(1-9-01)

DATA EVALUATION REPORT

TRITICONAZOLE
(RPA400727)

STUDY TYPE: SUBCHRONIC AND 4-WEEK PRELIMINARY ORAL TOXICITY STUDIES - RAT [OPPTS 870.3100 (§82-1a)]
MRIDs 44802101 & 44802102

Prepared for

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U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic and 4-Week Oral Toxicity Studies in Rats; [OPPTS 870.3100 (§82-1a)]

DP BARCODE: D261924
P.C. CODE: 125620

SUBMISSION CODE: S568827
TOX. CHEM. NO.: None given

TEST MATERIAL (PURITY): Triticonazole (Batch # YG 2156/1: 98.9% a.i.; Batch # YG 2160/1: 98.2% a.i.)

SYNONYMS: RPA400727; 2-(-4-chlorobenzylidene)-5,5-dimethyl-1-(1,2,4-triazolylmethyl)-1-cyclopentanol; 5-((4-chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazole-1-ylmethyl)cyclopentanol (from http://www.chemfinder.com)

CITATION: Aughton, P. (1991) RPA400727: Toxicity study by dietary administration to CD rats for 13 weeks. Life Science Research Limited, Eye, Suffolk, England, IP23 7PX. LSR Report No.: 91/RHA429/0793, December 10, 1991. MRID 44802102. Unpublished.

Aughton, P. (1991) RPA400727: Preliminary toxicity study by dietary administration to F-344 rats for four weeks. Life Science Research Limited, Eye, Suffolk, England, IP23 7PX. LSR Report No.: 90/RHA359/0947, March 1, 1991. MRID 44802101. Unpublished.

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

EXECUTIVE SUMMARY: In a 13-week subchronic toxicity study (MRID 44802102), Triticonazole (batch no. YG2156/1, 98.9% a.i.; and batch no. YG2160/1, 98.2% a.i.) was offered in feed for 13 weeks to 10 CD rats/sex at dose levels of 0, 25, 250, 12,500 or 25,000 ppm (males: 0, 2.0, 19.8, 1117.0, or 2309.3 mg/kg body weight/day; females: 0, 2.2, 22.3, 1183.5, or 2368.8 mg/kg/day). Prior to the subchronic study, a 4-week preliminary oral toxicity study (MRID 44802101) was conducted to identify appropriate dose ranges (see Appendix, this report).

Body weights and weight gains were markedly decreased by similar magnitudes in males and females receiving ≥ 12,500-ppm, with significantly decreased ($p \leq 0.01$) overall gains for these groups. Food efficiencies revealed a compound-related adverse effect, with notably decreased overall efficiencies for the groups receiving ≥ 12,500--ppm (80-88% of control values).

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Primary target organ effects involved the thymus, adrenals, liver, skin (hair loss), and possibly bone marrow. Centriacinar hepatocytic fatty vacuolation occurred at a clearly increased incidence in 12,500-ppm (7 affected) and high-dose (10 affected) females.. Dose-related periacinar hepatocytic hypertrophy was noted in males and females receiving ≥ 12,500 ppm of the compound ($p \leq 0.05$). At compound doses of ≥ 12,500 ppm, decreases in mean absolute and relative adrenal gland weights were slight in males and marked in females. Accompanying histopathological findings included adrenal cortical fatty vacuolation in males (incidence in control through high-dose: 1, 4, 8, 10, and 10, respectively) and in the corresponding female groups (0, 0, 1, 4, and 10, respectively). The increased cortical fatty vacuolation may have been related to a compound-related cytotoxic effect on the steroid synthesis pathway, leading to accumulation of cholesterol, which would be consistent with the elevated cholesterol levels noted in treated males and particularly in treated females. Statistically significant adrenal zona reticularis degeneration was noted in nine 12,500-ppm females and ten 25,000-ppm females. A generally dose-related hair loss from various regions in both sexes indicated an association with compound administration; males were affected more often than females.

Mild and generally dose-related decreases in hematocrit, hemoglobin, and red blood cells (RBC) were noted in the high-dose males and females. A generally positive correlation of compound dosage with the incidence and severity of anisocytosis and spherocytosis was consistent with an adverse systemic response to the compound. The marked decrease in thymic weights, mild anemia, and RBC morphological changes suggest a compound-related adverse effect on the immune system and bone marrow function.

The LOAEL in males is 25 ppm (19.8 mg/kg/day) based on a statistically significant increase in the incidence of adrenocortical fatty vacuolation in males receiving ≥ 250-ppm of the compound, with a NOAEL of 25 ppm (2.0 mg/kg/day) for males. The LOAEL in females is 12,500 ppm (1183.5 mg/kg/day) based on hair loss, decreased food efficiencies, adrenocortical fatty vacuolation, zona reticularis degeneration, centriacinar hepatocytic fatty vacuolation, and more severe anisocytosis and spherocytosis in females receiving ≥ 12,500 ppm, with a NOAEL of 250 ppm (22.3 mg/kg/day) for females.

This subchronic toxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral study in rats [OPPTS 870.3100 (§82-1a)].

COMPLIANCE: Signed and dated Good Laboratory Practice Compliance, Quality Assurance, Data Confidentiality and Flagging statements were provided for MRID 44802102.

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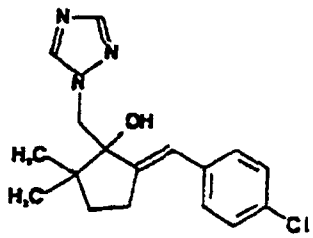
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triticonazole (RPA400727)

Description: Fine white or off-white powder
Batch #: YG 2156/1: 98.9% a.i.; Batch # YG 2160/1: 98.2% a.i.
Purity: See above
Stability of compound: Stability presumed on basis of previous studies
CAS #: 131983-72-7 (from http://www.chemfinder.com)
Structure:



2. Vehicle and/or positive control

The vehicle control material for diet preparations was powdered rodent diet, Laboratory Animal Diet No. 2 (Biosure, Manea, Cambridgeshire, England).

3. Test animals

Species: Rat
Strain: CD
Age at study initiation and weight at study week 0: Approximately 28 – 35 days of age; males: 106 – 140 g; females: 101 – 129 g
Source: Charles River (UK) Limited, Margate, Kent, England.
Housing: Five/sex/cage. Cages were made of 51 x 38 x 21 cm stainless steel body with a stainless steel mesh lid and floor. Cages were suspended above absorbent paper that was changed three times weekly.
Diet: Animals were given fresh powdered rodent diet, Laboratory Animal Diet No. 2 fresh meal-form *ad libitum* on a weekly basis, except prior to urine or blood collection.
Water: Public water supply available *ad libitum*.
Environmental conditions:
Temperature: Approximately 21 °C
Humidity: Approximately 55%
Air changes: At least 15 air changes/hour
Photoperiod: 12-hour light/dark cycle
Acclimation period: 7 days

B. STUDY DESIGN

1. In-life dates

Start: December 5, 1990; end: March 8, 1991 (necropsies completed)

2. Animal assignment

Animals were randomly allocated to groups using a computerized system based on random numbers. The distribution of animals in the room was designed to minimize the effect of any spatially variable environmental component. Table 1 shows the study design.

TABLE 1. Study design

Test group	Estimated dose (ppm)	Estimated dose (mg/kg/day)		Number of animals	
		Males	Females	Males	Females
1	0	0	0	10	10
2	25	2.0	2.2	10	10
3	250	19.8	22.3	10	10
4	12,500	1117.0	1183.5	10	10
5	25,000	2309.3	2368.8	10	10

Data taken from p. 17 and Table 5, p. 50, MRID 44802102.

3. Dose selection rationale

Dose selection was based upon a previous 4-week study (MRID 44802101, see Appendix, this review) in which RPA400727 was administered in the diet to F-344 rats at concentrations of 0, 500, 1500, 5000, 15,000, and 50,000 ppm (males: 0, 50.12, 152.3, 513.2, 1494, and 4802 mg/kg/day; females: 0, 52.44, 151.3, 489.4, 1476, and 4945 mg/kg/day). At concentrations of $\geq$ 5000 ppm, hepatic toxicity was evident to the study authors, and a NOAEL of 1500 ppm was established.

4. Test material preparation and analysis

Diets were prepared weekly by initial preparation of a premix of the compound and ground diet. The premix was passed through a Glen-Creston cross beater mill fitted with a 2 mm screen, followed by dilution with additional diet and mixing for 15 minutes in a Hobart mixer. The diet was divided for the high treatment level group and for preparation of the diets for the remaining groups by serial dilution. Due to the wide concentration range between the 250 and 12,500 ppm diets, an additional mixing stage was instituted for the 250-ppm diet, involving mixing in more diet for 10 minutes in a small planetary mixer. Prior to treatment initiation, samples of the low- and high-dose diets were taken from six positions in the mixer and analyzed for homogeneity. Dietary stability of the test compound was assumed on the basis of

studies performed on 10-ppm diet reported in LSR Report No. 90/RHA416/1345, and on the 500- and 50,000-ppm dietary mixes reported in MRID 44802101 (reported on p. 83, MRID 44802102). The stability samples for MRID 44802101 were collected by pooling the unused portions of the six homogeneity samples each from the 500- and 50,000-ppm groups. [The stability samples from the 10-ppm diet (LSR Report No. 90/RHA416/1345) were presumably collected in the same manner, although this was not specified.] The concentrations of two samples from the pooled test "rodent diet" were analyzed after 7 and 19 days of storage at 21 °C (MRID 44802101), and compared with the mean concentrations of the initial pooled samples. For the stability analysis from LSR Report No. 90/RHA416/1345, two samples from the 10-ppm diet were analyzed and compared with the mean initial concentrations at 7 and 14 days at 21 °C. Dietary concentrations of the compound used for study report MRID 44802102 were determined for three samples/dose at weeks 1 and 13 of treatment, with the exception of the 25-ppm diet, from which five samples were analyzed at week 13 (reason not specified).

Results -

Homogeneity analysis: The respective mean concentrations for the 25- and 25,000-ppm diets were 127% of nominal (range: 119-141% of nominal) and 103% of nominal (range: 100-106%).

Stability analysis: No dietary stability analysis was conducted as part of this study. Results from LSR Report No. 90/RHA416/1345 indicated mean concentrations of the two 10-ppm samples at 7 and 14 days, respectively, were 100% and 102% of the initial mean concentration of 10.4 ppm (range: 100-110% of nominal). Mean concentrations of the two 50,000-ppm samples from MRID 44802101 at 7 and 19 days, respectively, were 90% and 87% of the initial mean concentration of 56,850 ppm (range: 109-118% of nominal). Note: the initial mean concentration of 56,850 ppm was calculated by the reviewer; the study authors reported the mean as 56,900 ppm. In addition, the same stability analysis results for the same 50,000-ppm diet were reported in MRID 44802101 for day 19, and in MRID 44802102 for day 14; therefore, it was not possible to determine on which study day the analysis was conducted. Mean concentrations of the two 500-ppm samples from MRID 44802101 at 7 and 19 days, respectively, were each 97% of the initial mean concentration of 594 ppm (range: 105-133% of nominal).

Concentration analysis: The mean concentrations for the test diets at week 1 ranged from 96-101% of the nominal. At week 13, the low- to high-dose dietary concentrations were 147% (range: 138-154% of nominal), 102%, 103% and 103% of the nominal, respectively.

Because homogeneity analysis indicated that the mixing procedure was inadequate for the 25-ppm diet, the analysis should have been repeated on at least two subsequent 25-ppm dietary mixtures. Dietary stability results from previous studies suggested that the mixtures were most likely stable, with mean compound concentrations decreasing no more than 10% over 7 days; however, the results from the 10-ppm samples may

not have been applicable, as the type of rodent diet used in LSR Report No. 90/RHA416/1345 was not specified. The discrepant dates given for the last stability analysis of the 50,000-ppm diet were inconsequential as the diets were prepared fresh weekly and administered for no more than 7 days. Mean results from concentration analyses suggested that the variance between nominal and actual dosage to the animals was acceptable, with the exception of the 25-ppm diet at week 13.

5. Statistics

When appropriate, the sample statistic was used to calculate standard deviations. The significance of differences between group means for bodyweight gain; and hematologic, blood chemistry, and quantitative urinalysis parameters was assessed using Student's t-test using a pooled error variance. Statistical significance was not reported for eosinophil, basophil, monocyte, and large unstained cell counts, as those data were not normally distributed. Bartlett's test was used to test homogeneity of variance for organ weights. When statistically significant, a Behrens-Fisher test was then used to perform pairwise comparisons; otherwise, a Dunnett's test was employed. Incidence data were analyzed by the Fisher exact probability test.

C. METHODS

1. Observations

Animals were examined at least twice daily for response to treatment or morbidity. A more detailed examination, including palpation, was performed weekly on each animal. Cages were inspected daily for blood, loose feces or other evidence of poor health.

2. Body weight

Body weights were recorded on the day of treatment initiation, then weekly throughout the treatment period, and prior to necropsy. Animals exhibiting signs of illness were weighed more frequently, but the data were not included in study report MRID 44802102.

3. Food consumption and compound intake

Food consumption was estimated weekly by subtracting the amount of food remaining in each cage plus an estimate of spillage from the original weight of the food supplied. These values were used to calculate mean weekly consumption/rat/week. Allowance was made for intake by the 250-ppm female that was sacrificed on day 89. Overall (weeks 1-14) food intake values were calculated using the nonrounded weekly group mean measurements. Weekly group mean dosage values were derived using weekly body weights, the total food consumption per cage, and the nominal dietary concentrations of RPA400727. As with food intake, the calculation of compound intake was adjusted to account for the female sacrificed before study

termination. Dosages were kept constant throughout the treatment period, and not adjusted according to weight.

4. Food efficiencies

Weekly group mean food efficiencies were calculated. It is assumed that the formula

[weekly body weight change (g)/weekly food intake (g)](100)

was used by the study authors to derive the efficiencies, although this was not stated.

5. Ophthalmoscopic examination

Prior to treatment initiation, ophthalmologic examinations were conducted on both eyes of all rats, using an indirect ophthalmoscope. Rejected rats were replaced with those having no ocular abnormality. All control and 25,000-ppm group animals were re-examined after 12 weeks of treatment.

6. Blood was collected for hematology by retro-orbital sinus puncture after 12 weeks of treatment from fasted, ether-anesthetized rats, using ethylenediamine tetraacetic acid (EDTA) as anticoagulant. Additional samples were collected for prothrombin time (PT), using citrate as anticoagulant. At the same time, samples were taken from all rats in the same manner for blood chemistry analysis, using lithium heparin as anti-coagulant. Appendix 8A of the MRID 44802102 reports that sampling for PT was repeated for all males during week 14 due to an excessive number of clotted samples in week 13. One PT sample from each female group was clotted, and one from a 25,000-ppm female was of insufficient quantity, but these analyses were not repeated. Only individual reticulocyte counts were presented, as the data did not follow a normal distribution. The CHECKED (X) parameters were examined for all animals.

a. Hematology

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

* Recommended for subchronic studies, per Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	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 (ALP)		Triglycerides
	Cholinesterase (ChE)	X	Serum protein electrophoresis
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for subchronic studies, per Subdivision F Guidelines.

7. Urinalysis

After 11 weeks of treatment, urine collection was begun following water deprivation for approximately 4.7 hours. Urine was then collected from rats for an additional 16 hours in individual metabolism cages, while food and water were withheld. 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

8. Sacrifice and pathology

All animals were sacrificed by carbon dioxide inhalation after completion of treatment, with the exception of one 250-ppm female that was euthanized *in extremis* on day 89. The CHECKED (X) tissues were collected and examined histologically for all control and 25,000-ppm rats, and on the 250-ppm female rat sacrificed on day 89. Kidneys, liver, and lungs were examined microscopically on all rats, and the uteri from all females. Microscopic examination was conducted on the adrenals from all animals, excepting one 25-ppm male and one 250-ppm female. The (XX) organs were also weighed.

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 glands*
X	Colon*	XX	Kidneys*		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland
XX	Liver*+	XX	Testes*+	XX	Parathyroids*
	Gall bladder	X	Epididymides	XX	Thyroid*
X	Pancreas*	XX	Prostate		
		X	Seminal vesicle		**OTHER**
	RESPIRATORY	XX	Ovaries	X	Bone
X	Trachea*	XX	Uterus*	X	Skeletal muscle
XX	Lung*	X	Vagina	X	Skin
	Nose	XX	Cervix	X	All gross lesions and masses*
	Pharynx				
	Larynx				

* = Required for subchronic studies based on Subdivision F Guidelines.

+ = Organ weight required in subchronic studies based on Subdivision F Guidelines.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Hair loss – primarily from the head, and dorsal and ventral body surfaces – occurred in a dose-related manner in both sexes (with the exception of the control females), affecting all 12,500- and 25,000-ppm animals during the first week of treatment, and persisting until week 9 or later in 50% or more of the 12,500-ppm animals, and in all 25,000-ppm animals. Hindlimb hair loss occurred only in high-dose animals. Hair loss occurred in the control through high-dose males at an incidence of 40%, 50%, 60%, 100%, and 100%, respectively, and in the corresponding females groups at an incidence of 30%, 10%, 40%, 100% and 100%. With the exception of one 25-ppm male and 2 control females, the earlier the onset of hair loss, the greater the dose administered. At necropsy, the hair loss was most severe in high-dose females. Results are presented in Table 2.

TABLE 2. Hair loss in rats fed RPA400727 for 13 weeks

Hair Loss Location	Exposure concentration (ppm)					No. animals per affected site
	0	25	250	12,500	25,000	
No. affected males						**Males**
Number of males examined	10	10	10	10	10	
Body surface, dorsal	0	1	1	10	10	22
Body surface, ventral	0	0	3	4	10	17
Forelimb(s)	1	3	1	5	7	16
Head	4	2	3	7	10	22
Hindlimb(s)	0	0	0	0	4	4
Total no. affected	4	5	6	10	10	
Study week in which hair loss began, in (no. of animals)	12 (3)	4 (1)	10 (1)	1 (10)	1 (10)	
No. of affected animals with hair loss cessation in ≥ 1 locations at week 9	0	1 (20%)	0	5 (50%)	10 (100%)	
No. affected females						**Females**
Number of females examined	10	10	10	10	10	
Body surface, dorsal	3	1	0	9	10	20
Body surface, ventral	0	0	0	2	5	7
Forelimb(s)	0	0	4	0	4	8
Head	3	0	1	6	10	17
Hindlimb(s)	0	0	0	0	2	2
Total no. affected/no. females	3	1	4	10	10	
Study week in which hair loss began, in (no. of animals)	1 (2)	10 (1)	10 (4)	1 (10)	1 (10)	
No. of affected animals with hair loss cessation in ≥ 1 locations at week 9	0	0	0	4 (40%)	9 (90%)	

Data taken from Appendix 3, pp. 85-107, MRID 44802102.

2. Mortality

One 250-ppm female was sacrificed *in extremis* at week 13 due to significant body weight loss and generalized deteriorated condition. Findings included reduced body temperature, piloerection, hair loss from the forelimbs and head, skin and ocular pallor, abnormal gait, hunched posture, fast and labored respirations, and abnormal righting reflexes. Renal failure unrelated to treatment was considered the cause of death. All other animals survived to termination.

B. BODY WEIGHT AND WEIGHT GAIN

Body weight gains by the 12,500- and 25,000-ppm groups were markedly decreased throughout treatment, while gains by the lower dosage groups were not clearly affected. During the 13-week study, gains in the 12,500-ppm groups ranged from 74-85% (males) and 82-86% (females) of control values, and those in the 25,000-ppm groups ranged from 64-77% (males) and 78-80% (females). Overall (study weeks 0-13) weight gains for males, from low- through high-dose, were 103%, 98%, 80% ($p < 0.01$), and 70% ($p < 0.01$) of control values, respectively, and for the corresponding female groups were

96%, 103%, 75% ($p < 0.01$), and 70% ($p < 0.01$) of control values. Results are presented in Table 3.

TABLE 3. Group mean body weights and total body weight gains (g) in rats fed RPA400727 for 13 weeks[1]

Week of study	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
0	123	121 (98)	123 (100)	118 (96)	121 (98)
1	190	186 (98)	187 (98)	145 (76)	130 (68)
3	329	320 (97)	314 (95)	242 (74)	212 (64)
5	414	412 (100)	405 (98)	327 (79)	286 (69)
7	475	479 (101)	468 (96)	392 (83)	352 (74)
9	519	528 (102)	513 (99)	434 (84)	390 (75)
11	558	572 (103)	550 (99)	472 (85)	428 (77)
13	574	586 (102)	563 (98)	480 (84)	435 (76)
Total weight gain (Weeks 0-13)	451	465 (103)	440 (98)	362** (80)	314** (70)
Females					
0	117	116 (99)	114 (97)	117 (100)	115 (98)
1	160	157 (98)	159 (99)	137 (86)	125 (78)
3	231	222 (96)	230 (100)	195 (84)	185 (80)
5	272	262 (96)	271 (100)	223 (82)	216 (79)
7	303	289 (95)	303 (100)	248 (82)	241 (80)
9	323	311 (96)	318 (99)	267 (83)	257 (80)
11	343	326 (95)	337 (98)	285 (83)	274 (80)
13	344	332 (97)	349 (102)	289 (84)	275 (80)
Total weight gain (Weeks 0-13)	227	217 (96)	233 (103)	171** (75)	160** (70)

Data taken from Table 2, pp. 43-44, MRID 44802102.
[1] Numbers in parentheses represent percentage of control value, as calculated by the reviewer.
** Significantly different from control group mean at $p < 0.01$.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Initially, food consumption by the two higher-dose groups was sharply decreased, with the week 1 consumption by the 12,500- and 25,000-ppm males at 76% and 68% of control intakes, respectively, and at 86% and 78% (of controls) for the 12,500 and 25,000-ppm females. Following the first week of treatment, however, food consumption rates for the 12,500- and 25,000-ppm groups improved steadily, with all treated groups consuming 95-107% of the control consumption by week 13. Food intake was unaffected in animals receiving 25- and 250-ppm doses. Overall (weeks 1-13) mean food consumption for the low- to high-dosage males (expressed as percentage of control intake) was 101%, 99%, 94%, and 89%; and 98%, 101%, 93%, and 89% for the corresponding female groups. Results are presented in Table 4.

TABLE 4. Group mean food consumption (g/rat/day) in rats fed RPA400727 for 13 weeks[1]					
Week of study	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
1	186	194 (104)	192 (103)	145 (78)	129 (69)
3	214	215 (101)	210 (98)	178 (83)	167 (78)
5	217	216 (100)	210 (97)	199 (92)	195 (90)
7	208	210 (101)	203 (98)	207 (100)	193 (93)
9	199	205 (103)	195 (98)	198 (100)	187 (94)
11	203	204 (101)	195 (96)	204 (101)	202 (100)
13	183	183 (100)	183 (100)	180 (98)	174 (95)
Total food intake	2622	2651 (101)	2583 (99)	2475 (94)	2332 (89)
Females					
1	157	156 (99)	160 (102)	132 (84)	123 (78)
3	165	158 (96)	168 (102)	144 (87)	139 (84)
5	164	158 (96)	169 (103)	150 (92)	146 (89)
7	160	153 (96)	167 (104)	146 (91)	140 (88)
9	155	151 (97)	159 (103)	150 (97)	138 (89)
11	150	145 (97)	146 (97)	152 (101)	143 (95)
13	124	132 (107)	124 (100)	133 (107)	129 (104)
Total food intake	2004	1956 (98)	2027 (101)	1873 (93)	1784 (89)

Data taken from Table 3, pp. 45-46, MRID 44802102.
[1] Numbers in parentheses represent percentage of control value, as calculated by the reviewer.

2. Compound consumption

Animals were offered the compound *ad libitum* in fresh powdered rodent diet, Laboratory Animal Diet No. 2 on a weekly basis to reach the doses presented in Table 1. Because the dosages were not adjusted for increases in body weight, the achieved doses declined over time. Overall calculated dosages averaged 2.1, 21.1, 1150, and 2339 mg/kg/day for the low to high dosage groups, respectively.

3. Food efficiency

Mean food efficiency values were dramatically reduced in the 12,500- and 25,000-ppm male and female groups during the first study week. Food efficiencies for the 12,500-ppm males and females at week 1 were 53% and 55% of control values, respectively. Values for the high-dose males and females at week 1 were 18% and 27% of control efficiencies, respectively. Weekly food efficiencies quickly increased thereafter, and were not dose-related in either sex. However, the high-dose males usually had the lowest values relative to controls, and overall efficiencies for males were dose-related. Overall efficiencies for females showed a dose-related trend. Results are presented in Table 5.

TABLE 5. Group mean food efficiencies (%) in rats fed RPA400727 for 13 weeks[1,2,3]

Week of study	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
1	36.0	33.7 (94)	33.3 (93)	19.0 (53)	6.4 (18)
3	33.9	32.6 (96)	29.3 (86)	29.4 (87)	27.1 (80)
5	17.9	20.3 (113)	18.5 (103)	20.9 (117)	17.7 (99)
7	14.9	16.0 (107)	15.0 (101)	15.3 (103)	17.5 (118)
9	12.3	13.8 (112)	10.4 (85)	11.7 (95)	9.3 (76)
11	8.2	10.7 (131)	8.6 (105)	8.3 (101)	8.2 (100)
13	3.8	3.1 (82)	3.2 (84)	3.2 (84)	2.2 (58)
Overall (weeks 1-13)	17.0	17.3 (102)	16.8 (99)	14.9 (88)	13.6 (80)
Females					
1	27.5	26.8 (98)	28.0 (102)	15.0 (55)	7.5 (27)
3	21.9	20.6 (94)	21.4 (98)	19.5 (89)	21.1 (96)
5	13.0	11.8 (91)	12.1 (93)	8.1 (62)	10.8 (83)
7	10.9	8.8 (81)	11.0 (101)	7.7 (71)	10.3 (95)
9	7.6	7.5 (99)	4.3 (57)	4.7 (62)	7.2 (95)
11	8.3	4.8 (58)	8.4 (101)	7.9 (95)	5.9 (71)
13	1.9	2.7 (142)	11.3[4] (594)	2.5 (132)	3.0 (158)
Overall (weeks 1-13)	10.9	10.8 (99)	11.3[5] (104)	9.1 (84)	8.8 (81)

[1] Data from Table 4, pp. 47-48, MRID 44802102.
[2] Food efficiencies were calculated by study authors using formula: [weekly body weight change (g)/weekly food intake (g)](100)
[3] Numbers in parentheses represent percent of control value, as calculated by the reviewer.
[4] Calculated by reviewer. Table 4, p. 48, of MRID 44802102 provides no food efficiency value for this week, citing "bodyweight stasis or loss", although there was an increase in mean body weight of 14.0 g recorded on p. 44 of the study report for the 250-ppm females from weeks 12-13. The increase in the group mean body weight at week 13 was due to the exclusion of a body weight value for female no. 72, which was sacrificed *in extremis* that week.
[5] Value calculated by study reviewer, using the body weight value cited in footnote (4).

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related changes were observed upon ophthalmoscopic examination.

E. BLOOD WORK

1. Hematology

Slight alterations in several hematological parameters were observed in animals receiving 12,500- and 25,000-ppm RPA400727. The 12,500-ppm males had decreases in mean red blood cell counts (RBCs) (96% of controls, $p < 0.05$), and the 25,000-ppm males had decreases in hematocrit (HCT) (96% of controls, $p < 0.01$), hemoglobin (HGB) (96% of controls, $p < 0.01$), and RBCs (95% of controls, $p < 0.01$). Although mild, the decreases in HCT, HGB, and RBCs were generally dose-related in both sexes. Treated males, with the exception of the 250-ppm group experienced decreased platelet counts; the high-dose group had the lowest count (92% of the control value). Platelet counts were increased in treated females, relative to the control count, but there was no dose-response relationship.

Reticulocytes represented $\leq 2\%$ of the RBCs for all animals, with the exception of one 12,500-ppm male with a count of 3%. White blood cells were significantly increased in the 25-ppm males (127% of control, $p < 0.01$) and in the high-dose females (144% of controls, $p < 0.001$); no dose-response relationship was observed. Results are presented in Table 6.

TABLE 6. Selected mean hematologic values after 12 weeks in rats fed RPA400727[1]					
Parameter	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
Hematocrit (%)	45	46 (102)	46 (102)	45 (100)	43** (96)
Hemoglobin (g%)	15.8	16.2 (103)	16.1 (102)	15.8 (100)	15.2** (96)
MCV (μL^3)	51	52 (102)	52 (102)	53** (104)	51 (100)
Platelets (1000/cm)	909	836 (92)	913 (100)	840 (92)	833 (92)
PT (secs)	15.2	14.6 (96)	14.7 (97)	14.4* (95)	14.4 (95)
RBCs (x $10^6/mm^3$)	8.95	8.85 (99)	8.94 (100)	8.55* (96)	8.47** (95)
WBCs (x $10^3/mm^3$)	13.7	17.4** (127)	14.6 (107)	13.9 (102)	13.3 (97)
Females					
	0	25	250[2]	12,500	25,000
Hematocrit (%)	45	46 (102)	46 (102)	44 (98)	43** (96)
Hemoglobin (g%)	15.9	15.9 (100)	16.0 (101)	15.1* (95)	15.0** (94)
MCV (μL^3)	54	53 (98)	54 (100)	52** (96)	51*** (94)
Platelets (1000/cm)	886	1002* (113)	917 (104)	923 (104)	932 (105)
PT (secs)	14.2	15.5 (109)	15.6 (110)	14.5 (102)	14.2 (100)
RBCs (x $10^6/mm^3$)	8.40	8.59 (102)	8.59 (102)	8.40 (100)	8.28 (99)
WBCs (x $10^3/mm^3$)	8.8	7.4 (84)	7.2 (82)	10.2 (116)	12.7*** (144)

Data obtained from Table 6, pp. 51-52, and Appendix 8A, pp. 124-129, MRID 44802102.
[1] Numbers in parentheses represent percent of control values, as calculated by reviewer.
[2] Hematologic values for no. 72 (sacrificed *in extremis* during week 13) were not used in the derivation of the presented mean values for the 250-ppm females.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.
*** Significantly different from control group at $p < 0.001$.

Blood smear results revealed increases in the severity of anisocytosis and spherocytosis in males and females receiving doses ≥ 250 ppm of RPA400727. Although clear dose-response relationships were not observed, the severity of anisocytosis and spherocytosis was greatest in the high-dose males, and in females receiving ≥ 12,500 ppm of the compound. Slight macrocytosis was noted in 2 of 8 high-dose males. The blood smear from female no. 72 (250-ppm group), who was sacrificed *in extremis* during week 13, revealed marked anisocytosis and spherocytosis, moderate polychromasia, slight fragmentation (RBCs), and moderate hypersegmentation of neutrophils. Selected results are presented in Table 7.

TABLE 7. Morphological variations, severity, and incidence in blood smears after 12 weeks in rats fed RPA400727 for 13 weeks[1]					
Finding	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
No. males affected/No. samples					

TABLE 7. Morphological variations, severity, and incidence in blood smears after 12 weeks in rats fed RPA400727 for 13 weeks[1]					
Finding	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Anisocytosis					
Slight	7/10 (70)	8/10 (80)	10/10 (100)	7/9 (78)	3/8 (38)
Moderate	0/10 (0)	1/10 (10)	0/10 (0)	2/9 (22)	5/8 (62)
Macrocytosis					
Slight	0/10 (0)	0/10 (0)	0/10 (0)	0/9 (0)	2/8 (25)
Spherocytosis					
Slight	7/10 (70)	8/10 (80)	5/10 (50)	9/9 (100)	2/8 (25)
Moderate	1/10 (10)	0/10 (0)	2/10 (20)	0/9 (0)	6/8 (75)
Marked	0/10 (0)	1/10 (10)	0/10 (0)	0/9 (0)	0/8 (0)
No. females affected/No. samples					
Anisocytosis					
Slight	2/10 (20)	8/10 (80)	7/9 (78)	6/10 (60)	1/9 (11)
Moderate	0/10 (0)	0/10 (0)	1/9 (11)[2]	4/10 (40)	8/9 (89)
Marked	0/10 (0)	0/10 (0)	1/9 (11)[2]	0/10 (0)	0/9 (0)
Macrocytosis					
Slight	0/10 (0)	0/10 (0)	0/9 (0)[2]	0/10 (0)	0/9 (0)
Spherocytosis					
Slight	1/10 (10)	1/10 (10)	4/9 (44)	2/10 (20)	5/9 (56)
Moderate	0/10 (0)	0/10 (0)	1/9 (11)[2]	8/10 (80)	3/9 (33)
Marked	0/10 (0)	0/10 (0)	1/9 (11)[2]	0/10 (0)	0/9 (0)

Data taken from Appendices 4A and 8B, pp. 108 and 130-135, MRID 44802102.
[1] Numbers in parentheses are the percentage representations of the fractions shown, as calculated by the reviewer.
[2] Result for female no. 72.

2. Clinical chemistry

Glucose levels in were significantly decreased in males and females, 88 and 85%, respectively, at 25,000 ppm. Males and females receiving ≥ 12,500 ppm of RPA400727 had statistically increased cholesterol levels (133-204% of control values, $p \leq 0.01$); the effect was more pronounced in females. Mean alkaline phosphatase (ALP) activities were statistically increased in the high-dose males only (133% of controls, $p < 0.001$). Dose-response relationships were not observed for cholesterol or ALP. Results are presented in Table 8.

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TABLE 8. Selected mean clinical chemistry values after 12 weeks in rats fed RPA400727 for 13 weeks[1]

Parameter	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
ALP (IU/L)	90	93 (103)	104* (116)	97 (108)	120*** (133)
CHOL (mg%)	54	59 (109)	52 (96)	77*** (143)	72** (133)
GLUC (mg%)	138	155* (112)	150 (109)	133 (96)	121* (88)
Females					
	0	25	250	12,500	25,000
ALP (IU/L)	51	61 (120)	55 (108)	59 (116)	57 (112)
CHOL (mg%)	77	86 (112)	80 (104)	142*** (184)	157*** (204)
GLUC (mg%)	115	122 (106)	123 (107)	115 (100)	98** (85)

Data obtained from Table 7, pp. 53-56, MRID 44802102.
[1] Numbers in parentheses represent percent of control values, as calculated by reviewer.
[2] Female no. 72 was in the 250-ppm group, and sacrificed *in extremis* during week 13. Hematologic values for no. 72 were not included in deriving the presented mean values for the 250-ppm females.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.
*** Significantly different from control group at $p < 0.001$.
CHOL = cholesterol, GLUC = glucose

F. URINALYSIS

After 11 weeks of treatment, a generally dose-related decrease was observed in urinary pH in treated males and females, and high-dose males had more urinary crystals (not described) than any other study group. According to the grading system described by the authors, which measured numbers of crystals per field (see footnote # 1 for Table 9, this review), the mean group grade for males increased in a dose-related manner, except for those receiving 12,500 ppm. Treated females were not similarly affected. Control and treated males had more urinary protein than did females, but a dose-response relationship was not noted for either sex. Urinary crystal and pH results are presented in Table 9.

TABLE 9. Urinary crystals, pH, and numbers of affected animals after 11 weeks in rats fed RPA400727 for 13 weeks

Parameter	Exposure concentration (ppm)									
	0		25		250		12,500		25,000	
	M	F	M	F	M	F	M	F	M	F
Crystals										
0	1	2	1	3	1	4	5	2	1	3
1	8	2	6	4	5	5	2	6	1	7
2	1	6	3	3	4	1	3	2	3	0
3	0	0	0	0	0	0	0	0	5	0
Average grade[2]	**1.0**	**1.4**	**1.2**	**1.0**	**1.3**	**0.7**	**0.8**	**1.0**	**2.2**	**0.7**
pH	6.7	6.4	6.7	6.2*	6.6	6.0***	6.6	6.1**	6.5*	6.1**

Data obtained from Table 8, pp. 57-58, MRID 44802102.
[1] The study authors' grading system for presence of crystals was a "four-point scale ranging from 0 = none seen to 3 = many in all fields examined. The values for 1 and 2 were not defined.
[2] Average grade (i.e., relative score for numbers of crystals/field) was calculated by reviewer by multiplying the number of affected animals in each group by the severity level (0, 1, 2 or 3), summing the results for each group/sex, and dividing by the number of affected animals per group/sex.
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$.
*** Significantly different from controls, $p < 0.001$.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Because mean terminal body weights were highly variable among treated groups (76-102% of controls), brain weights, which ranged from only 96-106% of controls, were considered a more useful parameter for the derivation of relative organ weights. Several notable changes in absolute and relative organ weights were noted in one or both sexes. Dose-related decreased absolute and relative thymic weights in males and females were the most striking finding (relative weights at mid-high dose 75% and 76% of controls and high-dose: 66% and 73% of controls, respectively). Decreased absolute and relative cardiac weights were dose-related in males and females (relative high-dose weights: 84% and 88% of controls, respectively). Decreases in adrenal gland and lung weights were more pronounced in females; inexplicably, the 250-ppm groups had notable increases in adrenal gland weights. Kidney and spleen weights were decreased in a generally dose-related manner in males, while females were unaffected. In contrast, hepatic weights were markedly increased in a dose-related manner in females (high-dose absolute: 155% of controls, $p < 0.01$ and in 12,500 ppm group 138% of controls, $p < 0.01$), while only slight increases were noted in males. Ovarian weights were increased in the 12,500- and 25,000-ppm females. Results are provided in Table 10.

TABLE 10. Terminal weights and selected mean absolute (g) and relative (to brain) organ weights in rats fed RPA400727 for 13 weeks[1,2]

Organ	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Males					
No. Animals	**10**	**10**	**10**	**10**	**10**
Terminal BW	547.2	555.8 (102)	539.0 (99)	452.2** (83)	414.3** (76)
Brain	2.16	2.16 (100)	2.14 (99)	2.09 (97)	2.08 (96)
Adrenals					
Absolute	0.058	0.056 (97)	0.064 (110)	0.054 (93)	0.053 (91)
Relative (%)	2.7	2.6 (96)	3.0 (111)	2.6 (96)	2.6 (96)
Heart					
Absolute	1.63	1.59 (98)	1.56 (96)	1.49 (91)	1.32** (81)
Relative (%)	75.5	73.6 (98)	72.9 (97)	71.3 (94)	63.5 (84)
Kidneys					
Absolute	4.12	4.05 (98)	4.26 (103)	3.69 (90)	3.28** (80)
Relative (%)	190.7	187.5 (98)	199.1 (104)	176.6 (93)	157.7 (83)
Liver					
Absolute	17.4	17.7 (102)	17.3 (99)	19.1 (110)	18.6 (107)
Relative (%)	805.6	819.4 (102)	808.4 (100)	913.9 (113)	894.2 (111)
Lungs					
Absolute	1.97	1.97 (100)	1.86 (94)	1.82 (92)	1.71* (87)
Relative (%)	91.2	91.2 (100)	86.9 (95)	87.1 (96)	82.2 (90)
Spleen					
Absolute	0.907	0.891 (98)	0.866 (96)	0.871 (96)	0.751* (83)
Relative (%)	42.0	41.3 (98)	40.5 (96)	41.7 (99)	36.1 (86)
Thymus					
Absolute	0.516	0.508 (99)	0.508 (99)	0.385* (75)	0.329** (64)
Relative (%)	23.9	23.5 (98)	23.7 (99)	18.4 (77)	15.8 (66)
Females					
No. Animals	**10**	**10**	**9[3]**	**10**	**10**
Terminal BW	338.0	326.1 (97)	337.3 (100)	279.8** (83)	272.0** (81)
Brain	1.96	1.99 (102)	2.07** (106)	2.02 (103)	1.98 (101)
Adrenals					
Absolute	0.071	0.073 (103)	0.088** (124)	0.053** (75)	0.056** (79)
Relative (%)	3.6	3.7 (103)	4.3 (119)	2.6 (72)	2.8 (78)
Heart					
Absolute	1.16	1.15 (99)	1.14 (98)	1.06 (91)	1.03* (89)
Relative (%)	59.2	57.8 (98)	55.1 (93)	52.5 (89)	52.0 (88)
Kidneys					
Absolute	2.55	2.61 (102)	2.76 (108)	2.59 (102)	2.52 (99)
Relative (%)	130.1	131.1 (101)	133.3 (103)	128.2 (99)	127.3 (98)

TABLE 10. Terminal weights and selected mean absolute (g) and relative (to brain) organ weights in rats fed RPA400727 for 13 weeks[1,2]

Organ	Exposure concentration (ppm)				
	0	25	250	12,500	25,000
Liver					
Absolute	11.2	12.3 (110)	12.0 (107)	15.4** (138)	17.3** (155)
Relative (%)	571.4	618.1 (108)	579.7 (102)	762.4 (133)	873.7 (153)
Lungs					
Absolute	1.90	1.53 (81)	1.67 (88)	1.49 (78)	1.40* (74)
Relative (%)	96.9	76.9 (79)	80.7 (83)	73.8 (76)	70.7 (73)
Ovaries					
Absolute	0.090	0.098 (109)	0.105 (117)	0.120** (133)	0.114* (127)
Relative (%)	4.6	4.9 (107)	5.1 (111)	5.9 (128)	5.8 (126)
Spleen					
Absolute	0.662	0.605 (91)	0.695 (105)	0.719 (109)	0.696 (105)
Relative (%)	33.8	30.4 (90)	33.6 (99)	35.6 (105)	35.2 (104)
Thymus					
Absolute	0.481	0.434 (90)	0.463 (96)	0.365* (76)	0.353** (73)
Relative (%)	24.5	21.8 (89)	22.4 (91)	18.1 (74)	17.8 (73)

Data taken from Table 9A, pp. 59-61, MRID 44802102.
[1] Numbers in parentheses represent percent of control values, as calculated by reviewer.
[2] Relative organ to brain weights calculated by reviewer; statistics for relative weights were not calculated.
[3] Results in this column do not include those for the 250-ppm female, no. 72, which was sacrificed *in extremis* on day 89.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.

2. Gross pathology

By study termination, moderate to marked hair loss affected 1 male control and 2-3 males in each treated group, and 5 high-dose females. Ovarian pallor was noted in 1 high-dose female. Additional findings occurred in 1 or more treated animals, but their low incidence and/or similar incidence with controls precluded a determination of a probable causal relationship with the compound. Results are presented in Table 11.

TABLE 11. Selected macroscopic findings in rats fed RPA400727 for 13 weeks

Organ & Lesion	Exposure Dose (ppm)									
	Males					Females				
	0	25	250	12,500	25,000	0	25	250	12,500	25,000
Hair loss, marked	0	0	0	0	0	0	0	0	0	1
Hair loss, moderate	1	2	3	2	3	0	0	0	0	4
Liver, enlarged	0	0	0	0	0	0	0	0	0	4
Ovaries, pale	n.a.	n.a.	n.a.	n.a.	n.a.	0	0	0	0	1

Data taken from Table 10, pp. 65-69, MRID 44802102.
* Significantly different from control group at $p < 0.05$.
n.a. = not applicable

3. Microscopic pathology

Changes in the adrenal cortices and liver comprised the most notable findings in treated animals. The incidence of cortical fatty vacuolation of the adrenal glands was dose-related and statistically significant in both sexes, in male at ≥ 250 ppm and in females at ≥ 12,500 ppm, and more males were affected than females. Nine 12,500- and ten 25,000-ppm females experienced zona reticularis degeneration, while only one 25-ppm male was similarly affected. Renal congestion and cortical scarring were noted in no more than one animal from any one group; although no controls were affected, there was no dose-response relationship. Periacinar hepatocytic hypertrophy occurred in a dose-related manner, affecting males at doses of ≥ 250 ppm, and females receiving 25,000 ppm of RPA400727. Centriacinar hepatocytic fatty vacuolation appeared at a much greater incidence in females receiving ≥ 12,500 ppm, although no dose-response relationship was observed and three control females were affected. Hepatic extramedullary hematopoiesis was noted sporadically in treated males and females, but no high-dose animals were affected and the finding was not dose-related. Results are presented in Table 12.

TABLE 12. Selected histopathological findings in rats fed RPA400727 for 13 weeks

Organ & Lesion	Exposure Dose (ppm)									
	Males					Females				
	0	25	250	12,500	25,000	0	25	250	12,500	25,000
Adrenal cortex										
Cortical fatty vacuolation	1	4	8**	10***	10***	0	0	1	4	10***
Zona reticularis degeneration	0	1	0	0	0	0	0	0	9***	10***
Kidney										
Congestion	0	1	0	1	1	0	0	0	1	0
Cortical scar	0	1	1	0	0	0	0	0	1	0
Liver										
Periacinar hepatocytic hypertrophy	0	0	2	6*	10***	0	0	0	6*	9***
Centriacinar hepatocytic fatty vacuolation	0	3	1	1	2	3	1	4	7	10**
Extramedullary hematopoiesis	0	3	0	1	0	0	1	1	1	0

Data taken from Table 11, pp. 70-74, MRID 44802102.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.
*** Significantly different from control group at $p < 0.001$.

III. DISCUSSION

A. DISCUSSION

Primary target organ effects of RPA400727 involved the thymus, adrenals, liver, skin (hair loss), and possibly bone marrow. Thymic effects were similar for males and females, adrenal and hepatic effects were more pronounced in females, and the incidence of integumentary effects was higher in males.

Body weights and weight gains were markedly decreased by similar magnitudes for the 12,500- and 25,000-ppm males and females, with significantly decreased ($p \leq 0.01$) overall gains for these groups. The marginal decreases (89-94% of control values) in overall food consumption by groups receiving ≥ 12,500 ppm were inconsistent with poor palatability of the compound. Food efficiency results indicated a clear compound-related adverse effect, with notably decreased overall efficiencies for the 12,500- and 25,000-ppm groups (80-88% of control values).

Although a clear dose-response relationship was not observed, liver enlargement in females receiving ≥ 12,500 ppm of the compound ($p < 0.01$) suggested a female-specific effect at those doses. Centriacinar hepatocytic fatty vacuolation was not strictly dose-related in females, but occurred at a clearly increased incidence in females receiving 12,500 (7 affected) and 25,000 ppm (10 affected, $p < 0.01$). Because this finding also occurred in three control females, one 25-ppm female, and four 250-ppm females, the finding is of questionable toxicological significance in females at doses below 12,500 ppm. Centriacinar hepatocytic fatty vacuolation affected treated males, but the low incidence and lack of dose response obscured its toxicological significance. The slight

increases in cholesterol and ALP activity in high-dose males, and the dose-related increased incidence of periacinar hepatocytic hypertrophy in males receiving ≥ 250 ppm of RPA400727 ($p \leq 0.05$ at ≥ 12,500 ppm) may have been associated with a cholestatic effect of the compound, although hypertrophy alone is not considered an adverse effect. Dose-related periacinar hepatocytic hypertrophy was noted in females receiving ≥ 12,500 ppm of the compound ($p \leq 0.05$).

Decreased serum glucose levels in high-dose animals (88% and 85% of controls for males and females, respectively) were consistent with their reduced food consumption; alternatively, the lowered glucose levels and food efficiencies observed at 25,000 ppm may have reflected altered adrenal glucocorticoid secretion related to the adrenal gland changes noted in both sexes. At compound doses of ≥ 12,500 ppm, mean absolute and relative adrenal gland weights were decreased slightly in males and markedly in females. Accompanying histopathological findings included adrenal cortical fatty vacuolation in males (incidence in control through high-dose: 1, 4, 8, 10, and 10, respectively) and in the corresponding female groups (0, 0, 1, 4, and 10, respectively). Adrenal zona reticularis degeneration was noted in one 25-ppm male (not significant), nine 12,500-ppm females ($p < 0.001$), and ten 25,000-ppm females ($p < 0.001$).

The absolute and relative (to brain) weights of several organs were affected by compound consumption, but accompanied by few or no macroscopic or microscopic observations. Marked dose-related decreases in absolute and/or relative weights of the heart, lungs, and thymuses of both sexes were unaccompanied by consistent histopathology, obscuring their toxicological significance. Absolute and relative renal weights were decreased in males receiving ≥ 12,500 ppm (relative weights: 93% and 83% of control weights, respectively). Due to low incidences, the renal congestion noted in one treated male from each of the 25-, 12,500-, and 25,000-ppm groups, and the cortical scarring noted in one 25- and one 250-ppm male were of questionable toxicological significance. The dose-related increased numbers of crystals observed in treated males (with the exception of the 12,500-ppm group), and the slight, dose-related decreases in urinary pH noted in both sexes may have been compound-related.

Hair loss from the head, and dorsal and ventral body surfaces, forelimb(s) and/or hindlimb(s) in both sexes was generally dose-related, indicating an association with compound administration. Hair loss affected more males than females. All 12,500- and 25,000-ppm animals were affected within the first week of treatment. It is improbable that the hair loss resulted from compound-induced aggressive behavior, because site-specific (i.e., hindlimb) hair loss appeared only in the high-dose animals. In addition, there was a strong correlation with increasing dose and the cessation of hair loss in > 1 body site at study week 9. The reason for the timing of this phenomenon at week 9 is unexplained.

A generally positive correlation of compound dosage with the incidence and severity of anisocytosis and spherocytosis in females receiving ≥ 12,500 ppm, and with the incidence and severity of anisocytosis in males receiving ≥ 12,500 ppm was consistent with an adverse effect of RPA400727 on bone marrow function. The mild decreases in HCT,

HGB, and RBCs; marked decrease in thymic weights; and anisocytosis and spherocytosis were suggestive of a compound-related adverse effect on the immune system and bone marrow function of both sexes. Although the hepatic extramedullary hematopoiesis observed in some treated animals was also consistent with this scenario, the incidence distribution did not suggest a relationship with compound administration. The marginal decreases in food consumption by high-dose animals, and the absence of compound-related extramedullary hematopoiesis or hemosiderin in the spleen or liver suggested that the decreases in parameters of circulating RBCs were unrelated to diet or hemolysis.

Under the conditions of this study, the statistically significant adrenocortical fatty vacuolation in 250-ppm males indicates a LOAEL of 250 ppm (19.8 mg/kg/day) and a NOAEL of 25 ppm (2.0 mg/kg/day) for males. The hair loss, decreases in food efficiencies, increases in the severity of anisocytosis and spherocytosis, adrenocortical fatty vacuolation and zona reticularis degeneration and centriacinar hepatocytic fatty vacuolation in females receiving 12,500 ppm (1183.5 mg/kg/day) indicate a LOAEL of 12,500 ppm (1183.5 mg/kg/day) and a NOAEL of 250 ppm (22.3 mg/kg/day) for females.

This subchronic toxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral study in rats [OPPTS 870.3100 (§82-1a)].

B. STUDY DEFICIENCIES

Several deficiencies were identified in this study:

1. Homogeneity analysis indicated that the 25-ppm dietary admixture was not sufficiently homogeneous. Because the analysis was not repeated on subsequent admixtures, the homogeneity of the 25-ppm diet could not be definitively determined.

2. No stability analysis was conducted for this study.

3. Although recommended by OPPTS 870.3100, cholinesterase and triglycerides were not assayed.

4. Pharyngeal and epididymal weights were not obtained, as recommended by OPPTS 870.3100.

5. Page 26 of the text reported that the adrenals were examined microscopically for "all rats" receiving 25, 250, and 12,500 ppm. However, results of microscopic examination of the adrenals were presented for all study animals, excepting one 25-ppm male and one 250-ppm female.

6. The description of urine collection on p. 23 of study report MRID 44802102 was unclear. If food and water were actually withheld for 20 hours, the length of the fasting period seems excessive.

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7. The data presented in Table 1, p. 39 for numbers of animals affected with hair loss from various body sites were not in agreement with the data presented in Appendix 3, pp. 85-107.

8. Different strains of rats were used for the 4-and 13-week studies, impeding a direct comparison of the studies' results.

APPENDIX

CITATION: Aughton, P. (1991) RPA400727: Preliminary toxicity study by dietary administration to F-344 rats for four weeks. Life Science Research Limited, Eye, Suffolk, England, IP23 7PX. LSR Report No.: 90/RHA359/0947, March 1, 1991. MRID 44802101. Unpublished.

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

COMPLIANCE: Signed and dated GLP, Data Confidentiality and Flagging statements were provided for MRID 44802101, but no Quality Assurance statement.

STUDY DESIGN:

Five groups of Fischer 344 rats/sex were offered RPA400727 (99.5% a.i., lot no. YG2156/1) in feed at dose levels of 0, 500, 1500, 5000, 15,000, or 50,000 ppm for 4 weeks (Table 1) to identify appropriate dose levels for subsequent studies. The animals were approximately 5-6 weeks of age at treatment commencement. Respective mean body weights for males and females at treatment commencement were 98-100g and 88-90g. The animals were sacrificed on day 29 of the study (in-life dates: start, April 20, 1990; end: May 18, 1990).

TABLE 1. Study design

Test group	Estimated dose (ppm)	Estimated dose (mg/kg/day)		Number of animals	
		Males	Females	Males	Females
1	0	0	0	5	5
2	500	50.12	52.44	5	5
3	1500	152.3	151.3	5	5
4	5000	513.2	489.4	5	5
5	15,000	1494.0	1476.0	5	5
6	50,000	4802.0	4945.0	5	5

Data taken from Table 4, p. 40, MRID 44802101.

DOSE PREPARATION AND ANALYSIS:

An initial premix was prepared by incorporating the highest concentration of RPA400727 into the rodent diet. The admixture was passed through a cross beater mill and followed by dilution with further quantities of diet and mixing for 15 minutes in a planetary mixer. A portion of the diet was then given to the high-dose groups, and diets for the lower dosage groups were prepared by a process of serial dilution. Test diets were prepared fresh weekly.

Samples of the pretreatment 500- and 50,000-ppm diets were taken from six positions in the mixer and assayed for homogeneity. Mean respective concentrations for the low- and high-dose diets were 119% (range: 105-133% of nominal) and 114% (range: 109-118% of nominal) of the nominal concentrations. Stability analysis samples were taken from the lowest and highest dose pretreatment diet admixtures by pooling the unused portions of the six homogeneity samples for each group and calculating the mean concentration. Stability assays were conducted after 7 and

19 days of storage at room temperature (note: for stability results, and a description of a discrepancy between the stability analyses reported in MRIDs 44802101 and 44802102, refer to the Stability Analysis description on p. 5 of the attached subchronic study). Concentration analyses conducted at each dosage level for treatment weeks 1 and 4 indicated that all week 1 mean values were 97-103% of the nominal, and all week 4 mean concentrations were 91-99% of the nominal.

METHODS:

A powdered rodent diet [Laboratory Animal Diet No. 2 (Biosure, Manea, Cambridgeshire, England)] and water were provided *ad libitum*. Animals were observed ≥ 2 times daily for morbidity, mortality, or treatment-related effects. A detailed weekly examination was performed on each animal.

RESULTS:

All animals survived to termination. Primary toxicologically relevant target organs of RPA400727 were the integument, liver, uterus and, possibly, bone marrow. Moderate to marked hair loss on the head and dorsal body and/or dorsal cervical surfaces, thin body build, and piloerection were observed throughout the treatment period in high-dose animals only, with the exception of the absence of hair loss in one male. Marked hair loss occurred in females only. Data were not provided for thin body build and piloerection.

Dose-related decreases in mean body weights and body weight gains were noted for males and females (overall body weight gains for high-dose males and females: 45% and 47% of controls, respectively). Overall body weight gain decreases were biologically and statistically significant in males receiving ≥ 5000 ppm of RPA400727 and in females receiving ≥ 1500 ppm (with the exception of no statistical significance in 5000-ppm females). Weekly food consumption was decreased in a dose-related manner for males and females, with the most severe decreases occurring during the first week, followed by improvement thereafter. Decreases in overall food consumption were dose-related (high-dose males and females: 72% and 76% of control values, respectively). At doses of 15,000- and 50,000-ppm, males were affected slightly more than females. Weekly food conversion ratios (food consumption/body weight change) were variable for males and females. Overall food conversion ratios were generally increased in a dose-related manner for males and females, and were biologically relevant for males receiving ≥ 5000 ppm and for females receiving ≥ 1500 ppm of the compound. Statistical analysis was not conducted on food consumption or food conversion ratios.

Slight decreases were noted in hematocrit, hemoglobin, and red blood cell counts in high-dose males and females (all values 93-99% of controls). Blood smear results showed slight anisocytosis in all 50,000-ppm animals, and slight macrocytosis in one 50,000-ppm female. In addition, decreased white blood cell counts were biologically and statistically significant for the 50,000-ppm males (63% of control value) and 15,000- and 50,000-ppm females (66% and 79% of control value, respectively). As relative thymic weights were markedly decreased for males receiving ≥ 5000 ppm (89%, 83%, and 67% of control value, respectively), and for females receiving ≥ 15,000 ppm of the compound (80% and 60% of control, respectively), the blood

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smear and hematological findings may have been related to a thymic effect on bone marrow function in high-dose animals.

Decreased serum glucose was the only notable clinical chemistry observation and was most likely due to lowered food consumption. The decreases were dose-related in females, and showed a dose-response relationship in males receiving ≥ 5000 ppm. Presented as percentage of the control value, respective mean glucose levels for low- to high-dose males were 101%, 106%, 89% ($p < 0.01$), 81% ($p < 0.001$), and 62% ($p < 0.001$); and 98%, 92%, 89%, 79% ($p < 0.01$), and 60% ($p < 0.001$) for the corresponding female groups.

All high-dose males and females had high urinary ketone values, which was most likely related to their poor nutritional status and/or stress; ketonuria was absent in the other groups. No other biologically relevant urinary results were found.

Because of the wide variability in body weights among test groups, mean brain weight (93-99% of control value) was considered a more useful parameter for the determination of relative organ weights. Absolute and relative weights for several organs in males and/or females showed dose-related changes or marked changes in only high-dose animals. Many of the weights varied biologically and/or statistically from control weights. Relative weights for the adrenals, heart, kidneys, lungs, and spleen were decreased in high-dose females (78%, 85%, 88%, 78%, and 88% of control weights, respectively). For females, dose-related relative weight decreases were noted for the thymus and uterus with cervix, and a dose-related increase was noted for the liver (high-dose only: 60%, 36%, 175% of controls, respectively). Males receiving ≥ 15,000-ppm had dose-related decreases in relative weights for the heart, spleen, and thymus (high-dose only: 79%, 80%, and 67% of controls, respectively). Decreased relative renal and prostate weights were dose-related in males (high-dose only: 77% and 20% of controls, respectively), and only high-dose males had decreased relative lung and testicular weights (88% of controls for both). Only the liver in males and females, and the uterus had associated histopathological findings; however, it should be noted that an abnormally-shaped spleen was noted in one high-dose male and in one 15,000-ppm female.

Macroscopic findings included pale cecal contents in three high-dose males and two high-dose females, which were considered most likely compound residue. Four high-dose females were emaciated ($p < 0.05$).

Histopathologic findings were limited to the liver and the uterus (Table 2). Centriacinar and periacinar hepatocytic fatty vacuolation and "hepatocytic" fatty vacuolation affected one or more males and females receiving ≥ 500 ppm of RPA400727. Although clear dose-response relationships for each finding were not evident, the findings were related to dose in order of appearance in both sexes: periacinar hepatocytic fatty vacuolation was noted at a lower dose, followed by hepatocytic and then centriacinar hepatocytic fatty vacuolation.

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TABLE 2. Selected histopathologic findings in rats fed RPA400727 for 4 weeks

Organ/Lesion	Exposure concentration (ppm)					
	0	500	1500	5000	15,000	50,000
Males						
Liver						
Panacinar microvacuolation	0	0	0	0	1	5**
Focal necrosis	0	1	0	0	1	0
Hepatocyte necrosis	0	0	0	0	2	2
Centriacinar hepatocytic fatty vacuolation	0	0	0	0	0	5**
Periacinar hepatocytic fatty vacuolation	0	1	1	0	2	0
Hepatocytic fatty vacuolation	0	0	0	1	3	0
Hepatocyte hypertrophy	0	0	0	0	0	1
Females						
Liver						
Panacinar microvacuolation	0	0	0	0	5**	3
Focal necrosis	0	0	0	0	0	0
Hepatocyte necrosis	0	0	0	0	1	0
Centriacinar hepatocytic fatty vacuolation	0	0	0	0	3	1
Periacinar hepatocytic fatty vacuolation	0	0	0	3	0	0
Hepatocytic fatty vacuolation	0	0	0	2	0	3
Hepatocyte hypertrophy	0	0	0	0	0	4*
Uterus						
Reduced endometrial stroma	0	0	0	0	2	5**

Data taken from Table 11, pp. 60-61, MRID 44802101.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.

Under the conditions of this study, the periacinar hepatocytic fatty vacuolation, hepatocytic fatty vacuolation, and statistically and biologically significant increased liver weights noted in females receiving doses of 5000 ppm (489.4 mg/kg/day) and above indicate a LOAEL of 489.4 mg/kg/day and a NOAEL of 1500 ppm (151.3 mg/kg/day) for females. The panacinar microvacuolation, hepatic focal necrosis, hepatocyte necrosis, periacinar hepatocytic fatty vacuolation, and hepatocytic fatty vacuolation in males indicates a LOAEL of 15,000 ppm (1494.0 mg/kg/day) and a NOAEL of 5000 ppm (513.2 mg/kg/day) for males.

The study authors identified a NOAEL of 1500 ppm for male and female F-344 rats on the basis of "clear evidence of toxicity" at doses ≥ 5000 ppm.

STUDY DEFICIENCIES:

Several deficiencies were identified in this study:

1. Overall (week 1-4) food conversion ratios presented on page 39 of MRID 44802101 were not calculated correctly.

2. Homogeneity analyses indicated that the low- and high-dose diets were not sufficiently homogeneous.

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