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ISOXAFLUTOLE

Study Type: 83-1b; Chronic Toxicity to Dogs by Repeated Dietary Administration for 52 Weeks

Work Assignment No. 2-8C (MRID 43573218)

Prepared for

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

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
William Spangler, Ph.D

Signature: William J. Spangler
Date: 02/14/97

Secondary Reviewer:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 2/16/97

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 2/21/97

Quality Assurance:
Michael Norvell, Ph.D., D.A.B.T.

Signature: Michael Norvell
Date: 02/18/97

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ISOXAFLUTOLE (RPA 201772) **Chronic Oral Study (83-1b)**

EPA Reviewer: Sanjivani B. Diwan, Ph.D. S. Diwan, Date 2/26/97
Review Section I, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Jess Rowland, M.S. Jess Rowland, Date 2/27/97
Acting Section Head, Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [feeding] - dogs

OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

DP BARCODE: D224202 SUBMISSION CODE: S501233
P.C. CODE: 123000

TEST MATERIAL (PURITY): RPA 201772 (Isoxaflutole; 98.7% a.i.)

SYNONYMS: 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole

CITATION: Brooker, A.J. (1994) RPA 201772: Toxicity to Dogs by Repeated Dietary Administration for 52 Weeks. Huntingdon Research Centre Ltd., P.O. Box 2 Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project Study ID RNP/427. December 16, 1994. MRID 43573218. Unpublished.

SPONSOR: Rhône Poulenc Agriculture, Fyfield Road, Ongar, Essex, England.

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRID 43573218), RPA 201772 (Isoxaflutole 98.7% a.i.) was administered to five beagle dogs/sex/dose in the diet at dose levels of 0, 240, 1,200, 12,000, or 30,000 ppm (0, 8.56, 44.81, 453, or -- mg/kg/day, respectively, for males; 0, 8.41, 45.33, 498, or 1,254 mg/kg/day, respectively, for females) for 52 weeks. The 52 week mean intake value for males in the 30,000 ppm treatment group was not available because all dogs in that group were sacrificed after 26 weeks due to severe chronic reaction to the test substance.

Dogs in the ≥12,000 ppm treatment groups (both sexes) had lower mean body weights than dogs in the control group; significantly lower in females. Females in these treatment groups showed a significant decrease in red blood cell indices (hematocrit, RBC, and hemoglobin) compared to controls. Males in the 30,000 ppm group exhibited marked reduction in these parameters up to termination after 26 weeks of treatment. Males and females at 12,000 ppm and females at 30,000 ppm exhibited significant concomitant increases in platelet counts. At ≥12,000 ppm, males and females exhibited significantly increased absolute and

relative liver weights with friable surfaces and histopathological changes such as hepatocellular swelling, centrilobular clumping and margination of cytoplasmic staining, and centrilobular necrosis and fibrosis. There was an increased incidence of hypertrophy of thyroid follicular epithelium in males at 12,000 ppm and in males and females at 30,000 ppm. Evidence of prominent hematopoiesis was observed in the sterna and/or femurs and joints of males and females in these treatment groups and an increased degree of extramedullary hematopoiesis was apparent in spleens of males at 30,000 ppm only.

At treatment levels of 240 or 1,200 ppm, there were no observed treatment-related effects on mortality, clinical appearance, body weight changes, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, or gross and histopathology.

Generally the histopathological changes observed in livers, the evidence of prominent hematopoiesis in bone marrow, and the decreased red cell parameters seen in the hematology results, from males and females receiving 12,000 ppm or 30,000 ppm of RPA 201772, correlated well with classical symptoms of intravascular hemolysis (chronic hemolytic anemia).

The LOEL is 12,000 ppm (453 mg/kg/day for males; 498 mg/kg/day for females), based on reduced weight gains compared to controls and intravascular hemolysis with associated clinical chemistry and histopathological findings. The NOEL is 1,200 ppm (44.81 mg/kg/day for males; 45.33 mg/kg/day for females).

This chronic toxicity study in the dog is **Acceptable** and does **satisfy** the guideline requirements for a chronic oral study (§83-1b) 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: RPA 201772
   Description: Cream crystalline solid
   Lot/Batch #: 21 ADM 93
   Purity: 98.7% a.i.
   Stability of compound: 14 days in formulated diets at room temperature
   Storage: At room temperature, protected from light
   CAS #: 141112-29-0

   Structure: Not available

2. Vehicle: Basal diet

3. Test animals: Species: Dog
   Strain: Beagle
   Age and weight at study initiation: 22-26 weeks of age; body weight range 7.9-10.4 kg (49 dogs; one replacement dog weighed 13.6 kg)
   Source: Interfauna UK Limited, Wyton, Huntingdon, England
   Housing: Individual indoor kennels with up to two animals of the same sex and dose with partitioning to segregate for individual assessment of clinical signs and food consumption.
   Diet: SDS dog diet "A"; 400 g/day (residue weighed and discarded)
   Water: Tap water ad libitum
   Environmental conditions:
   Temperature: 15-23°C
   Humidity: Not provided
   Air changes: 12 per hour
   Photoperiod: 12-hour light/12-hour dark
   Acclimation period: 4-weeks minimum, except 2-weeks for replacement dog

B. STUDY DESIGN:

1. In life dates - Start: 05/06/93 End: 05/10/94

2. Animal assignment

   Dogs (25 of each sex) were assigned to the test groups in Table 1 on the basis of body weight employing a pseudo-random body weight stratification

procedure while avoiding inclusion of litter mates within the same group where possible.

TABLE 1: STUDY DESIGN[a]

Test Group	Conc. in Diet (ppm)	Achieved Intake[b] (mg/kg/day)		Animals Assigned	
		male	female	male	female
1	0	0	0	5	5
2	240	8.56	8.41	5	5
3	1,200	44.81	45.33	5	5
4	12,000	453	498	5	5
5	30,000	--[c]	1,254	5	5

[a] Data obtained from Table 3, pages 61-62 in the study report.
[b] Group means after 52 weeks of treatment.
[c] Group sacrificed in week 27 for humane reasons.

3. Dose selection rationale

No rationale was given for the doses selected.

4. Diet preparation and analysis

Diet was prepared weekly by grinding the test substance directly into untreated basal diet (SDS dog diet "A") and blending with a Turbula mixer for a minimum period of 5 minutes. The 12,000 and 30,000 ppm diets were then prepared by direct dilution of the premix with additional quantities of untreated diet. Homogeneity was achieved by mixing in a double cone blender for 7 minutes. A second premix was prepared by direct dilution of the first premix with untreated diet and was blended for 7 minutes in a double cone blender. The second premix was used to prepare the 240 and 1,200 ppm diets. The diets were stored at ambient temperature under animal room conditions. Dietary concentrations of RPA 201772 were analyzed from duplicate sub-samples (10 g) taken at discharge from the blender for analytical method validation. Homogeneity and stability were tested by taking random duplicate samples from specimen batches (35 kg) of 250 and 30,000 ppm diets collected from the top, center, and

bottom of the container. Stability was determined over a two week period at ambient temperature. Samples of the formulations prepared in the first week and Weeks 2, 3, 4, 13, 26, 39, and 52 were also analyzed to check the accuracy of preparation. Contingency samples of formulations prepared in Weeks 8, 17, 21, 30, 34, 43, and 47 were retained frozen for possible analysis. Because of poor analytical results obtained in Week 4, the contingency samples taken in Weeks 4 and 8 at 240 and 1,200 ppm were analyzed and additional contingency samples were taken from Week 13 to ensure samples were available each time the diets were prepared.

Results - Homogeneity Analysis: The mean of duplicate samples taken from the top, middle, and bottom of the mixer were 255 and 29,900 ppm for the 250 and 30,000 ppm nominal diets with respective coefficients of variation (CV) at 1.68 and 1.48%.

Stability Analysis: For the 250 and 30,000 ppm nominal diets, the RME (relative mean error, representing deviation from time zero) values for the stability results at 7 and 14 days were +5.9 and +1.6% (250 ppm) and +2.7 and -1.0% (30,000 ppm), respectively.

Concentration Analysis: Mean results were within +10%/-7% of nominal at all test intervals except for Week 4 Groups 2 and 3 which were approximately 40% below nominal. This result was confirmed by analysis of archived Week 4 diet samples. Additional analysis was performed on Group 2 and 3 diets prepared for Week 8 of the study and the results were within 2% of nominal . The procedural recovery data, obtained during method validation, indicate that the analytical method was both precise and accurate: a mean procedural recovery value of 98.7% ± 2.42 CV (n = 8) was obtained for the 250 ppm nominal diet and 100.1% ± 2.56 CV (n = 8) for the 30,000 ppm nominal diet.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics - The following sequence of statistical tests was used for food consumption, body weight gain, clinical chemistry, hematology, clinical

pathology, and organ weight (except organ-body-weight) for each sex, together and separately: If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analyzed by the methods of Fisher and Mantel. Otherwise, a Bartlett test was applied to test for heterogeneity of variance between treatments. Where significant (1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance (ANOVA) was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, an analysis of ranks (Kruskal-Wallis analysis) was used. Except for pre-dose data, analyses of variance were followed by Student's t-test and Williams' test for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported. The analysis of ranks (Kruskal-Wallis analyses) were followed by the non-parametric equivalents of these tests using Shirley's method. For pre-dose data a Student's t-test followed ANOVA to show any intergroup variability prior to treatment initiation.

C. METHODS:

1. Observations:

Animals were inspected at least once a day for signs of toxicity and mortality.

2. Body weight

Animals were weighed prior to feeding and once a week throughout the experimental period.

3. Food consumption and compound intake

Food consumption for each animal was determined daily and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg/day) values were calculated weekly as time-weighted

averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

Eyes were examined using a Keeler indirect ophthalmoscope and a Kowa hand held slit lamp biomicroscope prior to study initiation and during Weeks 4, 13, 26, and 52 of dosing.

5. Blood

Blood was collected prior to treatment (Weeks -2 and -1) and during Weeks 13, 26, 39, and 52. Samples were collected from the jugular or cephalic vein of all animals following an overnight fast. Hematology and clinical chemistry analyses were conducted on all samples. Additional blood samples were taken from one male dog (#733) in the 30,000 ppm group during Week 19 and from animals in the control, 12,000 and 30,000 ppm treatment groups during Weeks 22 and 24 for evaluation of specific hematological parameters. 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*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

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

* Required for chronic studies based on Subdivision F Guidelines

c. Bone Marrow Examination

Just prior to necropsy, the animals were weighed and a bone marrow smear obtained from each animal by sternebral puncture. Each smear was stained with a modified Wright's stain and a full differential marrow count was performed counting a minimum of 200 cells.

6. Urinalysis

At two- and one-weeks pretreatment and during Weeks 13, 26, 39, and 52, urine was collected for 16 hours from animals deprived of water for 5 hours prior to and during sampling. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bile pigments*
X	pH	X	Heme pigments*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

* Required for chronic studies

7. Sacrifice and Pathology

After 52-weeks of treatment all animals (except for the 30000 ppm male treatment group sacrificed after 26 weeks of treatment) were sacrificed on schedule and were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	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*		
X	Duodenum*	XX	Spleen*	XX	Pituitary*
X	Jejunum*	XX	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+		
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland
X	Gall bladder*	XX	Epididymides	X	Mammary gland*
XX	Pancreas*	XX	Prostate	X	Parathyroids*++
			Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries*+		
X	Trachea*	XX	Uterus*		
XX	Lung*	X	Vagina		OTHER
	Nose			X	Bone*
	Pharynx			X	Skeletal muscle*
	Larynx			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.
++ Organ weight required for non-rodent studies.

II. **RESULTS:**

A. Observations

1. Mortality - No animals died during the study. All males in the 30,000 ppm treatment group were sacrificed, for humane reasons, after 26 weeks of treatment due to apparent anemia suspected from pallor of the gums and confirmed by hematology.

2. Clinical signs of toxicity - Pale gums were recorded for one male and one female in the 12,000 ppm treatment group during Weeks 28-51 and 23-27, respectively. Three females in the 12,000 ppm and

two females in the 30,000 ppm treatment group were noted to have a thin appearance on occasion. At 30,000 ppm, thin appearance was noted in 3/5 males and pale gums were noted in all five males sacrificed after 26 weeks of treatment. One 30,000 ppm male was found prostrate twice in the week (Week 25) prior to sacrifice.

B. Body weight - There was a statistically significant decrease in mean body weight gain of females in the 12,000 and 30,000 ppm treatment groups over 52 weeks of treatment (Table 2). Mean body weight gain of males in the 12,000 ppm treatment group was lower than the controls although the difference did not attain statistical significance. Weight gains at 26 weeks were 65% and 56% of control gain in males and females in the 12,000 ppm group, respectively. In the 30,000 ppm group dogs, a mean weight loss of 0.58 kg was seen in males compared to a gain of 1.66 kg in control males and in females, the gain was 33% of the controls. At 52 weeks, mean weight gain in the 12,000 ppm group males and females was 60% and 55% of control gain, respectively. In the 30,000 ppm group dogs, the mean weight loss was 0.8 kg (36% of control gain) for females compared to g gain of 2.2 kg for the control group. There were no treatment-related effects on the overall body weight change at 240 or 1,200 ppm.

TABLE 2. MEAN BODY WEIGHTS AND BODY WEIGHT GAINS OF BEAGLES[a]

Conc. in Diet (ppm)	Body Weight (kg)				Total Body Weight Gain (kg) Weeks 0-52
	0 Weeks	13 Weeks	26 Weeks	52 Weeks	
Male					
0	9.3	10.6	11.0	11.4	2.0
240	9.5	11.1	11.5	11.8	2.3
1,200	9.4	10.7	10.8	11.3	1.8
12,000	9.6	10.6	10.7	10.8	1.2
30,000	9.3	9.6	9.5*	N/A	N/A
Female					
0	9.0	10.6	10.8	11.2	2.2
240	9.2	11.1	11.7	12.2	3.0
1,200	9.0	10.3	10.8	11.2	2.2
12,000	8.7	9.4	9.7*	9.9*	1.2*
30,000	9.0	9.5	9.6**	9.8**	0.8**

[a] Data obtained from Table 1, pages 57-58 in the study report.
* Significantly different ($p < 0.05$) from the control.
** Significantly different ($p < 0.01$) from the control.

C. Food consumption and compound intake

1. Food consumption - Food consumption was comparable between the control and treatment groups. Weekly average food consumption during the 52-week feeding study was 2,786-2,800 g/dog/week for males and females (Data obtained from Table 2, pages 59-60 in the study report). At sacrifice (study week 26) the weekly food consumption for males in the 30,000 ppm treatment group was 2,798 g/dog/week.

2. Compound consumption - Weekly dietary consumption of RPA 201772 (mg/kg/day) by male and female dogs is given in Table 1.

D. Ophthalmoscopic examination - No treatment-related ophthalmological abnormalities were noted.

E. Blood work

1. Hematology - Females in the 12,000 and 30,000 ppm treatment groups generally showed a significant decrease in mean red cell indices (hematocrit, RBC, and hemoglobin) compared to controls (Table 3) from Week 13 through Week 52. Males in the 12,000 ppm treatment group did not generally exhibit these decreases on the basis of mean value although individual animals did show decreased red cell indices from Week 22 (Appendix 4, pages 170-207 of the study report). Although terminated after 26 weeks due to treatment-related chronic toxicity, the 30,000 ppm group males exhibited marked reduction in red cell indices through the 26-week treatment period. Occasional reduction in red cell parameters in females in the 1,200 ppm treatment group was only slight and sporadic and was not statistically significant. An increase in platelet count was recorded for males and females (Tables 3 and 4) receiving 12,000 ppm and females receiving 30,000 ppm RPA 201722, with the differences compared to controls considered statistically significant at both dosages on all occasions with the exception of Week 39.

TABLE 3. RED BLOOD CELL, HEMOGLOBIN, PLATELET, AND HEMATOCRIT LEVELS IN FEMALE DOGS FOLLOWING 13, 26, 39, AND 52 WEEKS OF DOSING.[a]

Weeks of Dosing	Dose level (ppm)				
	0	240	1,200	12,000	30,000
Red Blood Cells (x 10^6/mm^3)					
13	6.9	5.9*	5.8*	6.1*	6.4*
26	6.9	6.3	6.4	5.8*	6.0**
39	6.7	6.5	6.1	5.7	6.0
52	7.2	6.4*	6.5*	6.0*	6.9*
Hemoglobin (g/dl)					
13	15.6	14.6	13.8	14.3	14.5
26	16.8	15.9	15.7	14.2*	14.0**
39	15.9	16.1	15.1	13.7*	14.0*
52	17.5	15.7	15.8	14.3*	15.5*
Hematocrit (%)					
13	56	51	48*	49*	50*
26	55	52	51	46**	46**
39	51	51	48	44*	46*
52	58	53	53	49*	54*
Platelets (x10^3/mm^3)					
13	297	320	320	419*	426*
26	308	324	334	459*	421*
39	328	319	306	439	425
52	305	356	320	471*	414*

[a] Data obtained from Table 5, pages 68-79, in the study report.
* Statistically different from the controls, $p < 0.05$.
** Statistically different from the controls, $p < 0.01$.

TABLE 4. RED BLOOD CELL, HEMOGLOBIN, PLATELET, AND HEMATOCRIT LEVELS IN MALE DOGS FOLLOWING 13, 26, 39, AND 52 WEEKS OF DOSING.[a]

Weeks of Dosing	Dose level (ppm)				
	0	240	1,200	12,000	30,000
Red Blood Cells (x 10^6/mm^3)					
13	5.4	5.7	5.9	6.0	4.9
26	5.8	6.0	6.2	5.8	3.3
39	6.2	6.1	6.2	6.0	N/A
52	6.2	6.3	6.6	6.6	N/A
Hemoglobin (g/dl)					
13	12.9	14.1	13.8	14.2	11.1
26	14.2	15.1	14.9	14.1	8.0
39	14.9	15.0	14.6	14.4	N/A
52	14.7	15.7	15.3	15.4	N/A
Hematocrit (%)					
13	46	49	50	50	40
26	47	49	49	47	28
39	47	48	47	47	N/A
52	49	52	52	53	N/A
Platelets (x10^3/mm^3)					
13	361	337	352	407	423
26	357	353	380	476	334
39	359	358	365	401	N/A
52	345	396	357	411	N/A

[a] Data obtained from Table 5, pages 68-71, in the study report.
No statistical difference from the controls, p >0.05.

TABLE 5. ALBUMIN, ALKALINE PHOSPHATASE, CALCIUM, AND GLUTAMIC-PYRUVATE TRANSAMINASE LEVELS IN DOGS (MALE AND FEMALE VALUES COMBINED) FOLLOWING 13, 26, 39, AND 52 WEEKS OF DOSING.[a]

Weeks of Dosing	Dose level (ppm)				
	0	240	1,200	12,000	30,000
Albumin (g/dl)					
13	2.8	2.8	2.6	2.3**	2.2**
26	2.8	2.9	2.8	2.3**	2.2**
39	2.8	2.9	2.7	2.3**	2.3**[b]
52	2.9	2.8	2.7	2.3**	2.3**[b]
Alkaline phosphatase (mU/ml)					
13	184	163	179	411**	555**
26	193	150	162	575**	902**
39	135	148	157	563**	813**[b]
52	121	123	150	500**	1029**[b]
Calcium (meq/l)					
13	5.4	5.4	5.3	5.1**	5.0**
26	5.4	5.4	5.4	5.1**	5.0**
39	5.2	5.1	5.1	5.0**	5.0[b]
52	5.3	5.1	5.1	4.9**	4.9**[b]
Glutamic-pyruvate transaminase (mU/ml)					
13	23	30	25	35*	60**
26	23	27	26	35**	39**
39	23	28	24	35**	43*[b]
52	22	26	23	30*	29[b]

[a] Data obtained from Table 6, pages 80-91, in the study report.
[b] Data for females only; males sacrificed after Week 26.
* Statistically different from the controls, $p < 0.05$.
** Statistically different from the controls, $p < 0.01$.

2. Clinical chemistry - Male and female beagles in the 12,000 ppm and females in the 30,000 ppm treatment groups had lower serum albumin levels from Week 13 on than controls. This also resulted in corresponding reductions in total protein and a lower albumin/globulin ratio. Both sexes in the 12,000 ppm group and females in the 30,000 ppm group had significantly higher group mean plasma glutamic-pyruvate transaminase and alkaline phosphatase levels than controls. Because there were no corresponding increases in other liver function indicator enzymes (γ-glutamyl transferase or 5'nucleotidase), these increases were not considered due to direct liver damage. Females in the 30,000 ppm treatment group exhibited a consistently high plasma urea level. From Week 13 on, a treatment-related lowering of serum calcium was observed in both sexes in the 12,000 and 30,000 ppm treatment groups. No treatment-related changes were found in bone marrow smears.

F. Urinalysis - During Week 39, males in the 240, 1,200, and 12,000 ppm treatment groups and females in the 1,200, 12,000 and 30,000 ppm treatment groups exhibited significant but sporadic increases in urinary pH during study week 13. Males in the 240, 1,200, 12,000, and 30,000 ppm treatment groups at 26-weeks and females in the 1,200, 12,000, and 30,000 ppm treatment groups at 13-weeks also exhibited significant but sporadic increases in urinary specific gravity. These changes were not considered to be toxicologically significant.

G. Sacrifice and Pathology

1. Organ weight - Absolute and relative liver weights were elevated for 2 males in the 240 ppm treatment group, 3 males, and 1 female in the 1,200 ppm treatment groups, and for all animals in the 12,000 and 30,000 ppm treatment groups. Increased liver weights in the 240 and 1,200 ppm group female dogs were only slightly higher than historical controls and were considered adaptive changes rather than due to toxicity. However, liver weight increases in the 12,000 and 30,000 ppm treatment groups were considered significant and treatment-related.

   Absolute and relative (to body weight) liver weight data are presented in Table 6.

TABLE 6. MEAN LIVER WEIGHTS(g) and LIVER-TO-BODY WEIGHT RATIOS IN DOGS FOLLOWING 52 WEEKS of DOSING[a]

Dose Level (ppm)	Males		Females	
	Grams	Percent	Grams	Percent
0	399.6	3.49	363.8	3.26
240	442.6	3.76	413.3*	3.43
1,200	457.1	4.09	417.2*	3.77
12,000	599.6**	5.54	539.1**	5.40
30,000	NA	NA	553.0**	5.72

[a] Data obtained from Table 8, pages 98 and 100, in the study report.
* Significantly different from controls, $p<0.05$.
** Significantly different from controls, $p<0.01$.

Kidney weights (relative and absolute) were elevated for both sexes in the 1,200 and 12,000 ppm treatment groups and for females receiving 30,000 ppm compared to non-treated controls, however these values were not considered elevated when compared to historical controls.

Elevated adjusted thyroid weights were noted for both sexes in the 12,000 ppm treatment group and in the 30,000 treatment group females. Again, these values were not elevated when compared to historical laboratory controls.

Mean spleen weights of males in the 12,000 ppm treatment group were higher than controls, however the total difference was attributable to one animal showing pale gums and other signs of anemia.

2. Gross pathology - Treatment-related gross pathological changes were limited to friable surfaces of livers from male and female dogs in the 12,000 and 30,000 ppm treatment groups. Other changes noted, such as thin ventricular heart walls and gelatinous foci in bile from 12,000 and 30,000 ppm females, were not corroborated by histochemical changes.

3. Microscopic pathology

a) Non-neoplastic - Histopathological abnormalities observed are given in Tables 7 and 8. Examination of thyroids revealed an increased incidence of hypertrophy of follicular epithelium in males and females (trace to minimum) in the 12,000 ppm and in males and females (trace to moderate) in the 30,000 ppm groups.

TABLE 8. LIVER, SPLEEN, THYMUS, THYROID, TESTES, EPIDIDYMIDES, AND BONE MARROW ABNORMALITIES OBSERVED IN MALE DOGS (AFFECTED/TOTAL) FOLLOWING 52 WEEKS OF TREATMENT WITH RPA 201772.[a]

Observation/severity	Treatment rate (ppm)				
	0	240	1,200	12,000	30,000[b]
Liver					
Hepatocellular swelling, trace	0/5	0/5	0/5	3/5	4/5
minimal	0/5	0/5	0/5	0/5	1/5
Clumping & margination of cytoplasmic staining					
trace	0/5	0/5	0/5	0/5	3/5
Centrilobular necrosis and fibrosis					
minimal	0/5	0/5	0/5	2/5	2/5
Dilated centrilobular sinusoids					
moderate	0/5	0/5	0/5	0/5	1/5
Occasional vacuolated hepatocytes	0/5	0/5	0/5	0/5	0/5
Extra medullary hematopoiesis					
trace	0/5	0/5	1/5	0/5	1/5
minimal	0/5	0/5	0/5	0/5	3/5
Spleen					
Extramedullary hematopoiesis					
trace	2/5	2/5	3/5	3/5	1/5
minimal	0/5	0/5	1/5	0/5	1/5
moderate	0/5	0/5	0/5	0/5	2/5
Thymus					
Involution minimal	0/5	0/5	0/5	4/5	2/5
moderate	0/5	0/5	1/5	0/5	3/5
Thyroid					
Hypertrophy of follicular epithelium					
trace	1/5	1/5	1/5	2/5	1/5
minimal	0/5	0/5	0/5	1/5	2/5
moderate	0/5	0/5	0/5	0/5	1/5
Sternum					
Prominent hematopoiesis	0/5	0/5	0/5	1/5	2/5
Femur and Joint					
Prominent hematopoiesis	0/5	0/5	0/5	1/5	4/5
Testes					
Multinucleate cells in tubules					
minimal	0/5	0/5	0/5	0/5	2/5
Reduced spermatogenesis	0/5	0/5	0/5	0/5	1/5
Epididymides					
Round spermatids minimal	0/5	0/5	0/5	0/5	3/5
Absence of spermatids in majority of tubules	0/5	0/5	0/5	0/5	2/5

[a] Data obtained from pages 37-41 in the study report.

[b] All sacrificed in extremis after 26 weeks of treatment.

TABLE 9. LIVER, SPLEEN, THYMUS, THYROID, OVARIES, AND BONE MARROW ABNORMALITIES OBSERVED IN FEMALE DOGS (AFFECTED/TOTAL) FOLLOWING 52 WEEKS OF TREATMENT WITH RPA 201772.[a]

Observation/severity	Treatment rate (ppm)				
	0	240	1,200	12,000	30,000
Liver					
Hepatocellular swelling, trace	0/5	0/5	0/5	2/5	0/5
minimal	0/5	0/5	0/5	2/5	2/5
moderate	0/5	0/5	0/5	0/5	3/5
Clumping & margination of cytoplasmic staining					
trace	0/5	0/5	0/5	4/5	3/5
minimal	0/5	0/5	0/5	0/5	2/5
Centrilobular necrosis and fibrosis	0/5	0/5	0/5	0/5	0/5
Dilated centrilobular sinusoids	0/5	0/5	0/5	0/5	0/5
Occasional vacuolated hepatocytes	0/5	1/5	0/5	3/5	5/5
Extra medullary hematopoiesis	0/5	0/5	0/5	0/5	0/5
Spleen					
Extramedullary hematopoiesis					
trace	3/5	4/5	3/5	2/5	2/5
minimal	0/5	0/5	1/5	0/5	0/5
Thymus					
Involution minimal	1/5	0/5	0/5	1/5	2/5
moderate	0/5	0/5	0/5	1/5	0/5
Thyroid					
Hypertrophy of follicular epithelium					
trace	0/5	0/5	0/5	1/5	3/5
minimal	0/5	0/5	0/5	0/5	1/5
moderate	0/5	0/5	0/5	0/5	1/5
Sternum					
Prominent hematopoiesis	0/5	0/5	0/5	2/5	0/5
Femur and Joint					
Prominent hematopoiesis	0/5	0/5	0/5	1/5	1/5
Ovaries					
Corpora lutea present	5/5	4/5	3/5	3/5	1/5

[a] Data obtained from pages 37-41 in the study report.

Liver changes were characterized by hepatocellular swelling in males and females in the 12,000 and 30,000 ppm treatment groups. Severity ranged from trace to minimum in males at both doses and in the 12,000 ppm females and minimum to moderate in the 30,000 ppm group females. Centrilobular clumping and margination of cytoplasmic staining were observed in 12,000 ppm group females and in males and females in the 30,000 ppm group.

Centrilobular necrosis and fibrosis (predominantly minimum) occurred in 2 males each in the 12,000 and 30,000 ppm treatment groups and dilated centrilobular sinusoids occurred in one male in the 30,000 ppm group. Occasional vacuolated hepatocytes were observed in females, but not in males. No microscopic changes were observed that might be related to increased liver weights in beagles receiving dietary levels of 240 or 1,200 ppm RPA 201772.

Evidence of prominent hematopoiesis was observed in the sternum and/or femur and joint of some males and females in the 12,000 and 30,000 ppm treatment groups. In the 30,000 ppm treatment group, trace to moderate extramedullary hematopoiesis was observed in the livers of 4 males and an increased degree of extramedullary hematopoiesis was evident in the spleens of 4 males and 2 females. These changes correlated with the decreased red cell parameters seen in the hematology results in both sexes in the 12,000 ppm group and females in the 30,000 ppm RPA 201772 treatment groups.

III. DISCUSSION

A. Investigator's Conclusions

The chronic LOEL of RPA 201772 is 12,000 ppm (453 mg/kg/day in males, 498 mg/kg/day in females), based on intravascular hemolysis with associated clinical chemistry and histopathological findings. The chronic NOEL is 1,200 ppm (44.81. mg/kg/day in males, 45.33 mg/kg/day in females).

At 30,000 ppm, the response of male beagles was sufficiently severe to necessitate sacrifice of all animals in the treatment group after 26 weeks of feeding.

B. Reviewer's Discussion

No animals died during the study, however males in the 30,000 ppm treatment group were euthanized after 26 weeks of treatment due to severe anemia. Clinical signs consisting of thin appearance (3/5) and pale gums (5/5) were observed and one dog was found prostrate twice in the week prior to sacrifice. Weight loss was observed in 3/5 males in the 30,000 ppm group; a total of 3.0 kg was lost by week 26. Mean weight loss for the group was -0.58 kg compared to a mean gain of 1.66 kg in controls at 26 weeks. One 30,000 ppm group male (#733) had severe decreases in red blood cell parameters by week 13 (e.g.,

HGB of 5.3g/dL and RBC of $2.2x10^6/mm^3$). This prompted monitoring hematology parameters in the control, 12,000 ppm and 30,000 ppm group dogs every 2 weeks during study weeks 20-26. Similar effects developed in two additional 30,000 ppm group males (weeks 22 and 26). At week 26, the reticulocyte counts were markedly elevated in all three dogs and red cell counts for all males, prior to sacrifice, ranged from 0.9 to $5.1x10^6/mm^3$. Bone myelograms on the sacrificed dogs (report p 283) showed a reduced myeloid:erythroblast ratio (M:E= 0.42 compared to a normal value of 1-2), indicating a greatly increased turnover of erythroblasts in partial compensation of the anemia. In the sacrificed group of males, spleen and liver weights were abnormally high.

Pale gums were observed in 1/5 males and 1/5 females in the 12,000 ppm treatment group. Three females in the 12,000 ppm and 2/5 females in the 30,000 ppm groups occasionally appeared thin.

There was a significant decrease in mean body weight gain of 12,000 and 30,000 ppm (55 and 36%, respectively) group females over 52 weeks of treatment compared to controls. Mean body weight gain in the 12,000 ppm group males was lower, but not statistically significant. Prior to sacrifice after 26 weeks, males in the 30,000 ppm treatment group showed significantly lower weight gains (60%) compared to controls. Lowered weight gains were not due to loss of appetite as evidenced by comparable food consumption between control and treatment groups.

No treatment-related ophthalmological abnormalities were noted.

Significant decreases in red cell indices (hematocrit, RBC, and hemoglobin) were exhibited by the 12,000 and 30,000 ppm group females compared to controls. The decreases in mean values were significant in females and toxicologically important, but not statistically significant in males. No changes in RBC parameters at the lower doses were considered of importance since all values were within their normal ranges. There were significant increases in platelet counts for both sexes in the 12,000 ppm and for females in the 30,000 ppm treatment group.

Dogs in the 12,000 ppm and females in the 30,000 ppm treatment groups had significantly lower serum albumin and calcium levels and significantly higher group mean glutamic-pyruvate transaminase and alkaline phosphatase levels than controls.

No toxicologically significant changes in urine parameters were observed in the 12,000 or 30,000 ppm group dogs.

Significant and treatment-related increases in absolute and relative liver weights were observed for dogs in the 12,000 ppm and 30,000 ppm treatment groups. Gross pathological changes in livers were limited to the friable surfaces.

Histopathological changes in livers in the 12,000 and 30,000 ppm group dogs were characterized by hepatocellular swelling in males and females in the 12,000 and 30,000 ppm groups. Centrilobular clumping and margination of cytoplasmic staining was observed in 12,000 ppm group females and in males and females in the 30,000 ppm treatment group. Centrilobular necrosis and fibrosis occurred in males in the 12,000 and 30,000 ppm groups and dilated centrilobular sinusoids occurred in a male in the 30,000 ppm group. Occasional vacuolated hepatocytes were observed in females in the 12,000 and 30,000 ppm treatment groups.

There was prominent evidence of hematopoiesis in the sterna and/or femurs and joints in dogs from the 12,000 and 30,000 ppm treatment groups. Increased incidences of extramedullary hematopoiesis were observed in the livers of 30,000 ppm males and in spleens of the 30,000 ppm group males and females.

Generally the histopathological changes observed in livers, the evidence of prominent hematopoiesis in bone marrow, and the decreased red cell parameters seen in the hematology results, from dogs receiving 12,000 ppm or 30,000 ppm RPA 201772, correlated well with classical symptoms of chronic hemolytic anemia.

Secondary changes observed in the reproductive organs of dogs in the 30,000 ppm group were dose-related decreases in numbers of corpora lutea for females and minimal reduced spermatogenesis as well as minimal multinucleate cells in tubules of testes, round spermatids (3/5), and absence of spermatids in the majority of tubules in epididymides of males (2/5).

No neoplastic tissue was observed in beagles in the treatment or control groups.

There were no observed treatment-related effects on mortality, clinical appearance, body weight changes, food consumption, ophthalmology, hematology, clinical

chemistry, urinalysis, organ weights, or gross and histopathological changes at treatment levels of 240 or 1,200 ppm.

The reviewer agrees with the study author that the chronic LOEL for RPA 201772 in the beagle dog is 12,000 ppm, based on intravascular hemolysis with associated clinical chemistry and histological findings, and that the chronic NOEL is 1,200 ppm.

IV. STUDY DEFICIENCIES

No significant deficiencies were noted in this study. Although the doses selected were adequate to assess the chronic toxicity of RPA 201772, the rationale for dose selection was not given in the study report.