(10-30-00)

DATA EVALUATION REPORT

TRITICONAZOLE

STUDY TYPE: CHRONIC ORAL TOXICITY CAPSULE – DOG
[OPPTS 870.4100 (§83-1)]
MRID 44802103

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-17M

Primary Reviewer: Claudia M. Troxel, Ph.D.	Signature: Date:	Claudia M. Troxel JUN 0 2 2000
Secondary Reviewers: H. Tim Borges, Ph.D., D.A.B.T.	Signature: Date:	HT Borges JUN 0 2 2000
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross JUN 0 2 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson JUN 0 2 2000

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EPA Reviewer: William Greear, M.P.H., D.A.B.T. William Greear, Date 10-25-00
Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Marion Copley, D.V.M., D.A.B.T. Mcopley, Date 10/30/00
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic oral toxicity capsule - dog [OPPTS 870.4100 (§83-1)]

DP BARCODE: D261924
P.C. CODE: 125620

SUBMISSION CODE: S568827
TOX. CHEM. NO.: none stated

TEST MATERIAL (PURITY): RPA400727 (Triticonazole) (96.5% a.i.)

SYNONYMS: Triticonazole; RPA591086; 2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1,2,4-triazolylmethyl)-1-cyclopentanol

CITATION: Broadmeadow, A. (1993) RPA400727: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks. Huntingdon Life Sciences Ltd, Eye, Suffolk, IP23 7PX, England. Report No. 92/RHA441/0782, February 10, 1993. MRID 44802103. Unpublished.

SPONSOR: Rhône-Poulenc Agrochimie SA, 14-20 rue Pierre Baizet, BP 9163, F-69263 Lyon Cedex 09, France.

SUBMITTED BY: ISK Biosciences Corporation, 5970 Heisley Road, Suite 200, Mentor, Ohio 44060.

EXECUTIVE SUMMARY: In a chronic oral toxicity study (MRID 44802103), RPA400727 (96.5% a.i., Lot/Batch #: DA646) was administered to 4 beagle dogs/sex/dose in capsules at dosages of 0, 2.5, 25, or 150 mg/kg/day for 52 weeks.

Treatment with 150 mg/kg/day resulted in ocular toxicity, neurological signs of toxicity, skin effects, decrements in body weights, hepatic effects, and adrenal toxicity. The moribund condition of one mid-dose female that was sacrificed during week 5 was not attributed to treatment. All other animals survived to study termination. Transient neurological signs were observed between treatment weeks 6-11 in 2/4 high-dose males and 4/4 high-dose females, and included tremors, hyperactivity or hypoactivity, convulsions, and ataxia. Reddened and thickened skin of the pinnae and other body regions was observed more frequently in high-dose males and females during treatment. Thickened skin, predominately of the pinnae and hocks, was noted during gross necropsy in 3/4 high-dose males and females. Cataract formation as confirmed by ophthalmoscopic and microscopic examination was observed in 4/4 high-dose males and 3/4 high-dose females by week 46. Although ophthalmoscopic examination suggested that uveitis accompanied the early stages of cataractogenesis in 3/4 males and 2/4 females, no histopathological correlates were found at necropsy. No ocular effects were observed in one high-dose female.

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Body weight gains were statistically decreased ($p<0.05$; 0.01; 0.001) during selected time intervals throughout most of the study in high-dose males (71-80% of controls), low-dose males (77-85% of controls), and high-dose females (31-54% of controls). Overall, high-dose males gained 27% less ($p<0.05$) and high-dose females gained 59% less ($p<0.01$) than controls. In high-dose females, decreases in body weight gains were accompanied by consistently decreased absolute body weights (approximately 73-84% of controls) beginning at week 9 and continuing throughout the study ($p<0.05$). Absolute body weights in low-, mid-, and high-dose males were comparable to controls. Although high-dose females generally consumed 8-15% less food than controls, the differences did not attain statistical significance.

Hepatotoxic effects were evident only as changes in clinical chemistry parameters. During the study, high-dose males and females exhibited increased activities ($p<0.05$; 0.01; 0.001) of alkaline phosphatase (643-1513% and 394-833% of controls, respectively) and alanine amino-transferase (162-192% and 174-214% of controls, respectively), and decreased levels of cholesterol (45-48% and 70-83% of controls, respectively), total proteins (85-90% and 88-93% of controls, respectively) and albumin (84-89% and 81-83% of controls, respectively). These changes were not accompanied by clear changes in liver weights or macro- or microscopic pathology.

Vacuolation of the cells of the *zona fascicularis* in the adrenals was observed in males at an incidence of 1/4, 1/4, 2/4, and 4/4 for the control, low-, mid-, and high-dose males, respectively, and in 1/3 and 3/4 mid- and high-dose females, respectively. Adrenal weights were increased in high-dose males (133-147% of controls), but the increases were not statistically significant.

A LOAEL of 150 mg/kg/day was identified for dogs based on decreased absolute body weights of female dogs, decreased weight gain by male and female dogs, and treatment-related toxicity to the eye, liver, and adrenals. The corresponding NOAEL is 25 mg/kg/day.

This chronic oral toxicity study in the dog is classified as **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a chronic oral toxicity study [OPPTS 870.4100 (§83-1)] in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: RPA400727

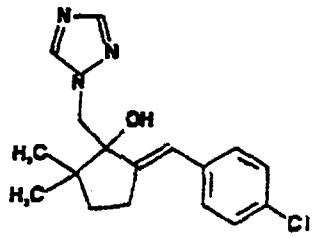
   Description: white to yellowish powder
   Lot/Batch #: DA646
   Purity: 96.5 a.i.
   Stability of compound: stable for the duration of study
   CAS #: Not available

2. Vehicle and/or positive control

The test chemical was administered in a gelatin capsule.

3. Test animals

Species: dogs
Strain: beagles
Age and weight at study initiation: approximately 23-25 weeks old, males: 6.3-8.8 kg, females: 6.3-8.3 kg
Source: Alpha Sirius Limited, Colwall, Worcestershire, England
Housing: housed individually in indoor kennels measuring approximately 2.6 x 0.9 m
Diet: 400 g of Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, England)
Water: tap water was available *ad libitum*
Environmental conditions:
Temperature: 21 °C (target)
Humidity: 55% (target)
Air changes: at least 12 changes/hour
Photoperiod: 12 hr dark/12 hr light
Acclimation period: approximately 4 weeks
Structure:



B. STUDY DESIGN

1. In life dates - start: June 12, 1991; end: June 12, 1992

2. Animal assignment

Groups of 4 animals were randomly assigned to the test groups in Table 1 based on body weights.

TABLE 1: Study design		
Test Group	Dose (mg/kg/day)	
	Male	Female
Control (1)	0	0
Low (2)	2.5	2.5
Mid (3)	25	25
High (4)	150	150

Data taken from p 15 and text table on p. 16, MRID 44802103.

3. Dose selection rationale

Dose selection was chosen by the Sponsor based on the results from a preliminary study conducted at the performing laboratory. In the preliminary study, beagle dogs were treated at dosages of 10, 30, 100, or 300 mg/kg/day for four weeks. One high-dose male exhibited transient clinical signs (description of signs not provided) and dogs receiving 100 or 300 mg/kg/day had changes in the liver (hepatic changes not described). No treatment-related effects were observed in animals dosed with 10 or 30 mg/kg/day.

4. Capsule preparation and analysis

The test material was weighed directly into gelatin capsules. The individual weights of the test material required for daily administration to each dog were calculated in advance, based on the most recently recorded body weight. The amount of test material necessary to fill the capsules for each group and the quantity actually used were determined for each day of treatment. The difference between those two amounts was checked before the capsules were dispensed.

The main consignment of the test material was stored protected from light and desiccated in a refrigerator at approximately 4 °C. Smaller amounts were stored in a desiccator in a cool store, not exceeding 15 °C, protected from light until use.

To insure the adequacy of the storage conditions, 10 g samples of the test material were returned to the Sponsor for analysis six months after receipt and at six-monthly intervals thereafter.

Dogs received the test material orally in gelatin capsules throughout the treatment period. All dogs were dosed, after feeding, in sequence of kennel-number, once each day, seven days per week. Dogs in Groups 1 and 4 weighing up to and including 13.5 kg each received one ¼ ounce capsule, above this weight they each received two ¼ capsules. Dogs in Groups 2 and 3 each received one 00 size capsule.

Results

The concentration (purity) of the test material was 971 g/kg before treatment commenced, 970 g/kg six months after commencement of treatment, and 965 g/kg approximately two and a half years after the commencement of treatment. The results demonstrate that the stability of the test material was adequate under the storage conditions employed.

5. Statistics

Inter-group differences in body weight gain, hematology, blood chemistry and urinalysis were analyzed by Student's t-test using a pooled error variance. Plasma alkaline phosphatase results were further assessed by Student's t-test after exclusion of Group 4. Statistical significance for reticulocyte, eosinophil, basophil, monocyte, and large unstained cell counts are not reported as these data are not normally distributed. Organ weights were analyzed by Behrens-Fischer test if Bartlett's test for homogeneity of variance was significant, or Dunnett's test if Bartlett's was not significant. Microscopic pathology and histopathology results were assessed using Fisher' Exact Test. The level of significance was $p>0.05$.

C. METHODS

1. Observations

Animals were observed at intervals throughout the working day for clinical signs. Individual daily observations of all animals were recorded before and shortly after each dose; whenever possible an additional observation was recorded several hours after dosing.

Animals were subjected to a veterinary examination before treatment commenced and after 12, 24, 38, and 50 weeks of treatment, in which particular attention was paid to teeth and gums, mucous membranes and skin, ears, eyes, superficial lymph nodes, abdomen, external genitalia and mammary glands, chest, gait and stance including palpitation of the legs, and general behavior and appearance.

2. Body weight

Each animal was weighed at weekly intervals throughout the acclimatization and treatment periods and before necropsy.

3. Food consumption

The quantity of food consumed by each animal was recorded for each day throughout the acclimatization and treatment periods. Food consumption per animal was calculated for the final two weeks of the acclimatization period and for each week throughout the study.

4. Water consumption: Water consumption was not measured.

5. Ophthalmoscopic examination

Eyes were examined in all animals before study initiation and after 12, 18, 24, 31, 38, 45, and 51 weeks of treatment by means of an indirect ophthalmoscope after the instillation of 1.0% tropicamide.

Additionally, dogs in Group 1 and 4 were administered the Schirmer tear test after 18 weeks of treatment. The test paper was inserted into the conjuctival sac of each eye, held for exactly one minute, and the tear film measured.

6. Blood was collected from the jugular vein from each dog, after overnight fasting, before study initiation and after 12, 24, and 50 weeks of treatment. from each group for hematology and clinical chemistry analyses. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		Nucleated red blood cell count
X	(Thromboplastin time)	X	Cell morphology
	(Clotting time)		
X	(Prothrombin time)		

* Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

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b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*		Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin*
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	x	Electrophoretic protein fractions
	Cholinesterase (ChE)		
	Creatine phosphokinase*		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Overnight urine samples were collected from all animals before commencement of treatment and after 11, 23, and 49 weeks of treatment. Each animals was placed in an individual metabolism cage for urine collection under conditions of food and water deprivations. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrite
X	Protein*	X	Urobilinogen
		X	Total reducing substances

* Required for combined chronic/oncogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and pathology

The animal sacrificed *in extremis* and those surviving to study termination after 52 weeks of treatment were given intravenous pentobarbitone anaesthesia and sacrificed by rapid exsanguination and were subjected to gross pathological examination. The CHECKED (X) tissues from all animals were collected for histological examination. All tissues collected from all animals sacrificed at termination were examined microscopically. Microscopic findings were either reported as "Present" or were assigned a severity grade of minimal, slight, moderate, marked, or severe. The (XX) organs, in addition, were weighed.

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X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)
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	Thyroid with parathyroids*
x	Pancreas*	xx	Prostate with urethra		Harderian gland
x	Gall bladder		sample		Zymbal's gland
			Seminal vesicle		
	RESPIRATORY	x	Epididymides		OTHER
x	Trachea	xx	Ovaries	x	Bone
x	Bronchi	xx	Uterus with cervix*	x	Skeletal muscle
xx	Lung*	x	Vagina	x	Skin
				x	All gross lesions and masses*

* Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The study authors reported that the group incidences of reddened pinnae and thickened skin of the pinnae in high-dose males and females tended to be higher than other treatment groups throughout the treatment period (see Table 2 for incidences for selected treatment intervals). Upon viewing the individual incidences, thickened skin of the pinnae was observed on a daily basis in 1 low-dose male, one control female, and 3 high-dose males and 3 high-dose females during the latter half of treatment. Reddened pinnae was a finding observed in all dose groups, but was not consistently noted in affected animals except for one high-dose female. Reddened skin for body regions other than the pinnae was observed more frequently in high-dose males and females, and 3 high-dose males and one high-dose female exhibited a thickening of the skin (for body regions other than the pinnae). High-dose females had a higher incidence of reddened gums during the study; this increased incidence may be primarily due to one high-dose female which consistently exhibited this finding starting at week 24 of treatment.

Ocular opacities were visible in all high-dose males at week 28, and in the 3 affected high-dose females at week 46. Eye discharge was transiently noted from almost all groups, but was more frequent from high-dose males and females. Additionally, partially closed eyelids were noted more frequently in high-dose males.

The study authors also reported neurological signs of toxicity in two high-dose males and all high-dose females between weeks 6 - 11 of treatment (reported in the Results section; MRID 44802103; data not found in summary table or appendix). Tremors, particularly of the head and neck, were occasionally observed in 2 males and all females approximately 30 minutes to 4 hours post-dosing. Hyperactivity was also occasionally observed in one male and one female post-dosing. Limited use of the hindlimbs was noted for 5 minutes in one male one hour post-dosing on the third day of week 6. During the second day of week 11, it was suspected that this male had a convulsion before dosing, and signs of convulsion, including paddling of the feet, vocalisation, muscle spasms, abnormal gait, prostration, and splayed legs, were observed in this male approximately one-hour post-dosing on the same day, with recovery completed the end of the following day. Two short-duration convulsions, with signs of paddling of the feet, ataxia, underactivity, muscle spasms, salivation, and resentment to touching, were also observed in one female before dosing and approximately 5 hours post-dosing on the fourth day of week 11. Another female exhibited signs of underactivity, tremor, ataxia, and reluctance to walk during the second half of week 9; recovery was observed by week 10.

TABLE 2. Selected group incidences of clinical signs in dogs treated with RPA400727 for 52 weeks [Expressed as a percentage of the maximum possible incidences per group]

Finding	Week	Dose (mg/kg/day)							
		Males				Females			
		0	2.5	25	150	0	2.5	25	150
Reddened pinnae	-2--1	0	0	0	0	0	0	0	0
	44	0	0	5	80	0	0	0	25
	52	0	25	0	25	0	0	0	30
Thickened pinnae	-2-12	0	0	0	0	0	0	0	0
	26-32	0	0	0	25	0	0	0	50
	48-52	0	25	0	75	25	0	0	75
Reddened skin (other than pinnae)	-1	0	25	0	0	0	0	0	0
	44	0	10	0	45	0	0	0	35
	52	0	0	0	50	0	25	0	50
Thickened skin (other than pinnae)	-2-17	0	0	0	0	0	0	0	0
	19-33	0	0	0	25	0	0	0	0
	40-52	0	0	0	75	0	0	0	25
Visible ocular opacities	1-17	0	0	0	0	0	0	0	0
	28	0	0	0	85	0	0	0	0
	46-52	0	0	0	100	0	0	0	75

Data taken from Table 1A-1H, pp. 45-59, MRID 44802103.

Veterinary examination revealed cataract formation in all high-dose males at week 39 and 3/4 high-dose females by week 51. Photophobia was also noted in 3 high-dose males and 1 high-dose female. Two high-dose males and 2 high-dose females were reported to have bodies that appeared thin. Thickened skin of the pinnae and other body regions was occasionally observed in some of the high-dose males and females.

2. Mortality

One female dog from the mid-dose group was euthanized during Week 5. Clinical signs of toxicity observed immediately before sacrifice included underactivity, reluctance to stand or walk, stiff gain, and pale mucous membranes. Prior to sacrifice, pain on manipulation of joints was observed. All other dogs survived to study termination.

B. BODY WEIGHT

Body weight gains were statistically decreased ($p<0.05$; 0.01; 0.001) during selected time intervals throughout most of the study in high-dose males (71-80% of controls), low-dose males (77-85% of controls), and high-dose females (31-54% of controls) (see Table 3). Overall, high-dose males gained 27% less ($p<0.05$) and high-dose females gained 59% less ($p<0.01$) than controls. In high-dose females, decreases in body weight gains were accompanied by consistently decreased absolute body weights (approximately 73-84% of

controls) beginning at week 9 and continuing throughout the study ($p<0.05$; statistical analysis performed by reviewer using ANOVA). Absolute body weights in all treated male dog groups were comparable to controls.

TABLE 3. Selected mean body weights and body weight gains of male and female dogs administered RPA400727 for up to 52 weeks

Weeks	Concentration (mg/kg/day)							
	0	2.5	25	150	0	2.5	25	150
	Males				Females			
Mean body weights (kg) [a]								
0	7.6 ± 0.9	7.7 ± 1.1	7.8 ± 0.6	8.0 ± 0.6	7.2 ± 0.8	7.3 ± 0.6	7.2 ± 0.8	7.1 ± 0.6
13	11.7 ± 0.6	11.2 ± 1.6	11.5 ± 0.7	10.9 ± 0.7	10.9 ± 0.4	10.8 ± 0.7	10.3 ± 1.0	9.1 ± 0.6† (83)
26	13.2 ± 0.6	12.3 ± 1.5	12.9 ± 0.5	12.1 ± 0.7	12.2 ± 0.4	11.8 ± 1.2	11.0 ± 0.8	9.4 ± 0.9† (77)
39	13.9 ± 0.6	12.8 ± 1.5	13.4 ± 0.4	12.5 ± 0.9	12.9 ± 0.6	12.3 ± 1.4	11.6 ± 1.0	9.5 ± 0.9† (74)
52	14.1 ± 1.0	12.8 ± 1.4	13.7 ± 0.6	12.9 ± 1.1	12.8 ± 0.8	12.2 ± 1.4	11.4 ± 0.9	9.4 ± 0.7† (73)
Body weight gain (kg)								
0-13	4.1 ± 0.3	3.5 ± 0.5*	3.7 ± 0.2	2.9 ±0.4*** (71) [a]	3.7 ± 0.7	3.5 ± 0.7 (95)	3.1 ± 0.5 (84)	2.0 ± 0.6** (54)
13-26	1.5 ± 0.4	1.1 ± 0.2	1.4 ± 0.2	1.2 ± 0.4 (80)	1.3 ± 0.3	1.0 ± 0.7 (77)	0.7 ± 0.3 (54)	0.4 ± 0.4* (31)
26-39	0.7 ± 0.3	0.5 ± 0.2	0.5 ± 0.3	0.5 ± 0.5 (71)	0.6 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	0.1 ± 0.1* (17)
39-52	0.2 ± 0.4	0.0 ± 0.2	0.3 ± 0.3	0.4 ± 0.3	-0.1 ± 0.4	-0.1 ± 0.2	-0.2 ± 0.2	-0.1 ± 0.3
0-26	5.7 ± 0.6	4.5 ± 0.5*	5.1 ± 0.2	4.0 ± 0.8** (70)	5.0 ± 1.0	4.6 ± 1.4 (92)	3.8 ± 0.6 (76)	2.3 ± 0.9** (46)
0-39	6.3 ± 0.9	5.0 ± 0.6*	5.6 ± 0.4	4.5 ± 1.1** (71)	5.7 ± 1.1	5.1 ± 1.7 (89)	4.4 ± 0.7 (77)	2.4 ± 0.9** (42)
0-52	6.6 ± 1.2	5.1 ± 0.6*	5.9 ± 0.6	4.8 ± 1.3* (73)	5.6 ± 1.3	5.0 ± 1.6 (89)	4.2 ± 0.5 (75)	2.3±0.6** (41)

Data taken from Table 2, pp. 60-63, MRID 44802103.
[a] Percentage of controls; calculated by reviewer.
† Significantly different from controls: $p<0.05$ as calculated by reviewer using ANOVA.
Significantly different from controls: *$p < 0.05$, **$p<0.01$; ***$p<0.001$ as analyzed using Student's t-test using a pooled error variance.

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C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Mean food consumption values for selected intervals are presented in Table 4. No statistically significant differences in food consumption were reported. High-dose females generally consumed 8-15% less food than controls, but these differences did not attain statistical significance.

TABLE 4. Selected mean food consumption of male and female dogs administered RPA400727 for up to 52 weeks (g/dog/week) [a]

Weeks	Concentration (mg/kg/day)							
	0	2.5	25	150	0	2.5	25	150
	Males				Females			
-2 - -1	2650	2650	2650	2600	2550	2550	2600	2350 (92) [b]
1-13	2790	2740	2760	2710	2780	2750	2723	2420 (87)
14-26	2800	2740	2790	2790	2800	2780	2710	2440 (87)
27-39	2800	2750	2800	2790	2770	2770	2680	2450 (88)
40-52	2800	2740	2800	2800	2790	2650	2520	2380 (85)
1-52	2800	2740	2790	2770	2780	2740	2660	2430 (87)

Data taken from Table 3, pp. 64-67, MRID 44802103.
[a] Calculated by reviewer: Total amount consumed for the time interval/ number of weeks in the time interval = average amount consumed in g/dog/week
[b] Percentage of control

2. Water consumption

Water consumption was not measured.

3. Compound consumption

The compound was administered in a capsule. Dosages of 0, 2.5, 25, or 150 mg/kg/day were administered to both male and female dogs (Table 1).

4. Food utilization

Food efficiency was not calculated by the study author.

D. OPHTHALMOSCOPIC EXAMINATION

Ocular changes were evident in all high-dose males and 3/4 high-dose females. Examination of the lens of the eyes revealed early cataract formation as evidenced by posterior and anterior capsular opacities following the suture line configuration of the lens in all high-dose males and 2 high-dose females at 13 weeks, and in the third high-dose female at week 19. As noted by the study authors, the opacities appeared slightly different in each

animal and progressed at varying rates. However, the opacities in affected animals all reached the endpoint of total cataract formation with a dense nucleus and equatorial vacuoles. Total cataract formation was reached by week 25 in 3/4 males and by week 32 in the fourth male; in females, total cataract formation was reached by week 32 in 2/4 dogs, and by week 46 in the third female.

In addition to the cataracts, examination of the iris also indicated uveitis in 3/4 males and 2/4 females as evidenced by an irregular edge with a nodular appearance, blepharitis, conjunctivitis, only partially dilated pupils following administration of a mydriatic, and photophobia.

Retinal changes could not be observed because the cataracts obscured the fundus.

No treatment-related differences were observed in the Schirmer tear test.

E. BLOOD WORK

1. Hematology

Elevated platelet counts were noted in high-dose females at 24 and 50 weeks (128 and 136% of control values, respectively). No other potential treatment-related differences in hematology were observed in groups of treated dogs as compared with the controls. Other statistically significant differences in group parameters observed during the study either were not biologically significant (<10% change relative to control animals), or did not show a dose- or time-response. The study authors did point out differences observed in individual high-dose male and female dogs: one male and two females had high packed cell volumes and hemoglobin concentrations at 12 weeks, with the male similarly affected at 24 weeks.

High neutrophil and monocyte counts were present in the mid-dose female killed at week 5.

2. Clinical chemistry

Selected clinical chemistry changes are presented in Table 5. Alkaline phosphatase activity increased in a time-related manner in high-dose males (643-1513% of controls; $p<0.01$; 0.001) and females (394-833%; $p< 0.001$) at weeks 12, 24, and 50. Alanine aminotransferase activity was also elevated ($p<0.05$; 0.01) in high-dose males at weeks 12, 24, and 50 (162-192% of controls) and in females at weeks 24 and 50 (174 and 214% of controls, respectively). High-dose males also exhibited a time-related decrease in total cholesterol at weeks 12, 24, and 50 (58, 48, and 45% of controls, respectively; $p<0.01$), and decreases in total plasma proteins (85 and 90%; $p<0.01$) and albumin (89 and 84%; $p< 0.05$; 0.01) at 24 and 50 weeks, respectively. Total serum cholesterol was reduced in high-dose females at weeks 12, 24, and 50 (83, 72, and 70% of controls, respectively), but the decrease was statistically significant only at week 24 ($p<0.05$). As in the males, high-dose females had decreased total plasma proteins (93 and 88%; $p<0.01$) and albumin (83 and 81%; $p< 0.01$; 0.001) at

24 and 50 weeks, respectively. At 24 weeks, mid-dose females had statistically decreased cholesterol levels (71% of controls; $p<0.05$) and total plasma protein levels (93% of controls, $p<0.01$).

Clinical chemistry analysis of serum from the mid-dose female killed at week 5 revealed elevated alkaline phosphatase activity, high plasma cholesterol and albumin concentrations, and slightly lowered alanine aminotransferase activity and potassium and chloride concentrations when compared to pretreatment values.

TABLE 5. Selected clinical chemistry changes

Finding	Week	Dose (mg/kg/day)							
		Males				Females			
		0	2.5	25	150	0	2.5	25	150
Alkaline phosphatase (IU/L)	0	111	110	109	127	118	100	106	93
	12	68	74	88	437***	83	71	111*	326***
	24	51	73	86	705***	63	64	107***	385***
	50	68	66	85	1029**	72	72	113**	600***
ALT (IU/L)	0	17	21	19	17	20	17	21	15
	12	24	32	33	44**	28	25	34	43
	24	27	29	32	52*	27	24	32	47*
	50	37	36	37	60**	27	32	36	58**
Total cholesterol (mg%)	0	184	153	163	174	148	153	152	170
	12	163	140	144	95**	145	143	137	121
	24	161	144	128	78**	146	138	103*	105*
	50	163	143	124	73**	159	163	157	112
Total plasma protein (g%)	0	5.3	5.1	5.3	5.4	5.4	5.2	5.5	5.3
	12	5.6	5.2	5.3	5.1	5.1	5.4	5.2	5.1
	24	5.9	5.6	5.4	5.0**	5.5	5.5	5.1**	5.1**
	50	6.0	5.8	5.7	5.4**	6.0	5.8	5.8	5.3**
Albumin (g%)	0	2.1	2.2*	2.2*	2.2	2.3	2.3	2.3	2.1
	12	2.8	2.6	2.6	2.6	2.8	2.9	2.9	2.8
	24	2.7	2.8	2.5	2.4*	3.0	2.9	2.7	2.5**
	50	3.1	3.1	2.8*	2.6**	3.2	3.3	3.2	2.6***

Data taken from Table 7A-7D, pp. 87-102, MRID 44802103.
Significantly different from control: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

F. URINALYSIS

Selected results for the urinalysis are presented in Table 6 Statistically significant ($p<0.05$; 0.01) increases in specific gravity were observed in high-dose females at weeks 11 and 23 and high-dose males at week 23. Urine volume was also decreased in high-dose females at weeks 23 and 49, statistically so at week 23 ($p<0.05$), and in low- and mid-dose males at week 49. No other potential treatment-related changes in the urinalysis were observed.

TABLE 6. Selected urinalysis changes

Finding	Week	Dose (mg/kg/day)							
		Males				Females			
		0	2.5	25	150	0	2.5	25	150
Volume (mL)	0	142	143	146	126	138	118	88	108
	11	158	159	129	139	138	182	197	100
	23	173	117	118	102	150	155	192	38*
	49	208	83*	26**	137	187	143	124	40
Specific gravity	0	1.041	1.041	1.041	1.038	1.045	1.046	1.044	1.043
	11	1.042	1.043	1.039	1.049	1.039	1.044	1.041	1.055**
	23	1.041	1.044	1.041	1.051*	1.040	1.044	1.042	1.049*
	49	1.036	1.041	1.039	1.047	1.036	1.036	1.041	1.045

Data taken from Table 8D, pp. 103-106, MRID 44802103.
Statistically significant as compared with controls: *$p<0.05$; **$p<0.01$.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Statistically significant differences in organ weights in males and females were generally confined to high-dose animals. Selected differences are presented in Table 7.

In males, terminal body weights of treated groups were comparable to controls. High-dose males had decreased absolute and relative (to body weight and brain weight) prostate weights (35-38%; $p<0.05$) and increased absolute and relative testes weights (139-153%; $p<0.05$) as compared with controls. Although liver weights and adrenal weights were increased in high-dose males compared with controls (109-120% and 133-147% of controls, respectively), the increases were not statistically significant.

High-dose females had terminal body weights that were 70% of controls ($p<0.001$); terminal weights of mid-dose females were 87% of controls but the decrease did not reach statistical significance. It was because of the decreased terminal body weights that the reviewer calculated organ weights relative to brain weights. High-dose females had increased kidney weights relative to body weights (133% of controls;

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$p<0.01$), but absolute kidney weights and kidney weights relative to brain weights were comparable to controls. Absolute lung weights and lung weights relative to brain weights were decreased in high-dose females (76 and 79% of controls, respectively; $p<0.05$; 0.01), while lung weights relative to body weights were increased (113%; $p<0.05$). Liver and adrenal weights relative to body weights were elevated in high-dose females (142% and 134%, respectively; $p<0.05$); absolute liver and adrenal weights and liver and adrenal weights relative to brain weights were comparable to controls. Increased brain weights relative to body weights were increased in mid- and high-dose females as compared with controls. These increases are likely due to the lower terminal body weights in these groups.

TABLE 7. Selected organ weights

Organ	Dosage (mg/kg/day)							
	Males				Females			
	0	2.5	25	150	0	2.5	25	150
Terminal body weight (g)	14495	13440	14260	13438	13708	12730 (93) [a]	11910 (87)	9630** (70)
Kidneys: absolute (g)	61	62	66	64	58	55	53	54
relative to b.w. (%)	0.42	0.46	0.46	0.47	0.42	0.44	0.45	0.56**
relative to brain wt (%) [b]	0.78	0.78	0.85	0.88	0.78	0.69	0.69	0.75
Liver: absolute (g)	496	417	504	539	472	424	415	475
relative to b.w. (%)	3.45	3.12	3.53	4.03	3.46	3.38	3.48	4.93*
relative to brain wt (%) [b]	6.26	5.30	6.52	7.49	6.39	5.24	5.38	6.50
Lungs:absolute (g)	114	108	108	107	98	91	93	74**
relative to b.w. (%)	0.79	0.81	0.75	0.80	0.71	0.72	0.78	0.80* (113)
relative to brain wt (%) [b]	1.44	1.37	1.38	1.49	1.31	1.13	1.21	1.04* (79)
Adrenals: absolute (g)	1.41	1.45	1.30	1.88	1.78	1.60	1.58	1.69
relative to b.w. (%)	0.0098	0.0108	0.0092	0.0139 (141)	0.0130	0.0125	0.0132	0.0174* (134)
relative to brain wt (%) [b]	0.0178	0.0184	0.0170	0.0261 (147)	0.0240	0.0200	0.0204	0.0237
Prostate: absolute (g)	8.18	8.93	8.92	2.86*	—	—	—	—
relative to b.w. (%)	0.0570	0.0651	0.0626	0.0211* (37)	—	—	—	—
relative to brain wt (%) [b]	0.104	0.113	0.116	0.0393* (38)	—	—	—	—
Testes:absolute (g)	17.3	19.6	20.0	24.0*	—	—	—	—
relative to b.w. (%)	0.120	0.147	0.140	0.179* (149)	—	—	—	—
relative to brain wt (%) [b]	0.218	0.248	0.261	0.333* (153)	—	—	—	—

Data taken from Tables 9A and 9B, pp. 107-112, MRID 44802103.
Statistical significant as compared with controls: *p<0.05; **p<0.01.
[a] Percentage relative to controls; calculated by reviewer.
[b] Organ weight relative to brain weight; calculated by reviewer using data from Appendices 13A and 13B, pp. 236-243; statistical analysis of data performed by reviewer using ANOVA.

2. Gross pathology

Selected macroscopic findings are presented in Table 8. Gross macroscopic evaluation revealed opaque eyes in 4/4 high-dose males and 3/4 high dose females. A large liver was observed in 2/4 mid-dose and 2/4 high-dose males and 2/4 high-dose females; 1/4 control males also exhibited an enlarged liver. Thickened skin, predominantly of the pinnae and hocks, was noted in 1/4 low-dose and 3/4 high dose males, and 1/4 control and 3/4 high-dose females. No other potential treatment-related findings were noted in high-dose animals.

Gross necropsy of the mid-dose female killed at week 5 revealed emaciation, pallor of internal tissues, dark coloration of several lymph nodes, and enlargement of the liver.

TABLE 8. Selected macroscopic findings in dogs administered RPA400727 for up to 52 weeks

Finding	Dosage (mg/kg/day)							
	Males				Females			
	0	2.5	25	150	0	2.5	25	150
n	4	4	4	4	4	4	3	4
Eyes - opaque	0	0	0	4	0	0	0	3
Liver - enlarged	1	0	2	2	0	0	0	2
Skin - thickened	0	1	0	3	1	0	0	3

Data taken from Table 10, pp. 113-116, MRID 44802103.

3. Microscopic pathology

a. Non-neoplastic

Microscopic evaluation revealed lenticular degeneration of the eyes in 4/4 high-dose males and 3/4 high-dose females, with one high-dose female additionally exhibiting retinal edema (see Table 9). No ocular changes were noted in the fourth high-dose female. Vacuolation of cells of the *zona fascicularis* in the adrenals was observed in males at an incidence of 1/4, 1/4, 2/4, and 4/4 for the control, low-, mid-, and high-dose males, respectively, and in 1/3 and 3/4 mid- and high-dose females, respectively. Acanthosis/hyperkeratosis was also observed in 3/4 and 2/4 high-dose males and females, respectively, but it was stated if the affected areas were the same as those noted with thickened skin during the macroscopic examination. Other findings were not considered to be an effect of treatment.

Microscopic evaluation of tissues from the mid-dose female killed at week 5 revealed myofiber degeneration; inflammation of skeletal muscle; slight, multifocal perivascular cuffing in the brain; and slight erythrocytosis in several lymph nodes.

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TABLE 9. Selected microscopic findings in dogs administered RPA400727 for up to 52 weeks

Finding	Dosage (mg/kg/day)							
	Males				Females			
	0	2.5	25	150	0	2.5	25	150
n	4	4	4	4	4	4	3	4
Eyes - lenticular degeneration	0	0	0	4	0	0	0	3
Eyes - retinal edema	0	0	0	0	0	0	0	1
Adrenal cortex - vacuolation	1	1	2	4	0	0	1	3
Acanthosis/hyperkeratosis	0	0	0	3	0	0	0	2

Data taken from Table 11, pp. 117-123, MRID 44802103.

b. Neoplastic

Neoplastic lesions were not evaluated in this study.

III. DISCUSSION

A. DISCUSSION

Although transient in nature, the neurological signs observed between weeks 6-11 in high-dose males and high-dose females were considered an effect of treatment. Neurological signs, comprised of tremors, hyperactivity or hypoactivity, convulsions, and ataxia, were only observed in the high-dose groups.

Because similar findings were not observed in other animals, the moribund condition and euthanization of the mid-dose female was not attributed to treatment.

Some skin effects were observed more frequently in high-dose males and females, and may have been related to treatment. Thickened skin of the pinnae and other skin regions and reddened skin for body regions other than the pinnae were observed more frequently in high-dose males and females. Veterinary examination and gross necropsy confirmed the observations of thickened skin in the high-dose animals. Microscopic evaluation of skin revealed ancanthosis/hyperkeratosis in 3/4 high-dose males and 2/4 high-dose females, but it was not stated if the affected areas were the same as those previously noted. The reviewer does not agree with the study authors that the increased group incidences of reddened pinnae in males and females and reddened gums in high-dose females were clearly associated with treatment because the incidences were not consistently noted in affected animals except for one individual animal which skewed the group incidences.

Ocular toxicity was clearly associated with treatment of 150 mg/kg/day. Cataractogenesis was observed early into treatment, with ophthalmoscopic examination revealing cataracts

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in all high-dose males and two high-dose females at week 13, and in the third affected high-dose female at week 19. Cataracts were later observed grossly, and finally by microscopic evaluation. Although ophthalmoscopic examination suggested that uveitis accompanied the early stages of cataractogenesis in some of the high-dose males and females, no histopathological correlates were found at necropsy. The retinal edema noted in a high-dose female cannot definitively be attributed to treatment since it was not observed in any other animals. No ocular effects were observed in one of the high-dose females; the reason for this is not known.

Body weight gains were affected by administration of the high dose in both males and females. Females, however, were more severely affected, and the decrements in body weight gains were accompanied by significantly decreased absolute body weights and terminal body weights. Although body weight gains were also decreased in low-dose males, the decrements were not considered an effect of treatment because of a lack of dose response: body weight gains of mid-dose males were not affected. Although high-dose females generally consumed 8-15% less food than controls, the differences did not attain statistical significance. No other potential treatment-related changes in food consumption were noted.

Treatment of males and females with the high-dose resulted in hepatotoxicity as indicated by changes in serum biochemistry measurements. High-dose males had increased alkaline phosphatase and alanine aminotransferase activities and decreased levels of total serum cholesterol, total plasma proteins, and albumin. Most of these plasma changes were evident as early as 12 weeks and remained altered throughout the study until termination at 52 weeks. Similar plasma changes were observed in high-dose females, but the changes were generally not as severe as those observed in the males. Changes in the plasma were not accompanied by other hepatic changes, however. Liver weights of treated males were comparable to controls. Although high-dose females had increased liver weights relative to body weights (142% of controls), the reviewer believes this increase is the result of the decreased body weights observed in this group. This is supported by the observation that liver weights relative to brain weights were comparable to controls. Although macroscopic examination revealed an enlarged liver in 2/4 mid-dose males, 2/4 high-dose males, and 2/4 high-dose females, 1/4 control males also had an enlarged liver, and no histopathological correlates were found during microscopic examination.

Adrenal toxicity was evident as vacuolation of cells of the *zona fascicularis* in the adrenal cortex. Vacuolation was observed in 1/4, 1/4, 2/4, and 4/4 control, low-, mid-, and high-dose males, respectively, and in 1/3 and 3/4 mid- and high-dose females, respectively. These microscopic changes were not clearly associated with changes in adrenal weights. Although adrenal weights were increased in high-dose males (133-147% of controls), the increases were not statistically significant. Adrenal weights relative to body weights were statistically increased in high-dose females, but again, the reviewer believes this is due to the decreased terminal body weights since absolute adrenal weights and adrenal weights relative to brain weights were comparable to controls.

Although some effects were observed in the kidneys, they were not considered an adverse effect of treatment. Urinalysis indicated that high-dose males and female produced a more concentrated urine: urine volume was generally decreased while specific gravity was generally increased. No other urinalysis findings were noted. Kidney weights were not affected. Although high-dose females had statistically increased kidney weights relative to body weights (133% of controls), the reviewer believes this increase is the result of the decreased body weights observed in this group. This is supported by the observation that kidney weights relative to brain weights were comparable to controls. No macroscopic or microscopic changes were observed in the kidneys of treated dogs.

High-dose males had significantly decreased prostate weights and increased testes weights. No histopathological correlates were found. The significance of these changes in organ weights is currently unknown.

A LOAEL of 150 mg/kg/day was identified for dogs based on decreased absolute body weights of female dogs, decreased weight gain by male and female dogs, and treatment-related toxicity to the eye, liver, and adrenals. The corresponding NOAEL is 25 mg/kg/day.

This chronic oral toxicity study in the dog is classified as **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a chronic oral toxicity study [OPPTS 870.4100 (§83-1)] in dogs.

B. STUDY DEFICIENCIES

No major study deficiencies were identified.