(1-15-03)

DATA EVALUATION REPORT

Antimicrobial AlphaSan® RC 2000

Study Type: 90-Day Oral Toxicity Study in Dogs (OPPTS 870.3150)

Prepared for

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Antimicrobial Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity in Nonrodents (Beagle dogs; capsule)
OPPTS 870.3150

DP BARCODE: D286393
SUBMISSION CODE: S623914
P.C. CODE: 072560

TEST MATERIAL (PURITY): Antimicrobial AlphaSan® RC 2000 (99% a.i.)

SYNONYMS: Silver Sodium Hydrogen Zirconium Phosphate (active ingredient); Experimental Additive 9823-37

CITATION: Kuhn, J.O. (2002) Final Report: 90-Day Oral Toxicity Study in Dogs (OPPTS 870.3150) Antimicrobial AlphaSan® RC 2000. STILLMEADOW, Inc. (12852 Park One Drive, Sugar Land, TX). Laboratory Study Number 6664-01, August 30, 2002. MRID 457694-01. Unpublished.

SPONSOR: Milliken Chemical
920 Milliken Road, M-206
Spartanburg, SC 29303

EXECUTIVE SUMMARY

In a subchronic toxicity study (MRID 457694-01), Antimicrobial AlphaSan® RC 2000 (99% a.i.) was administered in gelatin capsules to Beagle dogs (4/sex/dose) at dose levels of 200, 400, or 1000 mg/kg for 90 consecutive days (Groups II-IV, respectively). Due to extreme toxicity, the 1000-mg/kg dose level was reduced to 700 mg/kg on Day 43 in females and on Day 71 in males. Controls (Group I) were not treated.

One Group IV (1000/700 mg/kg) female was sacrificed prematurely (Day 42) due to morbidity. One Group IV male died just prior to necropsy. Clinical signs of toxicity observed in treated animals consisted mainly of diarrhea (sometimes red), soft feces, mucus or gel in feces, and decreased defecation in Group III and IV animals. The study author attributed these findings to excretion of undigested test substance and did not consider them to be toxicologically significant. Although our reviewers agree that these findings may be due to undigested test article, we do consider them toxicologically relevant since Group IV males and females demonstrated reduced body weight and body weight gain. Statistical analysis of body weights on Days 28, 56, and 84 revealed a significant decrease in Group IV female mean body weights compared to Group I females on Day 84. Our reviewers note that all Group IV

females had lower body weight than controls at study termination with only one of the dogs gaining weight over the course of the study. Decreased mean body weight and body weight gain also were observed in Group IV males, but the difference did not reach statistical significance. Group IV males had lower food consumption than Group I males beginning at Week 9, which continued throughout the remaining 4 weeks. Our reviewers note that there was a single Group IV male that ate very little food from Day 62 until the study termination, while the 3 remaining Group IV males ate amounts comparable to the controls. Group IV females consumed less food than the controls beginning at 2 weeks, and food consumption remained lower throughout the remainder of the study duration. Statistical analyses performed at Weeks 4, 8, and 12 revealed that food consumption was statistically significantly decreased at Week 4. By excluding from analysis the females that stopped eating, food consumption was significantly decreased during Week 12 as well. There were several occasions where some Group IV females were force fed, and it appears that the amount of food force fed was included in the food consumption results.

There were no statistically significant differences in hematology at Day 32. There were no statistically significant differences in hematology values in males at Day 60 and at study termination. MID cells (includes less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells) were statistically significantly higher in all female treatment groups than Group I females at Day 60 and in Group II and IV females at study termination. The study author reported that the results were within normal range and were unrelated to administration of the test article. Our reviewers disagree and note that these MID cells values were outside the reference range provided by the study author (p. 116). In addition, treated females demonstrated slightly elevated granulocytes and reduced lymphocytes at all time points. Only reduced lymphocytes in Group III females at Day 60 reached statistical significance. The study author reported that elevated granulocytes accompanied by a decrease in lymphocytes is often related to stress. Our reviewers believe that this may be the case (Karpinski, et al., 2000; Stefanski and Engler, 1999, abstracts only), but note that this was not observed in the male treatment groups. Our reviewers also note that administering empty gelatin capsules to the controls (Group I) may have aided in the interpretation of this stress-related observation.

The study author reported toxicologically relevant increases in alanine aminotransferase (ALT) and alkaline phosphatase in Group IV males and females over all time points, indicating hepatic injury in high dose animals. The study author stated, and our reviewers concur, that all other changes in clinical chemistry observed were not toxicologically significant or were found only in the dying animals. Group IV males had a statistically significant increase in ALT on Day 32 that fell within normal range, but ALT levels rose over time. Alkaline phosphatase in Group IV males was statistically significant on Days 60 and 90, with a trend of increase over time. The Group IV male that died had extremely high levels of ALT, alkaline phosphatase, GGT, and total bilirubin. When this male was removed from the analysis, there was still an increase in ALT and alkaline phosphatase. Group IV females had high values of CK, ALT, AST, alkaline phosphatase, BUN, and creatine on Day 32. One animal died before the 60 day measurement, but Group IV females still had statistically significant increases in ALT, AST, and alkaline phosphatase. ALT and alkaline phosphatase remained elevated in Group IV females at Day 89.

There were occasional variations in urinalysis parameters that the study author did not relate to treatment. Our reviewers agree that the occurrences were random and unrelated to treatment, but we note that the study did not measure urine volume.

There were occasional gross pathology findings in Groups I, II, and III that were not considered toxicologically significant. Group IV findings included discolored and/or thick/spongy pancreas and enlarged or discolored lymph nodes. Histopathology investigation pigmentation in the lamina propia of intestines, in liver macrophage, and in the glomerulus of the kidney of Group III and IV animals. These groups also exhibited chronic/granulomatous inflammation in the liver, which was accompanied by hepatic vacuolation and/or necrosis in Group IV animals. The study author attributed the pigmentation to the cosmetic effect of silver and did not consider it to be toxicologically significant; our reviewers agree. Debilitated dogs in Group IV also exhibited renal tubular dilation and necrosis, bronchoinsterstitial pneumonia, cerebral hemorrhage with thrombosis, and thymic atrophy with lymphoid depletion.

Based on the results of this study, the no-observable-adverse-effects-level (NOAEL) is determined to be 400 mg/kg/day, based on chronic granulomatous inflammation of the liver accompanied by vacuolization and necrosis observed at the 1000/700 mg/kg/day dose.

This subchronic toxicity study in dogs is classified as acceptable. Although the use of multiple doses at the high-dose level limits the ability to draw definitive conclusions regarding the toxicological effects of the test article at 1000 and/or 700 mg/kg/day in Beagle dogs, it can be conservatively interpreted that the 700 mg/kg/day dose resulted in significant toxic effects from oral administration of the test material. In general, the study was conducted reasonably well, with the exceptions noted in the study deficiencies section of this report, and it provides useful information regarding the effects of the undigested test substance, the cosmetic effects of the test substance, and target organ (i.e., liver) toxicological effects.

COMPLIANCE: Signed and dated GLP Compliance (p.3), Quality Assurance (p. 4), Data Confidentiality (p. 2), and Flagging Statements (p. 4a) are provided in the study report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Antimicrobial AlphaSan® RC 2000
   Description: White Powder
   Lot No.: N2547
   Purity: 99% active ingredient
   Stability: Not provided, declared to be the responsibility of the Sponsor
   Storage: Stored at ambient temperature
   CAS No.: None provided

2. Vehicle and/or Positive Control: Controls were untreated.

3. Test Animals:
Species: Dog
Strain: Beagle
Age: Approximately 5 months
Weight: 10.0-11.9 kg for males and 8.4-10.1 kg for females
Source: Ridglan Farms, Mt. Horeb, WI 53572
Housing: Individually housed in stainless steel cages (3 ft x 4 ft or larger)
Diet: Lab Diet®, High Density Canine Diet #5L18 (PMI Nutrition International, Inc.); reported to be presented in sufficient quantity to meet adequate nutritional requirements
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: 30-70%
Photoperiod: 12 hour light/dark cycle
Air changes: 10-12 times per hour
Acclimation period: 14 days

B. PROCEDURES AND STUDY DESIGN

1. In Life Dates
Start: 17 January 2002
End: 19 April 2002

2. Animal Assignment
Animals were assigned on Day -1 using a weight-stratified randomization procedure to the dose groups indicated in Table 1. Dogs were identified by numbered ear tags and cage cards. Dogs were untreated (controls, Group I) or administered Antimicrobial AlphaSan® RC 2000 orally in gelatin capsules (Torpac Inc., size 12) 5 days per week for 13 weeks. Administration took place in the morning 2 hours after food was presented.

3. Dose Preparation and Analysis
Dose levels of 200, 400, and 1000 mg/kg of the test substance were prepared weekly and administered in the form of gelatin capsules. The stability of the test article was stated to be the responsibility of the Sponsor. Homogeneity was unnecessary due to the means of administration. Verification of concentration was not addressed in the study report.

TABLE 1. Study Design

Test Group	Dose Levels (mg/kg/day)	Number of Animals	
		Males	Females
I (control)	0	4	4
II (low dose)	200	4	4
III (medium dose)	400	4	4
IV (high dose)	1000/700[a]	4	4

[a] The dosage for this group was reduced to 700 mg/kg on Day 43 in females and Day 71 in males due to severity of clinical signs and poor food consumption.

4. Statistics

Statistical analyses were performed separately for males and females. Mean values and standard deviations were calculated where appropriate. Treatment groups were compared to controls implementing a one-way analysis of variance (ANOVA) and Dunnett's t-test. Categorical data were not evaluated statistically.

C. OBSERVATIONS

1. Clinical Signs/Mortality

Animals were inspected daily for general appearance and health condition including hygiene, eyes and mucus membranes, respiratory system, circulatory system, sensory and motor behavior, and urine and feces. Mortality was checked once daily. Animals found in moribund condition (undefined criteria) were sacrificed.

2. Body Weight

Body weight was measured the day before study initiation, on Day 7, at weekly intervals thereafter, and at necropsy or at the time of discovery of death.

3. Food Consumption

Food consumption was measured daily by subtracting the amount of food remaining at the end of the day from the amount supplied in the morning. The amount of food was not specified but was reported to be increased as the animals grew. Animals that were not eating were supplied with moist food in an attempt to stimulate their appetite. These animals also were force-fed occasionally. It appears from the data that the amount administered via force-feeding is included in the food consumption total.

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4. Ophthalmoscopic Examination

Animals were examined pretest (Day -8) and on Day 84. Pupil dilation preceded examination with a slit lamp (biomicroscope) with 10-power magnification and indirect ophthalmoscopy with a 28-diopter lens.

5. Serology

Blood was collected via jugular venipuncture on Days -7 (pretest), 32, 60, and 89 from all surviving dogs. According to the study protocol, the dogs were fasted overnight, and blood was collected prior to feeding.

a. Hematology

The CHECKED (X) parameters were examined. In addition, MID cells were reported; these cells were defined as less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells.

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular hemoglobin (MCH)*
X	Leukocyte count (WBC)*		Mean corpuscular HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)*
X	Blood clotting measurements* [a]	X	Platelet count*
	(Activated partial thromboplastin time)		
	(Prothrombin time)		

* Recommended for non-rodent subchronic studies based on OPPTS 870.3150 Guidelines
[a] Not assessed pretest

b. Clinical Chemistry

The CHECKED (X) parameters were examined.

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin
X	Chloride*	X	Blood creatine*
X	Phosphorus*	X	Blood urea nitrogen (BUN)*
X	Potassium*	X	Total cholesterol*
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase (CK)		Serum protein electrophores
	Lactic dehydrogenase (LDH)	X	A/G ratio
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase*		

*Recommended for non-rodent subchronic studies based on OPPTS 870.3150 Guidelines

6. Urinalysis

Urine samples from all animals were collected (by free catch in females and sterile catheter in males) prior to study initiation (Day -7), on Day 61, and on Day 90. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

*Recommended for non-rodent subchronic studies based on OPPTS 870.3150 Guidelines

7. Sacrifice and Pathology

All surviving animals were sacrificed via injection of Fatal Plus® (Vortech Pharmaceuticals, Dearborn, MI 48126) on schedule and subjected to gross pathology examination. Animals found dead or sacrificed due to moribundity also were also examined. The CHECKED (X) tissues were collected for histological examination. The (XX) organs also were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain (3 levels)*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*+
X	Colon*	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	XX	Parathyroid gland*+
X	Gall bladder*+		Epididymides*+	XX	Thyroids*+
X	Pancreas*	X	Prostate*		
			Seminal vesicle*		OTHER
	RESPIRATORY	X	Ovaries*+	X	Bone
X	Trachea*	X	Uterus*+	X	Skeletal muscle
X	Lung*			X	Skin
	Nose*			X	All gross lesions and masses*
	Pharynx*				
	Larynx*				

*Collection recommended for non-rodent subchronic studies based on OPPTS 870.3150 Guidelines
+ Organ weight recommended for non-rodent subchronic studies based on OPPTS 870.3150 Guidelines

II. RESULTS

A. CLINICAL SIGNS/MORTALITY

Daily summarized clinical observations (Table 1, pp. 20-26) and individual animal data (Appendix F, pp. 117-129) are provided in the study report. The majority of the findings related to test articleadministration are associated with fecal excretion and include diarrhea, red diarrhea, soft feces, mucus or gel in the feces, and decreased defecation (see Table 2 below). There was an increase in the occurrence of fecal excretion-related findings in all treated females and in Groups III (400 mg/kg) and IV (1000/700 mg/kg) males. The study author attributed these findings to undigested test substance and did not consider the findings to be toxicologically significant. Although our reviewers agree that these findings may be due to undigested test article, we do consider them toxicologically relevant. In addition, there were several occurrences of vomiting in Group III and IV animals. One Group IV female exhibited various degrees of decreased activity and was considered moribund and sacrificed on Day 42. A second Group IV female also exhibited decreased activity from Day 42 until Day 86. A Group IV male began showing decreased activity on Day 68, which continued through the end of the study when the animal was found dead just prior to necropsy. This male also had green urine during the last two weeks accompanied by emaciation and occasional ptosis.

Therefore, our reviewers believe there are clear signs of toxicity in Group III and IV animals.

TABLE 2. Occurrences of Fecal Abnormalities

Treatment Group	Number of occasions of diarrhea, soft feces, mucus and/or gel in feces, and decreased defecation	
	Males	Females
Group I (untreated controls)	18	11
Group II (200 mg/kg)	19	44
Group III (400 mg/kg)	103	30
Group IV (1000/700 mg/kg)	55	124

Results were obtained from Table 1 in the study report by adding all the occurrences throughout the study duration.

B. BODY WEIGHT AND WEIGHT GAIN

Weekly mean body weights and body weight change over the study duration (Table 2, pp. 27-28, Figures 1 and 2) and individual animal data (Appendix A, pp. 72-73) are presented in the study report. Data for males and females also are summarized in Tables 3 and 4, respectively, of this evaluation (see Appendix). Statistical analysis was conducted only on body weight data from Days 28, 56, and 84; mean body weight was statistically significantly decreased in Group IV females on Day 84, compared to controls. Our reviewers note that all 4 Group IV females had lower body weights with only one of the dogs gaining weight over the course of the study. The high-dose males (Group IV; 1000/700 mg/kg) also demonstrated lower mean body weights at Day 28, 56 and 84, although not statistically significant from controls. Our reviewers and the study author note that there was no body weight gain over the entire 13-week period of treatment in the high-dose animals (males and females). The study author reported that weight loss was only notable in one Group IV male (5 kg loss), but even with that animal excluded, the mean weight of Group IV males was one kilogram (kg) lower than controls (12.3 kg Group IV vs. 13.3 kg Group I). When the animals that stopped eating (1 male and 2 females) were excluded from statistical analyses, the body weight gain was still lower in Group IV animals (2.7 kg in Group I males vs. 1.6 kg in Group IV males; 4.0 kg in Group I females vs. 1.7 kg in Group IV females).

C. FOOD CONSUMPTION

Daily and weekly mean food consumption (Table 4, pp. 33-37 and Figure 3 and 4), and individual animal data (Appendix B, pp. 74-99) are provided in the study report. Data for males and females also are summarized in Tables 5 and 6, respectively, of this evaluation (see Appendix). All groups had occasional days where some or all of the dogs

ate very little, as well as incidences of wet or spilled feed such that a reliable consumption measurement was not obtained. Statistical analysis was performed at Weeks 4, 8, and 12. Group IV males exhibited lower food consumption than Group I controls from Week 9 to the end of the study, but the decrease did not reach statistical significance. Our reviewers note that there was a single Group IV male that ate very little food from Day 62 until study termination. The 3 remaining Group IV males ate amounts comparable to Group I males. Group IV females had lower food consumption than their controls beginning at Week 2; consumption remained lower throughout the study duration and reached statistical significance during Week 4. When the females that stopped eating were excluded from analyses, statistical significance was also reached during Week 12. There were several occasions where the study author force fed a few of the Group IV females, and it appears that the amount of food force fed was included in the food consumption results. It is apparent in the individual data that two Group IV females were drastically affected and ate very little; although the two remaining Group IV females were less affected, they still ate less food on average than Group I females.

D. OPHTHALMOSCOPIC EXAMINATION

The ophthalmologic findings are presented in Table 3 (pp. 29-32) of the study report. The study author reported, and our reviewers concur, that there were no treatment-related findings.

E. SEROLOGY

1. Hematology

Mean hematology values (Table 6, pp. 56-63) and individual animal data (Appendix D, pp. 108-115) are presented in the study report. There were no statistically significant differences observed between treated and control animals at pretest and at Day 32. There were no statistically significant differences in males at Day 60 and at study termination. MID cells (includes less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts, and other precursor white cells) were statistically significantly higher than controls in all female treatment groups at Day 60 and in Group II and IV females at study termination. Our reviewers note that according to the data provided, the study author erroneously reported that Group IV females were significantly lower than controls at study termination. The study author concluded that these values were within normal range and were not toxicologically significant. Our reviewers disagree with the study author's observations since the MID cell levels were outside the reference range provided in the study report (p. 116). In addition, treated females demonstrated slightly elevated granulocytes and reduced lymphocytes at all time points. Only reduced lymphocytes in Group III females at Day 60 reached statistical significance. The study author reported that elevated granulocytes accompanied by a decrease in lymphocytes is often related to stress. Our reviewers believe that this may be the case based on our

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review of the scientific literature (Karpinski, et al., 2000; Stefanski and Engler, 1999, abstracts only), but we note that this effect was not observed in the male treatment groups. Our reviewers also note that administering empty gelatin capsules to the controls (Group I) may have aided in the interpretation of this stress-related observation.

2. Clinical Chemistry

Mean clinical chemistry values (Table 5, pp. 49-55) and individual animal data (Appendix C, pp. 100-107) are presented in the study report. The study author stated that on Day 32, sodium values in control animals were high, thereby causing a statistically significant differences for treated males. This finding was not considered to be toxicologically relevant by the study author; our reviewers agree since all values were within the normal range. Likewise, the low potassium level observed in Group IV females at Day 32 was also within the normal range and was not considered treatment-related by our reviewers.

Group IV males had a statistically significant increase in ALT at Day 32 that fell within normal range, but ALT levels increased over time. Alkaline phosphatase in Group IV males was only statistically significant higher than controls at Day 60, but it increased over time. One Group IV male had extremely high levels of ALT, alkaline phosphatase, GGT, and total bilirubin. This animal died just prior to scheduled necropsy. When this male was removed from the results, there was still an increase in ALT and alkaline phosphatase. Group IV females had high values of CK, ALT, AST, alkaline phosphatase, BUN, and creatine at Day 32 when compared to the controls. One animal died before the Day 60 measurement, but Group IV females still had statistically significant increases in ALT, AST, and alkaline phosphatase at Day 60 when this animal was excluded. On Day 89, there was still a statistically significant increase in ALT and alkaline phosphatase. GGT levels were also high in one of the females causing the mean to be high; the difference was not statistically significant. Although the females did not have an increase in ALT and alkaline phosphatase over time, our reviewers note that this finding may be due to: (1) the loss of one female between the Day 32 and Day 60 readings that had high values at Day 32 and (2) the reduction of the dose from 1000 to 700 mg/kg prior to the Day 60 measurement.

F. URINALYSIS

Individual urinalysis values are presented in Table 7 (pp. 64-69) of the study report. The study author concluded, and our reviewers agree, that there were no remarkable findings in urinalysis throughout the study. Normal values were observed with the exception of single occurrences for the presence of glucose, bilirubin, or ketones on Day 61. Our reviewers note that urine volume was not measured.

G. SACRIFICE AND PATHOLOGY

1. Organ weights

Mean organ weights (Table 9, p. 71) and individual animal data (Appendix H, pp. 132-133) are presented in the study report. The only statistically significant difference in organ weight was a reduction in the absolute kidney weight of Group II males. The study author concluded, and our reviewers agree, that this finding was not toxicologically significant. Our reviewers also note that the

absolute liver and spleen weights of Group IV animals were decreased compared to controls. This effect most likely was due to lower liver and spleen values in the debilitated Group IV animals, causing the mean to be low. Since these animals also had low body weight, the small organs may be related to lower body weight gain and not directly related to treatment. Group III females had a large (but not statistically significant) increase in gonad weight. Our reviewers note that this increase was due to a large measurement (8.6 g) in a single dog.

2. Gross pathology

Mean gross pathology (Table 8, p.70) and individual animal data (Appendix G, pp. 130-131) are presented in the study report. There were numerous external and internal findings in Group IV animals. One Group IV male was found dead just before necropsy and was emaciated. Internal findings in this male included enlarged salivary glands, engorged gall bladder, thickened stomach and small intestine, no subcutaneous fat, and discolored contents in the intestinal tract. One female was sacrificed due to morbidity on Day 42 and had food in the mouth, a small circular lesion on the tongue, a wet muzzle, feces at the anus, and lusterless hair and was dehydrated and emaciated. Internally, this animal exhibited a pale liver, stomach and intestine, a dark shrunken spleen, a discolored area and dark gel on the occipital region of the brain, and discolored contents in the intestinal tract. Group IV internal findings that occurred in both surviving animals and decedents included discolored and/or thick/spongy pancreas, pale or discolored lungs, and enlarged or discolored lymph nodes. Other occasional findings in surviving Group IV animals included a mass on the left lobe of the lung, a spot on the gall bladder, white foam in the esophagus, discolored liver, yellow gel in the duodenum, small spleen and pale stomach. Cherry eyes (single occurrences in Group I and II), pale lungs (both control and treatment groups), and prominent mesenteric lymph nodes and large nodes adjacent to trachea and abundant adipose tissue in the abdomen (Group III findings) were not considered by the study author to be toxicologically significant due to sporadic occurrence, lack of correlation to treatment, and/or lack of related histopathology findings. Our reviewers agree with this assessment.

3. Microscopic pathology

Histopathology findings (Appendix J, pp. 151-197) and summary data (Table 2) are presented in the study report.

a. Non-neoplastic

Group III and IV animals exhibited pigmentation in the lamina propia of the intestines, in liver macrophages, and in the glomerulus of the kidney. According to the study author, these findings were associated with the cosmetic effect of silver in the test substance and, therefore, were not toxicologically significant. Our reviewers concur with the study author. Group III and IV animals also had

chronic/granulomatous inflammation in the liver. The study author only considered this finding toxicologically significant in Group IV since it was accompanied by hepatic vacuolation and or necrosis in these animals. Debilitated dogs in Group IV also exhibited incidences of renal tubular dilation and necrosis, bronchoinsterstitial pneumonia, cerebral hemorrhage with thrombosis, and thymic atrophy with lymphoid depletion. The study author concluded that these findings were likely secondary to debilitation. Our reviewers agree, but we also note that the affect of the test substance on selenium and vitamin E could have contributed directly to the observed debilitation.

b. Neoplastic

There were no neoplastic findings observed.

III. DISCUSSION

A. CONCLUSIONS

In this subchronic toxicity study examining the effect of silver sodium hydrogen zirconium phosphate in dogs, there were no treatment-related effects on hematology and urinalysis parameters. Treatment-related effects were observed in clinical signs, mean body weight, mean body weight gain, food consumption, clinical chemistry, and gross and microscopic pathology. One Group IV male died just prior to sacrifice and one Group IV female was sacrificed due to morbidity on Day 42; both exhibited decreased activity prior to death. Clinical signs in Group III and IV dogs included diarrhea, red diarrhea, soft feces, mucus or gel in the feces, decreased defecation, and vomiting; these findings were considered to be a result of undigested test substance and were not considered toxicologically significant by the study author. Treatment-related reductions in mean body weight and body weight gains were observed in Group IV dogs. Food consumption also was reduced in Group IV dogs. Treatment-related effects on clinical chemistry included increased ALT and alkaline phosphatase in Group IV males and females. Group IV males demonstrated an increase in ALT and alkaline phosphatase over time. Treatment-related effects in gross pathology included incidences of discolored and/or thick/spongy pancreas, pale or discolored lungs, and enlarged or discolored lymph nodes in Group IV animals. Treatment-related effects in histopathology included pigmentation in the lamina propia of intestines, in liver macrophages, and in the glomerulus of the kidney in Group III and Group IV animals, chronic/granulomatous inflammation in the liver in Group III and Group IV, accompanied by hepatic vacuolation and or necrosis in Group IV animals. The study author associated the pigmentation with the cosmetic effect of silver and did not consider it to be toxicologically significant, and our reviewers agree. Debilitated dogs in Group IV also exhibited renal tubular dilation and necrosis, bronchoinsterstitial pneumonia, cerebral hemorrhage with thrombosis, and thymic atrophy with lymphoid depletion.

Based on the results of this study, the no-observable-adverse-effects-level (NOAEL) is determined to be 400 mg/kg/day, based on chronic granulomatous inflammation of the liver accompanied by vacuolization and necrosis observed at the 1000/700 mg/kg/day dose.

B. STUDY DEFICIENCIES

In comparing this study to the guideline requirements for a 90-day subchronic oral toxicity study in non-rodents [OPPTS 870.3150], several major and minor deficiencies were noted. There was no indication that concentration analyses were performed throughout the study to verify the accuracy of the dose levels. There also was no information provided regarding the methodology of dose preparation. Another major deficiency in this subchronic oral study is that the amount of food provided to the animals was not defined. The study author reported that a "sufficient quantity" was provided to the animals to assure adequate nutritional requirements. Also, several animals in the high-dose group were force fed. The quantities force fed were not indicated, and food spillage data were not provided. These issues make the food consumption data difficult to interpret, and the values within groups more variable. Problems with food consumption in the high-dose animals prompted the study laboratory to change dose levels during the study period. The time that the high dose was lowered differed in the male and female groups making it difficult to draw conclusions regarding the effect of a specific dose on toxicity observed in the animals, as well as possible different sensitivities of the sexes to the test substance. Another limitation of this study is the statistical analysis of only 3 time points for food consumption and body weight. Changes in these parameters are very relevant in this study. Statistical analyses of these parameters at weekly intervals would have been more desirable to enhance the ability to analyze trends in the data over the 13-week study period. Other minor study deficiencies that did not appear to affect the outcome of the study include the omission of several recommended hematology parameters including (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, pretest measurements of prothrombin activated partial thromboplastin time), urine volume, heart organ weight, and several organs normally obtained for histopathology examination. Our reviewers also note that control animals were not administered empty gelatin capsules, which potentially could have aided in the interpretation of stress-related observations.

IV. REFERENCES

Karpinski, J, Kidawa Z, Kocur E, Zeman K, Rogulski B, Wolkanin P, Pokoca L, Fornalczyk-Wachowska E, Pasnik J, Kaczmarek P (2000). Impact of chronic psychophysical stress on some neutrophil functions. Pol. Merkuriusz Lek. 9(52):697-9. (Abstract only; article in Polish)

Stefanski V and Engler H (1999). Social stress, dominance and blood cellular immunity. Neuroimmunol. 94(1-2):144-52. (Abstract only)

APPENDIX

TABLE 3. Mean Male Body Weight (kg)

Week	Dose Groups			
	I (0 mg/kg)	II (200 mg/kg)	III (400 mg/kg)	IV (1000/700 mg/kg)
0	10.6 ± 0.5	10.8 ± 0.7	10.8 ± 0.8	10.7 ± 0.6
1	9.8 ± 0.4	10.5 ± 0.3	10.7 ± 0.5	10.3 ± 0.5
2	9.9 ± 0.4	10.5 ± 0.2	10.8 ± 0.5	10.3 ± 0.4
3	10.4 ± 0.3	11.1 ± 0.4	11.3 ± 0.4	10.7 ± 0.4
4*	10.8 ± 0.3	11.5 ± 0.5	11.4 ± 0.9	10.3 ± 0.5
5	11.3 ± 0.2	11.8 ± 0.6	12.0 ± 0.8	11.1 ± 0.6
6	11.6 ± 0.2	12.0 ± 0.6	12.3 ± 0.8	11.3 ± 0.6
7	11.9 ± 0.2	12.3 ± 0.6	12.5 ± 0.7	11.5 ± 0.6
8*	12.3 ± 0.1	12.6 ± 0.6	12.8 ± 0.9	11.6 ±0.7
9	12.5 ± 0.1	12.9 ± 0.6	13.2 ± 0.9	11.5 ± 1.0
10	12.9 ± 0.2	13.1 ± 0.6	13.3 ± 0.9	11.3 ±1.8
11	13.1 ± 0.2	13.1 ± 0.6	13.6 ± 1.0	11.0 ± 2.4
12*	13.3 ± 0.1	13.4 ± 0.5	13.9 ± 1.0	10.8 ± 3.0
13	13.3 ± 0.3	13.4 ± 0.5	14.0 ± 1.3	10.7 ± 3.5
Body weight gain	2.7 ± 0.1	2.6 ± 0.1	3.2 ± 0.7	0.0 ± 3.3

Mean weights and standard deviations were extracted from Stillmeadow Study No. 6664-01 (p. 27).
* Statistical analysis performed
†Statistically different from the control (p value not specified)

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TABLE 4. Mean Female Body Weight (kg)

Week	Dose Groups			
	I (0 mg/kg)	II (200 mg/kg)	III (400 mg/kg)	IV (1000/700 mg/kg)
0	8.8 ± 0.5	9.0 ± 0.5	9.1 ± 0.7	9.0 ± 0.7
1	9.4 ± 0.5	9.4 ± 0.3	9.3 ± 0.9	9.1 ± 0.3
2	9.4 ± 0.5	9.2 ± 0.4	9.5 ± 0.9	8.9 ± 0.3
3	9.9 ± 0.4	9.5 ± 0.4	10.1 ± 0.9	9.4 ± 0.1
4*	10.2 ± 0.5	10.0 ± 0.4	10.2 ± 1.0	8.9 ± 0.6
5	10.5 ± 0.7	10.3 ± 0.5	10.9 ± 0.9	9.0 ± 1.4
6	10.9 ± 0.5	10.5 ± 0.6	11.2 ± 0.9	8.6 ± 1.8
7	11.3 ± 0.6	10.5 ± 0.4	11.3 ± 1.0	8.9 ± 1.7
8*	11.6 ± 0.5	10.9 ± 0.6	11.2 ± 1.2	9.0 ± 1.8
9	11.8 ± 0.5	11.2 ± 0.6	12.0 ± 1.1	8.7 ± 2.1
10	12.2 ± 0.6	11.3 ± 0.6	11.7 ± 1.2	8.7 ± 2.6
11	12.4 ± 0.6	11.3 ± 0.7	12.5 ± 1.1	8.7 ± 2.6
12*	12.7 ± 0.5	11.5 ± 0.7	12.8 ± 1.1	8.8 ± 2.7†
13	12.8 ± 0.9	11.5 ± 0.7	12.8 ± 1.3	9.1 ± 2.2
Body weight gain	4.0 ± 0.6	2.5 ± 0.2	3.6 ± 1.3	0.0 ± 3.0

Mean weights and standard deviations were extracted from Stillmeadow Study No. 6664-01 (p. 27).
* Statistical analysis performed
†Statistically different from the control (p value not specified)

TABLE 5. Mean Male Food Consumption (g/animal/day)

Week	Dose Groups			
	I (0 mg/kg)	II (200 mg/kg)	III (400 mg/kg)	IV (1000/700 mg/kg)
1	182 ± 36.9	255 ± 23.2	247 ± 36.4	266 ± 14.0
2	308 ± 16.8	299 ± 12.9	299 ± 13.7	307 ± 4.8
3	299 ± 54.3	339 ± 26.6	330 ± 27.3	331 ± 20.5
4*	305 ± 43.1	326 ± 67.9	319 ± 40.7	290 ± 43.2
5	302 ± 44.0	322 ± 39.6	343 ± 14.4	299 ± 40.4
6	349 ± 5.9	337 ± 30.7	348 ± 6.4	333 ± 22.8
7	322 ± 24.2	346 ± 14.4	347 ± 13.7	303 ± 50.9
8*	340 ± 25.9	339 ± 36.4	350 ± 14.5	306 ±38.3
9	326 ± 31.1	338 ± 29.7	349 ± 12.2	249 ± 83.4
10	334 ± 19.1	312 ± 52.9	356 ± 1.1	253 ±146.8
11	305 ± 16.0	326 ± 21.0	340 ± 15.9	239 ± 140
12*	338 ± 13.7	336 ± 3.5	325 ± 18.2	252 ± 149.3
13	347 ± 19.2	338 ± 12.1	342 ± 10.8	255 ± 150.5

Mean food consumption and standard deviations were extracted from Stillmeadow Study No. 6664-01 (pp. 33-37).
* Statistical analysis performed
†Statistically different from the control (p value not specified)

TABLE 6. Mean Female Food Consumption (g/animal/day)

Week	Dose Groups			
	I (0 mg/kg)	II (200 mg/kg)	III (400 mg/kg)	IV (1000/700 mg/kg)
1	252 ± 21.2	246 ± 25.8	250 ± 15.8	237 ± 37.2
2	318 ± 8.5	310 ± 2.6	299 ± 6.0	262 ± 60.1
3	330 ± 37.3	325 ± 15.3	331 ± 31.0	281 ± 58.5
4*	327 ± 38.0	312 ± 21.9	330 ± 32.1	215 ± 75.7†
5	330 ± 32.0	291 ± 39.6	311 ± 44.9	192 ± 103.0
6	341 ± 24.5	329 ± 18.5	331 ± 28.0	236 ± 125.9
7	336 ± 33.7	310 ± 10.4	317 ± 36.6	209 ± 108.7
8*	328 ± 46.6	333 ± 38.6	319 ± 27.4	217 ±91.4
9	337 ± 20.9	312 ± 34.5	325 ± 54.7	174 ± 107.9
10	350 ± 6.1	327 ± 24.7	334 ± 25.0	203 ±165.6
11	332 ± 14.9	274 ± 36.8	300 ± 31.3	193 ± 127.3
12*	325 ± 10.3	309 ± 14.3	329 ± 20.4	218 ± 116.8†
13	320 ± 44.9	297 ± 32.3	310 ± 51.3	261 ± 75.0

Mean food consumption and standard deviations were extracted from Stillmeadow Study No. 6664-01 (pp. 33-37).
* Statistical analysis performed
†Statistically different from the control (p value not specified)

APPENDIX B

DATA VALIDATION SUMMARY FOR
90-Day Oral Toxicity Study in Dogs
(OPPTS Guideline 870.3150)

Our data validator verified 20% of the data from this 90-day dog study, and followed 20% of the animals through the study. Twenty percent of the data from the body weight, food consumption, blood analysis clinical chemistry, and blood analysis hematology tables were verified. The individual data were presented in Appendices A, B, C, and D, respectively. Summary data were presented in Tables 2, 4, 5, and 6, respectively. The only inaccuracy found is as follows:

Appendix B, Food Consumption, Week 7 Average
Female 2474- reported as 83 grams, was calculated by our data validator as 86.4 grams

Data Validator

Peter S. Frantz, B.S. — Date: 1/16/03

Reviewer

Kara B. Altshuler, PhD. — Date: 1/16/03

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