012255

RPA 201772 Reproduction Study (§83-4)

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - [Rats] (§83-4)

DP BARCODE: D224202 SUBMISSION CODE: S501233

P.C. CODE: 123000 MRID NO.: 43904809

TEST MATERIAL (PURITY): RPA 201772 (98.7%)

SYNONYMS: Isoxaflutole

CITATION: Henwood, S.M. (1995). Two Generation Reproduction Study with RPA 201772 in Rats. Hazleton Wisconsin, Inc., Madison, Wisconsin. Report no. HWI 6224-202, July 10, 1995. MRID 43904809. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a 2-generation reproduction study, RPA 201772 (98.7% a.i.) was administered to Charles River Crl:CD®BR VAF/Plus® rats (30/sex/group) at nominal dietary levels of 0, 0.5, 2, 20 or 500 mg/kg/day (actual levels in males: 0, 0.45, 1.76, 17.4 or 414 mg/kg/day; females: 0, 0.46, 1.79, 17.7 or 437 mg/kg/day, respectively).

Evidence of toxicity was observed in the male and female parental rats of both generations: at 20 and 500 mg/kg/day, increased absolute and relative liver weights associated with liver hypertrophy was observed; at 500 mg/kg/day (HDT), decreased body weight, body weight gain and food consumption during premating and gestation, and increased incidence of subacute inflammation of the cornea of the eye in F_0 adults as well as keratitis in F_1 adults were reported. There were no other systemic effects that were attributed to treatment, nor was there any indication, at any treatment level, of an effect on reproductive performance of the adults. Treatment-related effects were observed in F_1 and F_2 offspring: at 20 and 500 mg/kg/day, reduction in pup survival was noted; at 500 mg/kg/day, decrease in body weights of F_1 and F_2 pups throughout lactation, increased incidence of chronic keratitis, low incidence of inflammation of the iris, as well as retinal and vitreous bleeding in F_2 pups and weanlings were observed. Necropsy of F_1 and F_2 pups culled on Day 4 revealed an increased number of pups with no milk in the stomach and underdeveloped renal papillae.

Systemic LOEL = 17.4 mg/kg/day for males and females, based upon increased liver weights and hypertrophy
Systemic NOEL = 1.76 mg/kg/day for males and females

Developmental LOEL = 17.4 mg/kg/day, based on decreased litter viability.
Developmental NOEL = 1.76 mg/kg/day

Reproductive LOEL = >17.4 mg/kg/day, based on lack of reproductive effects
Reproductive NOEL = ≥1.76 mg/kg/day

This study is classified as acceptable/Guideline and satisfies the guideline requirement for a 2-generation reproduction study (§83-4) in rats.

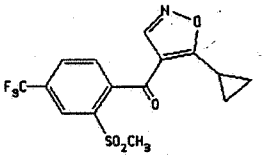
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: RPA 201772

Chemical Name: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole
Description: Yellow powder
Batch number: 21 ADM 93
Purity: 98.7%
Storage Conditions: At room temperature protected from light
CAS NO.: 141112-29-0
Structure:



3. Test animals: Species: Rat
Strain: Crl:CD®BR VAF/Plus®
Age at start of dosing: (P_1) 7 wks; (P_2) After weaning
Weight at start of dosing:
(P_1) Males: 205.6 - 258.1 g; Females: 138.1 - 177.1 g;
(P_2) Males: 97.0 - 203.7 g; Females: 84.4 - 167.7 g
Source: Charles River Laboratories, Inc., Portage, Michigan.
Housing: Individually in stainless steel cages; mated females beginning Day 15 of gestation and females with pups in polycarbonate cages
Diet: Certified Rodent Diet® #5002 Meal (PMI® Feeds, Inc.) ad libitum
Water: Tap water ad libitum
Environmental conditions: Temperature: 19-25°C (66-77°F)
Humidity: 50±20%
Air changes: not stated.
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period (P_1): 14 days

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Following at least 10 weeks of test substance administration, each F_0 male was housed with one female from the same treatment level for a maximum of three weeks. Females were examined daily for positive evidence of a

copulation plug and/or by the presence of sperm in a vaginal lavage. The day the plug was found was considered Day 0 of gestation. Following positive evidence of copulation, each mated female was returned to individual housing. Pregnancy was confirmed by the presence of a vascularized membrane in the vagina or by palpating uterine contents. F_1 weanlings that were selected randomly as second generation parental animals were maintained on test diet for at least 10 weeks prior to mating. They were then mated to non-siblings from the same dose level, using the same procedures described for the F_0 animals, to produce the F_2 liters.

2. Study schedule:The F_0 parental animals were given test diets for 10 weeks before they were mated, and the F_1 parental animals were not mated until at least 10 weeks after they were selected from the F_1 litters. Selection of parents for the F_2 generation was made on lactational day 4; pups were culled to 4/sex/litter and culled pups were sacrificed and discarded. Pups found dead were necropsied. Pups were weaned on day 21, and 30 male and 30 female F_1 pups were randomly selected as F_1 parental animals.

3. Animal assignment: F_0 and F_1 animals were assigned to five different groups (using computerized stratified randomization based on body weight) as seen in Table 1.

TABLE 1 Animal Assignment

Test Group	Dose in Diet (mg/kg/day)a	Animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	0.5	30	30	30	30
Mid (MDT)	2	30	30	30	30
Mid-High (MHDT)	20	30	30	30	30
High (HDT)	500	30	30	30	30

a Diets were administered continuously for two consecutive generations

4. Dose selection rationale: The basis for the selection of dose levels was not provided in the study report.

5. Dosage preparation and analysis: A specified amount of basal diet was weighed in a labeled Hobart® mixing bowl from which approximately 200 g were removed and placed in a Waring® blender. A weighed amount of test material was added to the blender and overlaid with 50 g of basal diet from the mixing bowl. This premix was thoroughly blended before transferring it to the mixing bowl. Approximately 100 g of basal diet left in the mixing bowl was blended with the residual test material from the blender and then returned to the mixing bowl. The contents in the mixing bowl were

mixed for at least 15 minutes. Samples of diet preparations were analyzed for concentration and homogeneity. Diets were prepared fresh weekly and were stored at room temperature until dispensed. Prior to analysis, all samples taken were stored at -20°C±10°.

The stability of the test substance in the diet was established in an earlier study (HWI 6224-203). Samples were taken from the top, left, right and bottom portions of the 4 and 10,000 ppm diet mixtures in triplicate and analyzed for homogeneity. The samples of all test diet blends were analyzed for concentration during first 8 weeks (Weeks 1 and 5 for F_1 males and Weeks 2 and 6 for F_1 females), at week 12 (mating of F_1 females) and once during lactation phase.

C. OBSERVATIONS

1. Parental: The following observations were performed:

Observation	Population Subset	Frequency
Mortality and signs of toxicity	All animals	twice daily during the study
Detailed clinical observations*	All animals	Pretest and at least weekly thereafter
Body weight	All animals	Pretest and weekly through premating
	Males after mating	Weekly until sacrifice
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0,4, 7, 14, and 21 post partum
Food consumption	All animals	Weekly during pre-mating period
	Males after mating	Weekly until sacrifice
	Maternal animals	Measured concurrently with body weights

*Ophthalmic examinations were performed on F_0 animals prior to initiation of treatment and for F_0 and F_1 adults during Week 10 premating using ophthalmoscopy and slitlamp biomicroscopy.

2. Offspring: The following observations were recorded for each litter:

Observation	Day of Observation (Lactation)				
	Day 0	Day 4	Day 7	Day 14	Day 21
No. live, dead, and missing/cannibalized pups	X	X[a]	X	X	X
Sex of each pup	X	X	X	X	X
Individual pup body weights	X	X	X	X	X
External alterations[b]	X				

a On day 4, litters were standardized randomly to 8 pups (4/sex) when possible.
b In addition, litters were observed daily for general appearance and mortality; ophthalmic examinations were performed on two F_2 pups/sex/litter and examined once between lactation Days 16 and 28. Additionally, F_2 weanlings (mid-high and high-dose groups) not examined during that period were examined once following weaning.

3. Postmortem Studies:

a. Sacrifice: For all generations, weanling and adult rats were anesthetized with sodium pentobarbital, followed by exsanguination and necropsy of adults.

b. Pathological evaluation:

• Parental animals: In each generation, all surviving parental males were sacrificed after the last litter was delivered. All F_0 and F_1 parental females were sacrificed after the last litter of each generation was weaned. These animals were subjected to postmortem examinations as follows:

Animals Examined	Macroscopic [a]	Microscopic [b]
Found dead	X	X
Unscheduled sacrifice	X	X
Scheduled sacrifice	X[b]	X

a = Uteri and ovaries examined for implantations and corpora lutea including a count of uterine implantation scars after staining with 10% ammonium sulfide solution
b = All preserved tissues from all F_0 and F_1 animals

The following tissues were preserved and examined histopathologically for all high-dose and control F_0 and F_1 adults. Tissues were preserved in 10% neutral buffered formalin and sections were stained with hematoxylin and eosin.

Male reproductive tissues: Testes (2) Epididymides (2) Prostate Seminal vesicles	Pituitary Liver Lesions Eyes
Female reproductive tissues: Ovaries (2) Uterus (with cervix) Vagina	

D. Offspring: The selected tissues and organs listed above from the F_1 and F_2 offspring were also preserved in 10% phosphate-buffered formalin. All F_1 offspring not selected for continuation to the next generation were sacrificed by an overdose of sodium pentobarbital after weaning. F_2 pups were euthanized on Day 21 of lactation. These animals, as well as all pups that were found dead, or culled on Day 4, were subjected to a gross external and internal examination. Microscopic pathological examination of pups was not performed.

E. Data Analysis

1. Statistical analyses: The following methods of statistical evaluation were employed when appropriate (extracted from report No. HWI 6224-202, page 23).

First Levene's test was performed to determine if groups had homogeneous variance. If the variances were equal, parametric procedure was performed; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA; if significant differences among the means were indicated, Dunnett's test was used (for pairwise comparisons between treated and control groups) to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Wilcoxon-Mann-Whitney two-sample rank test was used.

For continuous data (mean body weight, body weight change, food consumption, number of days required to mate, gestation length, organ weights, organ-to-body weight percentages, and litter data), statistical evaluation was made by the appropriate one-way ANOVA technique. Reproductive indices were analyzed by Cochran-Armitage test and Fisher-Irwin exact test. Pup weights, with the number of pups in the litter, were analyzed by One-way analysis of covariance (ANCOVA). All

statistical tests were conducted at the 5% and 1% level of significance.

2. Indices: The following reproductive indices were calculated from breeding, parturition, and lactation records of study animals:

$$\text{Male Mating Index} = \frac{\text{No. of males for which mating confirmed}}{\text{No. of males used for mating}} \times 100$$

$$\text{Male Fertility Index} = \frac{\text{No. of males impregnating females}}{\text{No. of males used for mating}} \times 100$$

$$\text{Female Fertility Index} = \frac{\text{No. of females pregnant}}{\text{No. of females paired}} \times 100$$

$$\text{Female Fecundity Index} = \frac{\text{No. of females pregnant}}{\text{No. of females for which mating confirmed}} \times 100$$

$$\text{Gestational Index} = \frac{\text{No. of females with live litters}}{\text{No. of females pregnant}} \times 100$$

$$\text{Live Birth Index} = \frac{\text{No. of live pups at birth}}{\text{No. of pups born}} \times 100$$

$$\text{Day 1 Survival Index} = \frac{\text{No. of live pups at day 1}}{\text{No. of live pups at day 0}} \times 100$$

$$\text{Day 4 Survival Index} = \frac{\text{No. of live pups at day 4 (precull)}}{\text{No. of live pups at day 1}} \times 100$$

$$\text{Day 7 Survival Index} = \frac{\text{No. of live pups at day 7}}{\text{No. of live pups at day 4 (postcull)}} \times 100$$

$$\text{Day 14 Survival Index} = \frac{\text{No. of live pups at day 14}}{\text{No. of live pups at day 7}} \times 100$$

$$\text{Day 21 Survival Index} = \frac{\text{No. of live pups at day 21}}{\text{No. of live pups at day 14}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of live pups at day 21}}{\text{No. of live pups at day 4 (postcull)}} \times 100$$

$$\text{Viability index} = \frac{\text{Mean \# live pups per litter on day 4}}{\text{Mean \# pups per litter born alive}} \times 100$$

$$\text{Weaning index} = \frac{\text{Mean \# pups per litter alive on day 21}}{\text{Mean \# pups per litter kept at day 4}} \times 100$$

3. Historical control data: Historical control data were not provided.

II. RESULTS

A. Analysis of Test Diets

Analysis of sample mixtures collected during first 8 weeks and at two other times throughout the study, indicated that the formulation procedures were adequate and produced mixtures that were homogeneous with reported ranges within ±12% of nominal (94.8%-113.0%; see p. 43 of the study report # HWI 6224202). Stability for samples of test substance in feed was confirmed during previous studies. Average recovery in test diets ranged between 88.4% and 115.0%; the analytical concentrations for the 0.5, 2.0, 20 and 500 mg/kg/day diets, ranged between 91.3%-108.0%, 90.9%-115.0%, 88.4%-114.0%, and 87.6%-114.0%, respectively (see p. 44-48 of the study report # HWI 6224202). Most analytical values, however, fell within ±10% of nominal concentration values, and were judged to be minimally acceptable.

B. Parental animals

1. Mortality and Clinical Signs

All F_0 and F_1 animals survived until scheduled termination. One control male was sacrificed at week 6 following mechanical injury. No compound-related clinical signs were noted in males and females from any dose group or generation. Common clinical signs occurred at a low frequency. These were not judged to be treatment-related.

2. Body Weight and Food Consumption

Selected body weight and food consumption data for parental animals prior to mating (Tables 2A and 2B, respectively) and for pregnant or nursing dams (Table 3) are summarized below.

a. Premating

Dietary administration of 500 mg/kg/day RPA 201772 caused decreases in premating body weight and body weight gain values in males and females of both generations

(Table 2A and 2B). Over the 10-week premating interval, decreases in body weight (4-9% in males and 5-9% in females; data not shown in this DER) and body weight gain (15 and 22%, respectively) were noted for F_0 high-dose (500 mg/kg/day) animals compared to controls (Table 2A). In addition, decreases in body weight and body weight gain at 500 mg/kg/day, from Week 11-19 for F_0 males and from Week 11-20 for F_1 males, were judged to be indicative of a toxic response to treatment (data not in this DER).

F_1 males and females at 500 mg/kg/day began the second generation premating period at significantly reduced mean body weights (Table 2B). These differences from control were observed in both sexes throughout the premating period. During the 10-week premating interval, mean body weight values for F_1 males were 18-26% less than control for the 500 mg/kg/day dose group; for F_1 females, mean body weight values were 11-20% less than control. For F_1 males, the body weight gain over the 10-week premating interval was significantly lower (15% during Weeks 0-10) at 500 mg/kg/day than control. For F_1 females, the body weight gain was lower (11% during weeks 0-10) at 500 mg/kg/day than control. Premating body weight and body weight gain values for F_0 and F_1 rats at lower dose levels were not adversely affected by treatment with RPA 201771.

The food consumption at 500 mg/kg/day was comparable between treated and control F_0 males (Table 2A), except during weeks 4-5 of treatment (8% decrease); for females, it decreased (6-12%) during Weeks 0-10 (Table 2A). Sporadic increases in food consumption among females at 20 mg/kg/day were not considered to be toxicologically significant.

Food consumption for the F_1 generation males at 500 mg/kg/day was 7-9% less than control (Table 2B). The mean food consumption among females, decreased (12%) significantly only during the first week of treatment at 500 mg/kg/day, compared to control. The reduction in body weight gain caused by decrease in food consumption noted during both generations.

b. Gestation and lactation:

Mean maternal gestational and lactational body weight and body weight gain values and mean gestational food consumption values for both generations are presented in Table 3.

In both the F_0 and F_1 generation dams, significant treatment-related decreases in mean gestational body weights (11-13% and 9-13%, respectively) and Day 0-20 body weight gains (8% in F_0 females only) were observed at 500 mg/kg/day. These findings were considered to be treatment-related. Food consumption during gestation was lower for F_0 (10%) and F_1 (9%) females during gestation day (GD) 7-14 and 0-7, respectively. During entire lactation period (postnatal day or PD 0-21) both the F_0 and F_1

generation dams had decrease in body weights (9-13% and 12-15%, respectively) at 500 mg/kg/day. Although, food consumption for F_0 and F_1 females at 500 mg/kg/day was lower (17-27% and 12-20%, respectively; p. 102 and 108 of the study report HWI 6224202) than control during entire lactation period (not shown in this DER), the overall body weight gain was higher compared to controls (14 g and 9 g versus 4 g and 5 g in controls, respectively).

TABLE 2 A. Body Weight and Food Consumption - Pre-mating (F0 Generation)

Observations/ study week	Dose Group (mg/kg/day)				
	Control	0.5	2	20	500
[F0] Generation Males - Pre-mating					
Mean body weight (g)					
Week 0	232	231	232	231	230
Week 5	423	417	424	421	391** (8)
Week 10	515	506	515	516	472** (8)
TBW (Week 19)	589	576	587	601	532**(10)
Mean weight gain (g)					
Week 0-10	283	275	283	285	242** (15)
Mean food consumption (g/animal/day)					
Weeks 0-1	25	25	24	24	23** (8)
Weeks 4-5	26	27	26	26	25
Weeks 9-10	26	27	26	27	26
[F0] Generation Females - Pre-mating					
Mean body weight (g)					
Week 0	156	158	158	157	155
Week 5	230	236	230	237	214**(7)
TBW Week 10	262	271	260	271	238**(9)
Mean weight gain (g)					
Week 0-10	106	113	103	114	83** (22)
Mean food consumption (g/animal/day)					
Weeks 0-1	17	17	17	17	15** (12)
Weeks 4-5	18	18	17	18	17* (6)
Weeks 9-10	17	18	17	18*	16 (6)

Extracted from Report number HWI 6224202, Tables 9, 15, and 21, pages P. 77-79, 87-89 and 97-100
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01
( ) = Values in parentheses represent % decrease from control
TBW = Terminal body weights

TABLE 2 B. Body Weight and Food Consumption - Pre-mating (F1 Generation)

Observations/ study week	Dose Group (mg/kg/day)				
	Control	0.5	2	20	500
[F1] Generation Males - Pre-mating					
Mean body weight (g)					
Week 0	170	170	175	167	125**(26)
Week 5	418	411	421	408	336**(20)
Week 10	520	514	524	505	425**(18)
TBW (Week 20)	605	612	617	583	485**(20)
Mean weight gain (g)					
Week 0-10	351	343	349	338	299**(15)
Mean food consumption (g/animal/day)					
Weeks 0-1	23	23	24	23	21** (9)
Weeks 4-5	27	27	27	27	25** (7)
Weeks 9-10	27	27	27	26	25 (7)
Weeks 19-20	27	28	27	27	25** (8)
[F1] Generation Females - Pre-mating					
Mean body weight (g)					
Week 0	137	139	141	134	109**(20)
Week 5	234	236	239	227	206**(12)
TBW Week 10	272	275	278	265	241**(11)
Mean weight gain (g)					
Week 0-10	135	136	137	131	132 (2)
Mean food consumption (g/animal/day)					
Weeks 0-1	16	17	17	17	18* (13)
Weeks 4-5	18	19	20	19	18
Weeks 9-10	17	18	18	17	17

Extracted from Report number HWI 6224202, Tables 12, 18 and 24, pages 82-84, 92, 94 and 103 -106
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.
( ) = Values in parentheses represent percent increase or decrease from control
TBW = Terminal Body Weight

Table 3. Maternal Body Weight/Change and Food Consumption During Gestation and Lactation

Maternal Observations mg/kg/day =	0	0.5	2	20	500
F0 Generation/Gestation					
Mean body weight (g) - Day 0	265	272	261	266	231**(13)a
Mean body weight (g) - Day 7	294	300	292	297	259**(12)
Mean body weight (g) - Day 14	321	323	317	320	278**(13)
Mean body weight (g) - Day 20	387	385	386	386	343**(11)
Mean body weight change (g) - Days 0-20	122	113	125	120	112 (8)
Food consumption (g/day) - Days 0-7	20	21	21	21	19 (5)
Food consumption (g/day) - Days 7-14	21	20	21	21	19**(10)
Food consumption (g/day) - Days 14-20	20	19	20	20	19 (5)
Lactation					
Mean body weight (g) - Day 0	301	301	294	301	264**(12)
Mean body weight (g) - Day 7	314	316	314	314	272**(13)
Mean body weight (g) - Day 14	320	326	323	322	278**(13)
Mean body weight (g) - Day 21	305	307	305	306	278**(9)
Mean body weight change (g) - Days 0-21	4	8	11	6	14
F1 Generation/Gestation					
Mean body weight (g) - Day 0	275	274	280	263	239**(13)
Mean body weight (g) - Day 7	305	307	313	291	265**(13)
Mean body weight (g) - Day 14	330	333	337	314	292**(12)
Mean body weight (g) - Day 20	391	395	403	382	356**(9)
Mean body weight change (g) - Days 0-20	116	121	123	119	117 (1)
Food consumption (g/day) - Days 0-7	22	23	23	21	20* (9)
Food consumption (g/day) - Days 7-14	23	23	23	22	22 (4)
Food consumption (g/day) - Days 14-20	21	21	21	21	23 (10)
Lactation					
Mean body weight (g) - Day 0	313	306	314	301	270**(14)
Mean body weight (g) - Day 7	323	323	326	305* (6)	284**(12)
Mean body weight (g) - Day 14	337	337	336	320* (5)	287**(15)
Mean body weight (g) - Day 21	317	318	314	308	280**(12)
Mean body weight change (g) - Days 0-21	5	10	-0.3	7	9

Extracted from Report number HWI 6224202, Tables 10, 11, 13, 14, 16, 17, 19, 20, 22, 23, and 25, pages 80, 81, 85, 86, 90, 91, 95, 96, 101, 102 and 107;
a = Percent decrease from control * = p≤0.05; ** = p≤0.01

3. Test Substance Intake

Based upon individual food consumption and body weight values, the mean intake of test substance during the premating period for F_0 and F_1 males and females (expressed as mg test substance/kg body weight/day) are summarized in Table 4. The lowest mean overall (Weeks 1-10) test substance intake values (Table 4) for the premating period of the F_0 generation are considered to be a conservative representative estimate for the overall study.

Table 4. Premating Test Substance Intake (mg/kg/day)[a]

Generation	Males/Dose in mg/kg/day)				Females/Dose in mg/kg/day			
	0.5	2	20	500	0.5	2	20	500
F0	0.45	1.77	17.7	423	0.45	1.76	17.9	432
F1	0.44	1.75	17.1	405	0.46	1.81	17.4	442

[a]Only the range of test material consumption values for each dose groups was provided; the values in the table are the lowest average mean values; no individual test material consumption was provided. The average test material consumption over two generations for 0.5, 2, 20 or 500 mg/kg/day dose groups was as follows: Males: 0.45, 1.76, 17.4 or 414 mg/kg/day, respectively; Females: 0.46, 1.79, 17.7 or 437 mg/kg/day, respectively)

Note: Data were extracted from report No. HWI 6224202, page 24.

4. Ophthalmic Examinations

At 500 mg/kg/day, a compound-related increased occurrence of chronic keratitis was noted in F_1 males (both eyes: 13/30; right eye: 5/30; left eye: 5/30) and females (both eyes: 14/30; right eye: 2/30; left eye: 3/30) (data not reported in this DER; see Report no. HWI 6224202, Table 30, page 112). This finding was not noted in controls.

5. Reproductive Performance

A summary of reproductive performance is presented in Table 5. There were no treatment-related effects on mating, fertility, mean number of days to mating, gestational and mating indices for either generation. In addition, the mean duration of gestation for F_0 females was comparable between treated and control groups; for F_1 females at 500 mg/kg/day, it was slightly increased compared to controls 22.4 versus 22.0 in control group). This finding was considered to be incidental.

Table 5. Reproductive Performance

Observation	0	0.5	2	20	500
F0 Generation					
# Females paired	30	30	30	30	30
# Females inseminated	30	30	30	29	30
# Females pregnant	27	29	29	27	29
Fertility index	90	97	97	90	97
# Females delivering (%)	27(90)	28(93)	29(97)	26(90)	29(97)
# Females with liveborn pups	27	27	29	26	29
# Females with stillborn pups	0	5	4	3	11
# Females with no liveborn pups	0	1	0	0	0
# Females with no pups del	0	1	0	1	0
Gestation index	100	93	100	96	100
Mean gestation duration (days)	21.9	22.1	22.0	22.0	22.1
F1 Generation					
# Females paired	30	30	29	30	29
# Females inseminated	29	30	28	30	28
# Females pregnant	25	29	23	30	27
Fertility Index	83	97	79	100	93
# Females delivering (%)	25(86)	29(97)	23(82)	30(100)	27(96)
# Females with liveborn pups	25	29	23	30	27
# Females with stillborn pups	6	5	2	5	6
# Females with no liveborn pups	0	0	0	0	0
# Females with no pups del	0	0	0	0	0
Gestation index	100	100	100	100	100
Mean gestation duration (days)	22.0	22.2	22.0	22.0	22.4*

* Significantly different from control value, $p \leq 0.05$
** Significantly different from control value, $p \leq 0.01$
Del = Delivered

Note: Data were extracted from report No. HWI 6224202, tables 35, 36, 38 and 39, pages 118-120 and 128-130.

B. OFFSPRING

1. Litter Size and Pup Viability: Mean litter size and viability (survival) results for pups during lactation are summarized from the report in Table 6. Maternal uterine implantation scar data and post-implantation losses were not provided in the study report. There were no biologically significant differences in mean litter size, mean number of live and dead offspring and sex ratio between treated and control groups in either generation. At 20 and 500 mg/kg/day, there were significant decreases in the viability indices and mean body weights of male and female offspring of both generations compared with controls (see Tables 7A and 7B).

Significant decreases in live birth indices observed at 0.5, 20, and 500 mg/kg/day in F_0 generation were not considered to be treatment-related because this effect was not seen in F_1 generation. There were no significant differences in gestation index, weaning index, or mean number of pups delivered. There were no differences in sex ratio. The viability index, however, was reduced at 20 and 100 mg/kg/day in F_1 pups and at 500 mg/kg/day in F_2 pups; the decreased viability index was associated with a dose-related increase in pup mortality during lactation days 0-4. Compound-related decrease in mean pup body weight of both the F_1 (11-35%) and F_2 (8-29%) pups was noted at 500 mg/kg/day from PD 0-21.

2. Pup Body Weight: Group mean pup body weight data are summarized from the report in Table 7 A and 7 B. Significant treatment-related decreases in mean pup body weight were noted for both generations at 500 mg/kg/day. Throughout lactation period, mean pup weights were 11-35% and 8-29% less than controls for F_1 and F_2 pups, respectively, at 500 mg/kg/day treatment level. High-dose pups were slightly smaller than control at birth and on Day 4; and continuing to weaning, significant ($p \leq 0.01$) differences in group mean pup weight were attained by 500 mg/kg/day pups.

3. Pup Clinical Observations: Clinical observations recorded for pups during lactation did not demonstrate treatment-related systemic toxicity. The incidental clinical observations noted during lactation included scabs, bent or short thread-like, enlarged eyes, and swollen abdomen (F_1 pups) and missing or black tail, sparse haircoat (F_2 pups).

4. Ophthalmic Examinations: Ophthalmic examination revealed chronic keratitis in F_2 male and female pups (Lactation day 16-28) and weanlings (Days 20-37 of age) 500 mg/kg/day. This finding was also noted in F_1 adults at the same dose level and, therefore, was considered to be compound-related. Additionally, F_2 pups at 500 mg/kg/day had a low incidence of inflammation of iris, as well as retinal and vitreous bleeding. The above findings are presented in Table 8.

Table 6. Selected Measurements of Litter Size and Viability

Observation	mg/kg/day				
	0	0.5	2	20	500
F0 Generation, F1 Litters					
No. of litters	27	28	29	26	29
Mean litter size	13.7	13.5	13.7	13.9	14.0
Total no. pups born	369	377	398	360	405
No. pups stillborn /uncertain	0	9	5	9	16
Mean no.live pups/litter					
Day 0	13.7 (27)a	13.6 (27)	13.6 (29)	13.5 (26)	13.4 (29)
Day 4 - Precull	13.5	13.4	13.3	13.5	12.0
Day 4 - Postcull	8.0	7.9	7.9	7.9	7.7
Day 7	8.0	7.9	7.9	7.9	7.7
Day 14	8.0	7.9	7.9	7.9	7.7
Day 21	8.0	7.9	7.9	7.9	7.7
Survival indices					
Live Birth index (%)	100	95*	99	97**	96**
Viability index (%)	99	98	98	93*	89**
Weaning Index	100	100	100	100	100
Pup survival-Day 21	216	213	230	197	223
Pup mortality (0-21)b	4	7	8	14	40
Day-0 Sex ratio (%) ♂/♀	47/53	52/48	51/49	52/48	50/50

* Significantly different from control value, $p \leq 0.05$
** Significantly different from control value, $p \leq 0.01$
a= Number of litters in parentheses; b= Dead/killed/cannibalized

Note: Data were extracted from report No. HWI 6224202, tables 35 and 36, pages 118, 119-123

Table 6. Selected Measurements of Litter Size and Viability (Continued)

Observation	Dose (mg/kg/day)				
	0	0.5	2	20	500
F1 Generation, F2 Litters					
No. of litters	25	29	23	30	27
Mean litter size	12.8	13.7	13.3	12.8	13.6
Total no. pups born	321	397	306	383	368
No. pups stillborn /uncertain	13	8	4	7	14
Mean no. live pups/litter					
Day 0	12.3 (25)	13.4 (29)	13.1 (23)	12.5 (30)	13.1 (27)
Day 4 - Precull	12.0	13.7	13.1	12.4	12.4
Day 4 - Postcull	7.8	8.0	7.9	7.9	7.6
Day 7	7.8	8.0	7.9	7.9	7.5
Day 14	7.8	8.0	7.9	7.8	7.5
Day 21	7.8	8.0	7.9	7.8	7.5
Survival indices					
Live Birth index (%)	96	96	99	98	96
Viability index (%)	98	96	100	99	93*
Weaning Index	100	100	100	99	99
Pup survival-Day 21	195	224	182	235	203
Pup mortality (0-21)a	7	5	1	7	11
Day-0 Sex ratio (%M/%F)	48/52	40/60	50/50	48/52	52/48

* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
a = Number of litters in parentheses; b= Dead/killed/cannibalized

Note: Data were extracted from report No. HWI 6224202, tables 38 and 39, pages 128, 129-133

Table 7 A. Mean Pup Weights (g)- F_1 Pups

Interval mg/kg=	0	0.5	2	20	500
F1 Male Pups					
Day 0	6.3	6.2	6.4	6.1	5.7**(11)a
Day 4 -Precull	9.9	9.9	9.9	9.5	7.6**(23)
-Postcull	10.0	10.0	10.0	9.7	7.6**(24)
Day 7	16.2	16.6	16.5	15.6	12.1**(25)
Day 14	33.5	33.9	34.0	32.8	23.4**(30)
Day 21	54.9	54.2	55.4	52.5	35.9**(35)
F1 Female Pups					
Day 0	5.9	5.9	6.1	5.9	5.4**(10)
Day 4 -Precull	9.4	9.6	9.7	9.2	7.1**(24)
-Postcull	9.4	9.6	9.6	9.2	7.2**(22)
Day 7	15.4	16.0	15.8	14.8	11.4**(26)
Day 14	32.2	32.8	33.0	31.6	22.5**(30)
Day 21	52.0	52.5	53.5	50.7	34.7**(33)

* Statistically significantly different from control value, $p \leq 0.05$.
** Statistically significantly different from control value, $p \leq 0.01$.
a Percent decrease from control

Note: Data were extracted from report No. HWI 6224202, table 36, page 121-123

Table 7 B. Mean Pup Weights (g)- F2 Pups

Interval mg/kg=	0	0.5	2	20	500
F2 Male Pups					
Day 0	6.6	6.4	6.4	6.5	6.0**(9)[a]
Day 4 -Precull	10.5	10.4	10.6	10.2	8.2**(22)
-Postcull	10.6	10.5	10.6	10.2	8.3**(22)
Day 7	17.3	16.9	17.2	16.3	13.2**(24)
Day 14	34.5	34.5	34.9	33.3	26.5**(23)
Day 21	56.8	56.2	57.2	54.2	40.7**(28)
F2 Female Pups					
Day 0	6.2	6.1	6.0	6.1	5.7**(8)
Day 4 -Precull	10.0	10.1	10.1	9.7	7.9**(21)
-Postcull	10.0	10.2	10.1	9.8	8.0**(20)
Day 7	16.5	16.4	16.4	15.7	12.5**(24)
Day 14	33.2	33.4	33.4	32.1	25.4**(23)
Day 21	54.6	54.2	54.7	51.7	39.1**(28)

* Statistically significantly different from control value, $p \leq 0.05$.
**Statistically significantly different from control value, $p \leq 0.01$.
a Percent decrease from control

Note: Data were extracted from report No. HWI 6224-202, table 39, page 131-133.

Table 8. Selected Ophthalmic Observations at 500 mg/kg/day-dose F_2 Pups and Weanlings

Observations	Number of Animals with findings	
	Males	Females
Slitlamp Examination	Total # Examined: Pups: 52;Weanlings: 40	Total # Examined: Pups:52;Weanlings:36
Chronic keratitis-		
Pups, Both eyes	14	8
Right eye	2	4
Left eye	2	2
Weanlings, Both eyes	13	20
Right eye	8	5
Left eye	6	1
Inflammation of eyes-		
Pups, Both eyes	1	1
Left eye	2	0
Weanling, Both eyes	1	0
Right eye	1	0
Indirect Examination		
Chronic keratitis-		
Pups, Both eyes	3	3
Left eye	1	0
Weanlings, Both eyes	10	18
Right eye	2	2
Left eye	1	0
Inflammation of eyes-		
Pups, Both eyes	3	3
Right eye	1	0
Left eye	2	1
Weanlings, Both eyes	3	1
Retinal vitreous bleeding-		
Pups, Both eyes	1	2
Right eye	5	0
Weanlings, Both eyes	0	2
Left eye	0	1
Vitreous hemorrhage-		
Pups, Both eye	1	0
Right eye	0	2
Weanlings, Both eyes	1	1
Left eye	1	0

Note: Data extracted from report number HWI 6224202, Tables 31-34, pages 113-117

5. Offspring Postmortem Results: At 500 mg/kg/day, there was increase in number of F_1 pups and litters, culled on PPD 4, with no milk in stomach. There was also a slight increase in the incidence of underdeveloped renal papilla in pups from both generations (see table 9 for selected observations). These observations were considered to be compound-related.

C. Parental Postmortem Data

a. Organ weights: The organ weight data are presented in Table 10. Terminal body weights of animals in both generations were lower (9-20%) than the controls. There was a compound-related significant increase in mean absolute and relative liver weights in F_0 adults at 20 and 500 mg/kg/day and F_1 adults at 500 mg/kg when compared to controls. The relative liver weight in F_1 males was increased (12%) at 20 mg/kg/day; the absolute liver weight was unaffected.

b. Pathology

1) Macroscopic examination: Necropsy observations reported at 500 mg/kg/day for F_0 and F_1 adult rats revealed treatment-related liver effect consisting of mottled liver (F_0: 6/30 and 4/30; F_1: 6/30 and 2/30, in males and females, respectively versus none in controls). Other macroscopic findings observed in treated F_0 and F_1 adults occurred at comparable incidence and severity to controls.

2) Microscopic examination: Selected microscopic findings are presented in Table 11. At 20 and 500 mg/kg/day, compound-related changes observed in the liver consisted of centrilobular hypertrophy in males and females as well as vacuolation in males from both generations. These changes were severe and frequent in males at 500 mg/kg/day. At 500 mg/kg/day, subacute bilateral or unilateral inflammation of cornea was noted in F_1 generation males and females. Other microscopic findings observed in treated F_0 and F_1 adults occurred at comparable incidence and severity to controls.

Table 9. Selected Necropsy Observations in Pups

Observations	Dose Groups (mg/kg)/Number of Pups with Observations				
	0	0.5	2	20	500
No. pups examined: F1	150	159	160	147	162
F2	119	172	123	141	155
No milk in stomach					
F1 pups (litter)	0	9 (6)	5 (4)	3 (3)	26 (14)
F2 pups (litter)	9 (4)	11 (7)	3 (2)	5 (5)	19 (9)
Underdeveloped Renal Papilla					
F1 pups (litter)	0	0	0	0	5 (5)
F2 pup (litter)	0	1 (1)	0	0	3 (2)

Note: Data extracted from report number HWI 6224202, table 37 and 40, pages 124, 125, 134, and 136.
( ) = # litters

TABLE 10. Selected Organ Weights (g)[a]

Observations	Dose Group (mg/kg)				
	Control	0.5	2	20	500
[F0] Generation Males and Females					
Males-					
Liver, Absolute	20.66	20.97	21.57	25.32** (23)a	32.41** (57)
Relative	3.52	3.66	3.64	4.17** (19)	6.09** (73)
Terminal Body Weight	585.8	573.0	590.2	605.6	532.5** (9)
Females-					
Liver, Absolute	11.16	11.29	11.03	12.14* (9)	16.10** (44)
Relative	3.71	3.68	3.70	3.98** (7)	6.11** (65)
Terminal Body Weight	300.1	307.3	298.0	305.4	263.7** (12)
[F1] Generation Males and Females					
Males-					
Liver, Absolute	21.49	21.34	22.00	23.11	28.71** (34)
Relative	3.52	3.49	3.54	3.94** (12)	5.90** (68)
Terminal Body Weight	609.6	611.0	621.4	586.0	487.7** (20)
Females-					
Liver, Absolute	11.48	11.49	11.87	11.67	15.12** (32)
Relative	3.65	3.65	3.69	3.85	5.59** (53)
Terminal Body Weight	315.9	314.6	322.5	303.7	271.7** (14)

Extracted from Report number HWI 6224202, Tables 41, 42, 47, and 48, pages P. 139, 141, 144, 159, 161, and 164

a Values in parentheses represent percent increase or decrease from control.

* Statistically different from control, p<0.05.

** Statistically different from control, p<0.01.

TABLE 11. Selected Microscopic findings

Observations	Dose Group (mg/kg)				
	0	0.5	2	20	500
[F0] Generation Males and Females (No. Examined =30/Group)					
Males-					
Eyes, Subacute corneal inflammation	0	0	0	0	0
Liver, - Hypertrophy, centrilobular	0	0	0	19	30
- Vacuolation	0	0	0	2	13
Females-					
Eyes, Subacute corneal inflammation, - bilateral	0	0	0	0	0
- unilateral	0	0	0	0	1
Liver, - Hypertrophy, centrilobular	0	0	0	5	30
- Vacuolation	1	0	0	0	0
[F1] Generation Males and Females (No. Examined = 29-30/Group)					
Males-					
Eyes, Subacute corneal inflammation, - bilateral	0	0	0	0	13
- unilateral	0	0	0	1	8
Liver, - Hypertrophy, centrilobular	0	0	0	29	30
- Vacuolation	0	0	0	7	17
Females-					
Eyes, Subacute corneal inflammation, - bilateral	0	0	0	0	8
- unilateral	0	0	0	0	4
Liver, - Hypertrophy, centrilobular	0	0	0	6	29
- Vacuolation	0	1	0	0	1

Note: Extracted from Report number HWI 6224202, Tables 44 and 50 pages 150 and 170

III. DISCUSSION

A. Investigator's/Reviewer's Conclusions

Dietary administration of RPA 201772 to male and females rats at levels of 0, 0.5, 2, 20 or 500 mg/kg/day over the duration of two generations resulted in treatment-related findings at the 20 and 500 mg/kg/day levels. These findings are described below.

1. Premating body weight and body weight gain values were adversely affected in the first generation adults (F_0) at 500 mg/kg/day; the mean body weight values of F_1 weanlings selected as the second generation parental animals were also significantly reduced compared to control.

This weight decrement was maintained throughout the 10-week F_1 premating period, and overall body weight gains were reduced significantly for the F_1 adults. At 500 mg/kg/day, weight gain decrements in F_0 and F_1 females during gestation were attributed to reductions in food consumption. Although reduced food consumption adversely affected body weights of dams during lactation, the overall body weight gain for both F_0 and F_1 females over entire the lactation period (PD 0-21) was unaffected.

The decrements in F_1 male and female premating body weight gain at 500 mg/kg/day could hypothetically be attributed to secondary developmental toxicity, since they occurred in animals which were developmentally compromised or may have resulted due to maternal toxicity. The evidence for developmental toxicity in the F_0 males and females included reduced weanling body weights. It is noteworthy that the premating body weight gains, were also adversely affected in males as well as females of the F_0 generation. There was also evidence of developmental effect in F_1 males and females offspring such as underdeveloped renal papilla. Therefore, although the exact etiology is not known, the significantly decreased premating body weight gain for high-dose F_1 males and females was designated as an indication of systemic as well as developmental toxicity. At 500 mg/kg/day, compound-related subacute inflammation of the cornea was noted in F_1 adults. At 20 and 500 mg/kg/day, increase in liver weights was associated with liver hypertrophy and vacuolation; there was a dose-related increase in the severity and frequency of these changes in males only.

2. No treatment-related effects on the reproductive performance of rats were noted.

3. Reduced viability indices were noted at 20 and 500 mg/kg/day for F_1 pups and at 500 mg/kg/day for F_2 pups. At 500 mg/kg/day, the F_1 and F_2 pup body weights were reduced throughout lactation; additional findings noted included increased incidences of chronic keratitis in F_2 pups and weanlings as well as absence of milk in the stomach and underdeveloped renal papilla in F_2 pups.

At 2 mg/kg/day, there were no treatment-related findings observed in either generation.

Systemic LOEL = 17.4 mg/kg/day for males and females, based upon increased liver weights, liver hypertrophy and vacuolation
Systemic NOEL = 1.76 mg/kg/day for males and females

Developmental LOEL = 17.4 mg/kg/day, based on reduced viability of offspring
Developmental NOEL = 1.76 mg/kg/day

Reproductive LOEL = >17.4 mg/kg/day, based on lack of reproductive effects
Reproductive NOEL = ≥1.76 mg/kg/day

B. Study Deficiencies

The following deficiencies were noted:

- The data on implantation scars and pre- and postimplantation loss as well as historical controls were not provided.

The above deficiencies were not judged to compromise the interpretation of study results or the overall acceptability of the study.