantly different from the controls (and were all below preexposure values).

Total protein: Significantly elevated at 24 hours for combined sexes at 5X (P=0.0322), with combined controls at 6.04 ± 0.34 and the 5X dogs at 6.36 ± 0.37 g/dL. However, the observed ranges for both groups (controls: 5.7 - 7.0; 5X: 5.9 - 7.0 g/dL) were essentially the same and within the normal reference range of 5.4 - 8.0 g/dL.

Other clinical chemistry parameters were not significantly changed, or if significance occurred it was not dose-related, and/or measurements tended to be within normal range considering the ages of the dogs, and/or were similar to preexposure values.

Total leukocyte counts were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, for the combined sexes at the 3X and 5X dose levels 24 hours postdose. Concurrently, neutrophils were increased 1.2-fold at the 3X and 1.5-fold at 5X. Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges." One reference gives the normal range for dog WBC counts as 5.9-16.6 K/mm^3; a number of 3X (4/6M, 1/6F) and 5X (4/6M, 2/6F) dogs at 24 hours (and also one female control) had WBC counts above 16.6 K/mm^3, and the mean for 5X males was 18.7 K/mm^3. An elevation in leukocyte counts is normally a response to infection. It is noteworthy that this proposed formulation has tested positive as a dermal sensitizer (MRID 46395810).

MCHC was significantly decreased in the 1X, 3X and 5X groups at 24 hours postdose in gender-pooled samples. There were mild decreases of erythrocytes (5%), hemoglobin (7%) and hematocrit (6%) in gender-pooled samples 24 hours postdose at 1X. MCV was significantly decreased 24 hours postdose for combined sexes at 1X, and significantly increased at 3X and 5X. MCV was also statistically increased for combined sexes at 3X on Day 22. MCH was decreased in males at 1X and 5X on Day 22, and in females at 1X. Absolute and percent reticulocytes were decreased in gender-pooled samples at all dose levels 24 hours postdose, but this was considered not physiologically relevant as there were no sustained effects on red cell parameters. However, as all individual values for these indices, at all dose levels, and at all intervals, fell within expected ranges, they were not physiologically relevant.

While the conduct and reporting of this companion animal (adult dog) safety study (OPPTS 870.7200) were adequate, the study does not demonstrate that there is an adequate margin of

safety (5X) associated with the proposed use of this formulation, and so does not support registration. This conclusion is based on the following:

1. A depressed righting reflex was seen in 4/12 5X dogs. In two of these dogs it was observed on Days 1, 2, 8 and 22, indicating the possibility of a permanent effect.

2. All dogs (including those at 0X) were very quiet and inactive ("little activity or barking compared to the previous day, even during repeated entries by the technical staff") on the afternoon of Day 1 (applications of test material or placebo were made during the morning). While the report states that this could be a result of increased handling which had occurred earlier (during application of the test substance or placebo), Amitraz can cause sedation. Although dogs in the placebo control group also showed the same or a similar effect, one or more of the inerts in this formulation have pharmacological activity, and these controls were being exposed to essentially a 7X dosage of the formulation without the actives.

3. The formulation has tested positive as a dermal sensitizer. Possibly associated with this, total leukocyte counts were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, for combined sexes at the 3X and 5X dose levels 24 hours postdose. Concurrently, neutrophils were increased 1.2-fold at the 3X and 1.5-fold at 5X. Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges. An elevation in leukocyte counts is normally a response to infection.

4. The increase in glucose levels for all dose groups (1X, 3X and 5X) at 24 hours is consistent with a known effect of Amitraz, and indicates pharmacologically significant quantities of this active are absorbed at even the 1X dose level.

The proposed label suggests that this would be an over-the-counter product, readily available to dog owners, particularly as the only mention of veterinarians is in the standardized statements under the heading HAZARDS TO DOMESTIC ANIMALS ("Consult a veterinarian before using this product on aged, debilitated, medicated, pregnant or nursing animals. Individual sensitivities while rare may occur after the use of any pesticide product. If signs persist or become more severe, consult a veterinarian immediately...").

The label for Mitaban® (a formulation containing 19.9% Amitraz, approved by the FDA for treating generalized canine demodicosis) includes the following WARNING statement: "Toxicology studies conducted in the dog and other species suggest amitraz may alter the animal's ability to maintain homeostasis. Animals treated with MITABAN (Amitraz) should not be subjected to stress for a period of at least 24 hours posttreatment. Adverse reactions including three fatalities were reported during the clinical studies. In excess of 1100 patients with generalized demodicosis were topically treated with MITABAN." In addition, the Mitaban® label includes the statement: "**Caution:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

The overall exposure level to Amitraz associated with the proposed use of this product seems to be comparable to that associated with the use of Mitaban® . However, there are indications that Amitraz from the Promeris formulation is absorbed more rapidly and/or to a greater extent than Amitraz in the 250 ppm Mitaban-use dilution. Publically available information on Mitaban® includes the following [see myweb.cableone.net/bdturner/**Mitaban**.pdf]: "Blood glucose values were elevated at 4 hours posttreatment in the 250 ppm female group, and in both sexes at the 1250 and 2500 ppm concentrations. ...glucose values returned to normal within 24 hours posttreatment. In another study, groups of healthy beagles were topically treated with either 250 ppm, 750 ppm or 1250 ppm of active drug at 14 day intervals and for 12 weeks. Blood glucose values were elevated at the 750 ppm concentration at 4 hours posttreatment after 3 of 6

treatments, and after 5 of 6 treatments at the 1250 ppm level. In the 750 ppm group, serum glucose values returned to normal at 24 hours posttreatment, however for the 1250 ppm group, at 24 hours and after 3 of 6 treatments the levels remained significantly elevated." In the adult dog Promeris study [MRID 46401003] 1/6 of the 3X females and 5/6 5X females had elevated glucose levels (≥120 mg/dL) at 24 hours, suggesting that Amitraz absorption from 5X Promeris treatment (7.0 mL) is greater than that from exposure to 2500 ppm Amitraz (or a 10X concentration of the use-dilution of Mitaban®), and a 1X (1.4 mL) Promeris treatment of an adult dog would then be associated with greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban® solution.

There is also potential for adverse drug reactions: from http://www.aava.org/pub/iatrogenic.html: "Ivermectin which is in Heartgard heart worm preventative and is sometimes used to treat "mange" and ear mites, causes increases in monoamine neurotransmitter metabolites which could result in important adverse drug reactions with amitraz (Mitaban topical mange treatment)..." At the very least, if this product is registered it should be available only through licensed veterinarians, as is Mitaban®.

COMPLIANCE: Signed and dated Quality Assurance (p. 8), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements were present.

A. MATERIALS

1. Test material: 15% w/v R-28153/15% w/v amitraz spot-on, with a label declaration of 14.34% Metaflumizone (also known as BAS 320 I) and 14.34% Amitraz. According to a certificate of analysis on p. 480 of MRID 46401003 the test formulation contained 14.7% w/w R-28153 and 14.65% Amitraz and had a specific gravity of 1.047 g/mL.
   Description: Pale yellow liquid
   Lot No.: 0381702 (manufactured August 5, 2003)
   Storage: Room Temperature, with protection from light

   Placebo: 0% w/v R-28153/% w/v amitraz spot-on. This formulation contained less than 0.15% w/v R-28153 and less than 0.15% w/v Amitraz. The specific gravity is reported as 0.9964-0.9967.
   Description: Clear colorless liquid
   Lot No.: 0381701 (manufactured July 15, 2003)
   Storage: Room Temperature, with protection from light

2. Administration: Topical (spot-on) on Day 1

3. Test animals
   Species: Dog
   Breed: Beagles
   Ages and weights at study initiation (Day 1, day of dosing): Males: 6 months, 1 day to 8 months, 25 days; 9.93 to 11.91 kg; Females: 7 months, 29 days to 8 months 25 days; 6.68 to 9.74 kg. Dogs were born between December 1, 2002 and February 25, 2003.
   Source: Covance Research Products, Kalamazoo, MI.
   Housing:
   Diet: PMI Canine High Density Diet 5L18.
   Water: Tap water, *ad libitum*

Environmental conditions:
Temperature: 22° ± 3°C
Humidity: 30 - 70%
Air changes: 10 - 12/hr
Photoperiod: 12 hr dark/12 hr light
Acclimation period: 2 weeks

II. STUDY DESIGN

A. IN LIFE DATES

From the report cover: study initiation date: 13 August 2003; study completion date: 20 October 2003.

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

There were a total of 12 (6 male, 6 female) dogs per dosage group. Dogs in the placebo control group received either 7.0 mL (dogs weighing less than 10.5 kg) or 17.0 mL (>10.5 kg) of the formulation less actives. As a result, they received an approximately 7X dose of the "other ingredients." Dogs in the 1X group received either 1.4 (<10.5 kg) or 3.4 (>10.5 kg) mL of the formulated product. Those in the 3X group received either 4.2 (<10.5 kg) or 10.2 (>10.5 kg) mL. Those in the 5X group received either 7.0 (<10.5 kg) or 17.0 (>10.5 kg) mL formulated product.

From p. 14 of MRID 46401003: "The test and placebo control substances were administered once on Day 1 (August 26, 2003) by topical application. The animal was held in a position such that the surface to be treated was easily accessible. A section of hair between the shoulder blades on the dorsal midline extending cranially and caudally was separated. A Becton Dickinson disposable syringe containing the appropriate amount of test substance was placed through the hair to the skin and the test substance was applied by dragging the syringe back slowly distally along the dorsal midline, while applying the dose. Due to varying dose volumes, a 1 mL syringe was used to administer the part of the dose that was less than 1 mL. A 5 mL syringe was used for the parts of the dose volumes at 1, 3 and 4 mL. A 10 mL syringe was used for dose volumes of 7 mL. A 20 mL syringe was used for the part of the dose volumes of 10 and 17 mL. Care was taken when applying the test substance to reduce the chance of run-off, and no run-off was observed. Separate syringes were used to apply test and control substances and gloves were changed between test and control substance application..."

TABLE 1. Study design								
Group & Weight Range (kg)		Number of dogs		Cumulative Dose/dog				
		Male	Female	Total/Dog	Mean Dog Wt ± S.D. (kg)	Mean mg/kg	Mean Dosage (mg/kg)	
							Metaflumi-zone	Amitraz
Placebo Control	<10.5	1	6	7.0 mL[a]	8.11 ± 1.11	860[b]	0	0
	>10.5	5	0	17.0 mL[a]	11.03 ± 0.44	1536[b]	0	0
1X	<10.5	2	6	1.4 mL[c]	8.51 ± 1.25	172[d]	25.3	25.2
	>10.5	4	0	3.4 mL[c]	11.38 ± 0.46	313[d]	46.0	45.9
3X	<10.5	3	6	4.2 mL[c]	8.64 ± 1.25	509[d]	74.8	74.6
	>10.5	3	0	10.2 mL[c]	11.50 ± 0.46	929[d]	136.6	136.1
5X	<10.5	2	6	7.0 mL	8.62 ± 1.21	850[d]	125.0	124.5
	>10.5	4	0	17.0 mL	11.22 ± 0.52	1586[d]	233.1	232.3

Data calculated from information on p. 482-485 of MRID 46401003.
[a] Placebo
[b] Based on a specific gravity for the placebo of 0.9965 g/mL (see p. 479 of MRID 46401003).
[c] Test material (with actives); amount delivered.
[d] Based on a specific gravity for the test material of 1.047 g/mL (see p. 480 of MRID 46401003)

C. DOSE SELECTION RATIONALE

From p. 14 of MRID 46401003: "The target minimum dose is 20 mg/kg R28153 and 20 mg/kg amitraz. Each mL contains 150 mg R28153 and 150 mg amitraz. Since the product was packaged for animal weight bands (4.6 to 10.5 kg and 10.6 to 25.5 kg), the test substance was administered at the same volume for each animal within the specified weight range. The dose levels were selected by the Sponsor to evaluate the safety of the test substance at up to five times the proposed *ad usum* rate in adult dogs. This was considered to provide an appropriate safety margin for the planned therapeutic dose."

According to the proposed label this product will be packaged as 6-packs of monthly unidose applicators with the following single dosages: 0.67 mL (for dogs less than 11 lb); 1.33 mL (for dogs weighing from 11 to 22 lb); 3.33 mL (for dogs weighing from 22 to 56 lbs); 5.33 mL (for dogs weighing 56 to 89 lbs); and 6.66 mL (for dogs weighing 89-111 lbs). Application directions include a specification to apply the entire content of the applicator on the dog's skin and not to apply to the surface of the dog's fur.

D. EXPERIMENTAL DESIGN

From p. 15 of MRID 46401003: "Clinical observations were conducted twice daily, approximately four hours apart during the acclimation period and Days 28 through 28, except on days that neurological and physical examinations were conducted (Days -1, 2, 8, and 22). On those days, clinical findings were conducted only in the morning. On the day of dosing (Day 1), clinical examinations were made predose and at 1, 2, and 3 hours postdose..."

Individual dogs were weighed on Days -14, -7, -1 (the day before dosing) and weekly thereafter. Body weights for Day -14 are not included in the report.

Individual food consumption was measured daily and reported weekly (beginning the last four days of the acclimation period and continuing through to the end of the study).

E. PATHOLOGICAL PARAMETERS

Blood samples were collected from each dog once pretest (Week -1), and on Days 2 (approximately 24 hours postdose), 8 and 22 by jugular venipuncture following an overnight fast. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
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	Absolutes reticulocytes
	Blood clotting measurements	X	Percent reticulocytes
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time [PT])*		
X	(Activated partial thromboplastin time [APTT])*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Blood creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
		X	Total bilirubin (T Bil)*
	ENZYMES		Direct bilirubin (D Bil)*
X	Alkaline phosphatase(ALPor ALK)*	X	Total serum protein (TP)*
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)	X	Albumin/Globulin (A/G) ratio
X	Serum alanine aminotransferase (ALT or SGPT)*	X	Lipase
X	Serum aspartate aminotransferase(AST or SGOT)*		
X	Gamma glutamyl transferase(GGT)		
X	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

A statistical summary report is found in Appendix M, running from pages 1288 to 1296 of MRID 4640103. From p. 16 of MRID 4640103: "Each of the four treatment groups contained one animal from each of six blocks for each sex. Data were summarized, in tabular form, for each group by mean, standard deviation, number of animals examined, minimal value, and maximum value."

For body weight: "Statistical analysis was performed on body weight change at Weeks 1, 2, 3, and 4 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% level

of significance. If the treatment by sex interaction was found significant, then the treated groups' LSMeans were compared to the control group LSMean for each sex by the 2-sided Student's t-test at the 10% level for that parameter. If the treatment by sex interaction was found not to be significant and treatment effect was found to be 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 found not significant for a parameter, no further analysis for that parameter was done at this step and treatments were considered to have no effect on body weight change."

For body weight and food consumption: "A repeated measure analysis was performed on body weight and food consumption parameters using the PROC MIXED procedure in SAS. The model contained pretreatment Day -1 body weight and Week -1 average food consumption values as corresponding covariates, and treatment, treatment by sex, week, sex by week, treatment by sex by week, and treatment by week as the fixed effects with week as the repeated fix effect... If treatment by sex by week interaction was found significant at the 5% level for a parameter, no further analysis was done for that parameter... If treatment by sex by week interaction was found not significant for a parameter, then treatment by sex interaction was tested at the 5% level of significance and treatment by week interactions...[was]...tested at the 10% level for that parameter. If treatment by sex interaction was found significant for the parameter, then the 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..."

"For all the quantitative hematology, coagulation and clinical chemistry parameters, pretreatment measurements were considered covariant in the analysis. For count data such as leukocyte count, etc., log-transformation was used. The individual parameters were analyzed by time (i.e. 24 hours postdose, Days 8 and 22) for hematology, coagulation and clinical chemistry parameters by the PROC MIXED procedure with pretreatment measurements as corresponding covariates, and treatment, sex and treatment by sex as fixed main effects and interaction..."

G. DISPOSITION OF ANIMALS

From p. 16 of MRID 46401003: "The animals were transferred to a stock colony on September 23, 2003. According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

H. COMPLIANCE

Signed and dated Quality Assurance [p. 8], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

Refer to Table 1 of this DER. Dogs in the placebo control group received either 7.0 mL (dogs weighing <10.5 kg) or 17.0 mL (>10.5 kg) of the formulation less actives. As a result, they received an approximately 7X dose of the "other ingredients." Dogs in the 1X group received either 1.4 (<10.5 kg) or 3.4 (>10.5 kg) mL of the formulated product. Those in the 3X group received either 4.2 (<10.5 kg) or 10.2 (>10.5 kg) mL. Those in the 5X group received either 7.0 (<10.5 kg) or 17.0 (>10.5 kg) mL formulated product.

B. MORTALITY

There was no mortality, with all dogs surviving the 28-day observation period.

C. CLINICAL SIGNS

It is stated (p. 20 of MRID 46401003) that: "No treatment related effect on clinical observations or Day 1 postdose observations was seen from treatment with R-28153/amitraz spot-on. Various observations were seen during the course of the study. These observations consisted of emesis, purulent ocular discharge, watery feces, sclera injected, lacrimation, abrasion, and skin discoloration. Several of the females also exhibited normal signs of estrus. All of these observations were considered to be within the normal variation for beagle dogs of this age and not related to treatment."

Examination of group observations indicates that few/absent feces were noted in a number of dogs of all exposure groups at the day 2 P.M. observation and again on the day 3 A.M. observation; otherwise it was an uncommon finding (noted in 1/6 control females on days 10 (A.M.) and 11 (A.M.) as well as in 1/6 5X females on day 15 (A.M.). Purulent ocular discharge was observed in one 3X male from the A.M. observation on day 2 through the P.M. observation on day 5, and in one 5X female, from the P.M. observation on day through through the A.M. observation on day 6; otherwise, this finding was not observed in this study.

TABLE 2. Effects Observed in Dogs Treated with R-28153 and Amitraz Spot-on In the Period Immediately Following Treatment[a]

Parameter	Placebo (Vehicle) Control	1X	3X	5X
Feces few/absent, day 2 P.M. observation	0/6M; 1[b]/6F	0/6M; 1[b]/6F	1[b]/6M; 1[b]/6F	3[b]/6M; 1[b]/6F
Feces few/absent, day 3 A.M. observation	0/6M; 1[b]/6F	0/6M; 0/6F	1[b]/6M; 1[b]/6F	2[b]/6M; 0/6F
Ocular discharge, purulent, day 2 P.M. observation	0/6M; 0/6F	0/6M; 0/6F	1[c]/6M; 0/6F	0/6M; 1[d]/6F

[a]Data taken from Table 1, Summary of Clinical Findings, pp. 26-57 of MRID 46401003.
[b]The following dogs had few/absent feces on days 2 and/or 3: 0XF: 138; 1XF: 140; 3XM: 141; 3XF: 139; 5XM: 118, 134, 144; 5XF: 123
[c]Dog 101; present for left eye from the A.M. observation on day 2 through the P.M. observation on day 5, also reported sclera injected for both eyes days 4-6 and 10-14.
[d]Dog 114, present for both eyes from the A.M. observation on day 2 through the A.M. observation on day 6.

D. NEUROLOGICAL EXAMINATIONS

From p. 21: "Treatment with R-28153/amitraz spot-on caused no treatment related effect on physical or neurological examinations. Observations noted were spread across all groups, including the placebo controls, and were all common findings for dogs of this age. During the neurological examinations, a depressed righting reflex was noted in two dogs at 5X on Days 1 and 2 [male #134 -see p. 680 - and female #137- see p. 776] and an additional 5X dog on Days 1, 2, 8 and 22 [male #128 - see p. 676 & 678; however, 5X female #147 also showed this effect, see p. 780 & 782]. One dog at 1X on Day 2 and one female at 3X on Day 22 had a depressed righting reflex. [Note: one female control - #108 - had a depressed righting reflex on Day 8, see p. 688]. Since this effect was transitory [? - depressed righting reflex persisted to Day 22, last neurological examination day, in male #128 and in female #147] and only seen in two males and two females in the 5X groups, it was not considered clinically significant."

The report then states (p. 21) that: "An additional neurological observation of interest was a general observation made during the afternoon examination session of Day 1. During this session, all of the dogs appeared unusually quiet and less active than usual. This observation included the placebo controls [which had been treated with vehicle at a dosage about 7X use exposure level] and could be a result of the increased handling, which occurred earlier on Day 1. This was a transient effect as all of the dogs had returned to a normal activity level the next day."

E. BODY WEIGHT AND WEIGHT GAIN

From p. 20 of MRID 46401003: "Treatment with R-28153/amitraz spot-on resulted in no treatment related effect on body weight. Body weight at the 1X, 3X, and 5X levels were generally comparable to the placebo controls. The only statistical significance noted was an increase in body weight at the 5X level for pooled sexes compared to the placebo control at week 2. This increase was not considered related to treatment."

The values in Table 3 are calculated from individual body weight data (p. 541-544 of MRID 46401003):

TABLE 3. Mean Body Weights for Dogs by Group

Group	kg ± S.D.				
	Day -1	Day +1	Day 8	Day 15	Day 22
Control Males	10.94 ± 0.63	10.89 ± 0.53	10.79 ± 0.53	11.14 ± 0.64	10.92 ± 0.57
Control Females	7.90 ± 0.66	7.77 ± 0.68	7.68 ± 0.59	7.94 ± 0.73	7.83 ± 0.60
1X Males	10.95 ± 0.79	10.98 ± 0.72	10.90 ± 0.84	11.10 ± 0.89	10.93 ± 0.95
1X Females	7.94 ± 0.81	7.95 ± 0.84	7.78 ± 0.78	8.03 ± 0.91	7.89 ± 0.88
3X Males	10.95 ± 0.73	10.88 ± 0.76	10.98 ± 0.78	11.01 ± 0.66	10.95 ± 0.56
3X Females	7.94 ± 0.56	7.83 ± 0.31	7.77 ± 0.38	7.88 ± 0.38	7.90 ± 0.39
5X Males	10.89 ± 0.64	10.87 ± 0.68	11.17 ± 0.80	11.16 ± 0.89	10.93 ± 0.92
5X Females	8.06 ± 0.78	8.09 ± 0.86	8.20 ± 0.89	8.27 ± 0.89	8.16 ± 0.88

Values calculated from data on p. 541-544 of MRID 46401003.

From p. 1288: "...in Week 2 [Day 8] there was a significance when comparing treatment group 5X to the Placebo Control (p=0.0033)." There was also a significant (p=0.0022) body weight change in the pooled (males + females combined) 5X group relative to the Placebo Control group for week 2. However, mean body weights for 5X males and 5X females were actually somewhat greater than those for their respective controls, and the difference is not biologically significant.

F. FOOD CONSUMPTION

From p. 21 of MRID 46401003: "No treatment related effects on food consumption were noted after treatment with R-28153/amitraz spot-on. Statistical significance was noted in several instances for pooled sexes in the treatment groups when compared to the placebo control. Food consumption was increased at 3X and 5X dose levels at Week 1 and at the 3X treated group at Week 4. Food consumption was decreased at 1X and 3X compared to the placebo control group at Week 2 and at 5X treated group at Week 3. Considering the sporadic nature of these effects, they were not considered related to treatment." This reviewer notes that mean daily food consumption for both sexes at 3X

and for females at 5X was very similar for week -1 and week 1, and slightly elevated for males at 5X for week 1 relative to week -1.

TABLE 4. Mean Food Consumption (g ± S.D./dog/day) by Group					
Group	Week -1	Week 1	Week 2	Week 3	Week 4
Control Males	355.7 ± 52.1	311.6 ± 70.5	349.6 ± 55.2	336.3 ± 35.4	353.6 ± 41.3
Control Females	284.6 ± 67.2	240.5 ± 50.3	274.3 ± 58.0	294.3 ± 66.9	278.5 ± 59.3
1X Males	385.4 ± 36.1	351.7 ± 49.5	315.6 ± 35.9	334.9 ± 27.7	362.9 ± 34.6
1X Females	286.7 ± 63.5	252.3 ± 55.1	259.1 ± 62.7	292.2 ± 65.4	288.0 ± 51.0
3X Males	361.3 ± 60.7	370.8 ± 51.7	310.0 ± 30.9	347.8 ± 49.0	377.7 ± 38.4
3X Females	288.1 ± 82.8	260.2 ± 72.1	252.0 ± 47.4	286.6 ± 47.7	304.5 ± 62.9
5X Males	332.1 ± 25.8	377.3 ± 47.0	341.5 ± 51.3	307.3 ± 33.1	344.7 ± 46.1
5X Females	292.3 ± 42.8	305.0 ± 39.6	241.3 ± 38.3	224.0 ± 30.7	264.3 ± 28.5

Values calculated from data on p. 567-570 of MRID 46401003.

TABLE 5. Mean Food Consumption (g ± S.D./dog/day) by Weight Group					
Group	kg ± S.D.				
	Week -1	Week 1	Week 2	Week 3	Week 4
Control <10.5	304.1 ± 80.2	260.3 ± 69.6	291.8 ± 70.4	307.7 ± 70.6	294.4 ± 68.6
Control >10.5	342.5 ± 45.8	298.1 ± 69.7	340.1 ± 56.0	325.9 ± 27.5	346.3 ± 41.6
1X < 10.5	317.7 ± 79.2	269.7 ± 59.1	266.0 ± 56.1	308.6 ± 63.5	308.0 ± 60.5
1X > 10.5	372.7 ± 36.3	366.6 ± 50.1	330.1 ± 30.1	323.4 ± 24.9	360.3 ± 31.3
3X < 10.5	302.9 ± 78.8	286.7 ± 74.2	270.1 ± 47.9	304.3 ± 50.0	321.3 ± 57.8
3X > 10.5	389.9 ± 32.1	402.0 ± 32.7	313.7 ± 41.9	356.0 ± 65.4	400.6 ± 33.7
5X < 10.5	302.5 ± 41.0	319.8 ± 47.1	257.8 ± 49.3	245.5 ± 48.9	292.1 ± 54.2
5X > 10.5	331.6 ± 33.0	383.8 ± 51.9	358.5 ± 46.8	305.9 ± 39.0	336.3 ± 54.7

Values calculated from data on p. 567-570 of MRID 46401003.

G. HEMATOLOGY

From p. 789 of MRID 46401003: "In gender-pooled samples, total leukocytes were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, at 3X and 5X dose levels 24 hours postdose. Neutrophils were concurrently increased 1.2-fold at 3X, and 1.5-fold at the 1.5X dose level 24 hours postdose. Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges."

TABLE 6. Ranges & Means ± S.D. for Leukocytes (thousands/mm^3)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	7.8-12.5; 9.9 ± 1.7	8.8-13.8; 11.1± 1.7	8.4-12.7; 11.1 ± 1.7	8.8-13.1; 10.9 ± 1.4
Placebo females	7.9-12.5; 10.6 ± 1.7	7.3-21.3; 13.1 ± 4.6	8.3-18.9; 12.8 ± 4.2	7.4-17.7; 12.4 ± 3.8
1X males	8.5-14.4; 10.4 ± 2.3	10.7-14.2; 12.3 ± 1.4	10.0-24.4; 13.7 ± 5.3	10.2-15.1; 11.8 ± 1.8
1X females	7.4-12.1; 9.5 ± 1.8	9.5-12.4; 10.9 ± 0.9	7.2-13.4; 10.4 ± 2.5	7.5-14.4; 9.5 ± 2.5
3X males	9.2-13.3; 11.8 ± 1.4	12.9-18.7; **16.6 ± 2.0**	10.4-15.5; 13.0 ± 2.0	10.8-14.0; 11.8 ± 1.2
3X females	8.9-14.2; 11.7 ± 2.3	8.2-21.9; **14.6 ± 4.7**	10.1-13.5; 11.5 ± 1.4	9.3-12.6; 10.7 ± 1.4
5X males	9.9-13.6; 11.3 ± 1.3	11.5-27.3; **18.7 ± 5.5**	12.3-14.2; 13.0 ± 0.7	10.5-47.6; 17.5 ± 14.8
5X females	9.8-18.0; 11.8 ± 3.1	13.4-20.8; **16.4 ± 2.7**	10.2-15.4; 12.7 ± 1.7	9.6-16.0; 13.4 ± 3.2

Individual data on p. 938, 939, 951, 952, 964, 965, 977, 978, 989, 990, 1002, 1003, 1014, 1015, 1027 and 1028; Means and Standard Deviations on p. 138 and 139.
P-value for 3X group (combined sexes) at 24 hrs: 0.0419; P-value for 5X group (combined sexes) at 24 hrs: 0.0009 [refer to p. 137 of MRID 464010-03].

According to http://www.ahc.umn.edu/rar/RefValues.html the normal reference range for WBC counts for dog is 5.9-16.6 (in thousands/mm^3). A number of individual values - particularly at 24 hours postdose in the 3X and 5X groups - were above 16.6 thousand/mm^3:

TABLE 7. Incidences of Leukocytes > 16.6 (thousands/mm^3)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	0/6	0/6	0/6	0/6
Placebo females	0/6	1/6	1/6	1/6
1X males	0/6	0/6	1/6	0/6
1X females	0/6	0/6	0/6	0.6
3X males	0/6	4/6	0/6	0/6
3X females	0/6	1/6	0/6	0/6
5X males	0/6	4/6	0/6	1/6
5X females	1/6	2/6	0/6	0/6

From individual data on p. 938, 939, 951, 952, 964, 965, 977, 978, 989, 990, 1002, 1003, 1014, 1015, 1027 and 1028.

There was an increase in neutrophils at 24 hours, consistent with the increase in total leukocytes at 24 hours.

TABLE 8. Ranges & Means ± S.D. for Neutrophils (thousands/mm^3)				
Group & sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X males	5.29-8.74; 6.67±1.31	6.07-10.25; 7.54±1.45	5.43-9.16; 7.45 ± 1.44	5.91-8.69; 7.36 ± 0.94
0X females	5.20-8,77; 7.20±1.34	4.92-17.05; 9.38 ±4.08	5.51-13.86;8.90 ± 3.32	4.85-14.18; 8.23 ± 3.13
1X males	4.65-9.60; 6.36±1.81	5.75-9.22; 7.76±1.54	6.25-19.53;9.10 ± 5.14	5.79-9.55; 7.32 ± 1.31
1X females	4.66-6.92; 5.57±1.00	5.35-7.13; 6.50±0.73	5.13-9.06; 6.57 ± 1.75	4.06-9.88; 5.95 ± 2.02
3X males	6.20-9.65; 7.86±1.41	9.81-12.12;**11.23±0.79**	6.79-9.49; 8.39 ± 1.24	5.00-8.98; 7.40 ± 1.46
3X females	5.14-10.54; 7.48±2.18	4.83-16.15; **9.85±3.93**	5.78-8.23; 7.11 ± 1.03	5.06-8.68; 6.73 ± 1.27
5X males	6.34-9.58; 7.56±1.13	8.01-22.14;**14.41±4.73**	7.50-9.58; 8.84 ± 0.73	6.98-42.68;13.43 ± 14.35
5X females	5.60-12.69; 7.61±2.61	8.66-15.32;**11.64±2.28**	5.30-9.87; 8.04 ± 1.61	6.12-13.35; 13.4 ± 3.2

Individual data on p. 944, 945, 957, 958, 970, 971, 983, 984, 995, 996, 1008, 1009, 1020, 1021, 1033, and 1034. Means and Standard Deviations on p. 231 and 232. P-value for 3X group (combined sexes) at 24 hrs: 0.0759; P-value for 5X group (combined sexes) at 24 hrs: 0.0005 [refer to p. 230 of MRID 464010-03].

According to http://www.ahc.umn.edu/rar/RefValues.html the normal reference range for neutrophils (segmented) as a percentage of leukocytes in the dog is 51-84; with one exception all individual values at all time points were within this range. The exception was male #104 on day 22 (89.7%; this dog also had the highest leukocyte count - 47.6 x $10^3/mm^3$ - observed in the study at this time point; consistent with an infection, although the only observational finding for this dog [refer to p. 498 & 503] was a red discoloration of both ears, present in several other dogs with normal hematology findings, including male #128 in the 5X group at this same time). Five males in the 5X group had relatively high normal readings (75.5-81.2%) at 24 hrs postdose

There was also an increase in monocytes at 24 hours.

TABLE 9. Ranges & Means ± S.D. for Monocytes (thousands/mm^3)				
Group & sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X males	0.29-0.87; 0.55 ± 0.20	0.49-0.97; 0.66 ± 0.18	0.50-1.00; 0.67 ± 0.19	0.50-0.75; 0.59 ± 0.10
0X females	0.00-0.67; 0.45 ± 0.24	0.26-0.96; 0.64 ± 0.24	0.36-1.43; 0.73 ± 0.41	0.00-0.92; 0.48 ± 0.33
1X males	0.51-0.71; 0.60 ± 0.09	0.54-1.03; 0.81 ± 0.17	0.54-1.97; 0.91 ± 0.54	0.35-1.43; 0.69 ± 0.38
1X females	0.25-0.83; 0.54 ± 0.23	0.58-0.79; 0.66 ± 0.07	0.39-0.75; 0.58 ± 0.15	0.33-0.82; 0.52 ± 0.20
3X males	0.00-0.95; 0.61 ± 0.33	0.71-1.68; **1.29 ± 0.36**	0.00-1.46; 0.71 ± 0.47	0.00-1.22; 0.63 ± 0.39
3X females	0.45-0.83; 0.59 ± 0.13	0.52-1.41; **0.92 ± 0.33**	0.51-0.81; 0.64 ± 0.11	0.49-0.61; 5.48 ± 0.05
5X males	0.39 -0.92; 0.65 ± 0.21	0.68-1.96; **1.37 ± 0.43**	0.62-1.14; 0.82 ± 0.18	0.50-2.33; 0.96 ± 0.68
5X females	0.46-0.92; 0.62 ± 0.18	0.89-1.50; **1.14 ± 0.24**	0.54-0.91; 0.71 ± 0.16	0.35-1.26; 0.75 ± 0.33

Individual data on p. 944, 945, 957, 958, 970, 971, 983, 984, 995, 996, 1008, 1009, 1020, 1021, 1033, and 1034. Means and Standard Deviations on p. 246 and 247.
P-value for 3X group (combined sexes) at 24 hrs: 0.0006; P-value for 5X group (combined sexes) at 24 hrs: 0.0000 [refer to p. 245 of MRID 464010-03].

"There were mild decreases of erythrocytes (5%), hemoglobin (7%) and hematocrit (6%) in gender-pooled samples 24 hours postdose at 1X. MCV was [significantly] decreased 24 hours postdose in gender-pooled samples at 1X, and [significantly] increased at 3X

and 5X. MCV was also statistically increased in gender-pooled samples on Day 22 at 3X. MCH was decreased in males at 1X and 5X on Day 22, and in females at 1X. MCHC was [significantly] decreased at all dose levels 24 hours postdose in gender-pooled samples. All individual values for these indices, at all dose levels, and at all intervals, fell within expected ranges and were not considered physiologically relevant or test substance-related. Absolute and percent reticulocytes were decreased in gender-pooled samples at all dose levels 24 hours postdose, but this was considered not physiologically relevant as there were no sustained effects on red cell parameters."

H. CLINICAL CHEMISTRY

From p. 1292: "Overall analysis for sodium resulted in a significant treatment effect in the 24 hour postdose interval (p=0.0045). Follow-up analyses in that period resulted in statistical significance when comparing the Control group to the 3X and 5X treatment groups (p=0.0363 and p=0.0010, respectively). Also, overall analysis results indicated that Day 8 had a significant treatment by sex interaction (p=0.0384). Thus, follow-up analyses were done in this period by sex and resulted in significance for the male data. There was a significant difference when comparing the Control group to both the 1X and the 5X groups (p=0.0212 and p=0.0022, respectively).

TABLE 10. Ranges & Means ± S.D. for Sodium (mEq/L)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	146-149; 147.2 ± 1.2	146-148; 147.0 ± 0.9	151-153; 151.7 ± 0.8	146-149; 147.7 ± 1.2
Placebo females	146-150; 147.8 ± 1.3	145-148; 146.0 ± 1.3	148-152; 150.2 ± 1.7	146-148; 146.7 ± 0.8
1X males	145-148; 146.5 ± 1.0	145-148; 146.8 ± 1.0	147-151; 149.5 ± 1.4	145-149; 146.5 ± 1.8
1X females	146-149; 147.8 ± 1.2	145-149; 147.0 ± 1.4	149-152; 150.7 ± 1.2	145-148; 146.8 ± 1.2
3X males	147-148; 147.2 ± 0.4	145-152; **148.5 ± 2.3**	148-153; 150.5 ± 2.1	145-150; 147.3 ± 1.8
3X females	146-148; 147.2 ± 0.8	145-150; **147.3 ± 1.6**	149-151; 150.0 ± 0.9	146-148; 147.2 ± 0.8
5X males	147-148; 147.3 ± 0.5	146-152; **149.3 ± 2.4**	147-151; 149.2 ± 1.5	146-150; 147.3 ± 1.4
5X females	147-149; 147.7 ± 0.8	147-150; **148.3 ± 1.0**	149-152; 150.7 ± 1.0	146-149; 147.0 ± 1.3

Individual values on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.
Means and Standard Deviations on p. 319 and 320.
P-value for 3X group (combined sexes) at 24 hrs: 0.0363; P-value for 5X group (combined sexes) at 24 hrs: 0.0010 [refer to p. 318 of MRID 464010-03].

TABLE 11. Ranges & Means ± S.D. for Sodium (mEq/L)				
Group and weight	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X < 10.5 kg	146-150; 147.6 ± 1.4	145-148; 146.0 ± 1.2	148-152; 150.4 ± 1.7	146-148; 146.6 ± 0.8
0X > 10.5 kg	146-149; 147.4 ± 1.1	146-148; 147.2 ± 0.8	151-153; 151.6 ± 0.9	147-149; 148.0 ± 1.0
1X < 10.5 kg	146-149; 147.8 ± 1.0	146-149; 147.1 ± 1.2	149-152; 150.6 ± 1.1	145-149; 147.1 ± 1.2
1X > 10.5 kg	145-147; 146.0 ± 0.8	145-147; 146.5 ± 1.0	147-150; 149.0 ± 1.4	145-148; 145.8 ± 1.5
3X < 10.5 kg	146-148; 147.1 ± 0.6	145-150; 147.6 ± 1.5	149-153; 150.4 ± 1.3	145-150; 147.3 ± 1.5
3X > 10.5 kg	147-148; 147.3 ± 0.5	145-152; 148.5 ± 2.9	148-153; 150.0 ± 2.2	146-148; 147.3 ± 1.0
5X < 10.5 kg	147-149; 147.5 ± 0.8	147-150; 148.3 ± 1.0	148-152; 150.4 ± 1.3	146-149; 147.0 ± 1.1
5X > 10.5 kg	147-148; 147.5 ± 0.6	146-152; 150.0 ± 2.7	147-150; 149.0 ± 1.4	146-150; 147.5 ± 1.7

Calculated from individual values on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for Na+ in the dog is 140-165 mEq/L. The range in this study (145-153) was within this reference range. In addition, mean values observed for the 3X and 5X groups at 24 hours were between those of controls at 24 hours and day 8. Therefore, it is concluded that the statistically significant differences observed in sodium levels were not biologically relevant.

From p. 1292: "A significant treatment effect was found for potassium at both the 24-hour postdose and Day 8 intervals (p=0.0034 and p=0.0132, respectively). Follow-up analyses were done for both of these periods with sexes combined. The follow-up analyses in the 24 hour postdose interval resulted in significance for all treatment groups compared to the Control with p=0.0082, 0.0006, and 0.0086, respectively for treatment groups 1X, 3X, and 5X. Day 8 follow-up also showed significance when comparing Control to treatment group 3X (p=0.0859) and 5X (p=0.0025)."

TABLE 12. Ranges & Means ± S.D. for Potassium (mEq/L)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	4.6 - 5.0; 4.7 ± 0.2	4.3 - 4.8; 4.6 ± 0.2	4.3 - 5.1; 4.7 ± 0.3	4.1 - 5.0; 4.4 ± 0.3
Placebo females	4.3 - 4.9; 4.7 ± 0.2	4.1 - 4.5; 4.4 ± 0.2	3.8 - 4.8; 4.4 ± 0.4	4.0 - 5.0; 4.5 ± 0.5
1X males	4.5 - 5.3; 4.8 ± 0.3	4.6 - 5.3; **5.0 ± 0.2**	4.2 - 5.1; 4.8 ± 0.3	4.3 - 4.6; 4.5 ± 0.1
1X females	4.4 - 5.4; 4.8 ± 0.4	4.3 - 5.2; **4.7 ± 0.3**	4.3 - 4.9; 4.7 ± 0.3	4.3 - 4.8; 4.5 ± 0.2
3X males	4.0 - 4.8; 4.5 ± 0.3	4.4 - 5.4; **4.8 ± 0.4**	4.4 - 5.4; **4.9 ± 0.4**	4.0 - 4.6; 4.4 ± 0.3
3X females	4.5 - 4.9; 4.7 ± 0.2	4.7 - 5.8; **5.0 ± 0.4**	4.4 - 5.0; **4.6 ± 0.2**	4.3 - 4.7; 4.5 ± 0.2
5X males	4.3 - 4.8; 4.6 ± 0.2	4.4 - 5.1; **4.8 ± 0.3**	4.6 - 5.4 ; **5.0 ± 0.3**	3.9 - 4.9; 4.4 ± 0.3
5X females	4.2 - 5.3; 4.9 ± 0.4	4.3 - 5.2; **4.8 ± 0.3**	4.2 - 5.5; **5.0 ± 0.5**	4.0 - 5.2; 4.5 ± 0.4

Individual values on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003. Means and Standard Deviations on p. 327 and 328.
P for 1X group (combined sexes) at 24 hrs: 0.0082; P for 3X group (combined sexes) at 24 hrs: 0.0006; P for 5X group (combined sexes) at 24 hrs: 0.0086; P for 3X group (combined sexes) for day 8: 0.0859; P for 5X (combined sexes) for day 8: 0.0025 [refer to p. 326 of MRID 464010-03].

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for K+ in the dog is 4.4-6.1 mEq/L. The range in this study (3.8-5.8) included values below this reference

range, but the exposed dogs at 24 hours and on day 8 showed a range of values (4.3-5.8) essentially within this reference range. In addition, control mean values at 24 hours were somewhat below control mean preexposure values at week -1. While exposure to the test material may have caused a slight increase in serum potassium, it is concluded that there are no indications that this would be of concern.

From p. 789: "In gender-pooled samples 24 hours postdose...calcium was mildly increased at 3X (1.03-fold) and 5X (1.07-fold)..."

TABLE 13. Ranges & Means ± S.D. for Calcium (mg/dL)				
Group & sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	10.7-11.2; 10.9 ± 0.2	10.9-11.9; 11.3 ± 0.3	10.6-11.4; 10.9 ± 0.3	10.5-11.3; 10.9 ± 0.3
Placebo females	10.5-11.4; 10.9 ± 0.3	10.8-11.6; 11.0 ± 0.3	10.2-10.9; 10.5 ± 0.2	10.2-11.2; 10.7 ± 0.3
1X males	10.8-11.6; 11.3 ± 0.3	11.1-12.0; 11.7 ± 0.3	10.5-11.4; 11.0 ± 0.4	10.6-11.3; 11.0 ± 0.3
1X females	10.5-11.1; 10.8 ± 0.2	10.7-11.5; 11.1± 0.3	10.2-11.1; 10.7 ± 0.3	10.4-11.1; 10.7 ± 0.3
3X males	10.5-11.1; 10.9 ± 0.2	11.4-12.1; **11.7 ± 0.3**	10.4-11.0; 10.8 ± 0.2	10.3-11.0; 10.7 ± 0.3
3X females	10.6-11.1; 10.8 ± 0.21	10.9-12.0; **11.3 ± 0.4**	10.3-10.9; 10.6 ± 0.2	10.2-10.8; 10.6 ± 0.3
5X males	10.8-11.5; 11.2 ± 0.3	11.6-12.9; **12.2 ± 0.6**	10.4-11.3; 10.9 ± 0.4	10.3-11.8; 10.9 ± 0.5
5X females	10.7-11.4; 11.0 ± 0.3	11.1-12.0; **11.7 ± 0.3**	10.7-11.0; 10.8 ± 0.1	10.7-10.9; 10.8 ± 0.1

Individual values on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003. Means and Standard Deviations on p. 343 and 344. P for 3X group (combined sexes) at 24 hrs: 0.0305; P for 5X group (combined sexes) at 24 hrs: 0.0001 [refer to p. 342 of MRID 464010-03].

Grouping the dogs by weights (<10.5 kg and >10.5 kg, correlating with dosage, as dogs >10.5 kg received a greater dose of test material on a bodyweight basis) rather than by sexes gives the values shown below:

TABLE 14. Ranges & Means ± S.D. for Calcium (mg/dL)				
Group and weight	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X < 10.5 kg	10.5-11.4; 10.9 ± 0.3	10.8-11.9; 11.2 ± 0.4	10.2-11.4; 10.6 ± 0.4	10.2-11.2; 10.7 ± 0.4
0X > 10.5 kg	10.7-11.2; 10.9 ± 0.2	10.9-11.4; 11.2 ± 0.2	10.6-11.0; 10.8 ± 0.2	10.5-11.3; 10.9 ± 0.3
1X < 10.5 kg	10.5-11.6; 10.9 ± 0.4	10.7-11.8; 11.3 ± 0.4	10.2-11.2; 10.8 ± 0.3	10.4-11.3; 10.8 ± 0.3
1X > 10.5 kg	10.8-11.4; 11.2 ± 0.3	11.1-12.0; 11.6 ± 0.4	10.5-11.4; 11.1 ± 0.4	10.8-11.2; 11.0 ± 0.2
3X < 10.5 kg	10.6-11.1; 10.8 ± 0.2	10.9-12.1; 11.4 ± 0.4	10.3-11.0; 10.7 ± 0.2	10.2-11.0; 10.7 ± 0.3
3X > 10.5 kg	10.5-11.1; 10.9 ± 0.3	11.4-12.1; 11.9 ± 0.4	10.4-11.0; 10.7 ± 0.3	10.3-10.9; 10.6 ± 0.3
5X < 10.5 kg	10.7-11.5; 11.1 ± 0.3	11.1-12.0; 11.7 ± 0.3	10.7-11.3; 10.9 ± 0.2	10.7-10.9; 10.8 ± 0.1
5X > 10.5 kg	10.8-11.5; 11.1 ± 0.3	11.6-12.9; 12.4 ± 0 .6	10.4-11.3; 10.8 ± 0.4	10.3-11.8; 10.9 ± 0.6

Values calculated from data on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.

Dogs >10.5 kg (which received both a greater absolute dose and a greater dose on a body weight basis) at the 3X and 5X levels showed a higher mean serum calcium value than dogs <10.5 kg. Dogs >10.5 kg also had a noticeably higher mean serum calcium than those <10.5 kg, and this is at least suggestive of an effect.

According to http://www.ahc.umn.edu/rar/RefValues.html normal values for calcium in the

dog are 9-11.4 mg/dL. However, in this study values as high as 11.6 mg/dL were observed at Week -1. Values > 11.6 mg/dL were observed in most groups (including controls <10.5 kg) at 24 hours postdose, but the only other occurrence was in one 5X male >10.5 kg on day 22.

TABLE 15. Incidences of Calcium >11.6 mg/dL

Group and weight	Week -1	24 hrs postdose	Day 8	Day 22
0X < 10.5 kg	0/7	1/7 (1/1M)	0/7	0/7
0X > 10.5 kg	0/5	0/5	0/5	0/5
1X < 10.5 kg	0/8	2/8 (2/2M)	0/8	0/8
1X > 10.5 kg	0/4	2/4	0/4	0/4
3X < 10.5 kg	0/9	1/9 (0/3M)	0/9	0/9
3X > 10.5 kg	0/3	2/3	0/3	0/3
5X < 10.5 kg	0/8	5/8 (2/2M)	0/8	0/8
5X > 10.5 kg	0/4	3/4	0/4	1/4

Values calculated from data on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.

The report (p. 1293 of MRID 46401003) states: "In the 24 hour postdose interval a significant treatment effect was found for calcium (p=0.0005). Follow-up analyses were done in this interval with sexes combined. This analysis showed significance for treatment groups 3X and 5X when compared to the Control with p=0.0305 and p<0.0001, respectively." Also, "at 24 hours, calcium was slightly increased at 3X and 5X (combined sexes: 1.03-fold and 1.07-fold respectively). However, there were indications of an effect at all dose levels, particularly in males, with the following incidences of calcium >11.6 mg/dL (normal reference values are 9-11.4 mg/dL) in males at 24 hours: 0X: 1/6; 1X: 4/6; 3X: 2/6; 5X: 5/6.

It is reported (p. 350) that the serum phosphorus level in the 5X group (combined sexes) was significantly lower than controls on day 22 [P-value compared with control = 0.0177; males: controls: 5.42 mg/dL; 5X: 4.95; females: controls:]. However, phosphate levels in the 5X group were not significantly lower than controls at 24 hours and on day 8 [24 hours: males: controls: 5.88; 5X: 5.77; females: controls: 5.15; 5X: 5.43; day 8: males: controls: 6.03; 5X: 6.00; females: controls: 5.28; 5X: 5.72]. This may be related to effects on alkaline phosphatase at 24 hours and 8 days (see below):

Alkaline phosphatase was significantly elevated at 24 hours in the 5X dose group (combined sexes) and at day 8 at 3X and 5X (combined sexes for both groups).

TABLE 16. Ranges & Means ± S.D. for Alkaline Phosphatase U/L)				
Group & sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	44-86; 63.0 ± 14.8	44-88; 63.2 ± 16.8	39-75; 56.0 ± 15.1	34-95; 58.5 ± 22.8
Placebo females	47-95; 70.0 ± 16.0	47-99; 75.0 ± 19.5	38-84; 61.8 ± 15.3	37-82; 59.3 ± 14.8
1X males	67-177; 97.3 ± 40.7	69-165; 96.7 ± 35.3	63-161; 95.8 ± 34.2	61-149; 89.8 ± 31.2
1X females	48-132; 88.7 ± 29.3	43-116; 78.2 ± 28.4	41-137; 82.3 ± 35.2	37-119; 74.3 ± 30.1
3X males	47-89; 67.3 ± 16.9	51-85; 69.0 ± 13.3	52-97; **75.0 ± 19.7**	48-89; 64.7 ± 15.1
3X females	57-105; 71.7 ± 17.3	56-108; 72.7 ± 18.2	63-87; **70.8 ± 8.8**	50-59; 55.8 ± 3.2
5X males	42-101; 69.2 ± 23.2	48-117; **84.3 ± 29.4**	50-109; **81.7 ± 25.7**	37-108; 66.0 ± 27.5
5X females	64-116; 80.7 ± 19.8	78-143; **94.0 ± 24.6**	75-118; **88.3 ± 15.6**	51-104; 70.5 ± 18.3

Individual values on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003. Means and Standard Deviations on p. 359 and 360. P for 5X group (combined sexes) at 24 hrs: 0.0039; P for 3X group (combined sexes) on day 8: 0.0060; P for 5X group (combined sexes) on day 8: 0.0000 [refer to p. 358 of MRID 464010-03].

From p. 789: "Aspartate aminotransferase (AST) was decreased at all dose levels 24 hours postdose..."

TABLE 17. Ranges & Means ± S.D. for AST (U/L)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	20-25; 22.7 ± 2.3	21-31; 24.7 ± 3.6	26-31; 28.8 ± 1.8	25-38; 28.3 ± 4.8
Placebo females	17-32; 26.5 ± 5.6	24-35; 29.2 ± 4.8	27-36; 31.2 ± 3.2	25-50; 33.2 ± 9.4
1X males	20-33; 25.8 ± 5.1	17-29; **23.3 ± 4.0**	24-34; 29.5 ± 3.8	22-34; 28.5 ± 4.3
1X females	23-34; 27.2 ± 3.9	16-31 ;**24.0 ± 5.2**	23-49; 33.5 ± 9.0	29-54; 35.3 ± 11.2
3X males	23-33; 26.7 ± 3.8	21-29; **24.0 ± 3.0**	29-33; 30.5 ± 1.6	29-34; 31.5 ± 1.9
3X females	25-36; 28.3 ± 3.9	18-26 ; **21.8 ± 3.3**	23-38; 28.7 ± 5.6	26-38; 31.7 ± 4.6
5X males	21-28; 23.5 ± 2.5	14-31; **21.3 ± 6.4**	22-30; 25.8 ± 3.3	23-31; 26.5 ± 3.0
5X females	21-32; 24.2 ± 4.4	15-27 ; **20.8 ± 4.3**	24-33; 28.8 ± 3.1	24-33; 26.5 ± 3.4

Individual values on p. 1211, 1212, 1217, 1218, 1223, 1224, 1229, 1230, 1235, 1236, 1241, 1242, 1247, 1248, 1253 and 1254 of MRID 46401003. Means and Standard Deviations on p. 381 and 382. P for 1X group (combined sexes) at 24 hours: 0.0373; P for 3X group (combined sexes): 0.0103; P for 5X group (combined sexes): 0.0029 [refer to p. 380 of MRID 464010-03].

Examining AST data by group and weight (<10.5 kg; >10.5 kg), the findings are unclear. Dogs within a dosage group weighing >10.5 kg did not necessarily show a greater mean decrease in AST than those weighing <10.5 kg.

TABLE 18. Ranges & Means ± S.D. for AST (U/L)				
Group and weight	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X <10.5 kg	17-32; 26.1± 5.2	24-35; 29.4 ± 4.4	27-36; 31.1 ± 2.9	25-50; 33.9 ± 8.8
0X >10.5 kg	20-25; 22.4 ± 2.4	21-26; 23.4 ± 1.9	26-30; 28.4 ± 1.7	25-28; 26.4 ± 1.1
1X <10.5 kg	17-32; 25.5 ± 5.3	16-31; 24.5 ± 4.8	23-49; 32.4 ± 8.0	22-54; 33.6 ± 10.0
1X >10.5 kg	22-33; 27.5 ± 5.3	17-26; 22.0 ± 3.7	24-34; 29.8 ± 4.6	22-34; 28.5 ± 5.5
3X <10.5 kg	23-36; 27.1 ± 3.9	18-26; 21.9 ± 2.7	23-38; 29.2 ± 5.4	26-38; 31.3 ± 3.7
3X >10.5 kg	25-33; 28.7 ± 4.0	23-29; 26.0 ± 3.0	29-33; 30.7 ± 2.1	30-34; 32.3 ± 2.1
5X <10.5 kg	21-32; 23.8 ± 3.9	15-27; 21.1 ± 4.5	23-33; 27.5 ± 3.6	24-33; 27.0 ± 3.3
5X >10.5 kg	22-28; 24.0 ± 2.8	14-31; 21.0 ± 7.2	22-30; 27.0 ± 3.5	23-29; 25.5 ± 2.6

Values calculated from data on p. 1211, 1212, 1217, 1218, 1223, 1224, 1229, 1230, 1235, 1236, 1241, 1242, 1247, 1248, 1253 and 1254 of MRID 46401003.

The significance (if any) of this transient finding is not evident. Like ALT (for which no effects were observed) AST is a leakage enzyme, not necessarily liver specific as it is also produced in the kidneys and striated muscle. Pathologies associated with a decrease in AST include a pyridoxine (vitamin B_6) deficiency and terminal stages of liver disease (the latter is obviously not the case in this study), but these would also be associated with decreases in ALT, not seen in this study. From http://www.ahc.umn.edu/rar/RefValues.html values below 105 U/L are normal in the dog.

From p. 789: "[In gender-pooled samples] Urea nitrogen was increased (1.4. 1.4 and 1.6-fold) at 1X, 3X and 5X, respectively, 24 hours postdose and 1.2-fold on Day 8 at 5X, but decreased on Day 22 at 1X (5%) and 5X (19%)..."

TABLE 19. Ranges & Means ± S.D. for Urea Nitrogen (mg/dL)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	10-11; 10.5 ± 0.5	10-14; 12.5 ± 1.5	10-15; 12.7 ± 1.6	13-17; 14.2 ± 1.6
Placebo females	12-17; 14.7 ± 1.8	9-17; 14.3 ± 3.3	13-18; 15.0 ± 2.1	14-18; 16.3 ± 1.6
1X males	11-21; 14.3 ± 4.2	15-24; **18.7 ± 3.1**	11-21; 15.0 ± 3.9	11-23; **15.3 ± 4.4**
1X females	11-17; 13.8 ± 2.6	14-27; **18.7 ± 5.0**	11-20; 14.8 ± 4.1	10-19; **13.8 ± 3.3**
3X males	10-16; 13.0 ± 2.1	17-26; **19.8 ± 3.2**	13-22; 15.7 ± 3.4	10-20; 14.5 ± 3.4
3X females	11-16; 14.0 ± 1.8	16-21; **18.5 ± 2.1**	11-15; 13.7 ± 1.5	11-19; 15.2 ± 2.8
5X males	11-15; 12.7 ± 1.6	16-27; **21.7 ± 5.2**	12-23; **17.5 ± 4.0**	11-15; **12.5 ± 1.6**
5X females	10-16; 13.0 ± 2.5	16-27; **21.3 ± 4.7**	10-21; **16.2 ± 3.8**	9-16; **12.3 ± 2.7**

Individual values on p. 1211, 1212, 1217, 1218, 1223, 1224, 1229, 1230, 1235, 1236, 1241, 1242, 1247, 1248, 1253 and 1254 of MRID 46401003. Means and Standard Deviations on p. 411 and 412. P for 1X group (combined sexes) at 24 hours: 0.0053; P for 3X group (combined sexes): 0.0006; P for 5X group (combined sexes): 0.0000; at day 8: P for 5X group (combined sexes): 0.0109; at day 22: P for 1X group (combined sexes): 0.0970; P for 5X group (combined sexes): 0.0036 [refer to p. 410 of MRID 464010-03].

According to http://www.ahc.umn.edu/rar/RefValues.html a normal range for BUN in the dog is 7-24 mg/dL; a number of dogs in the 1X, 3X and 5X groups had values slightly above 24 mg/dL at 24 hours (males: 0X: 0/6, 1X: 0/6: 3X: 1/6; 5X: 3/6; females: 0X: 0/6; 1X: 1/6; 3X: 0/6; 5X: 2/6), and this was the only time during the study that any dogs were outside (or above) this reference range.

A dose-relationship at 24 hours postdose was even more evident when the dogs were separated by group and weight. An effect was still present in dogs >10.5 kg at the 5X dose level on Day 8.

TABLE 20. Ranges & Means ± S.D. for Urea (mg/dL)

Group & weight	Week -1	24 hrs postdose	Day 8	Day 22
0X < 10.5 kg	11-17; 14.1 ± 2.1	9-17; 14.3 ± 3.0	12-18; 14.6 ± 2.2	14-18; 16.1 ± 1.6
0X > 10.5 kg	10-11; 10.4 ± 0.5	10-14; 12.2 ± 1.5	10-15; 12.8 ± 1.8	13-17; 14.0 ± 1.7
1X < 10.5 kg	11-21; 14.5 ± 3.5	14-27; 18.3 ± 4.4	11-20; 14.6 ± 3.9	10-23; 15.3 ± 4.2
1X > 10.5 kg	11-18; 13.3 ± 3.3	17-24; 19.5 ± 3.3	12-21; 15.5 ± 4.4	11-17; 13.3 ± 2.6
3X < 10.5 kg	10-16; 13.1 ± 2.0	16-21; 18.3 ± 1.7	11-17; 14.1 ± 1.7	10-20; 15.0 ± 3.4
3X > 10.5 kg	14-16; 14.7 ± 1.2	19-26; 21.7 ± 3.8	13-22; 16.3 ± 4.9	12-16; 14.3 ± 2.1
5X < 10.5 kg	10-16; 12.8 ± 2.3	16-27; 20.1 ± 4.5	10-21; 15.6 ± 3.5	9-16; 12.5 ± 2.5
5X > 10.5 kg	11-15; 13.0 ± 1.8	18-27; 24.3 ± 4.3	15-23; 19.3 ± 3.5	11-14; 12.3 ± 1.3

Values calculated from data on p. 1211, 1212, 1217, 1218, 1223, 1224, 1229, 1230, 1235, 1236, 1241, 1242, 1247, 1248, 1253 and 1254 of MRID 46401003.

Total serum protein was significantly increased at 5X (combined sexes) at 24 hours, although the effect appeared to involve only the males (however, all individual values were within the 5.4-8.0 g/dL range in http://www.ahc.umn.edu/rar/RefValues.html).

TABLE 21. Ranges & Means ± S.D. for total protein (g/dL)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	5.8-6.8; 6.0 ± 0.4	5.7-7.0; 6.1 ± 0.5	5.7-6.9; 6.1 ± 0.4	6.0-6.8; 6.3 ± 0.3
Placebo females	5.9-6.4; 6.2 ± 0.2	5.8-6.3; 6.0 ± 0.2	5.7-6.3; 5.9 ± 0.3	5.8-6.3; 6.0 ± 0.2
1X males	5.4-6.1; 5.7 ± 0.3	5.3-6.2; 5.8 ± 0.4	5.3-6.1; 5.8 ± 0.3	5.6-6.5; 5.9 ± 0.3
1X females	5.7-6.7; 6.2 ± 0.3	5.5-6.6; 5.9 ± 0.4	5.5-7.0; 6.0 ± 0.6	5.4-6.7; 5.9 ± 0.5
3X males	6.0-6.9; 6.3 ± 0.4	5.9-6.8; 6.3 ± 0.3	5.7-6.6; 6.1 ± 0.3	5.8-6.8; 6.2 ± 0.4
3X females	5.9-6.8; 6.2 ± 0.3	5.7-6.7; 6.1 ± 0.3	5.3-6.7; 6.0 ± 0.5	5.6-6.6; 6.1 ± 0.4
5X males	5.9-6.9; 6.3 ± 0.4	6.3-7.0; **6.6 ± 0.3**	5.6-6.9; 6.2 ± 0.4	5.9-6.7; 6.4 ± 0.3
5X females	6.0-6.8; 6.2 ± 0.3	5.9-6.3; 6.1 ± 0.1	5.4-6.1; 5.9 ± 0.3	5.8-6.4; 6.0 ± 0.3

Individual values on p. 1213, 1214, 1219, 1220, 1225, 1226, 1231, 1232, 1237, 1238, 1243, 1244, 1249, 1250, 1255 and 1256 of MRID 46401003. Means and Standard Deviations on p. 427 and 428. P for 5X group (combined sexes) at 24 hours: 0.0322 [refer to p. 426 of MRID 464010-03].

Cholesterol was significantly increased at 5X at 24 hours, and at 3X and 5X on day 8:

TABLE 22. Ranges & Means ± S.D. for Cholesterol (mg/dL)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	157-193; 169.7 ± 12.3	138-190; 169.0 ± 17.8	146-178; 165.2 ± 15.3	131-163; 149.0 ± 12.5
Placebo females	133-204; 160.7 ± 25.4	134-223; 170.2 ± 33.7	119-195; 152.5 ± 28.2	112-169; 138.3 ± 23.2
Placebo combined	133-204; 165.2 ± 19.6	134-223; 169.6 ± 25.7	119-195; 158.8 ± 22.6	112-169; 143.7 ± 18.6
1X males	149-198; 170.7 ± 18.5	156-215; 176.5 ± 22.2	146-228; 168.8 ± 30.3	127-187; 150.0 ± 22.7
1X females	128-166; 152.0 ± 13.0	129-185; 159.0 ± 20.6	128-215; 167.2 ± 33.1	126-217; 154.5 ± 40.4
1X Combined sexes	128-198; 161.3 ± 18.1	129-215; 167.8 ± 22.4	128-228; 168.0 ± 30.3	126-217; 152.3 ± 31.3
3X males	138-211; 180.2 ± 28.9	136-220; 183.5 ± 29.1	151-254; 194.5 ± 39.3	100-184; 147.2 ± 30.9
3X females	130-196; 157.0 ± 24.9	126-213; 167.5 ± 34.5	140-227; 172.8 ± 33.8	115-179; 147.5 ± 30.8
3X Combined sexes	130-211; 168.6 ± 28.5	126-220; 175.5 ± 31.6	140-254; **183.7 ± 36.7**	100-184; 147.3 ± 29.4
5X males	135-227; 178.7 ± 29.7	152-247; 205.0 ± 32.9	155-300; 215.8 ± 48.7	110-180; 157.3 ± 25.4
5X females	118-201; 149.3 ± 27.7	122-238; 171.5 ± 41.4	112-244; 171.8 ± 48.7	115-188; 138.0 ± 26.3
5X Combined sexes	118-227; 164.0 ± 31.4	122-247; **188.3 ± 39.7**	112-300; **193.8 ± 51.8**	110-188; 147.7 ± 26.6

Individual values on p. 1213, 1214, 1219, 1220, 1225, 1226, 1231, 1232, 1237, 1238, 1243, 1244, 1249, 1250, 1255 and 1256 of MRID 46401003. Means and Standard Deviations on p. 456, 457 and 461. P for 5X group (combined sexes) at 24 hours: 0.0110; P for 3X group (combined sexes) on day 8: 0.0431; P for 5X group (combined sexes) on day 8: 0.0008 [refer to p. 455 of MRID 464010-03].

However, from http://www.ahc.umn.edu/rar/RefValues.html the normal cholesterol range is from 110-330 mg/dL, and all values (even at 24 hours and 8 days postdose) were within this normal range.

Application of the test material resulted in an increase in serum glucose at 24 hours postdose, particularly obvious in the 3X and 5X groups. From p. 10 of MRID 46401003: "Glucose did exhibit a mild, dose-dependent increase 24 hours postdose but this effect was not seen at subsequent intervals. Individual glucose values slightly exceeded the upper range of expected values in males at 5X and females at 3X and more markedly in females at 5X."

From p. 789 of MRID46401003: "In gender-pooled samples 24 hours postdose, glucose was increased (1.3, 1.5-fold) at 3X and 5X..." However, an effect was present even at the 1X dose level, as all dogs in this group showed a glucose level $\geq$ 100 mg/dL, and this was in contrast to the week -1, day 8 and day 22 observations.

TABLE 23. Ranges & Means ± S.D. for Glucose (mg/dL)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	80-110; 92.7 ± 10.3	89-108; 97.7 ± 7.5	81-105; 95.0 ± 8.2	88-98; 93.3 ± 4.5
Placebo females	79-93; 87.2 ± 5.1	92-111; 98.7 ± 6.6	84-104; 94.7 ± 6.6	85-96; 91.5 ± 4.0
Combined sexes	79-110; 89.9 ± 8.3	89-111; 98.2 ± 6.8	81-105; 94.8 ± 7.1	85-98; 92.4 ± 4.2
1X males	85-105; 96.3 ± 6.7	100-125; 113.0 ± 10.2	87-105; 96.0 ± 6.4	86-105; 95.2 ± 7.4
1X females	79-93; 86.3 ± 5.9	100-116; 106.7 ± 6.1	89-104; 98.3 ± 5.3	82-106; 93.3 ± 9.1
1X Combined sexes	79-105; 91.3 ± 8.0	100-125; 109.8 ± 8.7	87-105; 97.2 ± 5.8	82-106; 94.3 ± 8.0
3X males	85-96; 90.7 ± 4.3	110-148; **131.2 ± 15.7**	84-105; 93.2 ± 7.1	80-97; 91.7 ± 6.2
3X females	77-101; 88.3 ± 7.8	104-126; **116.2 ± 7.6**	92-106; 98.3 ± 6.2	89-102; 95.5 ± 5.8
3X Combined sexes	77-101; 89.5 ± 6.2	104-148; 123.7 ± 14.1	84-106; 95.8 ± 6.9	80-102; 93.6 ± 6.1
5X males	87-95; 91.2 ± 2.9	117-182; **144.2 ± 21.7**	72-101; 89.5 ± 10.3	65-107; 90.0 ± 16.6
5X females	82-98; 87.8 ± 6.6	119-222; **159.2 ± 36.2**	88-106; 99.8 ± 6.7	85-108; 97.5 ± 9.1
5X Combined sexes	82-98; 89.5 ± 5.1	117-222; **151.7 ± 29.5**	72-106; 94.7 ± 9.9	65-109; 93.8 ± 13.4

Individual values on p. 1213, 1214, 1219, 1220, 1225, 1226, 1231, 1232, 1237, 1238, 1243, 1244, 1249, 1250, 1255 and 1256 of MRID 46401003. Means and Standard Deviations on p. 464, 465 and 469. P for 1X group (combined sexes) at 24 hours: 0.1229; P for 3X group (combined sexes) at 24 hours: 0.0006; P for 5X group (combined sexes) at 24 hours: 0.0000 [refer to p. 463 of MRID 464010-03].

Grouping the dogs by weights (<10.5 kg and >10.5 kg, correlating with dosage, as dogs >10.5 kg received a greater dose of test material on a bodyweight basis) rather than by sexes gives the values shown in Table 7. At the 1X and 3X dose levels (but not at 5X) dogs weighing >10.5 kg tended to show a higher mean level of serum glucose at 24 hours postdose than those <10.5 kg (receiving both a lower absolute dose of test material and a lower dose of test material on a bodyweight basis):

TABLE 24. Ranges & Means ± S.D. for Glucose (mg/dL)				
Group and weight	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
0X < 10.5 kg	79-93; 87.7 ± 4.9	91-111; 97.6 ± 6.7	89-104; 97.6 ± 5.3	85-96; 91.0 ± 3.9
0X > 10.5 kg	80-110; 93.0 ± 11.5	89-108; 99.0 ± 7.5	81-105; 95.4 ± 9.1	89-98; 94.4 ± 4.2
1X < 10.5 kg	79-105; 90.3 ± 8.9	100-117; 107.3 ± 6.8	89-104; 97.6 ± 5.3	82-106; 94.1 ± 8.4
1X > 10.5 kg	85-98; 93.5 ± 6.1	100-125; 115.0 ± 10.7	87-105; 96.3 ± 7.5	86-105; 94.5 ± 8.3
3X < 10.5 kg	77-101; 88.3 ± 6.5	104-126; 116.7 ± 7.0	91-106; 97.9 ± 6.1	89-102; 94.6 ± 5.0
3X > 10.5 kg	89-96; 93.0 ± 3.6	140-148; 144.7 ± 4.2	84-95; 89.3 ± 5.5	80-97; 90.7 ± 9.3
5X < 10.5 kg	82-98; 89.0 ± 6.0	117-222; 152.6 ± 34.0	88-106; 98.3 ± 6.5	65-108; 94.1 ± 14.2
5X > 10.5 kg	87-95; 90.5 ± 3.3	133-182; 149.8 ± 22.2	72-101; 87.5 ± 12.4	79-107; 93.0 ± 13.6

Individual values on p. 1213, 1214, 1219, 1220, 1225, 1226, 1231, 1232, 1237, 1238, 1243, 1244, 1249, 1250, 1255 and 1256 of MRID 46401003.

From p. 1293: "ALT...[showed]...a significant treatment effect in the 24-hour postdose interval, (p=0.0144). Follow-up analyses were done at this period, with sexes combined. One significance resulted in this follow-up analysis...when comparing the 3X treatment

group to Control (p=0.0350)." However, this was not dose-related as the 5X dogs had mean values below controls (the 3X dogs were higher) and mean values for 3X dogs at 24 hours postdose were similar to those at Week -1. It is concluded that there was no observed effect on ALT.

TABLE 25. Ranges & Means ± S.D. for ALT (U/L)				
Group	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	22-36; 28.7 ± 5.5	21-35; 27.0 ± 4.9	23-35; 28.5 ± 4.5	24-36; 29.3 ± 5.0
Placebo females	24-64; 32.7 ± 15.5	21-38; 27.7 ± 6.0	25-51; 31.3 ± 9.9	27-35; 30.0 ± 3.0
1X males	20-29; 24.5 ± 2.9	19-25; 22.3 ± 2.7	19-27; 22.5 ± 3.1	21-27; 24.3 ± 2.3
1X females	22-43; 29.8 ± 7.3	18-38; 25.2 ± 7.5	21-48; 30.0 ± 11.2	23-48; 31.7 ± 9.1
3X males	22-39; 30.7 ± 6.0	24-43; 31.7 ± 6.3	22-33; 27.7 ± 4.0	24-45; 33.2 ± 7.3
3X females	28-37; 32.2 ± 3.7	24-40; 30.7 ± 5.4	20-33; 27.8 ± 4.8	26-37; 31.8 ± 4.1
5X males	20-36; 26.5 ± 7.1	19-35; 24.5 ± 6.8	17-36; 25.5 ± 6.9	18-36; 25.3 ± 7.8
5X females	18-31; 25.2 ± 4.3	18-26; 22.7 ± 3.3	19-30; 24.0 ± 4.4	22-34; 26.3 ± 4.5

Values calculated from data on p. 1211, 1212, 1217, 1218, 1223, 1224, 1229, 1230, 1235, 1236, 1241, 1242, 1247, 1248, 1253 and 1254 of MRID 46401003.

TABLE 26. Ranges & Means ± S.D. for Potassium (mEq/L)				
Group and Sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	4.6-5.0; 4.7 ± 0.2	4.3-4.8; 4.6 ± 0.2	4.3-5.1; 4.7 ± 0.3	4.1-5.0; 4.4 ± 0.3
Placebo females	4.3-4.9; 4.7 ± 0.2	4.1-4.5; 4.4 ± 0.2	3.8-4.8; 4.4 ± 0.4	4.0-5.0; 4.5 ± 0.5
1X males	4.5-5.3; 4.8 ± 0.3	4.6-5.3; 5.0 ± 0.2	4.2-5.1; 4.8 ± 0.3	4.3-4.6; 4.5 ± 0.1
1X females	4.4-5.4; 4.8 ± 0.4	4.3-5.2; 4.7 ± 0.3	4.3-4.9; 4.7 ± 0.4	4.3-4.8; 4.5 ± 0.2
3X males	4.0-4.8; 4.5 ± 0.3	4.4-5.4; 4.8 ± 0.4	4.4-5.4; 4.9 ± 0.4	4.0-4.6; 4.4 ± 0.3
3X females	4.5-4.9; 4.7 ± 0.2	4.7-5.8; 5.0 ± 0.4	4.4-5.0; 4.6 ± 0.2	4.3-4.7; 4.5 ± 0.2
5X males	4.3-4.8; 4.6 ± 0.2	4.4-5.1; 4.8 ± 0.3	4.6-5.4; 5.0 ± 0.3	3.9-4.9; 4.4 ± 0.3
5X females	4.2-5.3; 4.9 ± 0.4	4.3-5.2; 4.8 ± 0.3	4.2-5.5; 5.0 ± 0.5	4.0-5.2; 4.5 ± 0.4

Values calculated from data on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.

Statistical significance at 24 hours in the 1X, 3X and 5X groups was due to lower mean potassium levels in the controls at this time. Potassium levels were still relatively low in controls on day 8, although 5X dogs had slightly higher means than usual. However, the range of individual values (3.8-5.8 mEq/L) in this study included values slightly lower than those in the normal reference range (4.4-6.1 mEq/L) as given in http://www.ahc.umn.edu/rar/RefValues.html so dogs in the treated groups had values more within this normal reference range at 24 hours and 8 days. It is concluded that the statistically significantly different potassium means are not biologically relevant.

From p. 1292: "Chloride also showed a significant treatment effect at the 24-hour postdose interval (p=0.0046) and at Day 22 (p=0.0979). Follow-up analyses were done in this period with sexes combined. The analysis at the 24-hour postdose period showed significance for treatment groups 1X and 3X compared to the Control (p=0.0005 and p=0.0332, respectively). At Day 22, no differences were significantly significant when comparing the Control group to each treatment group."

TABLE 27. Ranges & Means ± S.D. for Chloride (mEq/L)				
Group and Sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	108-111; 109.5 ± 1.2	108-111; 109.8 ± 1.2	110-113; 112.5 ± 1.2	108-112; 110.8 ± 1.5
Placebo females	110-113; 110.7 ± 1.2	108-111; 109.8 ± 1.2	111-116; 113.0 ± 2.1	110-112; 111.0 ± 1.1
1X males	108-110; 109.2 ± 0.8	110-113; 111.5 ± 1.0	107-114; 111.5 ± 2.6	108-113; 110.3 ± 2.0
1X females	109-111; 110.2 ± 1.0	111-114; 112.5 ± 1.0	111-115; 113.0 ± 1.7	111-113; 111.8 ± 1.0
3X males	108-112; 110.0 ± 1.4	110-113; 111.3 ± 2.1	109-115; 112.0 ± 2.2	109-114; 110.8 ± 2.1
3X females	109-111; 109.7 ± 1.0	109-113; 110.8 ± 1.6	110-114; 112.0 ± 1.4	109-112; 111.0 ± 1.3
5X males	109-112; 110.7 ± 1.0	109-112; 110.7 ± 1.2	110-115; 111.7 ± 2.0	109-112; 110.8 ± 1.0
5X females	109-112; 110.5 ± 1.0	108-113; 110.7 ± 2.1	112-115; 113.5 ± 1.0	108-112; 110.3 ± 1.4

Values calculated from data on p. 1209, 1210, 1215, 1216, 1221, 1222, 1227, 1228, 1233, 1234, 1239, 1240, 1245, 1246, 1251 and 1252 of MRID 46401003.

Although there was statistical significance to a slight elevation in the mean chloride level at 24 hours, it involved only the 1X and 3X levels and not the 5X level. Also, the normal reference range is given in http://www.ahc.umn.edu/rar/RefValues.html as (109-122 mEq/L), and the individual range observed in this study was 108-116 mEq/L, so that the differences observed between groups in this study were not biologically relevant.

I. NECROPSY FINDINGS

As there were no mortalities, there were no necropsy findings.

IV. DISCUSSION

This is the first application for registration that the Agency has received for a spot-on formulation containing Amitraz.

According to proposed label directions the product would be applied as a spot-on with the following dosage rates: dogs ≤ 5 kg: 0.67 mL; 5-10 kg: 1.33 mL; 10-25 kg: 3.33 mL; 25-40 kg: 5.33 mL; and 40-50 kg: 6.66 mL.

Amitraz (Mitaban®) has been approved by the Food and Drug Administration for treatment of canine demodicosis, with application on a biweekly basis of a solution containing 0.025 percent (250 ppm; 10.6 ml concentrate in 7.6 L or 2 gallons of water). For a 10 kg dog exposure would then be to (2.0 g amitraz)/(10 kg) = 200 mg amitraz/kg, although some would be lost to runoff. Assuming 25% retention, a 10.5 kg dog would be exposed to approximately 50 mg Amitraz/kg, consistent with the 1X dose (approximately 50 mg Amitraz/kg) for a 10.5 dog receiving 3.4 mL formulation (as proposed for this product). However, a more accurate quantitative comparison is difficult because absorption (and absorption rates) would also depend on factors such as concentration, solvents, and differences in type of dermal exposure (localized spot-on versus whole-body).

TAll animals were observed at least twice daily. On the day of dosing (Day 1: there was no Day 0) clinical observations were made predose and at 1, 2 and 3 hours postdose. Otherwise, clinical observations were twice daily, approximately 4 hours apart, during both the acclimation period and then from Days 2 through 28, except on days of neurological and physical examinations (Days -1, 1, 2, 8 and 22).

Dogs were individually weighed on Days -7, -1, 1 (just before dosing) and then postdose on Days 8, 15 and 22. Food consumption is reported (g/dog/day) for weeks -1, 1, 2, 3 and 4. Blood samples were taken (following overnight fasts) once pretest (Week -1) and then on Days 2 (about 24 hours postdose), 8 and 22.

All dogs survived and there was no indication of any effects on body weight or food consumption.

Neurologically, two dogs at the 5X dose level had a depressed righting reflex on Days 1 and 2, and an additional two dogs (one male, one female) at this dose level had a depressed righting reflex on Days 1, 2, 8 and 22, not observed before exposure. Although the report states that this was "transitory" and not considered to be clinically significant, no subsequent (after Day 22) observations are reported for these two dogs. Other occurrences of this finding (a control female on Day 8, a 1X female on Day 2, another 1X female on Day 8, a 3X female on Day 22) were sporadic.

In addition, all dogs (including those at 0X) were very quiet and inactive ("little activity or barking compared to the previous day, even during repeated entries by the technical staff") on the afternoon of Day 1 (applications of test material or placebo were made during the morning). While the report states that this could be a result of increased handling which had occurred earlier (during application of the test substance or placebo), Amitraz can cause sedation. From http://www.medi-vet.com/Mitaban.aspx: "The most frequently observed adverse reaction in the [Amitraz] clinical studies was transient sedation, which occurred in approximately 8% of the generalized demodicosis patients. This effect was observed within 2 to 6 hours posttreatment, and usually dissipated within 24 to 72 hours. In approximately 40% of the affected generalized demodicosis patients, the effect dissipated in less than 24 hours." While the report states that this effect was seen in the 0X group dogs, the CSF indicates there are one or more "inactive" ingredients present with potential pharmacological activity.

For clinical observations, few or absent feces were observed in 4/12 dogs in the 5X group on day 2, and purulent discharge was observed in one eye of a 3X male on days 2-5, and from both eyes of a 5X female on days 2-6.

The following clinical chemistry and hematology effects were noted:

Glucose: Significantly increased at 24 hours postdose in the 3X and 5X groups, with a dose relationship [Males: controls: mean 97.7 mg/dL; 1X: mean 113.0 (+15.7%); 3X: mean 131.2 (+34.3%); 5X: mean 144.2 (+47.6%); Females: controls: mean: 98.7; 1X: mean: 106.7 (+8.1%); 3X: 116.2 (+17.7%); 5X: 159.2 (+61.3%)]. P values (combined sexes) were: 1X: 0.1229 [not considered significant]; 3X: 0.0006; 5X: 0.0000. The report (p. 22 of MRID 46401003) states: "Glucose was increased 24 hours postdose at 3X and 5X. This effect was not seen at 1X or at subsequent intervals." However, all 1X dogs at 24 hours had glucose values $\geq$ 100 mg/dL, not seen in any group pretest, nor on Days 8 or 22. Of the four 1X males >10.5 kg treated with 3.4 mL formulation one had an elevated glucose level (125 mg/dL) outside the normal range (80-120 mg/dL, as given in http://www.ahc.umn.edu/rar/RefValues.html) and two showed high normal values (116 and 119 mg/dL). Mitaban treatment of dogs is known to cause a transient hyperglycemia. In an Amitraz 2-year feeding study in beagle dogs (MRIDs 00030493; 00040856) blood samples collected during weeks 40 and 53 showed significant increases in mean blood sugar concentration 3 hours after dosing in the 1 mg/kg/day group, but no effects at lower doses (0.1 and 0.25 mg/kg/day).

Calcium: "In the 24 hour postdose interval a significant treatment effect was found for

calcium (p=0.0005). Follow-up analyses were done in this interval with sexes combined. This analysis showed significance for...groups 3X and 5X when compared to the Control with p=0.0305 and p<0.0001, respectively." Also, "at 24 hours, calcium was slightly increased at 3X and 5X (combined sexes: 1.03-fold and 1.07-fold respectively). However, there were suggestions of an effect in males at all dose levels, with the following incidences of calcium >11.6 mg/dL (normal range: 9-11.4 mg/dL) in males at 24 hours: 0X: 1/6; 1X: 4/6; 3X: 2/6; 5X: 5/6.

BUN: At 24 hours postdose there were significant increases in urea nitrogen at 1X, 3X and 5X (combined sexes: 1.4. 1.4 and 1.6-fold, P values of 0.0053, 0.0006 and 0.0000 respectively), and 1.2-fold on Day 8 at 5X, with decreases on Day 22 at 1X (5%) and 5X (19%). A normal range for BUN in the dog is 7-24 mg/dL; a number of dogs in the 1X, 3X and 5X groups (males: 0X: 0/6, 1X: 0/6: 3X: 1/6; 5X: 3/6; females: 0X: 0/6; 1X: 1/6; 3X: 0/6; 5X: 2/6) had values slightly above 24 mg/dL (up to 27 mg/dL) at 24 hours, and this was the only time during the study that any dogs were outside (or above) this reference range.

Aspartate aminotransferase (AST) was decreased at all dose levels 24 hours postdose (combined mean for both sexes as percentage of control mean: 1X: 87.8%; 3X: 85.0%; 5X: 78.1%). The significance (if any) of this finding is not evident, as there was no concurrent dose-related change in ALT. In any case, all values observed in this study for AST were below 105 U/L, and so were normal for the dog.

Cholesterol: Significantly increased at 5X at 24 hours and at 3X and 5X on day 8. The mean increases (relative to preexposure values) at 24 hours for the 5X group were: males: 14.7%; females: 14.8%; combined: 14.8%; day 8: 3X: males: 7.9%; females: 10.1%; combined: 9.0%; 5X: males: 20.8%; females: 15.1%; combined: 18.2%. However, the range of values (112-300 mg/dL) seen at 24 hours and on Day 8 was within the normal reference range (110-330 mg/dL), and the means for the 1X, 3X and 5X groups on Day 22 were not significantly different from the controls (and were all below preexposure values).

Total protein: Significantly elevated at 24 hours for combined sexes at 5X (P=0.0322), with combined controls at 6.04 ± 0.34 and the 5X dogs at 6.36 ± 0.37 g/dL. However, the observed ranges for both groups (controls: 5.7 - 7.0; 5X: 5.9 - 7.0 g/dL) were essentially the same and within the normal reference range of 5.4 - 8.0 g/dL.

Other clinical chemistry parameters were not significantly changed, or if significance occurred it was not dose-related, and/or measurements tended to be within normal range considering the ages of the dogs, and/or were similar to preexposure values.

Total leukocyte counts were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, for the combined sexes at the 3X and 5X dose levels 24 hours postdose. Concurrently, neutrophils were increased 1.2-fold at the 3X and 1.5-fold at 5X. Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges." One reference gives the normal range for dog WBC counts as 5.9-16.6 K/mm^3; a number of 3X (4/6M, 1/6F) and 5X (4/6M, 2/6F) dogs at 24 hours (and also one female control) had WBC counts above 16.6 K/mm^3, and the mean for 5X males was 18.7 K/mm^3. An elevation in leukocyte counts is normally a response to infection. It is noteworthy that this proposed formulation has tested positive as a dermal sensitizer (MRID 46395810).

MCHC was significantly decreased in the 1X, 3X and 5X groups at 24 hours postdose in gender-pooled samples. There were mild decreases of erythrocytes (5%), hemoglobin

(7%) and hematocrit (6%) in gender-pooled samples 24 hours postdose at 1X. MCV was significantly decreased 24 hours postdose for combined sexes at 1X, and significantly increased at 3X and 5X. MCV was also statistically increased for combined sexes at 3X on Day 22. MCH was decreased in males at 1X and 5X on Day 22, and in females at 1X. Absolute and percent reticulocytes were decreased in gender-pooled samples at all dose levels 24 hours postdose, but this was considered not physiologically relevant as there were no sustained effects on red cell parameters. However, as all individual values for these indices, at all dose levels, and at all intervals, fell within expected ranges, they were not physiologically relevant.

While the conduct and reporting of this companion animal (adult dog) safety study (OPPTS 870.7200) were adequate, the study does not demonstrate that there is an adequate margin of safety (5X) associated with the proposed use of this formulation, and so does not support registration. This conclusion is based on the following:

1. A depressed righting reflex was seen in 4/12 5X dogs. In two of these dogs it was observed on Days 1, 2, 8 and 22, indicating the possibility of a permanent effect.

2. All dogs (including those at 0X) were very quiet and inactive ("little activity or barking compared to the previous day, even during repeated entries by the technical staff") on the afternoon of Day 1 (applications of test material or placebo were made during the morning). While the report states that this could be a result of increased handling which had occurred earlier (during application of the test substance or placebo), Amitraz can cause sedation. Although dogs in the placebo control group also showed the same or a similar effect, one or more of the inerts in this formulation have pharmacological activity, and these controls were being exposed to essentially a 7X dosage of the formulation without the actives.

3. The formulation has tested positive as a dermal sensitizer. Possibly associated with this, total leukocyte counts were increased, 1.3 and 1.5-fold [relative to concurrent controls], respectively, for combined sexes at the 3X and 5X dose levels 24 hours postdose. Concurrently, neutrophils were increased 1.2-fold at the 3X and 1.5-fold at 5X. Monocytes were increased 24 hours postdose at 3X and 5X with [a] few elevated above expected ranges. An elevation in leukocyte counts is normally a response to infection.

4. The increase in glucose levels for all dose groups (1X, 3X and 5X) at 24 hours is consistent with a known effect of Amitraz, and indicates pharmacologically significant quantities of this active are absorbed at even the 1X dose level.

The proposed label suggests that this would be an over-the-counter product, readily available to dog owners, particularly as the only mention of veterinarians is in the standardized statements under the heading HAZARDS TO DOMESTIC ANIMALS ("Consult a veterinarian before using this product on aged, debilitated, medicated, pregnant or nursing animals. Individual sensitivities while rare may occur after the use of any pesticide product. If signs persist or become more severe, consult a veterinarian immediately...").

The label for Mitaban® (a formulation containing 19.9% Amitraz, approved by the FDA for treating generalized canine demodicosis) includes the following WARNING statement: "Toxicology studies conducted in the dog and other species suggest amitraz may alter the animal's ability to maintain homeostasis. Animals treated with MITABAN (Amitraz) should not be subjected to stress for a period of at least 24 hours posttreatment. Adverse reactions including three fatalities were reported during the clinical studies. In excess of

1100 patients with generalized demodicosis were topically treated with MITABAN." In addition, the Mitaban® label includes the statement: "**Caution:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

The overall exposure level to Amitraz associated with the proposed use of this product seems to be approximately the same order of magnitude as that associated with the use of Mitaban® . The overall exposure level to Amitraz associated with the proposed use of this product seems to be comparable to that associated with the use of Mitaban® . However, there are indications that Amitraz from the Promeris formulation is absorbed more rapidly and/or to a greater extent than Amitraz in the 250 ppm Mitaban-use dilution. Publically available information on Mitaban® includes the following [see myweb.cableone.net/bdturner/**Mitaban**.pdf]: "Blood glucose values were elevated at 4 hours posttreatment in the 250 ppm female group, and in both sexes at the 1250 and 2500 ppm concentrations. ...glucose values returned to normal within 24 hours posttreatment. In another study, groups of healthy beagles were topically treated with either 250 ppm, 750 ppm or 1250 ppm of active drug at 14 day intervals and for 12 weeks. Blood glucose values were elevated at the 750 ppm concentration at 4 hours posttreatment after 3 of 6 treatments, and after 5 of 6 treatments at the 1250 ppm level. In the 750 ppm group, serum glucose values returned to normal at 24 hours posttreatment, however for the 1250 ppm group, at 24 hours and after 3 of 6 treatments the levels remained significantly elevated." In the adult dog Promeris study [MRID 46401003] 1/6 of the 3X females and 5/6 5X females had elevated glucose levels ($\geq$120 mg/dL) at 24 hours, suggesting that Amitraz absorption from 5X Promeris treatment (7.0 mL) is greater than that from exposure to 2500 ppm Amitraz (or a 10X concentration of the use-dilution of Mitaban®), and a 1X (1.4 mL) Promeris treatment of an adult dog would then be associated with greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban® solution.

There is also potential for adverse drug reactions: from http://www.aava.org/pub/iatrogenic.html: "Ivermectin which is in Heartgard heart worm preventative and is sometimes used to treat "mange" and ear mites, causes increases in monoamine neurotransmitter metabolites which could result in important adverse drug reactions with amitraz (Mitaban topical mange treatment)..." At the very least, if this product is registered it should be available only through licensed veterinarians, as is Mitaban®.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D311482
2. **PC CODES:** 106201 [Amitraz], 281250 [Metaflumizone]
3. **CURRENT DATE:** May 24, 2005
4. **TEST MATERIAL:** 15% w/v R-28153/15% w/v Amitraz Spot-on Lot No. 0381702, a pale yellow liquid with a specific gravity of 1.047 g/mL containing (from p. 480 of MRID 46401003) 14.7% R-28153 (Metaflumizone) and 14.65% Amitraz; PROMERIS SPOT-ON FOR DOGS.

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal/adult dog (beagle)/MPI Research, Inc. Mattawan, MI/Study No. 817-007; Sponsor Study No. 0817-C-US-03-03/19-MAR-2004	46401003	4 groups of young adult beagles (M: 9.99-12.02 kg; F: 6.66-9.54 kg) were treated at 0X (7 or 17 mL formulation without actives), 1X (1.4 or 3.4 mL spot-on formulation), 3X (4.2 or 10.2 mL spot-on), or 5X (7 or 17 mL spot-on). Dogs weighing >10.5 kg (no females were in this category) received the higher doses. Spot-on was applied along the spine, starting between shoulder blades and then continuing caudally. Dogs were then observed for 28 days. All survived, no indication of any effects on body weight or food consumption. Two dogs (1M, 1F) had a depressed righting reflex on Days 1, 2, 8 & 22, not observed before exposure. In addition, all dogs (including 0X) were very quiet and inactive on the afternoon of Day 1 (application of placebo or test material was in morning). Few or absent feces were observed in 4/12 5X dogs on day 2. Glucose was elevated at 24 hours postdose; of the four 1X males >10.5 kg one had a glucose level (125 mg/dL) outside normal (80-120 mg/dL) range. Glucose means (mg/dL) at 24 hours: Males: 0X: 97.7; 1X: 113.0; 3X: 131.2; 5X: 144.2; Females: 0X: 98.7; 1X: 106.7; 3X: 116.2; 5X: 159.2. Also at 24 hrs: increases in serum calcium in males; significant increases in BUN (normal range for BUN in dog: 7-24 mg/dL; incidences above normal at 24 hrs were: 0X: 0/12; 1X: 1/12; 3X: 1/12; 5X: 5/12). Total leukocytes were increased 1.3X and 1.5X relative to controls at 3X and 5X. Study does not demonstrate 5X safety factor. 1/6 of the 3X females & 5/6 5X females had elevated (>120 mg/dL) glucose levels at 24 hrs, suggesting that Amitraz absorption from 5X (7.0 mL) Promeris treatment is greater than that from a 10X (2500 ppm Amitraz) use-concentration Mitaban treatment; a 1X (1.4 mL) Promeris treatment of an adult dog would then be associated with greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban treatment.	N/A	N/A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**

EPA Primary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Technical Review Branch, Registration Division (7505C) Date 5/27/05
EPA Secondary Reviewer: William Dykstra, Ph.D. Signature: W. Dykstra
Health Effects Division (7509C) Date 5/25/05
EPA Tertiary Reviewer: Kit Farwell, DVM, DABT Signature: K Farwell
Health Effects Division (7509C) Date 5-26-05

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Dogs (Puppies) OPPTS 870.7200
PC CODES: 106201 (Amitraz), 281250 (Metaflumizone)
DP BARCODE: D311482 **RISK MANAGER:** (EPA): 07 **DECISION NO.:**351841

PRODUCT AND TEST MATERIAL: 15% w/v R-28153/15% w/v Amitraz spot-on Lot No. 0381702, a pale yellow liquid with a specific gravity of 1.047 g/mL containing (from p. 480 of MRID 464010-03) 14.70% R-28153 (Metaflumizone) and 14.65% Amitraz.

CITATION: Lindahl, R.G. (2004) Safety Evaluation Study of a Topically Applied Spot-On Formulation of R-28153 and Amitraz in Eight Week Old Puppies. Study No. 817-008; Sponsor Study No. 0817-C-US-09-03. Unpublished study prepared by MPI Research, Inc. 54943 North Main St. Mattawan, MI 49071-9399. Study Completion Date: 10 March 2004; Date of Revised Final Report: 17 March 2004. MRID 46401004.

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

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 46401004), 4 groups, each containing 12 (6/sex) 53 to 58-day (approximately 8-week) old beagle puppies (source: Covance Research Products; males: 1.82-2.97 kg; females: 1.72-2.89 kg) were treated at 0X (3.0 mL of the formulation without active ingredients, so that each of these puppies received 6.74X the dose of "inert" ingredients that 1X puppies were exposed to, and approximately 1.35X the dose of "inert" ingredients that 5X puppies were exposed to); 1X: (0.6 mL of the proposed formulation); 3X (1.8 mL of the proposed formulation); and 5X (3.0 mL of the proposed formulation). Each puppy was treated in a single application using a syringe along the dorsal midline starting between the shoulder blades and then moving caudally.

According to proposed label directions the product would be applied as a spot-on with the following dosage rates: dogs ≤ 5 kg: 0.67 mL; 5-10 kg: 1.33 mL; 10-25 kg: 3.33 mL; 25-40 kg: 5.33 mL; and 40-50 kg: 6.66 mL. The 1X dose rate in this study is slightly less than the 0.67 mL specified for dogs [puppies?] less than 5 kg.

All animals were observed at least twice daily. On the day of dosing (Day 1; note: there was no Day 0) clinical observations were made predose, immediately postdose and at 1, 2 and 3 hours postdose. Otherwise, clinical observations were conducted twice daily, approximately 4 hours apart, during both the acclimation period and then from Days 2 through 28, except on days of neurological and physical examinations (Days -1, 2, 8 and 22).

Individual body weights were measured on Days -7, -1, and then at Weeks 1, 2, 3 and 4 (Days 7, 14, 21 and 28?) postdose. Individual food consumption was measured on a daily basis from Day -4; mean values (by group and sex) are reported for the 4-day predose period and then on a post-dose weekly basis. Blood samples were taken once pretest (Week -1) and then on Days 2 (approximately 24 hours postdose), 8 and 22 following overnight fasts.

One 1X male (#125) was found dead on Day 9 and a 3X female (#122) was euthanized *in extremis* on Day 9. No definitive cause of death could be determined for either. Male #125 had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, and had continued to lose weight in the postexposure period (Day 2: 1.79 kg; Day 4: 1.78 kg; Day 7: 1.67 kg), consistent with poor food consumption (150.8 g/day preexposure; 141.3 g/day during week 1). However, the possibility that Amitraz may have contributed to this puppy's death cannot be ruled out.

Female 122 was the only female puppy in the 3X group showing a weight loss (0.01 kg) in the period from Day -8 to -4, but then gained 0.18 kg from day -4 to day -1 (see p. 550). Although most (controls: 5/6; 1X: 3/6; 3X: 5/6; 5X: 3/6) female puppies lost weight in the interval from day -1 to 2, #122 had the greatest individual weight loss (0.2 kg; (#115 in the controls had the second highest weight loss, 0.15 kg; otherwise, the range was from 0.01 to 0.09 kg). The appetite of #122 had been good during week -1 (actually, food consumption was calculated for only 4 days) at 318 g/day, but then fell to 142.5 g/day for week 1). The report states (p. 11) that: "Macroscopic and microscopic evaluations, organ weights, and bone marrow evaluations did not implicate the test substance or indicate a definitive cause of death, and the deaths were not considered related to treatment." While 1X male #125 clearly had preexposure problems (as indicated by a considerable weight loss on days -4 to -1), the situation with 3X female #122 is not so obvious. This puppy weighed 1.99 kg on Day -1 and was dosed with 1.8 mL of formulation (containing 276 mg Amitraz, it was dosed with 138.7 mg Amitraz/kg) on Day 1 (there was no day zero).

Use directions for the FDA-approved Amitraz-containing formulation [MITABAN] specify application of a dilution (2 gallons of water containing 250 ppm or 1.9 g of Amitraz). The dose for a 10 kg dog is 190 mg Amitraz/kg [incidentally, in http://www.medi-vet.com/**Mitaban**.aspx it is stated that: "The safety of MITABAN has not been established for dogs less than four months of age."]. While no fatalities occurred in the 5X puppies in this study, 3X female #122 may have been representative of a more sensitive population subgroup. Dermally, #122 was dosed with 1.8 mL of Promeris, containing 276 mg Amitraz. As this puppy weighed 1.99 kg at application, dosage was 139 mg Amitraz/kg. This dosage, although dermal, is over 100 mg/kg, shown to be potentially lethal in the dog by the oral route. According to http://myweb.cableone.net/bdturner/Mitaban.pdf#search='mitaban%20amitraz' : "Death occurred in one of two dogs given a single oral dose of 100 mg/kg. Clinical signs included CNS depression, ataxia, hypothermia, bradycardia, muscular weakness, vomition, uncontrolled vocal spasm and micturition. Clinical laboratory data indicated a hemoconcentration, and transient elevations in blood glucose, blood urea nitrogen, serum potassium and alkaline phosphatase values." While puppy #122 had a normal serum glucose value (86 mg/dL) at 24 hours, the BUN was elevated (22 mg/dL at 24 hours, and 33 mg/dL on Day 8). The serum potassium level at 24 hours (6.7 mEq/L) was the highest postexposure value (another female had a preexposure value of 6.8 mEq/L); the serum potassium on Day 8 (4.0 mEq/L; other electrolyte values were also low) was the lowest seen in any puppy during this study.

There are indications that Amitraz in the Promeris formulation is absorbed more rapidly and/or to a greater extent than Amitraz in the Mitaban-use dilution. Public information on Mitaban includes the following [see myweb.cableone.net/bdturner/**Mitaban**.pdf]: "Blood glucose values were elevated at 4 hours posttreatment in the 250 ppm female group, and in both sexes at the 1250 and 2500 ppm concentrations. ...glucose values returned to normal within 24 hours posttreatment. In another study, groups of healthy beagles were topically treated with either 250 ppm, 750 ppm or 1250 ppm of active drug at 14 day intervals and for 12 weeks. Blood glucose values were elevated at the 750 ppm concentration at 4 hours posttreatment after 3 of 6 treatments, and after 5 of 6 treatments at the 1250 ppm level. In the 750 ppm group, serum glucose values returned to normal at 24 hours posttreatment, however for the 1250 ppm group, at 24 hours and after 3 of 6 treatments the levels remained significantly elevated." In the adult dog Promeris study [MRID 46401003] 1/6 of the 3X females and 5/6 5X females had elevated glucose levels ($\geq$120 mg/dL) at 24 hours, suggesting that Amitraz absorption from a 5X (in this case, 7.0 mL) Promeris treatment is greater than that from exposure to 2500 ppm Amitraz (or a 10X concentration of the use-dilution of Mitaban), which would mean that a 1X (1.4 mL) Promeris treatment of an adult dog results in greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban solution.

Although serum glucose was significantly elevated at the 3X and 5X dose levels in the adult study [MRID 46401003] a similar finding was not reported by the laboratory for the puppy study. However, at 24 hours postdose the incidences of elevated (>120 mg/dL) glucose were dose-related: Placebo: 1/12; 1X: 2/12, 3X: 3/12; 5X: 5/12. Incidences on Day 8 were: Placebo: 1/12; 1X: 0/12; 3X: 0/12; and 5X: 1/12. Incidences on Day 22 were similar to those on Day 8. The dose-related incidences for elevated serum glucose at 24 hours, in conjunction with the low incidences of elevated glucose on Days 8 and 22, indicate that there was an effect (probably involving the hypothalamus, consistent with a known effect of Amitraz). The lack of significance for serum glucose levels at 24 hours postdose relative to predose was due to a large number of serum glucose values >120 for week -1 (Placebo: 5/12; 1X: 2/12; 3X: 4/12; and 5X: 3/12). One possible explanation: Some puppies were not adequately fasted before predose (Week -1) blood samples were taken.

The mean weight of the 5X females on Day -1 in the adult study was 8.06 (range: 7.34-9.54) kg, and they were treated with 7.0 mL test material (mean Amitraz dosage: 133.1 mg/kg). The mean weight of the 5X females on Day -1 in the puppy study was 2.25 (range: 1.72-2.89) kg, and they were treated with 3.0 mL test material (mean Amitraz dosage: 204.5 mg/kg).

Except for 1X male #125 and 3X female #122 all puppies survived to study termination.

There is no indication that body weight and/or food consumption were affected by treatment with R-28153/amitraz spot-on.

The only clinical findings on Day 1 (the day of dosing) were lacrimation (not observed at 5X) and soft and/or mucoid feces (and, in one vehicle control female, clear discharge from the nose and muzzle), with no indication of a dose-response.

Physical and neurological examinations showed no effect as a result of treatment with R-28153/amitraz spot-on. However, no temperature measurements were made. While these are not specified in the 870.7200 Guidelines, treatment with Mitaban at 1250 and 2500 ppm

Amitraz was associated with significant depression of rectal temperatures at 4 hours posttreatment (returning to normal at 24 hours), and this would have provided additional information.

Several clinical chemistry and hematology parameters were affected. While there were some cases in which there were no significant differences between controls and test material-treated groups at 24 hours, there were significant differences between pretreatment and 24-hour postdose values, suggesting the possibility of pharmacological activity involving one or more of the inerts.

Urea nitrogen was significantly increased at 24 hours postdose in 1X males and in both sexes at 3X and 5X, although it was not a dose-related trend (perhaps because 1X male #125 and 3X female #122 had more elevated readings than most of the other puppies). A similar increase in was also observed in the adult study, and is consistent with a known effect of Amitraz.

While the statistical summary (see pages 1226 and 1227) does not indicate an effect on serum glucose, this reviewer has examined the data in somewhat more detail, and is convinced that there was an effect. According to http://www.ahc.umn.edu/rar/RefValues.html the normal range for glucose in the dog is 80-120 g/dL. On Day 8 1X male #125 (found dead day 9) had a value of <10 mg/dL, while 3X female #122 (sacrificed *in extremis* day 9) had a value of 23 mg/dL. However, 1X female #116 had a value of 20 mg/dL. A number of puppies had values below 80 mg/dL at 24 hours postdose, including 1X male #120 (64 mg/dL), 3X female #148 (65 mg/dL) , 5X male #117 (48 mg/dL), and 5X females #107 (59 mg/dL) and #129 (64 mg/dL). These values suggest a hypoglycemic effect in puppies, in contrast to the hyperglycemic effect observed in adult dogs (MRID 46401003). However, examining the incidences of glucose values >120 mg/dL, a somewhat different (and dose-related) picture emerges, as at 24 hours postdose 1/12 placebo puppies, 2/12 1X puppies, 3/12 3X puppies and 5/12 5X puppies had elevated levels, and these results are in striking comparison with Day 8 and Day 22 incidences (no more than 1/12 per group). Perhaps the occurrence of either hypoglycemia and/or hyperglycemia (at the 5X dose level only 4/12 puppies were within normal glucose limits) at 24 hours postdose is a manifestation of a temporary inhibition of a metabolic control process, possibly involving the hypothalamus. The relatively high glucose levels for Week -1 suggest the possibility that at least some of the puppies may not have been adequately fasted before that blood collection. Hyperglycemia is a known effect of Amitraz, and is consistent with what was observed in the adult dog study.

Phosphorus was significantly elevated for the 3X and 5X levels at 24 hours postdose.

For leukocyte counts there were significant differences between pretreatment and 24-hour postdose [24 hours lower with P=0.0002] and pretreatment and Day 8 [Day 8 higher with P=0.0007]. At 24 hours postdose means were lower in all groups (including controls) from their predose means. At 8 days means were noticeably higher in all groups (including controls) from their predose means. On Day 22 means were essentially the same as predose. These results are consistent with a physiological effect involving one or more of the "inert" ingredients in this formulation, with a depression in leukocyte at 24 hours postdose and a "rebound" effect by Day 8, and a return to preexposure values on Day 22. One placebo control female [#145] and one 5X female [#146] had below-normal leukocyte counts at 24 hours [5.5 and 5.4 K/mm^3, respectively; the normal range in the dog is 5.9-16.6 K/mm^3].

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for K+ in the dog is 4.4-6.1 mEq/L. The postdose range in this study (4.0-6.7) included values below and above this reference range. However, the only values below this range were on Day 8, in 1X male 125 (4.2; found dead on Day 9) and 3X female 122 (4.0; euthanized *in extremis* on Day 9). The significance at 24 hours postdose was largely due to a more pronounced drop from the preexposure mean in controls than those occurring in the 1X, 3X and 5X dose groups. The magnitude of the drops at 24 hours from preexposure was Placebo > 5X > 3X >1X, suggesting an effect from one or more of the "inerts" in this formulation.

Aspartate aminotransferase (AST) was decreased from preexposure means for controls and at all dose levels at 24 hours postdose (combined mean for both sexes as percentage of preexposure mean for that group: 0X: 88.0%; 1X: 72.7%; 3X: 72.0%; 5X: 69.8%). The significance (if any) of this finding is not evident, as there was no concurrent dose-related change in ALT. In any case, all values observed preexposure and at 24 hours postdose for AST were considerably below 105 U/L, and so were normal for the dog. A drop in AST was noted in the adult dog study.

TRB concludes that this companion animal safety study (OPPTS 870.7200) does not demonstrate an adequate margin of safety (at least 5X) between the exposure associated with the proposed use level for this formulation in puppies and that at which significant adverse systemic effects may occur, and does not support this particular use. The greatest concern involves the death of 3X female #122, sacrificed *in extremis* on Day 9.. This animal had a urea nitrogen of 8 mg/dL pretest, 22 mg/dL at 24 hours postdose, and 33 mg/dL at 8 days postdose, as well as an elevated serum potassium at 24 hours, consistent with known effects of Amitraz. In addition, 1X male #125 was found dead on Day 9. While this puppy had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, the possibility that exposure to Amitraz may have contributed to death cannot be ruled out. Increases in blood urea nitrogen (BUN) and a dose-related trend of increasing incidences (Placebo: 1/12; 1X: 2/12; 3X: 3/12; 5X: 5/12) of elevated glucose (>120 mg/dL) at 24 hours postexposure indicate that physiologically significant amounts of Amitraz had been absorbed.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2), and Good Laboratory Practice Compliance (p. 3) Statements were present.

I. MATERIALS

A. MATERIALS

1. Test material: 15% w/v R-28153/15% w/v amitraz spot-on, with a label declaration of 14.34% Metaflumizone (also known as BAS 320 I) and 14.34% Amitraz. According to information on p. 484 of MRID 46401004 the test formulation contained 14.7% w/w R-28153 and 14.65% Amitraz and had a specific gravity of 1.047 g/mL.
   Description: Pale yellow liquid
   Lot No.: 0381702 (manufactured August 5, 2003)
   Storage: Room Temperature, with protection from light

Placebo: 0% w/v R-28153/0% w/v amitraz spot-on. From information on p. 483 of MRID 46401004 this formulation contained less than 0.01% w/v R-28153 and less than 0.01% w/v Amitraz. The specific gravity is reported as 0.9964-0.9967.
Description: Clear colorless liquid
Lot No.: 0381701 (manufactured July 15, 2003)
Storage: Room Temperature, with protection from light

2. Administration: Topical (spot-on) on Day 1

3. Test animals
Species: Dog
Breed: Beagles
Ages and weights at study initiation: Males: 1 month & 22 days to 1 month & 27 days [53-58 days]: 1.82 to 2.97 kg; Females: also 1 month & 22 days to 1 month & 27 days [53-58 days]: 1.72 to 2.89 kg. Note: randomization was on day -4; at that time males weighed 2.01 to 2.81 kg and females weighed 1.64 to 2.67 kg). Puppies were born between August 3, 2002 and August 8, 2003, and were treated on September 30, 2003.
Source: Covance Research Products, Kalamazoo, MI.
Housing: Individually housed from Day -4 and for the entire experimental period.
Diet: Hill's Prescription Diet (PD) Puppy Food
Water: "drinking water," *ad libitum*
Environmental conditions:
Temperature:
Humidity:
Air changes:
Photoperiod:
Acclimation period: 21 days (September 9 to September 30, 2003)

II. STUDY DESIGN

A. IN LIFE DATES

Arrival of the puppies: 9 September 2003; Treatment date: 30 September 2003; Study termination: 28 October 2003.

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

There were a total of 12 puppies (6 males & 6 females) per dosage group. Each puppy in the Placebo Control Group was treated with 3.0 mL of the placebo formulation (which contained the same "inert" ingredients but was lacking the two actives - R-28153 and Amitraz - present in the test substance formulation). As a result, placebo group puppies were exposed to approximately 6.74X the dose of "inert" ingredients that the 1X group puppies were exposed to (and to approximately 1.35X the dose of "inert" ingredients that the 5X group puppies were exposed to).

From p. 16 of MRID 46401004: "The test substance and Placebo Control were administered once on Day 1 (September 30, 2003) by topical application. The animal

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was held in a position such that the surface to be treated was easily accessible. A section of hair between the shoulder blades on the dorsal midline extending cranially and caudally was separated. A Becton Dickinson disposable syringe containing the appropriate amount of test substance was placed through the hair to the skin and the test substance was applied by dragging the syringe back slowly while administering the dose. Due to varying dose volumes, a 1 mL syringe was used to administer the part of the dose that was less than 1 mL. A 5 mL syringe was used for the parts of the dose volumes at 1 and 3 mL. Care was taken when applying the test substance to reduce the chance of run-off, and no run-off was observed. Separate syringes were used to apply test and control substances and gloves were changed between test and control substance application. The dose volume for each animal was based on body weight recorded on the day prior to dosing (Day -1)..."

TABLE 1. Study design

		Number of Puppies	Amount Applied to Each Puppy	Mean Puppy Wt ± S.D. (kg) on Day -1	Mean Dose mg/kg	Mean Dosage (mg/kg)	
						Metaflumizone	Amitraz
Placebo Control	Male	6	3.0 mL[a]	2.50 ± 0.29	1196[b]	0	0
	Female	6	3.0 mL[a]	2.21 ± 0.31	1353[b]	0	0
1X	Male	6	0.6 mL[c]	2.48 ± 0.39	253[d]	37.2	37.1
	Female	6	0.6 mL[c]	2.24 ± 0.39	280[d]	41.2	41.1
3X	Male	6	1.8 mL[c]	2.51 ± 0.41	751[d]	110.4	110.0
	Female	6	1.8 mL[c]	2.25 ± 0.26	838[d]	123.1	122.7
5X	Male	6	3.0 mL[c]	2.51 ± 0.33	1251[d]	184.0	183.3
	Female	6	3.0 mL[c]	2.25 ± 0.40	1396[d]	205.2	204.5

Individual body weights given on p. 486-489 of MRID 46401004; means (with standard deviations) are presented on p. 73 and 74.

[a] Placebo

[b] Based on a specific gravity for the placebo of 0.9965 g/mL (see p. 483 of MRID 46401004).

[c] Test material (with actives); amount delivered.

[d] Based on a specific gravity for the test material of 1.047 g/mL (see p. 484 of MRID 46401004)

C. DOSE SELECTION RATIONALE

From p. 16 of MRID 46401004: "The dose levels were selected by the Sponsor to evaluate the safety of the test substance at up to five times the proposed *ad usum* rate in puppies. This was considered to provide an appropriate safety margin for the planned therapeutic dose."

According to the proposed label this product will be packaged as 6-packs of monthly unidose applicators with the following single dosages: 0.67 mL (for dogs less than 11 lb); 1.33 mL (for dogs weighing from 11 to 22 lb); 3.33 mL (for dogs weighing from 22 to 56 lbs); 5.33 mL (for dogs weighing 56 to 89 lbs); and 6.66 mL (for dogs weighing 89-111 lbs). Application directions include a specification to apply the entire content of the applicator on the dog's skin and not to apply to the surface of the dog's fur.

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D. EXPERIMENTAL DESIGN

From p. 16 of MRID 46401004: "All animals were observed at least twice a day for morbidity, mortality, injury, and the availability of food and water..."
From p. 17 of MRID 46401004: "Detailed clinical examinations were conducted on Day 1 predose, immediately postdose and at 1, 2, and 3 hours postdose. Detailed clinical examinations were also conducted twice daily approximately four hours apart during the acclimation period, prior to randomization (Day -1), and continued three times a week during the course of the study. Body weights recorded at the end of each week were used for body weight and body weight change statistical analysis, and the body weights recorded on the other days were used to monitor the health of the animals... Body weights collected on Day -1 were used as the baseline measurement for statistical analysis."

Individual puppies were weighed on Days -1 (the day before dosing; Day 1 was the day of dosing so there was no Day 0), 7, 14, 21 and 28.

From p. 17: "Food consumption was measured daily and is reported beginning the last four days of the pretest period and continuing weekly through study termination. The pretest collection was used as the baseline measurement for statistical analysis."
Individual food consumption was measured daily and reported weekly (beginning the last four days of the acclimation period and continuing through to the end of the study).

E. PATHOLOGICAL PARAMETERS

Blood samples were collected from each puppy once pretest (Week -1), and on Days 2 (approximately 24 hours postdose), 8 and 22 by jugular venipuncture following an overnight fast. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
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	Absolutes reticulocytes
	Blood clotting measurements	X	Percent reticulocytes
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time [PT])*		
X	(Activated partial thromboplastin time [APTT])*		
	Erythrocyte morphology		

*Recommended in OPPTS 870.7200 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Blood creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
		X	Total bilirubin (T Bil)*
	ENZYMES		Direct bilirubin (D Bil)*
X	Alkaline phosphatase(ALPor ALK)*	X	Total serum protein (TP)*
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)	X	Albumin/Globulin (A/G) ratio
X	Serum alanine aminotransferase (ALT or SGPT)*	X	Lipase
X	Serum aspartate aminotransferase(AST or SGOT)*		
X	Gamma glutamyl transferase(GGT)		
X	Amylase		
	Glutamate dehydrogenase		

*Recommended in OPPTS 870.7200 Guidelines.

F. STATISTICS

A statistical summary report is found in Appendix O, running from pages 1222 to 1228 of MRID 4640104. From p. 19 of MRID 4640104: "Each of the four treatment groups contained one animal from each of six blocks for each sex. Data were summarized, in tabular form, for each group by mean, standard deviation, number of animals examined, minimal value, and maximum value."

For body weight: "Statistical analysis was performed on body weight change at Weeks 1, 2, 3, and 4 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% level of significance. If the treatment by sex interaction was found significant, then the treated groups' LSMeans were compared to the control group LSMean for each sex by the 2-sided Student's t-test at the 10% level for that parameter. If the treatment by sex interaction was found not to be significant and treatment effect was found to be 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 found not significant for a parameter, no further analysis for that parameter was done at this step and treatments were considered to have no effect on body weight change."

For body weight and food consumption: "A repeated measure analysis was performed on body weight and food consumption parameters using the PROC MIXED procedure in SAS. The model contained pretreatment measurements averaged over Week -1 for food consumption and collected on Day -1 for body weight as the corresponding covariate, and treatment, sex, treatment by sex, week, sex by week, treatment by sex by week, and treatment by week as the fixed effects and interactions with week as the repeated fixed effect..."

"...The interactions, treatment by sex and treatment by sex by week, were tested at the 5% level, whereas the interactions of sex by week and treatment by week were tested at the 10% level."

"For all the quantitative hematology, coagulation and clinical chemistry parameters, pretreatment measurements were considered covariant in the analysis. For count data such as leukocyte count, etc., log-transformation was used. The individual parameters were analyzed by time (i.e. 24 hours postdose, and Days 8 and 22) for hematology, coagulation and clinical chemistry parameters by the PROC MIXED procedure with pretreatment measurements as corresponding covariates, and treatment, sex and treatment by sex as fixed main effects and interaction..."

G. DISPOSITION OF ANIMALS

From p. 18 of MRID 46401004: "The surviving animals were transferred to a stock colony on October 28, 2003." According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

For those puppies which died: "A complete necropsy examination was performed under procedures according to SOP...approved by a veterinary pathologist on a single 1X male...that was found dead and the single 3X female...euthanized *in extremis*..."

H. COMPLIANCE

Signed and dated Quality Assurance [p. 10], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

Mean doses of active ingredients by group and sex were the following: 1X: Males: 37.2 mg/kg R-28153; 37.1 mg/kg Amitraz; Females: 41.2 mg/kg R-28153; 41.1 mg/kg Amitraz; 3X: Males: 110.4 mg/kg R-28153; 110.0 mg/kg Amitraz; Females: 123.1 mg/kg R-28153; 122.7 mg/kg Amitraz; 5X; Males: 184.0 mg/kg R-28153; 183.3 mg/kg Amitraz; Females: 205.2 mg/kg R-28153; 204.5 mg/kg Amitraz. Each puppy in the Placebo Control Group was treated with 3.0 mL placebo formulation (containing the same "inert" ingredients as the test substance formulation but lacking the two actives - R-28153 and Amitraz).. As a result, placebo group puppies were exposed to approximately 6.74X the dose of "inert" ingredients that 1X group puppies were exposed to (and to approximately 1.35X the dose of "inert" ingredients that 5X group puppies were exposed to).

B. MORTALITY

From p. 23 of MRID 46401004: "A single male at 1X (animal number 125) was found dead on Day 9 and a single female at 3X (animal number 122) was euthanized *in extremis* on Day 9. All other animals survived to study termination..."

"The single male at 1X...which was found dead on Day 9, was observed with decreased

activity, soft feces, and pale gums on Day 8 at both the last clinical observation and the physical examination prior to death. At necropsy, the only notable macroscopic observation was of a mildly pale liver, which corresponded to mild hepatocellular vacuolation observed microscopically. In addition, this male was observed with a severely atrophied thymus upon microscopic examination. No definitive cause of death could be determined."

The single female at 3X...euthanized *in extremis* on Day 9 was observed with decreased activity, feces mucoid and discolored red, and pale gums. At necropsy, this animal was noted to have a moderately pale liver, which corresponded to mild hepatocellular vacuolation, observed microscopically. Moderate tan foci were also observed for the stomach macroscopically, while a severely atrophied thymus was noted upon microscopic examination. No definitive cause of death could be determined..."

From p. 546 male 125 had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, and had continued to show weight losses in the postexposure period (Day 2: 1.79 kg; Day 4: 1.78 kg; Day 7: 1.67 kg). Only one other male (#119 in the 3X group) showed a substantial weight loss (2.03 to 1.83 kg) from Day -4 to -1, but this puppy subsequently showed weight gains (including in the period from Day -1 to 2, during which the test material was applied).

Female 122 was the only female puppy in the 3X group showing a weight loss (0.01 kg) in the period from Day -8 to -1 (see p. 550). C. CLINICAL SIGNS

Clinical observations on Day 1 (the day of dosing) were made predose, immediately postdose, and at 1, 2 and 3 hours postdose. The only findings were lacrimation and soft and/or mucoid feces (and, in one vehicle control female, clear discharge from the nose and muzzle), with no indication of a dose-response.

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TABLE 2. Clinical Observations in Puppies Treated with R-28153 and Amitraz Spot-on Immediately Prior and Following Treatment[a]				
Time and Observation	**Placebo (Vehicle) Control**	**1X**	**3X**	**5X**
Predose:				
Soft and/or mucoid feces:	0/6M, 0/6F	1/6M, 1/6F	2/6M, 2/6F	1/6M, 0/6F
Lacrimation:	0/6M, 0/6F	0/6M, 1/6F	0/6M, 0/6F	0/6M, 0/6F
Immediate postdose:				
Soft and/or mucoid feces:	2/6M, 2/6F	2/6M, 3/6F	2/6M, 0/6F	2/6M, 1/6F
Lacrimation:	1/6M, 0/6F	1/6M, 0/6F	1/6M, 1/6F	0/6M, 0/6F
Clear discharge, nose + muzzle:	0/6M, 1/6F	0/6M, 0/6F	0/6M, 0/6F	0/6M, 0/6F
1-hr postdose:				
Soft and/or mucoid feces:	1/6M, 1/6F	1/6M, 2/6F	1/6M, 0/6F	2/6M, 0/6F
Lacrimation:	1/6M, 0/6F	1/6M, 0/6F	1/6M, 1/6F	0/6M, 0/6F
Clear discharge, nose + muzzle:	0/6M, 1/6F	0/6M, 0/6F	0/6M, 0/6F	0/6M, 0/6F
2-hr postdose:				
Soft and/or mucoid feces:	1/6M, 2/6F	1/6M, 4/6F	2/6M, 1/6F	2/6M, 3/6F
Lacrimation:	0/6M, 0/6F	1/6M, 0/6F	1/6M, 2/6F	0/6M, 0/6F
Clear discharge, nose + muzzle:	0/6M, 1/6F	0/6M, 0/6F	0/6M, 0/6F	0/6M, 0/6F
3-hr postdose:				
Soft and/or mucoid feces:	1/6M, 2/6F	1/6M, 4/6F	2/6M, 1/6F	2/6M, 3/6F
Lacrimation:	0/6M, 1/6F	1/6M, 0/6F	1/6M, 2/6F	0/6M, 0/6F
Clear discharge, nose + muzzle:	0/6M, 1/6F	0/6M, 0/6F	0/6M, 0/6F	0/6M, 0/6F

[a]Data taken from Table 2, Summary of Day 1 Clinical Findings, pp. 64-67 of MRID 46401004 with additional information from Individual Day 1 Clinical Findings pp. 517-536

Puppies which showed soft and/or mucoid feces were the following (from pp. 517-536):
Predose Males: Placebo: (none); 1X: #142; 3X: #136, #143; 5X: #102;
Postdose Males: Placebo: #104, #144; 1X: #125, 142; 3X: #112, #119, #136; 5X: #102, #141;
Predose Females: Placebo: (none); 1X: #139; 3X: #130, #138; 5X: none;
Postdose Females: Placebo: #115, #124, #132; 1X: #116, #123, #131, #139, #147; 3X: #122; 5X: #107, #113, #121, #129

Puppies which showed lacrimation were the following (from pp. 517-536):
Predose Males: Placebo: (none); 1X: (none); 3X: (none), #143; 5X: (none)
Postdose Males: Placebo: #104; 1X: #101; 3X: #112; 5X: (none)
Predose Females: Placebo: (none); 1X: #147; 3X: (none); 5X: (none);
Postdose Females: Placebo: #132; 1X: (none); 3X: #122, #148; 5X: (none)

Only one female puppy (#124 in the placebo control group) showed clear discharge from the nose and muzzle.

D. BODY WEIGHT AND WEIGHT GAIN

From p. 24 of MRID 46401004: "Body weight was not affected by treatment with R-28153/amitraz spot-on. There was a significant sex by period interaction but follow-up analysis by sex and by period showed no significant treatment effects."

TABLE 3. Mean Body Weights for Puppies by Group					
Group	kg ± S.D.				
	Day -1	Day 7	Day 14	Day 21	Day 28
Control Males	2.497 ± 0.292	2.703 ± 0.586	3.187 ± 0.760	3.783 ± 0.875	4.422 ± 0.827
Control Females	2.208 ± 0.308	2.455 ± 0.335	2.793 ± 0.384	3.243 ± 0.381	3.703 ± 0.564
1X Males	2.478 ± 0.388	2.747 ± 0.603	3.436 ± 0.374	3.916 ± 0.420	4.592 ± 0.563
1X Females	2.240 ± 0.392	2.513 ± 0.487	2.922 ± 0.566	3.435 ± 0.650	3.883 ± 0.824
3X Males	2.508 ± 0.406	2.908 ± 0.379	3.350 ± 0.544	3.918 ± 0.855	4.570 ± 0.962
3X Females	2.253 ± 0.256	2.465 ± 0.464	2.938 ± 0.235	3.448 ± 0.307	3.930 ± 0.299
5X Males	2.513 ± 0.329	2.892 ± 0.371	3.350 ± 0.372	3.903 ± 0.548	4.408 ± 0.606
5X Females	2.250 ± 0.397	2.493 ± 0.574	2.843 ± 0.570	3.183 ± 0.566	3.800 ± 0.683

Values from data p. 73-74 of MRID 46401004

There was no significant difference between groups for mean body weight gains:

TABLE 4. Mean Body Weight Gains for Puppies by Group				
Group	kg ± S.D.			
	Day -1 to 7	Day 7 to 14	Day 14 to 21	Day 21 to 28
Control Males	0.207 ± 0.341	0.690 ± 0.492	1.287 ± 0.613	1.925 ± 0.563
Control Females	0.247 ± 0.186	0.585 ± 0.195	1.035 ± 0.186	1.495 ± 0.325
1X Males	0.268 ± 0.225	0.826 ± 0.189	1.306 ± 0.284	1.982 ± 0.413
1X Females	0.273 ± 0.203	0.682 ± 0.262	1.195 ± 0.386	1.643 ± 0.519
3X Males	0.400 ± 0.229	0.842 ± 0.382	1.410 ± 0.643	2.062 ± 0.737
3X Females	0.212 ± 0.286	0.632 ± 0.228	1.142 ± 0.209	1.624 ± 0.266
5X Males	0.378 ± 0.156	0.837 ± 0.213	1.390 ± 0.316	1.895 ± 0.422
5X Females	0.243 ± 0.183	0.593 ± 0.200	0.933 ± 0.281	1.550 ± 0.358

Values from data p. 83-84 of MRID 46401004

A number of puppies of both sexes of all groups (including Placebo Control) showed slight weight losses in the period from Day -1 to 2 (controls: 4/6M; 5/6F; 1X: 4/6M; 3/6F; 3X: 1/6M; 5/6F; 5X: 2/6M; 3/6F). This may have been due, in part, to overnight fasting prior to collecting blood on Day 2, although it is also possible that exposure to the test material actives and/or inerts played a part.

E. FOOD CONSUMPTION

From p. 24 of MRID 46401004: "Food consumption was comparable to the Placebo Controls for all of the treatment groups. No effect from treatment with R-28153/amitraz spot-on was evident."

TABLE 5. Mean Food Consumption (g/puppy/day) by Group					
Group	g ± S.D.				
	Week -1	Week 1	Week 2	Week 3	Week 4
Control Males	312.7 ± 88.5	380.1 ± 151.2	440.8 ± 135.0	511.2 ± 81.3	610.0 ± 101.0
Control Females	286.0 ± 36.1	362.7 ± 60.3	395.7 ± 52.0	456.3 ± 47.0	520.1 ± 89.2
1X Males	319.3 ± 71.7	383.4 ± 134.4	466.9 ± 51.1	504.1 ± 80.9	630.0 ± 84.1
1X Females	279.0 ± 79.0	384.9 ± 102.5	395.9 ± 84.1	465.6 ± 67.3	493.2 ± 98.9
3X Males	321.2 ± 101.4	461.8 ± 106.4	461.4 ± 119.8	555.4 ± 202.9	615.9 ± 151.7
3X Females	306.3 ± 19.0	366.8 ± 113.3	391.2 ± 69.3	490.3 ± 77.0	523.9 ± 46.6
5X Males	335.4 ± 65.4	443.6 ± 68.5	454.7 ± 45.1	515.4 ± 106.7	549.1 ± 140.4
5X Females	304.5 ± 86.1	371.7 ± 117.1	404.7 ± 68.9	418.6 ± 120.6	555.3 ± 78.6

Values from p. 93-94 of MRID 46401004.

F. HEMATOLOGY

For leukocyte counts there were no significant differences between the placebo and test-material exposed groups at 24 hours postdose, on Day 8, or on Day 22. However, significant differences are reported [p. 136 of MRID 46401004] between Pretreatment and 24-hour postdose [P = 0.0002] and Pretreatment and Day 8 [P = 0.0007].

TABLE 6. Ranges & Means ± S.D. for Leukocytes (K/mm^3)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	9.7-17.7; 13.45 ± 2.86	7.5-16.5; 10.83 ± 3.44	11.4-27.8; 19.30±6.14	10.4-22.4; 14.32±4.53
Placebo females	14.5-18.3; 15.88±1.31	5.5-19.0; 12.80 ± 5.73	14.0-24.8; 19.75±3.96	11.0-16.8; 13.83±2.18
Placebo combined	9.7-18.3; 14.67± 2.47	5.5-19.0; **11.82± 4.62**	11.4-27.8; **19.53±4.93**	10.4-22.4; 14.08±3.40
1X males	13.9-28.6; 20.27±4.96	9.1-28.9; 15.10 ± 7.50	15.9-32.9; 21.47±5.98	11.5-18.7; 15.32±3.07
1X females	12.6-20.2; 15.10±2.74	11.0-15.4; 12.53±1.58	16.0-24.9; 19.27±3.13	11.4-17.3; 14.67±2.16
1X combined	12.6-28.6; 17.68±4.68	9.1-28.9; **13.82 ± 5.34**	15.9-32.9; **20.37±4.69**	11.4-18.7; 14.96±2.49
3X males	9.3-27.5; 19.10± 7.05	8.6-21.1; 12.55 ± 4.87	9.9-31.1; 18.93 ± 6.96	8.8 - 17.0; 12.53±2.82
3X females	10.2-15.7; 14.27±2.08	6.8-15.7; 9.57± 3.29	12.4-25.1; 17.70±4.92	12.4-20.9; 14.96±3.73
3X combined	9.3-27.5; 16.68 ± 5.56	6.8-21.1; **11.06 ± 4.26**	9.9-31.1; **18.32 ± 5.78**	8.8-20.9; 13.64 ± 3.34
5X males	11.8-26.2; 15.87±5.28	8.3-20.7; 13.50 ± 4.30	13.7-29.9; 21.68±5.74	12.0-16.2; 13.90±1.69
5X females	9.2-25.6; 15.62 ± 6.07	5.4-22.3; 12.15 ± 5.74	13.8-32.1; 20.05±6.40	10.3-25.9; 17.07±6.07
5X combined	9.2-26.2; 15.74±5.43	5.4-22.3; **12.83 ± 4.89**	13.7-32.1; **20.87±5.86**	10.3-25.9; 15.48±4.56

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942;
Means and Standard Deviations on p. 137, 138 and 142
Bold values indicate noticeable (overall significant) differences with week -1 means.

At 24 hours postdose means were noticeably lower in all groups (including controls) from their predose means. At 8 days means were noticeably higher in all groups (including controls) from their predose means. On Day 22 means were essentially the same as predose.

These results suggest the possibility of a physiological effect involving one or more of the "inert" solvents in this formulation, with a depression in leukocyte counts at 24 hours postdose, a "rebound" effect by day 8, and a return to preexposure values by day 22.

According to http://www.ahc.umn.edu/rar/RefValues.html the normal reference range for leukocytes in the dog is 5.9-16.6 K/mm^3; [http://www.vet.purdue.edu/vm525/fall 2002/reference_ranges.pdf, gives a similar range of 6.0-17.0 K/mm^3]. As these were puppies their range might be somewhat different. However, it is noteworthy that the only values below 5.9 K/mm^3 occurred in one placebo control female puppy [145 - see p. 892] and one 5X female puppy [146 - see p. 893] (i.e., those which were dosed with the maximum amounts of inerts) at 24 hours postdose. Values of 30 K/mm^3 and above were observed only on Day 8 in three test-material exposed animals (1X: male 125, which died on Day 9; 3X: male 119; and 5X: female 107; although a 5X male [109] had a value of 29.9; see p. 905, 906, 918). From the way statistical calculations were done, the P value for the pooled 5X group = 0.0081, but the mean and SD are 5.24 and 0.44 respectively. The mean of 5.24 M/mm^3 is greater than the pooled mean of the 3X group (5.20 M/mm^3, P=0.0724) and is closer to the pooled mean (5.53 M/mm^3) of the controls.

From p. 1225: "Large Unstained Cells had a significant treatment effect in the 24 hour postdose interval (p=0.0642). The follow-up analysis results indicated that there was a significant effect in this interval when comparing control to the 5X groups, (p=0.0143)."

TABLE 7. Ranges & Means ± S.D. for Large Unstained Cells (X10.e2/uL)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	0.7-2.5; 1.53 ± 0.75	0.7-2.0; 1.50 ± 0.44	0.5-5.8; 3.18 ± 1.89	0.3-1.3; 0.92 ± 0.45
Placebo females	0.8-5.4; 1.87 ± 1.76	1.3-2.2; 1.73 ± 0.35	1.0-3.7; 2.18 ± 0.98	0.5-1.5; 0.95 ± 0.41
Placebo both	0.7-5.4; 1.70 ± 1.30	0.7-2.2; 1.62 ± 0.40	0.5-5.8; 2.68 ± 1.53	0.3-1.5; 0.93 ± 0.41
1X males	0.8-3.7; 2.12 ± 1.09	1.0-4.1; 2.47 ± 1.41	1.6-2.5; 2.0 ± 0.34	0.7-1.5; 1.06 ± 0.37
1X females	0.0-1.9; 1.18 ± 0.65	0.0-2.3; 1.33 ± 0.88	0.9-5.9; 3.22 ± 1.73	0.7-1.2; 0.95 ± 0.21
1X combined	0.0-3.7; 1.65 ± 0.99	0.0-4.1; 1.90 ± 1.27	0.9-5.9; 2.61± 1.35	0.7-1.5; 1.0 ± 0.28
3X males	0.7-7.4; 3.2 ± 2.41	1.1-5.9; 2.92 ± 1.77	0.7-4.6; 2.45 ± 1.74	0.7-1.8; 1.27 ± 0.43
3X females	0.9-2.6; 1.65 ± 0.71	0.6-3.5; 1.68 ± 1.03	1.4-2.9; 2.22 ± 0.63	0.7-1.5; 1.04 ± 0.30
3X combined	0.70-7.4; 2.43 ± 1.88	0.6-5.9; 2.30 ± 1.52	0.7-4.6; 2.33 ± 1.25	0.7-1.8; 1.16 ± 0.38
5X males	0.8-2.3; 1.45 ± 0.57	0.0-3.5; 1.55 ± 1.40	1.1-4.0; 2.15 ± 1.03	0.6-1.7; 0.98 ± 0.45
5X females	0.3-4.3; 1.82 ± 1.38	1.2-3.3; 2.08 ± 0.79	1.6-5.3; 2.78 ± 1.52	0.8-1.2; 1.02 ± 0.17
5X combined	0.3-4.3; 1.63 ± 1.02	0.0-3.5; 1.82 ± 1.12	1.1-5.3; 2.47± 1.28	0.6-1.7; 1.0 ± 0.33

Individual data on p. 862, 863, 874, 875, 887, 888, 900, 901, 913, 914, 925, 926, 937, 938, 949 and 950;
Means and Standard Deviations on p. 261, 262 and 266

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From the reported values for this parameter, this reviewer sees no indication of any statistical significance in the 5X group relative to placebo controls at 24 hours postdose.

From p. 1225 of MRID 46401004: "Erythrocytes had a significant treatment effect in the Day 8 postdose interval only (p=0.0572). Follow-up analysis results indicated that there was a significant effect when comparing control to all three treatment groups (p=0.0899 for 1X, p=0.0724 for 3X, p=0.0081 for 5X, respectively)."

From p. 143 there were significant differences with respect to the pretreatment covariate at 24 hours, on day 8, and again on day 22 (reported P values of 0.0000, 0.0000, and 0.0000 respectively). However, these were associated with increases, rather than decreases, in RBC counts. According to http://www.vet.purdue.edu/vm525/fall 2002/reference_ranges.pdf the normal RBC range for dog is 5.5 to 8.5 M/mm^3, so the postexposure measurements were more consistent with "normal" values.

TABLE 8. Ranges & Means ± S.D. for Erythrocytes (M/mm^3)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	4.28-5.66; 4.74 ± 0.54	4.45-5.48; 4.93 ± 0.49	4.92-5.93; 5.61 ± 0.39	5.07-5.83; 5.47 ± 0.33
Placebo females	4.01-5.75; 4.68 ± 0.59	4.39-5.59; 4.78 ± 0.45	4.80-6.49; 5.45 ± 0.58	5.46-6.39; 5.87 ± 0.36
Placebo combined	4.01-5.75; 4.71 ± 0.54	4.39-5.59; 4.85 ± 0.45	4.80-6.49; 5.53 ± 0.47	5.07-6.39; 5.67 ± 0.39
1X males	4.01-5.43; 4.74 ± 0.55	4.16-5.39; 4.77 ± 0.51	4.72-5.83; 5.29 ± 0.42	4.95-5.98; 5.47 ± 0.37
1X females	4.24-5.24; 4.79 ± 0.32	4.29-5.66; 4.82 ± 0.50	4.84-5.66; 5.36 ± 0.30	5.03-6.03; 5.69 ± 0.36
1X combined	4.01-5.43; 4.77 ± 0.43	4.16-5.66; 4.79 ± 0.48	4.72-5.83; **5.32 ± 0.35**	4.95-6.03; 5.59 ± 0.36
3X males	4.23-4.76; 4.55 ± 0.18	4.08-4.96; 4.55 ± 0.36	4.98-5.46; 5.19 ± 0.20	5.32-6.20; 5.65 ± 0.34
3X females	4.03-5.03; 4.61 ± 0.37	4.42-5.65; 4.79 ± 0.45	4.48-5.81; 5.20 ± 0.52	5.23-6.05; 5.67 ± 0.30
3X combined	4.03-5.03; 4.58 ± 0.28	4.08-5.65; 4.67 ± 0.41	4.48-5.81; **5.20 ± 0.37**	5.23-6.20; 5.66 ± 0.30
5X males	4.19-5.07; 4.83 ± 0.32	4.08-4.92; 4.63 ± 0.29	4.68-5.54; 5.03 ± 0.36	4.92-5.84; 5.55 ± 0.34
5X females	4.52-5.41; 4.90 ± 0.36	4.28-5.68; 4.83 ± 0.50	4.71-5.99; 5.45 ± 0.43	5.26-6.06; 5.74 ± 0.32
5X combined	4.19-5.41; 4.86 ± 0.33	4.08-5.68; 4.73 ± 0.40	4.68-5.99; **5.24 ± 0.44**	4.92-6.06; 5.65 ± 0.33

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942;
Means and Standard Deviations on p. 145, 146 and 150
On p. 144 it is reported that the P values for the pooled means on Day 8 were: 1X: 0.0899; 3X: 0.0724; and 5X: 0.0081 [however the values above indicate only the males showed an apparent dose-relationship].

From page 201: percent reticulocytes showed a significant difference at 24 hours postdose from the pretreatment covariate (P = .0008).

TABLE 9. Ranges & Means ± S.D. for Percent Reticulocytes				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	2.3 - 7.5; 5.17 ± 1.78	1.3 - 4.7; 2.98 ± 1.34	0.3 - 3.8; 2.28 ± 1.19	1.8 - 5.3; 2.73 ± 1.36
Placebo females	1.5 - 4.3; 2.82 ± 1.09	1.8 - 3.4; 2.82 ± 0.56	2.0 - 4.9; 3.42 ± 1.02	1.4 - 3.8; 2.57 ± 0.94
Placebo combined	1.5 - 7.5; 3.99 ± 1.87	1.3 - 4.7; 2.90 ± 0.98	0.3 - 4.9; 2.85 ± 1.21	1.4 - 5.3; 2.65 ± 1.12
1X males	1.8 - 6.4; 3.40 ± 1.69	1.1 - 4.0; 2.27 ± 1.08	0.2 - 4.3; 2.87 ± 1.49	0.9 - 3.5; 2.14 ± 1.05
1X females	2.7 - 5.4; 3.83 ± 1.15	1.6 - 3.0; 2.18 ± 0.46	1.7 - 3.6; 2.77 ± 0.76	1.7 - 3.4; 2.38 ± 0.67
1X combined	1.8 - 6.4; 3.62 ± 1.40	1.1 - 4.0; 2.23 ± 0.79	0.2 - 4.3; 2.82 ± 1.13	0.9 - 3.5; 2.27 ± 0.83
3X males	2.3 - 5.4; 3.87 ± 1.18	0.8 - 3.1; 2.12 ± 0.92	1.7 - 5.5; 3.65 ± 1.48	0.4 - 3.1; 1.97 ± 0.89
3X females	2.6 - 5.7; 3.87 ± 1.06	2.4 - 3.5; 3.08 ± 0.45	0.3 - 4.9; 3.35 ± 1.61	1.2 - 3.4; 2.76 ± 0.90
3X combined	2.3 - 5.7; 3.87 ± 1.07	0.8 - 3.5; 2.60 ± 0.86	0.3 - 5.5; 3.50 ± 1.48	0.4 - 3.4; 2.33 ± 0.94
5X males	3.4 - 6.3; 4.65 ± 1.32	1.7 - 3.7; 2.82 ± 0.84	1.6 - 5.4; 3.45 ± 1.47	1.3 - 3.3; 2.38 ± 0.77
5X females	2.4 - 4.5; 3.80 ± 0.78	2.0 - 3.0; 2.52 ± 0.39	0.2 - 5.1; 2.70 ± 1.57	0.6 - 5.4; 2.28 ± 1.82
5X combined	2.4 - 6.3; 4.23 ± 1.12	1.7 - 3.7; 2.67 ± 0.64	0.2 - 5.4; 3.08 ± 1.50	0.6 - 5.4; 2.33 ± 1.33

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942;
Means and Standard Deviations on p. 202, 203 and 207
On p. 201 it is reported that P=0.0008 for the 24 hour postdose compared to the pretreatment covariate; the corresponding P value for day 8 was 0.6573 and for day 22 was 0.0731.

For hemoglobin (HGB) there were significant differences with respect to pretreatment covariates at 24 hours, on day 8 and day 22 (from p. 151: P = 0.0002, 0.0008 and 0.0000, respectively), associated with increases in HGB, as would be expected from RBC count increases.

TABLE 10. Ranges & Means ± S.D. for HGB g/dL				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	8.5-11.1; 9.52 ± 0.88	8.8-10.7; 9.68 ± 0.79	10.0-11.3; 10.75±0.56	9.8-11.8; 10.88± 0.77
Placebo females	8.4-10.3; 9.45 ± 0.68	9.1-9.9; 9.40 ± 0.36	9.7-11.4; 10.52 ± 0.56	11.3-12.5;11.80± 0.47
Placebo combined	8.4-11.1; 9.48 ± 0.75	8.8-10.7; 9.54 ± 0.60	9.7-11.4; 10.63 ± 0.55	9.8-12.5; 11.34 ± 0.77
1X males	8.6-11.3; 9.83 ± 0.94	8.6-11.2; 9.68 ± 1.01	9.7-11.8; 10.53 ± 0.84	10.2-12.6; 11.22± 0.88
1X females	9.3-11.0; 10.10 ± 0.57	9.1-11.6; 9.87± 0.94	10.1-11.4;10.75 ± 0.51	10.8-12.2; 11.77± 0.54
1X combined	8.6-11.3;9.97 ± 0.75	8.6-11.6; 9.78 ± 0.93	9.7-11.8; 10.64 ± 0.67	10.2-12.6;11.52 ± 0.74
3X males	8.7-10.4; 9.62 ± 0.55	8.5-10.4; 9.37 ± 0.67	9.6-11.2; 10.43±0.57	10.9-12.9; 11.62± 0.89
3X females	7.7-10.4; 9.70 ± 1.04	9.3-11.4; 9.87 ± 0.81	9.2-11.9; 10.45 ± 0.96	10.9-12.3; 11.78± 0.55
3X combined	7.7-10.4; 9.66 ± 0.79	8.5-11.4; 9.62 ± 0.75	9.2-11.9; 10.44 ± 0.75	10.9-12.9; 11.69 ±0.72
5X males	9.1-11.0; 9.93 ± 0.63	8.7-10.1; 9.43 ± 0.46	9.2-11.0; 10.02±0.60	10.8-11.9; 11.35 ±0.44
5X females	8.4-11.3; 10.05 ± 0.99	8.5-11.0; 9.72 ± 1.09	9.2-11.8; 10.68 ± 0.92	10.6-12.8;11.62± 0.88
5X combined	8.4-11.3; 9.99 ± 0.79	8.5-11.0; 9.58 ± 0.81	9.2-11.8; 10.35 ± 0.82	10.6-12.8; 11.48± 0.68

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942;
Means and Standard Deviations on p. 152, 153 and 157.

According to http://www.ahc.umn.edu/rar/RefValues.html the normal reference range for HGB in dogs is from 14.2-19.2 g/dL. The day 22 values are closer to the normal reference range than are those of week -1.

Hematocrit showed similar increases at 24 hours, on day 8, and again on day 22 (from p. 158 P values of 0.0036, 0.0205, and 0.0002, respectively). Pretreatment values for HCT ranged from 25.3 to 35.8, and posttreatment values from 26.8 to 40.8%. One reference (http://www.vet.purdue.edu/vm525/fall 2002/reference_ranges.pdf) gives the normal range for dog as 37-55%; the increase in values may simply have been part of a maturation process. However, many of the puppies showed significant levels of preexposure anisocytosis [variation in size of red blood cells, sometimes caused by low vitamin B 12, folic acid and/or iron].

TABLE 11. Ranges & Means ± S.D. for HCT (%)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	27.6-34.0; 30.08±2.27	28.5-33.8; 30.70±2.35	32.3-36.6; 34.92± 2.04	32.3-36.3; 34.18± 1.77
Placebo females	26.2-32.0; 29.52±1.89	28.4-31.7; 29.83±1.16	31.7-37.9; 34.73± 2.16	35.0-38.1; 36.50± 1.14
Placebo combined	26.2-34.0; 29.80±2.01	28.4-33.8; 30.27±1.82	31.7-37.9; 34.83± 2.00	32.3-38.1; 35.34± 1.86
1X males	27.9-34.3; 30.73±2.62	27.8-34.3; 30.27±2.53	31.0-36.8; 33.48± 2.40	31.7-38.7; 34.60± 2.60
1X females	29.6-34.1; 31.63±1.51	29.3-35.7; 31.02±2.46	32.4-36.3; 34.65± 1.41	33.1-37.4; 36.18± 1.56
1X combined	27.9-34.3; 31.18±2.09	27.8-35.7; 30.64±2.41	31.0-36.8; 34.07± 1.97	31.7-38.7; 35.46± 2.15
3X males	28.2-31.5; 29.85±1.29	27.6-32.8; 29.07±2.01	31.1-35.6; 33.47± 1.51	33.3-40.8; 35.88± 3.19
3X females	25.3-32.2; 30.07±2.58	29.5-35.9; 31.03±2.44	29.0-38.1; 33.48± 3.20	34.1-38.5; 36.52± 1.78
3X combined	25.3-32.2; 29.96±1.95	27.6-35.9; 30.05±2.37	29.0-38.1; 33.48± 2.38	33.3-40.8; 36.17± 2.54
5X males	28.4-34.1; 31.47±1.88	26.8-31.4; 29.67±1.59	30.7-35.6; 32.35± 1.84	32.4-36.6; 35.00± 1.60
5X females	27.0-35.8; 32.00±2.92	26.9-35.7; 31.13±3.13	29.7-38.3; 35.13± 2.98	32.9-39.9; 36.42± 2.53
5X combined	27.0-35.8; 31.73±2.35	26.8-35.7; 30.40±2.49	29.7-38.3; 33.74± 2.77	32.4-39.9; 35.71± 2.15

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942;
Means and Standard Deviations on p. 159, 160 and 164.

From p. 1225 of MRID 46401004: "MCH had a significant treatment effect in the 24 hour postdose interval (p=0.0868). The follow-up analysis indicated that there was a significant effect in this interval when comparing control to the 3X and the 5X groups (p=0.0515 and p=0.0260, respectively)." [See also p. 173].

TABLE 12. Ranges & Means ± S.D. for MCH (pg)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	19.0-21.1; 20.15±0.78	19.0-20.8; 19.63±0.70	18.5-20.4; 19.25±0.68	18.9-20.7; 19.88±0.65
Placebo females	17.8-21.6; 20.32±1.40	17.8-20.8; 19.80±1.09	17.6-20.2; 19.42±0.97	19.5-20.8; 20.15±0.56
Placebo combined	17.8-21.6; 20.23±1.09	17.8-20.8; 19.72±0.88	17.6-20.4; 19.33±0.80	18.9-20.8; 20.02±0.59
1X males	19.5-22.2;20.82±0.92	19.0-21.3; 20.28±0.79	18.7-20.9; 19.90±0.81	19.7-21.3; 20.52±0.69
1X females	20.0-22.0; 21.13±0.70	19.9-21.3; 20.53±0.52	19.1-20.8; 20.05±0.59	20.2-21.5; 20.73±0.45
1X combined	19.5-22.2; 20.98±0.80	19.0-21.3; 20.41±0.65	18.7-20.9; 19.98±0.68	19.7-21.5; 20.64±0.55
3X males	20.0-22.3; 21.15±0.77	19.2-22.1; 20.65±0.98	18.7-21.2; 20.08±0.82	19.4-21.6; 20.50±0.75
3X females	19.2-21.9; 21.03±1.08	19.2-21.4; 20.67±0.80	19.1-20.6; 20.15±0.67	20.2-21.4; 20.74±0.50
3X combined	19.2-22.3; 21.09±0.90	19.2-22.1; **20.66±0.85**	18.7-21.2; 20.12±0.72	19.4-21.6; 20.61±0.63
5X males	19.5-21.8; 20.63±0.91	19.4-21.2; 20.40±0.71	18.7-21.0; 19.93±0.91	19.8-22.0; 20.55±0.82
5X females	18.6-22.3; 20.50±1.34	18.5-21.8; 20.13±1.15	18.2-20.8; 19.60±0.92	18.9-21.1; 20.20±0.78
5X combined	18.6-22.3; 20.57±1.10	18.5-21.8; **20.27±0.92**	18.2-21.0; 19.77±0.89	18.9-22.0; 20.38±0.78

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942; Means and Standard Deviations on p. 174, 175 and 179

According to http://www.ahc.umn.edu/rar/RefValues.html the normal reference range for MCH in the dog is 12.2-25.4 pg; none of the values observed at 24 hours postdose in the 3X and 5X groups (range: 18.5-22.1 pg) or even in this study (range: 17.6-22.3 pg) was outside this range.

On p. 173 it is reported that the P values for the pooled (combined sex) means at 24 hours relative to controls were: 1X: 0.4322; 3X: 0.0515; and 5X: 0.0260. This reviewer is concerned about the statistical calculations, as the P value for the pooled 5X group is reported as 0.0260, but the mean and SD are 20.27 and 0.92 respectively. The mean of 20.27 pg is closer to the pooled mean (19.72) of the controls, and is less than the pooled mean (20.41 pg) of the 1X group, reported as not statistically significant with respect to controls.

For MCHC the range of individual values was 28.6 to 33.4 g/dL (for placebo controls alone it was 29.6 to 33.2 g/dL); this range is slightly below the normal reference range of 32-36 g/dL in http://www.ahc.umn.edu/rar/RefValues.html, but there was no evidence of any significant difference between the 4 groups.

For platelets, there was a significant drop in counts between pretest and 24 hours postdose across all groups, with recovery by day 8.

TABLE 13. Ranges & Means ± S.D. for Platelets (thousands/mm^3)				
Group & sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo M	419-1018;692.0±237.0	217-667; 408.3±168.4	296-816; 579.0±179.3	429-875; 651.8±158.8
Placebo F	297-650; 490.3±130.7	173-556; 400.2±144.5	473-749; 594.0±102.3	503-776; 616.7±108.8
Placebo com.	297-1018; 591.2±210.7	173-667; 404.3±149.7	296-816; 586.5±139.4	429-875; 634.3±131.1
1X males	465-686; 581.8± 84.0	374-530; 453.0± 74.3	394-879; 644.8±166.8	557-814; 655.4±112.6
1X females	291-757; 463.5±156.0	237-472; 372.0± 88.5	383-614; 517.7± 83.6	431-877; 594.2±170.4
1X combined	291-757; 522.7±134.5	237-530; 412.5± 88.7	383-879; 581.3±142.2	431-877; 622.0±143.6
3X males	358-614; 475.7±106.3	299-814; 425.3±200.8	533-786; 629.0± 99.3	507-699; 621.3± 73.3
3X females	401-775; 534.8±146.9	210-546; 361.2±135.8	418-614; 525.2 ± 78.2	491-745; 612.6±101.2
3X combined	358-775; 505.3±126.1	210-814; 393.3±166.8	418-786; 577.1±101.0	491-745; 617.4± 82.5
5X males	390-731; 497.0±121.1	222-407; 338.7± 64.5	325-677; 502.5±160.0	523-744; 605.7± 81.9
5X females	377-743; 520.0±152.4	183-528; 338.8±140.3	413-728; 550.0±112.3	516-853; 642.0±126.4
5X combined	377-743; 508.5±131.8	183-528; 338.8±104.1	325-728; 526.3±134.1	516-853; 623.8±103.3

Individual data on p. 854, 855, 866, 867, 879, 880, 892, 893, 905, 906, 917, 918, 929, 930, 941 and 942; Ranges, Means and Standard Deviations on p. 188, 189 and 193.

The only values below 250 (thousands/mm^3 or K/mm^3) observed in the course of this study were at 24 hours in 1 male placebo control [#104; value 217 K/mm^3]; one male at 5X [#117; value 222 K/mm^3]; one female placebo control [#145 - at 173 K/mm^3 the lowest value observed during this study]; one female at 1X [#131; value 237 K/mm^3; two 3X females [#114 and #130, values of 210 and 216 K/mm^3 respectively], and one 5X female [#107, value 183 K/mm^3].

The drop in platelet counts suggests an effect from one or more of the inert ingredients in this formulation. However, according to http://www.ahc.umn.edu/rar/RefValues.html the normal range for platelet counts in the dog is 160-525, so that even at 24 hours all values were above the lower limit. This effect was not seen in adult dogs (MRID 46401003).

From p. 1225: "APTT resulted in a significant treatment by sex interaction on the Day 22 interval (p=0.0310). There was also a significant treatment effect (p=0.0887), however, since the interaction was statistically significant, the follow-up analysis was done by sex for this interval. The follow-up analysis resulted in a significant result for males only, when comparing control to treatment group 1X (p=0.0082)."

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From information on p. 208 there was a highly significant difference between pretreatment values [for all exposure levels including controls] and those at 24 hours postdose (P value 0.0001). This may be related to the decrease in platelet counts at 24 hours. At Day 22 (but not Day 8) APTT was significantly different (p=0.0000) from the pretreatment covariant; however, in contrast to the situation at 24 hours postdose, this significance involved more rapid time. An APTT test primarily screens for deficiencies in the intrinsic pathway, such as factors VIII, IX, XI, XII, prekallikrein and high molecular weight kininogen. It is also used to detect inhibitors of the coagulation system.

TABLE 14. Ranges & Means ± S.D. for APTT (sec)

Group & sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo M	12.2-13.9; 12.82± 0.65	12.4-15.3; 13.55± 1.14	10.8-16.1; 12.90± 1.86	10.9-13.9; 12.17± 1.02
Placebo F	12.7-14.5; 13.62± 0.59	12.0-15.2; 14.00± 1.18	11.1-13.6; 12.33± 0.89	11.8-13.8; 12.60± 0.77
Placebo com.	12.2-14.5; 13.22± 0.73	12.0-15.3; 13.78± 1.13	10.8-16.1; 12.62± 1.42	10.9-13.9; 12.38± 0.89
1X males	12.6-14.4; 13.62± 0.75	13.2-15.1; 14.02± 0.73	11.7-21.7; 16.30± 3.98	10.7-12.4; 11.76± 0.68
1X females	11.5-14.0; 12.68± 0.87	12.4-15.7; 14.00± 1.16	12.0-14.1; 13.12± 0.81	11.9-13.9; 12.53± 0.75
1X combined	11.5-14.4; 13.15± 0.91	12.4-15.7; 14.01± 0.92	11.7-21.7; 14.71± 3.20	10.7-13.9; 12.18± 0.79
3X males	11.7-14.7; 13.53± 1.07	13.0-16.6; 14.40± 1.19	11.0-14.2; 13.05± 1.29	10.9-13.5; 12.18± 0.98
3X females	12.1-14.7; 13.73± 0.99	13.0-16.5; 14.58± 1.26	12.0-27.9; 15.52± 6.18	11.9-12.4; 12.04± 0.22
3X combined	11.7-14.7; 13.63± 0.99	13.0-16.6; 14.49± 1.18	11.0-27.9; 14.28± 4.45	10.9-13.5; 12.12± 0.71
5X males	12.0-17.8; 14.13± 2.01	12.9-15.4; 14.30± 1.07	10.2-19.5; 14.23± 3.07	11.1-16.5; 13.05± 1.94
5X females	12.1-14.9; 13.57± 0.90	13.5-17.8; 15.08± 1.82	10.4-15.3; 13.42± 1.79	11.8-14.7; 13.05± 0.93
5X combined	12.0-17.8; 13.85± 1.51	12.9-17.8; 14.69± 1.48	10.2-19.5; 13.83± 2.43	11.1-16.5; 13.05± 1.45

Individual data on p. 858, 859, 870, 871, 883, 884, 896, 897, 909, 910, 921, 922, 933, 934, 945 and 946; Ranges, Means and Standard Deviations on p. 210, 211 and 215.

On day 8 for 1X males #125 (found dead on day 9) had an APTT of 21.7 seconds, male 110 had an APTT of 19.4 seconds and male 101 had an APTT of 18.0 seconds. For 3X females #122 (euthanized *in extremis* day 9) had an APTT of 27.9 seconds; the others ranged from 12.0-15.3 seconds. In the 5X males #102 had a value of 19.5 seconds; the others ranged from 10.2 to 15.1 seconds. According to http://www.vet.purdue.edu/vm525/fall%202002/reference_ranges.pdf#search='APTT%20dog' the reference range is 11.4-16.4 seconds.

From p. 1225: "Prothrombin Time had a significant treatment effect in the Day 22 postdose interval only (p=0.0420). Follow-up analysis results resulted in a significant effect when comparing control to treatment group 1X in this time interval (p=0.0082).

From information on p. 216 at 24-hours postdose Prothrombin Time was significantly different (p=0.0000) from the pretreatment covariant. There was a similar finding on Day 8 (p=0.0001).

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TABLE 15. Ranges & Means ± S.D. for Prothrombin Time (sec)				
Group & sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo M	8.5-9.2; 8.72 ± 0.26	8.4-8.6; 8.47 ± 0.08	8.3-9.3; 8.80 ± 0.32	8.3-8.8; 8.50 ± 0.18
Placebo F	8.5-9.2; 8.85 ± 0.27	8.4-9.1; 8.62 ± 0.26	8.6-9.2; 8.90 ± 0.22	8.6-9.2; 8.82 ± 0.26
Placebo com.	8.5-9.2; 8.78 ± 0.26	8.4-9.1; 8.54 ± 0.20	8.3-9.3; 8.85 ± 0.27	8.3-9.2; 8.66 ± 0.27
1X males	8.5-9.5; 8.90 ± 0.38	8.2-9.0; 8.55 ± 0.30	8.2 - 11.0; 9.20 ± 0.99	7.8-8.8; 8.34 ± 0.46
1X females	8.6-9.4; 8.77± 0.31	8.3-8.9; 8.53 ± 0.23	8.6-9.4; 8.88 ± 0.29	7.4-8.7; 8.23 ± 0.47
1X combined	8.5-9.5; 8.83 ± 0.34	8.2-9.0; 8.54 ± 0.25	8.2-11.0; 9.04 ± 0.71	7.4-8.8; 8.28 ± 0.45
3X males	8.7-9.5; 9.02 ± 0.32	8.4-8.9; 8.60 ± 0.21	8.2-9.2; 8.87 ± 0.39	8.2-9.2; 8.65 ± 0.41
3X females	8.6-9.1; 8.87± 0.18	8.5-8.9; 8.70 ± 0.17	8.5-9.3; 8.90 ± 0.28	8.3-8.7; 8.54 ± 0.18
3X combined	8.6-9.5; 8.94 ± 0.26	8.4-8.9; 8.65 ± 0.19	8.2-9.3; 8.88 ± 0.32	8.2-9.2; 8.60 ± 0.32
5X males	8.3-9.1; 8.85 ± 0.29	8.5-8.8; 8.68 ± 0.10	8.6-9.3; 8.97 ± 0.27	8.6-9.1; 8.80 ± 0.18
5X females	8.7-9.2; 8.88 ± 0.22	8.2-9.0; 8.63 ± 0.27	8.3-9.2; 8.83 ± 0.42	8.0-8.8; 8.47 ± 0.27
5X combined	8.3-9.2; 8.87 ± 0.25	8.2-9.0; 8.66 ± 0.20	8.3-9.3; 8.90 ± 0.34	8.0-9.1; 8.63 ± 0.28

Individual data on p. 858, 859, 870, 871, 883, 884, 896, 897, 909, 910, 921, 922, 933, 934, 945 and 946; Ranges, Means and Standard Deviations on p. 218, 219 and 223.

According to http://www.vet.purdue.edu/vm525/fall%202002/reference_ranges.pdf#search='APTT%20dog' the reference range for Prothrombin Time in the [adult?] dog is 5.5-7.9 seconds. However, these were puppies, and this may have been a factor in the values obtained from them. Interestingly, the only values that were within this range were from the 1X group on Day 22 (significantly different from the control group at that time interval with p=0.0082).

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From p. 1226: "Sodium showed a significant overall treatment effect in the Day 22 interval (p=0.0670). The follow-up analysis for that time period did not reveal any significant comparisons to control."

TABLE 16. Ranges & Means ± S.D. for Sodium (mEq/L)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	141-145; 143.3 ± 1.6	140-145; 141.3 ± 2.2	141-145; 143.3 ± 1.6	142-144; 142.8 ± 0.8
Placebo females	143-144; 143.5 ± 0.6	139-143; 142.0 ± 1.6	142-144; 142.7 ± 0.8	141-144; 142.7 ± 1.0
Placebo both	141-145; 143.4 ± 1.2	139-145; 141.7 ± 1.8	141-145; 143.0 ± 1.3	141-144; 142.8 ± 0.9
1X males	142-144; 143.7 ± 0.82	133-143; 139.8 ± 4.3	127-146; 140.7 ± 6.9	142-145; 143.6 ± 1.1
1X females	142-145; 142.8 ± 1.2	141-144; 142.7 ± 1.2	140-146; 143.5 ± 2.4	142-144; 142.7 ± 0.8
1X both	142-145; 143.3 ± 1.1	133-144; 141.3 ± 3.3	127-146; 142.1 ± 5.1	142-145; 143.1 ± 1.0
3X males	143-146; 144.2 ± 1.2	137-145; 141.7 ± 2.9	142-146; 143.7 ± 1.4	141-143; 142.3 ± 0.8
3X females	142-145; 143.3 ± 1.0	136-146; 142.7 ± 3.5	124-146; 141.0 ± 8.4	142-143; 142.2 ± 0.5
3X both	142-146; 143.8 ± 1.1	136-146; 142.2 ± 3.1	124-146; 142.3 ± 5.9	141-143; 142.3 ± 0.7
5X males	140-145; 142.8 ± 1.7	138-145; 142.3 ± 2.4	142-145; 143.7 ± 1.0	142-145; 143.5 ± 1.4
5X females	142-145; 143.8 ± 1.2	139-144; 141.8 ± 1.7	141-145; 143.3 ± 1.4	141-144; 142.5 ± 1.1
5X both	140-145; 143.3 ± 1.5	138-145; 142.1 ± 2.0	141-145; 143.5 ± 1.2	141-145; 143.0 ± 1.3

Individual values on p. 1123, 1124, 1129. 1130, 1135, 1136, 1141, 1142, 1147, 1148, 1153, 1154, 1159, 1160, 1165 and 1166 of MRID 46401004.
Means and Standard Deviations on p. 312, 313 and 317.

From p. 1226: "Sodium showed a significant overall treatment effect in the Day 22 interval (p=0.0670). The follow-up analysis for that time period did not reveal any significant comparisons to control. However, from information on p. 310 there was a P value of 0.0022 associated with 24-hour postdose readings relative to the pretreatment covariate.

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for Na+ in the dog is 140-165 mEq/L. The range at Week -1 and Day 22 of this study (140-146) was within this reference range. On Day 8 1X male #125 (found dead on Day 9) had a value of 127 mEq/L; the other 1X males ranged from 141-146 mEq/L. On Day 8 3X female 122 (euthanized *in extremis* on Day 9) had a Na+ value of 124 mEq/L; the other 3X females ranged from 143-146 mEq/L. At 24 hours postdose in 1X males #120 had a value of 133 mEq/L and 125 had a value of 136 mEq/L; 3X male #137 had a value of 137 mEq/L and 5X male #126 had a value of 138 mEq/L. For females, placebo control #132 had a value of 139 mEq/L; at 3X #122 had a value of 136 mEq/L, while at 5X #121 had a value of 139 mEq/L. It is concluded then that there was an effect on serum sodium at 24 hours postdose, although it was not dose-related. The value of 133 mEq in 1X male #120 at 24 hours (other values for this puppy were 144 pretest, 144 on Day 8, and 142 on Day 22) is of particular concern.

From p. 1226: "Potassium showed a significant overall treatment effect in the 24 hour

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postdose interval (p=0.0580). Follow-up analysis indicated that there was significance in comparisons to control in this interval when compared to all three treatment groups (p=0.0176 for 1X, p=0.0267 for 3X and p=0.0374 for 5X)."

TABLE 17. Ranges & Means ± S.D. for Potassium (mEq/L)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	5.6 - 6.5; 6.05 ± 0.34	5.0 - 6.0; 5.47 ± 0.40	5.4 - 6.2; 5.80 ± 0.35	4.8 - 6.1; 5.50 ± 0.44
Placebo females	5.1 - 6.0; 5.55 ± 0.39	4.8 - 5.6; 5.33 ± 0.30	5.6 - 6.2; 5.83 ± 0.23	4.6 - 5.6; 5.22 ± 0.34
Placebo both	5.1 - 6.5; 5.80 ± 0.44	4.8 - 6.0; 5.40 ± 0.34	5.4 - 6.2; 5.82 ± 0.28	4.6 - 6.1; 5.36 ± 0.40
1X males	4.9 - 6.2; 5.72 ± 0.46	5.3 - 6.1; 5.72 ± 0.29	4.2 - 6.2; 5.60 ± 0.74	5.1 - 6.1; 5.50 ± 0.39
1X females	5.3 - 5.9; 5.57 ± 0.24	5.2 - 5.8; 5.55 ± 0.21	5.3 - 6.3; 5.72 ± 0.35	4.9 - 5.7; 5.35 ± 0.26
1X combined	4.9 - 6.2; 5.64 ± 0.36	**5.2 - 6.1; 5.63 ± 0.25**	4.2 - 6.3; 5.66 ± 0.56	4.9 - 6.1; 5.42 ± 0.32
3X males	5.3 - 6.2; 5.77 ± 0.31	5.1 - 6.4; 5.70 ± 0.44	5.2 - 6.0; 5.73 ± 0.29	5.2 - 5.8; 5.47 ± 0.27
3X females	5.0 - 6.5; 5.73 ± 0.57	5.3 - 6.7; 5.65 ± 0.53	4.0 - 5.7; 5.17 ± 0.61	4.8 - 5.4; 5.16 ± 0.23
3X combined	5.0 - 6.5; 5.75 ± 0.44	**5.1 - 6.7; 5.68 ± 0.47**	4.0 - 6.0; 5.45 ± 0.54	4.8 - 5.8; 5.33 ± 0.29
5X males	5.2 - 6.0; 5.68 ± 0.27	5.2 - 6.4; 5.70 ± 0.46	5.2 - 6.0; 5.62 ± 0.34	5.0 - 5.6; 5.37± 0.20
5X females	5.0 - 6.8; 5.93 ± 0.60	5.0 - 6.4; 5.68 ± 0.56	5.3 - 6.0; 5.75 ± 0.26	5.1 - 5.9; 5.37 ± 0.28
5X combined	5.0 - 6.8; 5.81 ± 0.46	**5.0 - 6.4; 5.69 ± 0.49**	5.2 - 6.0; 5.68 ± 0.30	5.0 - 5.9; 5.37 ± 0.23

Individual values on p. 1123, 1124, 1129. 1130, 1135, 1136, 1141, 1142, 1147, 1148, 1153, 1154, 1159, 1160, 1165 and 1166 of MRID 46401004.
Means and Standard Deviations on p. 320, 321 and 325.
1X, 3X and 5X pooled values were significantly different (p=0.0176, 0.0267 and 0.0374 respectively) from controls at 24 hours postdose.

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for K+ in the dog is 4.4-6.1 mEq/L. The postdose range in this study (4.0-6.7) included values below and above this reference range. However, the only values below this range were on Day 8, in 1X male 125 (4.2; found dead on Day 9) and 3X female 122 (4.0; euthanized *in extremis* on Day 9). The significance at 24 hours postdose was largely due to a more pronounced drop from the preexposure mean in controls than those occurring in the 1X, 3X and 5X dose groups. The magnitude of the drops at 24 hours from preexposure was Placebo > 5X > 3X >1X, suggesting an effect from one or more of the "inerts" in this formulation, although elevated potassium levels are known to be an effect of Amitraz.

No statistical significance between groups (or pretreatment covariate) was seen for chloride. From http://www.ahc.umn.edu/rar/RefValues.html the normal range for Cl- in the dog is 109-122 mEq/L; the only two values that were below 100 mEq/L were on Day 8, in 1X male 125 (92 mEq/L; found dead on Day 9) and 3X female 122 (87 mEq/L; euthanized *in extremis* on Day 9). At 24 hours postdose 1X male #120 had a value of 102 mEq/L; otherwise, postexposure values ranged from 106-115 mEq/L.

No statistical significance between groups (or pretreatment covariate) was seen for calcium. From http://www.ahc.umn.edu/rar/RefValues.html the normal range for Ca++

in the dog is 9.0-11.4 mg/dL. The lowest observed values were on Day 8 in 1X male 125 (7.3 mg/dL; found dead on Day 9) and 3X female 122 (6.8 mg/dL; euthanized *in extremis* on Day 9). Otherwise the range was 9.0-12.5 mg/dL.

From p. 1226: "Phosphorous resulted in a significant treatment effect in the 24 hour postdose interval (p=0.0688). When follow-up analysis was done in this interval with sexes combined, significance resulted for treatment groups 3X and 5X when compared to the control (p=0.0245 and p=0.0247, respectively)."

TABLE 18. Ranges & Means ± S.D. for Phosphorous (mg/dL)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	7.3 - 8.8; 8.00 ± 0.62	7.0 - 8.4; 7.95 ± 0.50	5.6 - 8.2; 6.83 ± 0.85	7.3 - 7.9; 7.68 ± 0.21
Placebo females	6.9 - 7.6; 7.25 ± 0.32	7.1 - 8.4; 7.72 ± 0.45	6.6 - 7.9; 7.25 ± 0.49	6.9 - 7.8; 7.55 ± 0.34
Placebo both	6.9 - 8.8; 7.63 ± 0.61	7.0 - 8.4; 7.83 ± 0.47	5.6 - 8.2; 7.04 ± 0.69	6.9 - 7.9; 7.62 ± 0.28
1X males	7.6 - 8.4; 7.93 ± 0.31	8.2 - 8.9; 8.57 ± 0.26	5.0 - 8.1; 7.25 ± 1.15	7.5 - 8.3; 8.04 ± 0.33
1X females	7.2 - 8.4; 7.88 ± 0.44	7.0 - 8.4; 7.85 ± 0.50	7.2 - 7.9; 7.53 ± 0.26	7.4 - 8.7; 7.92 ± 0.48
1X combined	7.2 - 8.4; 7.91 ± 0.36	7.0 - 8.9; 8.21 ± 0.53	5.0 - 8.1; 7.39 ± 0.81	7.4 - 8.7; 7.97 ± 0.41
3X males	7.4 - 8.5; 8.00 ± 0.36	7.8 - 8.6; 8.32 ± 0.37	6.6 - 7.7; 7.30 ± 0.42	7.0 - 8.5; 7.83 ± 0.48
3X females	6.2 - 8.3; 7.43 ± 0.68	7.6 - 9.9; 8.42 ± 1.02	5.0 - 7.8; 6.88 ± 0.97	6.9 - 7.9; 7.32 ± 0.39
3X combined	6.2 - 8.5; 7.72 ± 0.60	**7.6 - 9.9; 8.37 ± 0.73**	5.0 - 7.8; 7.09 ± 0.75	6.9 - 8.5; 7.60 ± 0.50
5X males	6.9 - 8.8; 7.82 ± 0.65	7.5 - 8.8; 8.35 ± 0.51	6.6 - 7.4; 7.08 ± 0.35	7.2 - 8.2; 7.85± 0.36
5X females	7.6 - 8.4; 8.03 ± 0.33	7.9 - 9.4; 8.62 ± 0.56	5.4 - 7.9; 7.13 ± 0.91	6.5 - 8.7; 7.68 ± 0.78
5X combined	6.9 - 8.8; 7.93 ± 0.50	**7.5 - 9.4; 8.48 ± 0.53**	5.4 - 7.9; 7.11 ± 0.66	6.5 - 8.7; 7.77 ± 0.59

Individual values on p. 1123, 1124, 1129. 1130, 1135, 1136, 1141, 1142, 1147, 1148, 1153, 1154, 1159, 1160, 1165 and 1166 of MRID 46401004.
Means and Standard Deviations on p. 342, 343 and 347.
3X and 5X pooled values were significantly different (p=0.0245 and 0.0247 respectively) from controls at 24 hours postdose.

While the mean 1X combined at 24 hours was not significantly (p=0.2816) different relative to the placebo control value, it was still noticeably elevated, being above 8.0 mg/dL. According to http://www.ahc.umn.edu/rar/RefValues.html the normal range for phosphorous in the dog is 2.5-7.7 mg/dL, but as these were puppies with active bone growth it would be anticipated they would have somewhat greater values.

From information on p. 348 alkaline phosphatase was significantly different from the pretreatment covariant at 24 hours postdose (p=0.0000), on Day 8 (p=0.0103) and on Day 22 (p=0.0000).

TABLE 19. Ranges & Means ± S.D. for Alkaline Phosphatase (U/dL)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	78-149; 123.5 ± 31.0	68-128; 104.3 ± 26.2	72-290; 129.2 ± 81.5	88-192; 136.3 ± 41.3
Placebo females	107-204; 147.2 ± 35.3	80-169; 120.8 ± 33.4	79-189; 135.5 ± 47.8	110-240; 176.5 ± 49.8
Placebo both	78-204; 135.3 ± 34.0	68-169; 112.6 ± 29.9	72-290; 132.3 ± 63.8	88-240; 156.4 ± 48.4
1X males	132-188; 153.8 ± 21.7	79-164; 124.8 ± 27.8	53-150; 121.7 ± 35.9	121-234; 162.0 ± 44.7
1X females	118-150; 135.3 ± 14.2	90-144; 119.7 ± 21.9	95-150; 115.5 ± 22.6	122-166; 142.7 ± 20.1
1X combined	118-188; 144.6 ± 20.0	79-164; 122.3 ± 24.0	53-150; 118.6 ± 28.8	121-234; 151.5 ± 33.2
3X males	115-184; 135.0 ± 26.8	82-132; 105.8 ± 20.9	98-140; 117.2 ± 15.1	97-160; 132.7 ± 28.4
3X females	77-139; 109.5 ± 23.5	69-145; 108.3 ± 26.5	69-122; 106.8 ± 20.1	77-132; 115.2 ± 22.6
3X combined	77-184; 122.3 ± 27.5	69-145; 107.1 ± 22.8	69-140; 112.0 ± 17.8	77-160; 124.7 ± 26.3
5X males	104-152; 128.3 ± 24.9	93-140; 113.5 ± 21.8	101-138; 116.7 ± 14.6	88-206; 137.5 ± 40.2
5X females	137-178; 161.0 ± 13.7	122-148; 132.3 ± 11.1	81-162; 125.2 ± 26.9	111-177; 141.2 ± 30.6
5X combined	104-178; 144.7 ± 25.6	93-148; 122.9 ± 19.2	81-162; 120.9 ± 21.1	88-206; 139.3 ± 34.1

Individual values on p. 1123, 1124, 1129. 1130, 1135, 1136, 1141, 1142, 1147, 1148, 1153, 1154, 1159, 1160, 1165 and 1166 of MRID 46401004.; Means and Standard Deviations on p. 349, 350 and 354.
Alkaline phosphatase was significantly different from the pretreatment covariant at 24 hours postdose (p=0.0000), on Day 8 (p=0.0103) and on Day 22 (p=0.0000).

From information on p. 355 total bilirubin was significantly different from the pretreatment covariant at 24 hours postdose (p=0.0007) and Day 22 (p=0.0269), but not on Day 8 (p=0.3499). Individual values observed in this study were between 0.1 and 0.4 mg/dL, and group means (by group and sex) ranged from 0.10 to 0.20 mg/dL; from http://www.ahc.umn.edu/rar/RefValues.html the normal range in the dog is from 0.2 to 0.8 mg/dL, so it is concluded that this variation was not biologically significant.

From p. 1226: "GGT resulted in a significant treatment by sex interaction on Day 8 (p=0.0252). A follow-up analysis was done by sex for this interval. The follow-up analysis resulted in a significant result for males only, when comparing control to treatment group 1X and treatment group 3X (p=0.0098 and p=0.0010, respectively)."

TABLE 20. Ranges & Means ± S.D. for GGT (U/L)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	2 - 8; 3.7 ± 2.2	2 - 4; 2.8 ± 0.8	3 - 10; 5.0 ± 2.7	2 - 11; 3.8 ± 3.5
Placebo females	2 - 4; 2.7 ± 0.8	1 - 3; 2.3 ± 0.8	2 - 4; 2.5 ± 0.8	1 - 3; 2.2 ± 0.8
Placebo both	2 - 8; 3.2 ± 1.6	1 - 4; 2.6 ± 0.8	2 - 10; 3.8 ± 2.3	1 - 11; 3.0 ± 2.6
1X males	2 - 5; 2.7 ± 1.2	2 - 3; 2.3 ± 0.5	2 - 4; 2.7 ± 1.0	2 - 4; 2.8 ± 1.1
1X females	2 - 3; 2.7 ± 0.5	1 - 3; 2.3 ± 0.8	1 - 3; 2.3 ± 0.8	1 - 4; 2.7 ± 1.0
1X combined	2 - 5; 2.7 ± 0.9	1 - 3; 2.3 ± 0.7	1 - 4; 2.5 ± 0.9	1 - 4; 2.7 ± 1.0
3X males	2 - 4; 3.0 ± 0.9	2 - 3; 2.5 ± 0.6	2 - 4; 2.5 ± 0.8	2 - 4; 2.5 ± 0.8
3X females	2 - 4; 3.0 ± 0.9	2 - 4; 2.8 ± 0.8	2 - 4; 3.2 ± 0.8	2 - 4; 3.2 ± 0.8
3X combined	2 - 4; 3.0 ± 0.9	2 - 4; 2.7 ± 0.7	2 - 4; 2.8 ± 0.8	2 - 4; 2.8 ± 0.9
5X males	2 - 3; 2.7 ± 0.5	2 - 3; 2.3 ± 0.5	2 - 6; 3.3 ± 1.5	2 - 4; 2.8 ± 0.8
5X females	2 - 4; 2.8 ± 1.0	2 - 2; 2.0 ± 0.0	2 - 3; 2.3 ± 0.5	2 - 3; 2.2 ± 0.4
5X combined	2 - 4; 2.8 ± 0.8	2 - 3; 2.2 ± 0.4	2 - 6; 2.8 ± 1.2	2 - 4; 2.5 ± 0.7

Individual values on p. 1123, 1124, 1129. 1130, 1135, 1136, 1141, 1142, 1147, 1148, 1153, 1154, 1159, 1160, 1165 and 1166 of MRID 46401004.
Means and Standard Deviations on p. 364, 365 and 369.

From information on p. 370 the overall 24-hour postdose values for AST were significantly different from (and lower than) the pretreatment covariate (p=0.0113). The P value associated with treatment at 24 hours was 0.1165.

TABLE 21. Ranges & Means ± S.D. for AST (U/L)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	25 - 35; 29.2 ± 3.5	18 - 30; 21.8 ± 5.3	27 - 163; 54.5 ± 53.5	27 - 68; 42.3 ± 14.5
Placebo females	22 - 27; 24.0 ± 1.7	18 - 35; 25.0 ± 7.5	20 - 44; 32.5 ± 8.5	30 - 56; 41.2 ± 9.1
Placebo both	22 - 35; 26.6 ± 3.8	18 - 35; 23.4 ± 6.4	20 - 163; 43.5 ± 38.3	27 - 68; 41.8 ± 11.6
1X males	22 - 45; 30.2 ± 7.8	15 - 31; 22.0 ± 6.3	24 - 82; 40.7 ± 22.5	30 - 42; 38.2 ± 4.8
1X females	24 - 31; 26.2 ± 2.5	13 - 21; 19.0 ± 3.2	20 - 34; 27.3 ± 4.7	32 - 41; 34.5 ± 3.6
1X combined	22 - 45; 28.2 ± 5.9	13 - 31; 20.5 ± 5.0	20 - 82; 34.0 ± 17.0	30 - 42; 36.2 ± 4.4
3X males	18 - 33; 27.7 ± 5.3	16 - 30; 19.8 ± 5.3	24 - 47; 33.5 ± 8.8	32 - 43; 37.2 ± 4.4
3X females	20 - 46; 30.8 ± 9.7	20 - 30; 22.3 ± 4.1	25 - 98; 41.2 ± 28.2	30 - 45; 35.2 ± 6.1
3X combined	18 - 46; 29.3 ± 7.6	16 - 30; 21.1 ± 4.7	24 - 98; 37.3 ± 20.3	30 - 45; 36.3 ± 5.0
5X males	23 - 32; 26.2 ± 3.4	17 - 25; 20.3 ± 2.7	18 - 35; 27.8 ± 5.6	31 - 39; 34.2 ± 2.9
5X females	24 - 31; 28.8 ± 2.5	10 - 22; 18.0 ± 4.6	24 - 41; 32.0 ± 5.6	21 - 45; 34.5 ± 8.5
5X combined	23 - 32; 27.5 ± 3.2	10 - 25; 19.2 ± 3.8	18 - 41; 29.9 ± 5.8	21 - 45; 34.3 ± 6.0

Individual values on p. 1125, 1126, 1131, 1132, 1137, 1138, 1143, 1144, 1149, 1150, 1155, 1156, 1161, 1162, 1167 and 1168 of MRID 46401004.
Means and Standard Deviations on p. 371, 372 and 376.

From p. 1226: "Amylase resulted in a significant treatment by sex interaction on Day 22 (p=0.0042). A follow-up analysis was done by sex for this interval. The follow-up analysis resulted in a significant result for females only, when comparing control to treatment group 3X (p=0.0141)."

However, from information on p. 384 the overall 24-hour postdose values were significantly different from (and lower than) the pretreatment covariate (p=0.0000). On Days 8 and 22 values were significantly different from (and higher than) the pretreatment covariate, with p=0.0000 and 0.0000, respectively.

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TABLE 22. Ranges & Means ± S.D. for Amylase (U/L)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	267-684; 419.8±146.0	245-591; 376.5±124.2	286-695; 482.5±150.3	370-762; 523.0±153.2
Placebo females	292-559; 374.2±99.5	282-524; 372.0±87.9	340-570; 416.0±83.1	360-651; 458.0±107.9
Placebo both	267-684; 397.0±121.5	245-591; 374.3±102.6	286-695; 449.3±120.9	360-762; 490.5±130.8
1X males	418-619; 500.0±78.5	299-557; 400.3±111.8	353-859; 564.8±176.2	481-733; 612.0±99.1
1X females	303-656; 445.2±132.7	284-793; 444.0±185.7	237-775; 481.7±203.8	379-590; 480.7±76.6
1X combined	303-656; 472.6±107.8	284-793; 422.2±147.9	237-859; 523.3±186.8	379-733; 540.4±107.5
3X males	307-452; 351.2±53.3	299-397; 345.7±31.4	321-625; 411.2±112.3	357-492; 408.0±52.7
3X females	345-635; 459.7±106.4	331-577; 412.8±97.9	378-782; 534.2±176.1	412-800; 639.2±174.6
3X combined	307-635; 405.4± 98.2	299-577; 379.3±77.7	321-782; 472.7±154.8	357-800; 513.1±167.8
5X males	290-766; 456.8±179.7	283-627; 400.8±127.1	288-633; 456.3±137.1	346-842; 516.8±182.4
5X females	267-606; 379.8±125.1	201-567; 358.5±124.8	358-587; 449.8±84.9	369-684; 495.7±116.1
5X combined	267-766; 418.3±153.0	201-627; 379.7±122.1	288-633; 453.1±108.7	346-842; 506.3±146.2

Individual values on p. 1125, 1126, 1131, 1132, 1137, 1138, 1143, 1144, 1149, 1150, 1155, 1156, 1161, 1162, 1167 and 1168 of MRID 46401004.
Means and Standard Deviations on p. 386, 387 and 391.

According to http://www.ahc.umn.edu/rar/RefValues.html the normal range for amylase activity in the dog is 220-1070 U/L. The only value lying outside (below) this range was in 5X female #107 at 24 hours postdose.

From p. 1226: "Urea Nitrogen resulted in a significant treatment and treatment by sex effects at 24 hour postdose interval ($p<0.0001$ and $p=0.0437$, respectively). Follow-up analysis was done for each sex in this period. The resulting follow-up analysis indicated significance in most treatment groups when comparing to control. Specifically, for the male data, difference from control in all treatment groups ($p=0.0027$, $p=0.0118$, and $p=0.0070$, respectively for treatment groups 1X, 3X and 5X). For the female data, difference from control in the 3X and 5X groups only ($p<0.0001$ and $p=0.0001$, respectively)."

TABLE 23. Ranges & Means ± S.D. for Urea Nitrogen (mg/dL)				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	4 - 7; 6.0 ± 1.3	4 - 10; 6.8 ± 2.3	5 - 14; 9.0 ± 3.1	5 - 10; 8.0 ± 2.0
Placebo females	6 - 11; 8.3 ± 1.8	6 - 9; 7.0 ± 1.3	6 - 10; 8.2 ± 1.5	7 - 12; 9.0 ± 2.0
Placebo both	4 - 11; 7.2 ± 1.9	4 - 10; 6.9 ± 1.8	5 - 14; 8.6 ± 2.4	5 - 12; 8.5 ± 2.0
1X males	5 - 9; 7.0 ± 1.7	**10 - 19; 13.5 ± 3.5**	6 - 10; 7.7 ± 1.6	6 - 9; 7.6 ± 1.1
1X females	5 - 11; 8.0 ± 2.2	7 - 14; 10.0 ± 2.9	5 - 17; 9.2 ± 4.2	8 - 10; 8.7 ± 1.0
1X combined	5 - 11; 7.5 ± 1.9	7 - 19; 11.8 ± 3.6	5 - 17; 8.4 ± 3.2	6 - 10; 8.2 ± 1.2
3X males	4 - 11; 6.7 ± 2.6	**7 - 15; 12.3 ± 2.9**	6 - 9; 8.2 ± 1.2	7 - 18; 10.0 ± 4.1
3X females	5 - 10; 7.5 ± 1.8	**11 - 24; 17.0 ± 5.6**	5 - 33; 11.5 ± 10.8	6 - 10; 7.8 ± 1.6
3X combined	4 - 11; 7.1 ± 2.2	7 - 24; 14.7 ± 4.9	5 - 33; 9.8 ± 7.5	6 - 18; 9.0 ± 3.3
5X males	5 - 8; 6.2 ± 1.3	**7 - 19; 12.8 ± 4.8**	6 - 10; 7.5 ± 1.5	6 - 12; 8.3 ± 2.1
5X females	4 - 11; 7.3 ± 2.8	**10 - 21; 16.3 ± 4.0**	6 - 11; 8.3 ± 1.9	7 - 13; 9.2 ± 2.1
5X combined	4 - 11; 6.8 ± 2.2	7 - 21; 14.6 ± 4.6	6 - 11; 7.9 ± 1.7	6 - 13; 8.8 ± 2.1

Individual values on p. 1125, 1126, 1131, 1132, 1137, 1138, 1143, 1144, 1149, 1150, 1155, 1156, 1161, 1162, 1167 and 1168 of MRID 46401004.
Means and Standard Deviations on p. 401, 402 and 406.
From information on p. 400 at 24 hours postdose the following were the p values (compared to their respective placebo controls) for: 1X males: 0.0027; 3X males: 0.0118; 5X males: 0.0070; 1X females: 0.1734; 3X females: 0.0000; 5X females: 0.0001.

On Day 8 3X female #122 (euthanized on Day 9) had a value of 33 mg/dL; the other 5 females in this group ranged from 5-11 mg/dL for this parameter (mean: 7.2, S.D. 2.7).

The finding of elevated BUN in all dose groups at 24 hours postdose was also observed in the adult study (MRID 46401003). From http://www.ahc.umn.edu/rar/RefValues.html the normal range in the dog is 7-24 mg/dL.

For total protein at 24 hours postdose values were significantly different (and lower than) pretreatment covariates with p=0.0034. On Day 8 values were nearly significantly different (and lower than) pretreatment covariates with p=0.0548.

TABLE 24. Ranges & Means ± S.D. for Total Protein (g/dL)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	3.9 - 4.7; 4.40 ± 0.32	4.0 - 4.7; 4.33 ± 0.23	3.0 - 4.7; 4.33 ± 0.66	4.2 - 5.2; 4.80 ± 0.34
Placebo females	4.0 - 4.7; 4.40 ± 0.24	3.7 - 5.0; 4.45 ± 0.47	4.3 - 4.7; 4.53 ± 0.15	4.4 - 4.9; 4.72 ± 0.21
Placebo both	3.9 - 4.7; 4.40 ± 0.27	3.7 - 5.0; 4.39 ± 0.36	3.0 - 4.7; 4.43 ± 0.47	4.2 - 5.2; 4.76 ± 0.28
1X males	4.2 - 4.8; 4.53 ± 0.22	4.3 - 4.7; 4.48 ± 0.15	3.0 - 4.8; 4.33 ± 0.67	4.5 - 5.0; 4.74 ± 0.25
1X females	4.1 - 4.8; 4.62 ± 0.27	4.2 - 4.9; 4.48 ± 0.26	3.8 - 4.9; 4.45 ± 0.46	4.6 - 5.1; 4.80 ± 0.21
1X combined	4.1 - 4.8; 4.58 ± 0.24	4.2 - 4.9; 4.48 ± 0.20	3.0 - 4.9; 4.39 ± 0.55	4.5 - 5.1; 4.77 ± 0.22
3X males	4.4 - 4.9; 4.68 ± 0.20	4.3 - 5.0; 4.60 ± 0.24	4.1 - 4.9; 4.50 ± 0.27	4.2 - 5.1; 4.77 ± 0.31
3X females	3.9 - 4.7; 4.42 ± 0.33	3.8 - 4.8; 4.43 ± 0.37	3.0 - 5.0; 4.22 ± 0.73	4.8 - 5.1; 4.96 ± 0.11
3X combined	3.9 - 4.9; 4.55 ± 0.30	3.8 - 5.0; 4.52 ± 0.31	3.0 - 5.0; 4.36 ± 0.55	4.2 - 5.1; 4.85 ± 0.25
5X males	4.6 - 4.9; 4.72 ± 0.12	4.3 - 4.9; 4.53 ± 0.23	4.1 - 4.8; 4.53 ± 0.24	4.5 - 5.4; 4.92 ± 0.34
5X females	4.3 - 4.8; 4.52 ± 0.17	3.6 - 4.9; 4.35 ± 0.44	3.7 - 5.0; 4.45 ± 0.54	4.0 - 5.4; 4.70 ± 0.45
5X combined	4.3 - 4.9; 4.62 ± 0.18	3.6 - 4.9; 4.44 ± 0.35	3.7 - 5.0; 4.49 ± 0.40	4.0 - 5.4; 4.81 ± 0.40

Individual values on p. 1127, 1128, 1133, 1134, 1139, 1140, 1145, 1146, 1151, 1152, 1157, 1158, 1163, 1164, 1169 and 1170 of MRID 46401004.
Means and Standard Deviations on p. 415, 416 and 420.

The lowest values observed were on Day 8 in control male #104 (3.0 g/dL; other males in this group ranged from 4.4 - 4.7 g/dL, with a mean of 4.6 and a S.D. of 0.12); 1X male 125 (found dead on day 9; reported on p. 1151 as <3 g/dL; other males in this group ranged from 4.4 - 4.8 g/dL, with a mean of 4.6 and a S.D. of 0.16); and 3X female 122 (sacrificed *in extremis* day 9; other females in this group ranged from 3.7 to 5.0, with a mean of 4.46 g/dL and a S.D. of 0.48).

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for total protein in the dog is 5.4 - 8.0 g/dL. These values may have been somewhat lower than adults.

For albumin, at 24 hours and 8 days postdose values were significantly different (and lower than) their pretreatment covariates (from p. 421 p = 0.0007 and 0.0163, respectively).

TABLE 25. Ranges & Means ± S.D. for Albumin (g/dL)

Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	2.1 - 2.6; 2.40 ± 0.21	1.9 - 2.6; 2.15 ± 0.27	1.5 - 2.4; 2.12 ± 0.32	1.8 - 2.7; 2.28 ± 0.33
Placebo females	2.0 - 2.6; 2.42 ± 0.26	1.8 - 2.5; 2.13 ± 0.26	1.9 - 2.5; 2.25 ± 0.24	2.0 - 2.6; 2.33 ± 0.23
Placebo both	2.0 - 2.6; 2.41 ± 0.22	1.8 - 2.6; 2.14 ± 0.25	1.5 - 2.5; 2.18 ± 0.28	1.8 - 2.7; 2.31 ± 0.28
1X males	2.1 - 2.8; 2.55 ± 0.27	2.0 - 2.5; 2.27 ± 0.23	1.5 - 2.7; 2.30 ± 0.45	2.1 - 2.5; 2.36 ± 0.15
1X females	2.2 - 2.7; 2.57 ± 0.20	2.0 - 2.7; 2.25 ± 0.24	1.9 - .8; 2.27 ± 0.32	2.0 - 2.7; 2.33 ± 0.25
1X combined	2.1 - 2.8; 2.56 ± 0.23	2.0 - 2.7; 2.26 ± 0.23	1.5 - 2.8; 2.28 ± 0.37	2.0 - 2.7; 2.35 ± 0.20
3X males	2.4 - 2.7; 2.52 ± 0.13	2.1 - 2.4; 2.22 ± 0.12	2.0 - 2.6; 2.25 ± 0.22	1.7 - 2.9; 2.38 ± 0.39
3X females	2.1 - 2.7; 2.38 ± 0.21	1.5 - 2.5; 2.17 ± 0.36	1.5 - 2.6; 2.12 ± 0.38	2.3 - 2.7; 2.48 ± 0.15
3X combined	2.1 - 2.7; 2.45 ± 0.18	1.5 - 2.5; 2.19 ± 0.25	1.5 - 2.6; 2.18 ± 0.30	1.7 - 2.9; 2.43 ± 0.30
5X males	2.5 - 2.7; 2.62 ± 0.08	2.2 - 2.4; 2.27 ± 0.08	1.9 - 2.5; 2.30 ± 0.21	2.0 - 2.8; 2.45 ± 0.31
5X females	2.2 - 2.8; 2.58 ± 0.24	1.7 - 2.6; 2.18 ± 0.33	1.8 - 2.6; 2.13 ± 0.34	1.8 - 2.5; 2.25 ± 0.30
5X combined	2.2 - 2.8; 2.60 ± 0.17	1.7 - 2.6; 2.23 ± 0.23	1.8 - 2.6; 2.22 ± 0.28	1.8 - 2.8; 2.35 ± 0.31

Individual values on p. 1127, 1128, 1133, 1134, 1139, 1140, 1145, 1146, 1151, 1152, 1157, 1158, 1163, 1164, 1169 and 1170 of MRID 46401004.
Means and Standard Deviations on p. 422, 423 and 427.

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for albumin in the dog is 2.6-4.0 g/dL. Most of the values (including pretreatment) were below this range. The lower values at 24 hours postdose and on day 8 relative to week -1 including controls and showed no indication of a dose relationship.

From information on p. 450 of MRID 46401004 glucose measurements on Day 8 were significantly different from the pretreatment covariate at p=0.0184.

TABLE 26. Ranges & Means ± S.D. for Glucose (mg/dL)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	104-123; 114.5 ± 8.5	95-110; 102.7 ± 4.9	93-126; 108.2 ± 11.6	89-118; 103.0 ± 11.9
Placebo females	107-124; 116.2 ± 7.4	82 - 124; 100.8 ± 13.6	81-111; 97.8 ± 12.5	61-138; 106.2 ± 26.0
Placebo both	104-124; 115.3 ± 7.6	82-124; 101.8 ± 9.8	81-126; 103.0 ± 12.7	61 - 138; 104.6 ± 19.4
1X males	108-129; 114.5 ± 8.0	64-117; 98.2 ± 19.6	10-119; 89.5 ± 40.2	103-120; 112.0 ± 6.0
1X females	99-152; 116.7± 19.3	98-124; 110.7 ± 10.5	20-110; 82.8 ± 32.3	80-120; 100.2 ± 16.4
1X combined	99-152; 115.6 ± 14.1	64-124; 104.4 ± 16.3	10-119; 86.2 ± 34.91	80-120; 105.5 ± 13.7
3X males	65-121; 104.5 ± 19.9	89-127; 104.7 ± 15.9	82-113; 102.0 ± 12.0	79-118; 106.2 ± 14.2
3X females	99-134; 117.0 ± 12.1	65-123; 90.0 ± 18.9	23-111; 93.2 ± 34.7	103-113; 108.2 ± 4.6
3X combined	65-134; 110.8 ± 17.0	65-127; 97.3 ± 18.3	23-113; 97.6 ± 25.2	79-118; 107.1 ± 10.5
5X males	112-156; 123.2 ± 17.2	48-133; 107.0 ± 30.3	96-119; 105.5 ± 10.3	79-122; 106.3 ± 14.4
5X females	103-125; 112.5 ± 8.3	59-133; 101.5 ± 32.3	84-122; 105.7 ± 14.5	92-115; 102.8 ± 8.1
5X combined	103-156; 117.8 ± 14.0	48-133; 104.3 ± 30.0	84-122; 105.6 ± 12.0	79-122; 104.6 ± 11.3

Individual values on p. 1127, 1128, 1133, 1134, 1139, 1140, 1145, 1146, 1151, 1152, 1157, 1158, 1163, 1164, 1169 and 1170 of MRID 46401004.
Means and Standard Deviations on p. 451, 452 and 456.

According to http://www.ahc.umn.edu/rar/RefValues.html the normal range for glucose in the dog is 80-120 g/dL. On Day 8 1X male 125 (found dead day 9) had a value of <10 mg/dL, while 3X female 122 (sacrificed in *extremis* day 9) had a value of 23 mg/dL. However 1X female 116 had a value of 20 mg/dL. A number of puppies had values clearly below 80 mg/dL at 24 hours postdose, including 1X male #120 (64 mg/dL), 3X female #148 (65 mg/dL), 5X male #117 (48 mg/dL), 5X females #107 (59 mg/dL) and #129 (64 mg/dL). These values suggest a hypoglycemic effect in puppies, in contrast to the hyperglycemic effect observed in adult dogs (MRID 46401003). However, examining the incidences of glucose values >120 mg/dL, a somewhat different picture emerges:

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TABLE 27. Incidences of Glucose >120 mg/dL				
Group and sex	**Week -1**	**24 hrs postdose**	**Day 8**	**Day 22**
Placebo males	3/6	0/6	1/6	0/6
Placebo females	2/6	1/6	0/6	1/6
1X males	1/6	0/6	0/6	0/5
1X females	1/6	2/6	0/6	0/6
3X males	1/6	2/6	0/6	0/6
3X females	3/6	1/6	0/6	0/5
5X males	2/6	2/6	0/6	1/6
5X females	1/6	3/6	1/6	0/6

Table taken from individual values on p. 1127, 1128, 1133, 1134, 1139, 1140, 1145, 1146, 1151, 1152, 1157, 1158, 1163, 1164, 1169 and 1170 of MRID 46401004.

At 24 hours postdose incidences of glucose >120 mg/dL showed a clear dose response (Placebo group: 1/12; 1X group: 2/12; 3X: 3/12; 5X: 5/12), and the results are striking in comparison with Day 8 and Day 22 incidences (no more than 1/12 per group). The relatively high glucose levels at Week -1 suggest the possibility that at least some of the puppies may not have have been adequately fasted at that time before blood collection.

A dose-related hyperglycemic effect in puppies at 24 hours postdose would be consistent with what was observed in adult dogs (MRID 46401003).

From information on p. 457 of MRID 46401004 lipase measurements at 24 hours postdose, on Day 8 and on Day 22 were significantly different from the pretreatment covariate at p=0.0000, 0.0000, and 0.0000, respectively.

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TABLE 28. Ranges & Means ± S.D. for Lipase (U/L)				
Group and sex	Week -1	24 hrs postdose	Day 8	Day 22
Placebo males	74-228; 155.5 ± 56.3	71-166; 114.0 ± 33.3	63-275; 135.3 ± 74.4	53-210; 139.3 ± 61.4
Placebo females	99-203; 151.5 ± 39.1	74-166; 123.7 ± 34.1	107-256; 176.0 ± 53.9	86-285; 181.8 ± 67.3
Placebo both	74-228; 153.5 ± 46.3	71-166; 118.8 ± 32.5	63-275; 155.7 ± 65.5	53-285; 160.6 ± 65.3
1X males	83-234; 159.2 ± 51.5	35-238; 131.5 ± 68.5	117-325; 215.2 ± 69.9	49-166; 124.2 ± 46.0
1X females	62-257; 149.8± 86.1	46-383; 151.8 ± 130.0	42-309; 155.7 ± 110.7	52-292; 138.0 ± 112.0
1X combined	62-257; 154.5 ± 67.8	35-383; 141.7 ± 99.7	42-325; 185.4 ± 93.6	49-292; 131.7 ± 84.7
3X males	34-486; 220.5 ± 182.8	33-457; 151.8 ± 155.9	39-433; 219.3 ± 168.9	18-239; 163.2 ± 91.3
3X females	138-739; 267.2±232.4	93-314; 159.8 ± 80.2	144-326; 200.5 ± 68.3	135-555; 277.8±167.4
3X combined	34-739; 243.8 ± 200.8	33-457; 155.8 ± 118.3	39-433; 209.9 ± 123.3	18-555; 215.3 ± 137.7
5X males	112-279; 182.5 ± 57.5	99-209; 147.2 ± 43.4	74-209; 157.7 ± 53.3	106-322; 183.3 ± 89.2
5X females	127-249; 203.5 ± 44.9	114-269; 165.0 ± 57.2	127-285; 211.2 ± 57.5	135-321; 204.0 ± 75.3
5X combined	112-279; 193.0 ± 50.4	99-269; 156.1 ± 49.3	74-285; 184.4 ± 59.8	106-322; 193.7 ± 79.5

Individual values on p. 1127, 1128, 1133, 1134, 1139, 1140, 1145, 1146, 1151, 1152, 1157, 1158, 1163, 1164, 1169 and 1170 of MRID 46401004.
Means and Standard Deviations on p. 458, 459 and 463.

The high mean in pretreatment 3X females was due largely to the value from a single female (#114; 739 U/L); without this value the range was from 138-202 U/L with a mean of 172.8 and a S.D. of 26.7. Except for 1X females all groups showed a drop in Lipase activity at 24 hours postdose from predose means. The greatest drop was in the 3X group (combined sexes: -36.1%), followed by controls (combined sexes: -22.6%), the 5X group (combined sexes: -19.12%) and the 1X group (-8.27%). However, most values were within the normal range (117-578 U/L) for the dog; an elevated lipase activity in conjunction with elevated amylase would suggest pancreatitis, but there is no indication of this.

H. NECROPSY FINDINGS

The two puppies dying in the course of this study were #125 (1X male) and #122 (3X female). The report includes (p. 1172) bone marrow values obtained from #122. The pathologist's interpretation (p. 1177) states: "There were no significant macroscopic observations. Pale livers, which corresponded microscopically to hepatocellular vacuolation, were possibly due to lipid accumulation, due to mobilization of the fat stores in the body in the face of anorexia." In addition: "Lymphoid depletion of Peyer's Patches (animal number 122) and lymphoid necrosis of the spleen, bone marrow depletion, and thymic atrophy (in both animals) may have been due to stress and/or anorexia. The cause of death for the two animals that died on study was not determined since the microscopic lesions observed were most likely secondary to stress and/or anorexia, and not primary lesions of toxicity."

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From information on p. 546 puppy #125 (1X male) had a weight loss in the period from day -4 (2.01 kg) to day -1 (1.82 kg); from p. 550 puppy #122 showed essentially no weight gain between day -8 (1.82 kg) and day -4 (1.81 kg), lost weight between day -1 (1.99 kg) and day 2 (1.79 kg). From p. 580 food consumption for male #125 in week -1 was only 150.8 g/day (the lowest of all males, which otherwise ranged from 178.8 to 425 g/day in this period); during week 1 food consumption for this male was 141.3 g/day (again the lowest for all males, which otherwise ranged from 151.3 to 628.2 g/day). From p. 582 food consumption for 3X female #122 was 318 g/day during week -1, and then dropped to 142.5 g/day during week 1.

IV. DISCUSSION

In a companion animal safety study (MRID 46401004), 4 groups, each containing 12 (6/sex) 53 to 58-day (approximately 8-week) old beagle puppies (source: Covance Research Products; males: 1.82-2.97 kg; females: 1.72-2.89 kg) were treated at 0X (3.0 mL of the formulation without active ingredients, so that each of these puppies received 6.74X the dose of "inert" ingredients that 1X puppies received, and approximately 1.35X the dose of "inert" ingredients that 5X puppies received); 1X: (0.6 mL of the proposed formulation); 3X (1.8 mL of the proposed formulation); and 5X (3.0 mL of the proposed formulation). Each puppy was treated in a single application using a syringe along the dorsal midline starting between the shoulder blades and then moving caudally.

According to proposed label directions the product would be applied as a spot-on with the following dosage rates: dogs $\leq$ 5 kg: 0.67 mL; 5-10 kg: 1.33 mL; 10-25 kg: 3.33 mL; 25-40 kg: 5.33 mL; and 40-50 kg: 6.66 mL. The 1X dose rate in this study is slightly less than the 0.67 mL specified for dogs [puppies?] less than 5 kg.

All animals were observed at least twice daily. On the day of dosing (Day 1; note: there was no Day 0) clinical observations were made predose, immediately postdose and at 1, 2 and 3 hours postdose. Otherwise, clinical observations were conducted twice daily, approximately 4 hours apart, during both the acclimation period and then from Days 2 through 28, except on days of neurological and physical examinations (Days -1, 2, 8 and 22).

Individual body weights were measured on Days -7, -1, and then at Weeks 1, 2, 3 and 4 (Days 7, 14, 21 and 28?) postdose. Individual food consumption was measured on a daily basis from Day -4; mean values (by group and sex) are reported for the 4-day predose period and then on a post-dose weekly basis. Blood samples were taken once pretest (Week -1) and then on Days 2 (approximately 24 hours postdose), 8 and 22 following overnight fasts.

One 1X male (#125) was found dead on Day 9 and a 3X female (#122) was euthanized *in extremis* on Day 9. No definitive cause of death could be determined for either. Male #125 had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, and had continued to lose weight in the postexposure period (Day 2: 1.79 kg; Day 4: 1.78 kg; Day 7: 1.67 kg), consistent with poor food consumption (150.8 g/day preexposure; 141.3 g/day during week 1). However, the possibility that Amitraz may have contributed to this puppy's death cannot be ruled out.

Female 122 was the only female puppy in the 3X group showing a weight loss (0.01 kg) in the period from Day -8 to -4, but then gained 0.18 kg from day -4 to day -1 (see p. 550). Although most (controls: 5/6; 1X: 3/6; 3X: 5/6; 5X: 3/6) female puppies lost weight in the interval from day -1 to 2, #122 had the greatest individual weight loss (0.2 kg; (#115 in the controls had the second highest weight loss, 0.15 kg; otherwise, the range was from 0.01 to 0.09 kg). The appetite of #122 had been good during week -1 (actually, food consumption was calculated for only 4 days) at 318 g/day, but then fell to 142.5 g/day for week 1). The report states (p. 11) that: "Macroscopic and microscopic evaluations, organ weights, and bone marrow evaluations did not implicate the test substance or indicate a definitive cause of death, and the deaths were not considered related to treatment." While 1X male #125 clearly had preexposure problems (as indicated by a considerable weight loss on days -4 to -1), the situation with 3X female #122 is not so obvious. This puppy weighed 1.99 kg on Day -1 and was dosed with 1.8 mL of formulation (containing 276 mg Amitraz, it was dosed with 138.7 mg Amitraz/kg) on Day 1 (there was no day zero).

Use directions for the FDA-approved Amitraz-containing formulation [MITABAN] specify application of a dilution (2 gallons of water containing 250 ppm or 1.9 g of Amitraz). The dose for a 10 kg dog is 190 mg Amitraz/kg [incidentally, in http://www.medi-vet.com/**Mitaban**.aspx it is stated that: "The safety of MITABAN has not been established for dogs less than four months of age."]. While no fatalities occurred in the 5X puppies in this study, 3X female #122 may have been representative of a more sensitive population subgroup. Dermally, #122 was dosed with 1.8 mL of Promeris, containing 276 mg Amitraz. As this puppy weighed 1.99 kg at application, dosage was 139 mg Amitraz/kg. This dosage, although dermal, is over 100 mg/kg, shown to be potentially lethal in the dog by the oral route. According to http://myweb.cableone.net/bdturner/Mitaban.pdf#search='mitaban%20amitraz' : "Death occurred in one of two dogs given a single oral dose of 100 mg/kg. Clinical signs included CNS depression, ataxia, hypothermia, bradycardia, muscular weakness, vomition, uncontrolled vocal spasm and micturition. Clinical laboratory data indicated a hemoconcentration, and transient elevations in blood glucose, blood urea nitrogen, serum potassium and alkaline phosphatase values." While puppy #122 had a normal serum glucose value (86 mg/dL) at 24 hours, the BUN was elevated (22 mg/dL at 24 hours, and 33 mg/dL on Day 8). The serum potassium level at 24 hours (6.7 mEq/L) was the highest postexposure value (another female had a preexposure value of 6.8 mEq/L); the serum potassium on Day 8 (4.0 mEq/L; other electrolyte values were also low) was the lowest seen in any puppy during this study.

There are indications that Amitraz in the Promeris formulation is absorbed more rapidly and/or to a greater extent than Amitraz in the Mitaban-use dilution. Public information on Mitaban includes the following [see myweb.cableone.net/bdturner/**Mitaban**.pdf]: "Blood glucose values were elevated at 4 hours posttreatment in the 250 ppm female group, and in both sexes at the 1250 and 2500 ppm concentrations. ...glucose values returned to normal within 24 hours posttreatment. In another study, groups of healthy beagles were topically treated with either 250 ppm, 750 ppm or 1250 ppm of active drug at 14 day intervals and for 12 weeks. Blood glucose values were elevated at the 750 ppm concentration at 4 hours posttreatment after 3 of 6 treatments, and after 5 of 6 treatments at the 1250 ppm level. In the 750 ppm group, serum glucose values returned to normal at 24 hours posttreatment, however for the 1250 ppm group, at 24 hours and

after 3 of 6 treatments the levels remained significantly elevated." In the adult dog Promeris study [MRID 46401003] 1/6 of the 3X females and 5/6 5X females had elevated glucose levels (≥120 mg/dL) at 24 hours, suggesting that Amitraz absorption from a 5X (in this case, 7.0 mL) Promeris treatment is greater than that from exposure to 2500 ppm Amitraz (or a 10X concentration of the use-dilution of Mitaban), which would mean that a 1X (1.4 mL) Promeris treatment of an adult dog results in greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration Mitaban solution.

The mean weight of the 5X females on Day -1 in the adult study was 8.06 (range: 7.34-9.54) kg, and they were treated with 7.0 mL test material (mean Amitraz dosage: 133.1 mg/kg). The mean weight of the 5X females on Day -1 in the puppy study was 2.25 (range: 1.72-2.89) kg, and they were treated with 3.0 mL test material (mean Amitraz dosage: 204.5 mg/kg).

Except for 1X male #125 and 3X female #122 all puppies survived to study termination.

There is no indication that body weight and/or food consumption were affected by treatment with R-28153/amitraz spot-on.

The only clinical findings on Day 1 (the day of dosing) were lacrimation (not observed at 5X) and soft and/or mucoid feces (and, in one vehicle control female, clear discharge from the nose and muzzle), with no indication of a dose-response.

Physical and neurological examinations showed no effect as a result of treatment with R-28153/amitraz spot-on. However, no temperature measurements were made. While these are not specified in the 870.7200 Guidelines, treatment with Mitaban at 1250 and 2500 ppm Amitraz was associated with significant depression of rectal temperatures at 4 hours posttreatment (returning to normal at 24 hours), and this would have provided additional information.

Several clinical chemistry and hematology parameters were affected. While there were some cases in which there were no significant differences between controls and test material-treated groups at 24 hours, there were significant differences between pretreatment and 24-hour postdose values, suggesting the possibility of pharmacological activity involving one or more of the inerts.

Urea nitrogen was significantly increased at 24 hours postdose in 1X males and in both sexes at 3X and 5X, although it was not a dose-related trend (perhaps because 1X male #125 and 3X female #122 had more elevated readings than most of the other puppies). A similar increase in was also observed in the adult study, and is consistent with a known effect of Amitraz.

While the statistical summary (see pages 1226 and 1227 of MRID 46401004) does not indicate an effect on serum glucose, this reviewer has examined the data in somewhat more detail, and is convinced that there was an effect. According to http://www.ahc.umn.edu/rar/RefValues.html the normal range for glucose in the dog is 80-120 g/dL. On Day 8 1X male #125 (found dead day 9) had a value of <10 mg/dL, while 3X female #122 (sacrificed *in extremis* day 9) had a value of 23 mg/dL. However,

1X female #116 had a value of 20 mg/dL. A number of puppies had values below 80 mg/dL at 24 hours postdose, including 1X male #120 (64 mg/dL), 3X female #148 (65 mg/dL) , 5X male #117 (48 mg/dL), and 5X females #107 (59 mg/dL) and #129 (64 mg/dL). These values suggest a hypoglycemic effect in puppies, in contrast to the hyperglycemic effect observed in adult dogs (MRID 46401003). However, examining the incidences of glucose values >120 mg/dL, a somewhat different (and dose-related) picture emerges, as at 24 hours postdose 1/12 placebo puppies, 2/12 1X puppies, 3/12 3X puppies and 5/12 5X puppies had elevated levels, and these results are in striking comparison with Day 8 and Day 22 incidences (no more than 1/12 per group). Perhaps the occurrence of either hypoglycemia and/or hyperglycemia (at the 5X dose level only 4/12 puppies were within normal glucose limits) at 24 hours postdose is a manifestation of a temporary inhibition of a metabolic control process, possibly involving the hypothalamus. The relatively high glucose levels (including a number of values >120 mg/dL) for Week -1 suggest the possibility that at least some of the puppies may not have been adequately fasted before that blood collection. Hyperglycemia is a known effect of Amitraz, and is consistent with what was observed in the adult dog study.

Phosphorus was significantly elevated for the 3X and 5X levels at 24 hours postdose.

For leukocyte counts there were significant differences between pretreatment and 24-hour postdose [24 hours lower with P=0.0002] and pretreatment and Day 8 [Day 8 higher with P=0.0007]. At 24 hours postdose means were lower in all groups (including controls) from their predose means. At 8 days means were noticeably higher in all groups (including controls) from their predose means. On Day 22 means were essentially the same as predose. These results are consistent with a physiological effect involving one or more of the "inert" ingredients in this formulation, with a depression in leukocyte at 24 hours postdose and a "rebound" effect by Day 8, and a return to preexposure values on Day 22. One placebo control female [#145] and one 5X female [#146] had below-normal leukocyte counts at 24 hours [5.5 and 5.4 K/mm^3, respectively; the normal range in the dog is 5.9-16.6 K/mm^3].

From http://www.ahc.umn.edu/rar/RefValues.html the normal range for K+ in the dog is 4.4-6.1 mEq/L. The postdose range in this study (4.0-6.7) included values below and above this reference range. However, the only values below this range were on Day 8, in 1X male 125 (4.2; found dead on Day 9) and 3X female 122 (4.0; euthanized *in extremis* on Day 9). The significance at 24 hours postdose was largely due to a more pronounced drop from the preexposure mean in controls than those occurring in the 1X, 3X and 5X dose groups. The magnitude of the drops at 24 hours from preexposure was Placebo > 5X > 3X >1X, suggesting an effect from one or more of the "inerts" in this formulation.

Aspartate aminotransferase (AST) was decreased from preexposure means for controls and at all dose levels at 24 hours postdose (combined mean for both sexes as percentage of preexposure mean for that group: 0X: 88.0%; 1X: 72.7%; 3X: 72.0%; 5X: 69.8%). The significance (if any) of this finding is not evident, as there was no concurrent dose-related change in ALT. In any case, all values observed preexposure and at 24 hours postdose for AST were considerably below 105 U/L, and so were normal for the dog. A drop in AST was noted in the adult dog study.

TRB concludes that this companion animal safety study (OPPTS 870.7200) does not demonstrate an adequate margin of safety (at least 5X) between the exposure associated with the proposed use level for this formulation in puppies and that at which significant adverse systemic effects may occur, and does not support this particular use. The greatest concern involves the death of 3X female #122, sacrificed *in extremis* on Day 9. This animal had a urea nitrogen of 8 mg/dL pretest, 22 mg/dL at 24 hours postdose, and 33 mg/dL at 8 days postdose, as well as an elevated serum potassium at 24 hours, consistent with known effects of Amitraz. In addition, 1X male #125 was found dead on Day 9. While this puppy had shown a substantial weight loss (from 2.01 to 1.82 kg) in the preexposure period from Day -4 to -1, the possibility that exposure to Amitraz may have contributed to death cannot be ruled out. Increases in blood urea nitrogen (BUN) and a dose-related trend of increasing incidences (Placebo: 1/12; 1X: 2/12; 3X: 3/12; 5X: 5/12) of elevated glucose (>120 mg/dL) at 24 hours postexposure indicate that physiologically significant amounts of Amitraz had been absorbed.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D311482
2. **PC CODES:** 106201 [Amitraz], 281250 [Metaflumizone]
3. **CURRENT DATE:** May 23, 2005
4. **TEST MATERIAL:** 15% w/v R-28153/15% w/v Amitraz Spot-on Lot No. 0381702, a pale yellow liquid with a specific gravity of 1.047 g/mL containing (from p. 480 of MRID 46401003) 14.7% R-28153 (Metaflumizone) and 14.65% Amitraz; PROMERIS SPOT-ON FOR DOGS

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal/8-wk old puppies/MPI Research, Inc. Mattawan, MI/Study No. 817-008; Sponsor Study No. 0817-C-US-09-03/10-MAR-2004; revised final report: 17-MAR-2004	46401004	4 groups of 53-58 day old puppies (M: 1.82-2.97 kg; F: 1.72-2.89 kg) were treated at 0X (3.0 mL formulation without actives, so each puppy was given 6.74X the dose of inerts that 1X puppies received); 1X (0.6 mL test material); 3X (1.8 mL test material) and 5X (3.0 mL) applied to dorsal midline starting between shoulder blades. Puppies were observed for 28 days. 1X male #125 was found dead on Day 9 and 3X female #122 was euthanized *in extremis* on Day 9. Cause(s) of death were not established. #122 had a very slight wt loss from Day -8 to -4, but then gained wt from Day -4 to Day -1. While none of the 5X puppies died, 3X female #122 may have been representative of a more sensitive population subgroup. This puppy had a urea nitrogen of 8 mg/dL pretest, 22 mg/dL at 24 hours, and 33 mg/dL at 8 days postdose, as well as an elevated serum potassium at 24 hrs, consistent with known effects of Amitraz. Increases in blood urea nitrogen (all test material exposed groups) and a dose-related trend of increasing incidences (0X: 1/12; 1X: 2/12; 3X: 3/12; 5X: 5/12) of elevated glucose (>120 mg/dL) at 24 hours postexposure indicate that physiologically significant amounts of Amitraz had been absorbed. Serum phosphorus was significantly elevated at 3X and 5X at 24 hours postdose. Physical and neurological examinations showed no effect as a result of treatment; however, no rectal temperatures were taken. From adult study in MRID 46401003 1/6 3X females and 5/6 5X females had glucose levels >120 mg/dL; Amitraz absorption at 5X in adult study is greater than that from exposure to 2500 ppm topically applied Amitraz, indicating 1X Promeris treatment of an adult dog would be associated with greater Amitraz absorption than that from exposure to a 2X (500 ppm Amitraz) use-concentration (250 ppm Amitraz) Mitaban solution.	N/A	N/A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**