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DATA EVALUATION REPORT

RPA400727 (TRITICONAZOLE)

STUDY TYPE: CHRONIC TOXICITY/ONCOGENICITY ORAL STUDY - RAT [OPPTS 870.4300 (§83-5)] MRID 44802107

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding- rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D261924
P.C. CODE: 125620

SUBMISSION CODE: D568827
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): RPA400727 (Triticonazole) (97.1%, a.i.)

SYNONYMS: 2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1,2,4-triazolylmethyl)-1-cyclopentanol (IUPAC)

CITATION: Aughton, P. (1994) RPA400727: Combined oncogenicity and toxicity study by dietary administration to CD rats. Pharmaco LSR, Ltd., Eye, Suffolk, IP23 7PX, England. Laboratory report number, 94/RHA445/0134, June 28, 1994. MRID 44802107. Unpublished.

SPONSOR: Rhone-Poulenc Secteur Agrochimie, Centre de Recherche, 355 rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France.

EXECUTIVE SUMMARY: In a chronic toxicity/oncogenicity study (MRID 44802107), RPA400727 (97.1% a.i., Batch No. DA646) was administered continuously in the diet to groups of 50 male and 50 female CD rats at concentrations of 0, 5, 25, 750, or 5000 ppm for 99 or 100 weeks. Weight-normalized doses were 0, 0.2, 1.0, 29.4, and 203.6 mg/kg/day, respectively, for males and 0, 0.3, 1.3, 38.3, and 286.6 mg/kg/day, respectively, for females. Additional groups of 15 male and 15 female rats per sex per dose were similarly administered the test material and sacrificed at 26 and 53 weeks for interim evaluations.

Clinical signs of toxicity, mortality, hematology parameters, and clinical chemistry parameters were not affected by treatment with any dose. Excessive mortality occurred in control and the lower dose groups prompting early termination of the study. Except for mean body weights and body weight gain in high-dose females, rats at all doses had mean body weights, body weight gain, food consumption, and food conversion efficiency values similar to those of controls. Females administered the 5000-ppm diet weighed up to 18% ($p<0.01$ or <0.05) less than controls between week 0 and week 86 of the study and gained 29% ($p<0.01$) less weight during the first week of treatment, 22% less during the first year, and 10% less over the entire study.

Postmortem evaluations showed no treatment-related effects on organ weights, and the only gross finding associated with microscopic changes was areas of change in the lungs of high-dose females (11/50 vs 3/50 controls, $p<0.05$). Liver and adrenal cortical toxicity and accumulation of

alveolar macrophage in the lungs were observed in female rats. The incidences of multinucleated cells in the zona fascicularis in the adrenal cortex was 9/15 ($p<0.01$), 3/14 (N.S.), and 3/50 (N.S.) high-dose females at 26 weeks, 53 weeks, and in the main study group, respectively, compared with none of the controls. In addition, 4/14 ($p<0.05$) high-dose females sacrificed at 53 weeks had chronic inflammation of the zona fascicularis compared with none of the controls. Centriacinar fatty vacuolation of liver hepatocytes was noted in 33/50 ($p<0.01$) high-dose females compared with 16/50 controls. Accumulation of alveolar macrophages was observed in 7/50 ($p<0.05$) high-dose females compared with 0/50 controls. No treatment-related microscopic lesions occurred in male rats at any dose level or in female rats at ≤750 ppm.

In conclusion, the lowest-observed-adverse-effect level (LOAEL) is 5000 ppm (286.6 mg/kg/day) based on decreased body weight and body weight gain and adrenal cortical and liver toxicity in females; adverse effects were not observed in male rats. The no-observed-adverse-effect level (NOAEL) was 750 ppm (38.3 mg/kg/day) for females and ≥5000 ppm (203.6 mg/kg/day) for males.

At the doses tested, RPA400727 showed no carcinogenic activity in either male or female rats after administration for up to 100 weeks. Dosing was considered adequate based on adverse effects on body weights and body weight gain, liver toxicity, and adrenal cortical toxicity in female rats. Male rats could have tolerated a higher dose. However, since females were adequately dosed; the lack of adverse effects in male rats is not considered a deficiency.

This chronic toxicity/oncogenicity study in the rat is **Acceptable/Guideline** and does satisfy the guideline requirement for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. The variance between the actual and measured concentration for the 5-ppm dietary group was much higher than acceptable, resulting in a higher dose than reported in the study. Because, the NOAEL was higher than 5 ppm, this deficiency had no impact on the conclusions of this study.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: RPA400727

   Description: white to yellowish powder
   Lot/Batch #: DA646
   Purity: 97.1% a.i.
   Stability of compound: for duration of the study
   CAS #: none reported

2. Vehicle and/or positive control

The test material was administered in the diet (powdered Laboratory Animal Diet No. 2, Special Diets Services Ltd., Witham, Essex, England); no positive control was used in this study

3. Test animals

Species: rat
Strain: CD
Age and weight at study initiation: 35 - 42 days old; males: 179 ± 13.3 - 182 ± 12.9 g; females; 152 ± 11.4 - 155 ± 10.6 g
Source: Charles River (UK) Ltd., Margate, Kent, England
Housing: Housed five of one sex per cage (stainless steel measuring 36 × 51 × 21 cm with stainless-steel mesh lid and floor).
Diet: powdered Laboratory Animal diet No. 2, *ad libitum*
Water: public water supply (Suffolk Water Company, Lowestoft, England), *ad libitum*
Environmental conditions:
Temperature: 21 °C (target)
Humidity: 55% (target)
Air changes: 15 changes/hour (target)
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates

Start: June 12, 1991; end: May 15-21, 1993

2. Animal assignment

Animals were randomly assigned to the test groups listed in Table 1 based on computer-generated random numbers. Allocation was such that all individual body weights were within 20% of the mean for each sex, and the group means were not statistically different from each other. An additional 10 animals per sex were provided for baseline clinical pathology evaluations. The animals in the 52-week interim sacrifice group were sacrificed at 53 weeks.

TABLE 1: Study design									
Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)[a]		Interim (26 weeks)		Interim (52 weeks)		Main Study (99/100 weeks)	
		Male	Female	Male	Female	Male	Female	Male	Female
1 - Control	0	0	0	15	15	15	15	50	50
2- Low	5	0.2	0.3	15	15	15	15	50	50
3 - Low-intermediate	25	1.0	1.3	15	15	15	15	50	50
4 - High-intermediate	750	29.4	38.3	15	15	15	15	50	50
5 - High	5000	203.6	286.6	15	15	15	15	50	50

Data taken from page 23 and 41, MRID 44802107.
[a]Based on nominal concentration of RPA400727.

3. Dose selection rationale

Dose selection was based on a 4-week study in F-344 rats and a 13-week study in CD rats. Hepatotoxicity was observed at dietary concentrations ≥5000 ppm and adrenal changes at ≥250 ppm, and no effects at 25 ppm.

4. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of test substance with powdered Laboratory Animal Diet No. 2 to prepare two premixes, one for the 5- and 25-ppm diets and one for the 750- and 5000-ppm diets. The premixes were diluted with appropriate amounts of food and mixed in an electrically grounded mixer. The prepared diets were stored in sealed polyethylene bags. Mixing procedures for the 5- and 25-ppm diets were established after three trial mixes. Homogeneity and stability were tested before study initiation, and homogeneity was again analyzed during week 75 (low concentrations only). Homogeneity was tested on six samples taken from regularly spaced positions in the mixer of the 5- 25-, and 50,000-ppm diets. Stability was tested on a composite of six samples from the 5-ppm diet stored at ambient temperature for 7 or 16 days. Stability of a 50,000-ppm preparation was tested in another study (LSR Report No. 90/RHA359/0947). During the study, samples of treated food were analyzed for concentration weekly for the first 4 weeks, every 8 weeks from week 12 to 84, and at weeks 94 and 100.

Results –
Homogeneity Analysis: **5 ppm**: One of the six samples was only 85.6% of the target concentration; the remaining samples taken pretest and at week 75 were within ±11% of the target concentration. **25 ppm**: all samples were within ±10% of the target. **5000 ppm**: One of the six samples in one trial mixtures was 112% of nominal; the remaining samples in the two trials were within ±10% of the nominal concentration.

Stability Analysis: 5 ppm: the concentration after storage at ambient temperature for 7 days (83-87% of day 0 concentration) was less than after storage for 16 days (93-94% of day 0 concentration), suggesting a problem with measurement accuracy rather than stability. **50,000 ppm**: the concentration after storage for 7 days was 89-90% of day 0 and 86-87% after storage for 16 days.

Concentration Analysis: 5 ppm: the concentration of test material in all except four dietary preparations exceeded the target by more than 10%; almost all exceeded the target by more than 20% and ranged up to 66% greater than the target. Concentration of test material in all **25-, 750-, and 5000-ppm** dietary preparations tested were within ±10% of the target except for two at the 25-ppm level (+27% and +14%).

The analytical data indicated that the mixing procedure for the 25-, 750-, and 5000-ppm diets was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The mixing procedure for the 5-ppm diet was inadequate because the concentration of test material was significantly higher than the target throughout the study; therefore, the weight-normalized doses based on nominal concentrations are lower than the actual doses received by the rats.

5. Statistics

Mortality: Cox's proportional hazards model and Tarone's partition of the chi-square statistics into linear trend on dose and deviation from linearity – two-tailed.

Hematology (excluding morphology from blood smears), blood chemistry, urinalysis (pH, specific gravity, and volume): Student's t-test.

Organ weights, food consumption, body weights: Bartlett's test for homogeneity of variance was performed; if Bartlett's test was significant then Behrens-Fisher test was performed for pairwise comparisons, otherwise Dunnett's test was used.

Ophthalmoscopic, macroscopic, and microscopic findings: Fisher's Exact test.

Statistical significance was indicated by $p<0.05$.

C. METHODS

1. Observations

Animals were inspected twice a day for signs of toxicity (ill health); debilitated animals were isolated to prevent cannibalism, and animals *in extremis* were sacrificed. Detailed physical examinations with palpations were conducted weekly.

2 Body weight

Animals were weighed during acclimatization, the day treatment was initiated, weekly for the first 14 weeks, and every 2 weeks thereafter.

3. Food consumption and compound intake

Food consumption for each cage was measured each week throughout the study. Mean weekly diet consumption was calculated as g food/animal/week. Weekly food efficiency values ((body weight gain in kg/food consumption in kg per unit time) × 100) were calculated for the first 14 weeks, and mean compound intake (mg/kg/day) values were calculated for the same weeks as body weights were determined.

4. Ophthalmoscopic examination

Eyes were examined in all animals before initiation of treatment and in all surviving control and 5000-ppm group animals killed at 24 weeks, 50 weeks, and study termination

5. Blood was collected from animals fasted overnight for hematology and clinical analysis. Blood samples were collected from ten male and ten females before treatment began, and from ten animals per sex per group at weeks 24, and 52 (interim phase animals) and 76 and 97 (main study animals). The CHECKED (X) parameters were examined.

a. Hematology

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

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

b. Clinical chemistry

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

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

6. Urinalysis

Urine was collected from 10 male and 10 female animals before study initiation and from 10 rats per sex per group at weeks 23 and 49 weeks (interim sacrifice animals) and weeks 75 and 97 (main study animals). Water and food were removed during collection of urine. 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

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

7. Sacrifice and pathology

All animals that died and those sacrificed moribund or at scheduled times were subjected to gross pathological examination. The animals were sacrificed by carbon dioxide asphyxiation. The CHECKED (X) tissues were collected for microscopic examination. Except as noted, tissues from animals sacrificed after 26 and 53 weeks, control and 5000-ppm main study groups, all animals dying before study termination, and all animals sacrificed *in extremis* were examined microscopically. In addition, the liver, kidney, and lungs from all animals in the 25- and 750-ppm dose groups were

examined microscopically. The following tissues were held in fixative but not examined microscopically: aorta, right eye and optic nerve, Harderian glands, cranial mammary gland, right submandibular salivary gland, right sciatic nerve, seminal vesicle, thigh skeletal muscle, skin, and tongue. The [XX] organs were weighed.

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

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity/oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No treatment related clinical signs were observed upon examination of male or female rats receiving any dose of the test material. The most common findings in main study animals included hair loss on at least 74% of rats in all groups and brown staining on at least 94%.

2. Mortality

Mortality and survival rates are summarized in Table 2. The mortality rate in control males and females was greater than that of the treated groups, but no treatment-related effect was observed. Sufficient animals were alive at 78 weeks to assess the effect of treatment with RPA400727 on late-developing lesions. Both male and female groups were terminated early because of excessive mortality; only 28% of males and 38% of females in the control groups were alive at weeks 99 and 100, respectively.

TABLE 2. Mortality/survival rates in male and female rats fed RPA400727 for up to 100 weeks -main Study

Week of study	Dietary concentration (ppm)				
	0	5	25	750	5000
Males					
Week 52 – mortality	4 (8%)	4 (8%)	2 (4%)	1 (2%)	2 (4%)
Week 78 – mortality	20 (40%)	11 (22%)	16 (32%)	12 (24%)	8 (16%)
Week 99 – mortality	36 (72%)	27 (54%)	36 (72%)	33 (66%)	29 (58%)
Week 99 – survival	14 (28%)	23 (46%)	14 (28%)	17 (34%)	21 (42%)
Females					
Week 52 – mortality	1 (2%)	0 (0%)	4 (8%)	1 (2%)	2 (4%)
Week 78 – mortality	11 (22%)	13 (26%)	8 (16%)	15 (30%)	6 (12%)
Week 100 – mortality	31 (62%)	36 (72%)	34 (68%)	32 (64%)	22 (44%)
Week 100 – survival	19 (38%)	14 (28%)	16 (32%)	18 (36%)	28 (56%)

Data taken from Table 3C, pages 75-77, MRID 44802107.

B. BODY WEIGHT

Selected mean body weights and body weight gain data are summarized in Table 3. Mean body weights of male rats receiving 5000 ppm of the test material were generally lower but within 7% (N.S.) of that of the control group throughout the study. Mean body weights of females receiving the 5000-ppm diet were significantly less than those of the control group throughout most of the study. High-dose females weighed 5% ($p<0.05$) less than controls at week 1, 15% ($p<0.01$) less at week (52), 18% ($p<0.05$) less at week 86, and 8% (N.S.) less at week 100. High-dose males gained 20% ($p<0.01$) less weight than controls during the first week of treatment, gained 6% less during the first year, and gained more weight than controls during the second year; weight gain over the entire study was similar in high-dose and control males. High-dose females gained 29% ($p<0.01$) less weight than controls during the first week of the study, 22% less during the first year, 39% more during the second year, and 10% less than controls over the entire study. The test material administered at 5, 25, or 750 ppm had no effect on body weights or body weight gain in either sex.

TABLE 3. Selected mean body weight, body weight gain, food consumption, and food efficiency data for male and female rats fed RPA400727 for up to 99/100 weeks

Week	Dietary Concentration (ppm)									
	0	5	25	750	5000	0	5	25	750	5000
Mean Body Weights (g)										
0	180	179	182	179	180	153	155	154	152	152
1	244	244	247	243	231	184	184	183	182	174* (95)[a]
8	509	509	514	516	497	299	299	293	294	276* (92)
13	586	585	594	592	569	334	335	326	330	303* (91)
26	721	720	727	727	693	384	385	377	379	344* (90)
54/52[b]	886	876	924	917	844	483	488	464	486	411* (85)
78	954	963	1045	983	914	584	579	542	562	487* (83)
88	958	941	1044	1029	917	619	607	547	562	519 * (84)
98/100[b]	877	913	930	946	866	558	571	570	623	515 (92)
Body Weight Gain (g)										
0-1	64	65	65	64	51** (80)	31	29	29	31	22** (71)
0-54/52[b,c]	706	697	742	738	664 (94)	330	333	310	331	259 (78)
54/52-term[c]	-9	37	6	29	22	75	83	106	137	104 (139)
0- term.	698	732	748	771	690	404	423	417	472	363 (90)
Food Consumption (g/animal)										
1-99/100	20,725	20,522	20,505	20,329	20,136	16,004	15,537	15,318	15,435	15,437
Food Conversion Efficiency (%)										
1-14	14.4	14.5	14.6	14.7	14.0	8.5	8.5	8.3	8.5	7.3

Data taken from Table 4A and 4B (pp. 78-84), 5 (pp. 85-94), and 6 (p. 95), MRID 44802107.
[a]Numbers in parentheses are percent of control calculated by the reviewer.
[b]Week of body weight measurement, male/female
[c]Body weight gain calculated by the reviewer.
*$p \leq 0.05$, $p \leq 0.01$, statistically significant, treated group compared with the control group calculated by the reviewer except for body weight gain (0-1 week).

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Overall food consumption values are presented in Table 3. Administration of RPA400727 had no effect on weekly or overall food consumption at any dose in either sex compared with control rats.

2. Compound consumption

Compound intake values are summarized in Table 1.

3. Food efficiency

Overall food conversion efficiency values are presented in Table 3. Food efficiency conversion values were calculated for the first 14 weeks of the study. Food efficiency for high-dose male rats was 17% less than that of controls during the first week of treatment and similar to that of controls for the remaining 13 weeks and for the 14 weeks overall. Food efficiency for high-dose females was 27% less than that of controls during the first week of treatment and 14% less over the 14 weeks.

D. OPHTHALMOSCOPIC EXAMINATION

The only notable ophthalmoscopic finding in male and female rats receiving the test material was the significantly increased incidence of sclerosis (9/22 vs 1/14 for controls, $p<0.05$) of the lens in high-dose males at 98 weeks.

E. BLOOD WORK

1. Hematology

No treatment-related hematologic changes were observed in male or female rats receiving any dose of the test material. Statistically significant differences were observed for some parameters in high-dose animals, but were transient or not biologically significant. The changes did not exceed ±4% for the erythrocyte parameters. The lymphocyte count was depressed by 19% in high-dose males at 76 weeks. Platelet counts were depressed in high-dose males by 10% at 24 weeks and 15% at 76 weeks compared with controls and in high-dose females by 16% at 76 weeks and by 19% at 97 weeks compared with controls. Prothrombin time was slightly increased ($p<0.05$ or <0.01) in 25-, 750-, and 5000-ppm group females at week 76 compared with controls and at 5000 ppm at week 97 compared with controls.

2. Clinical chemistry

Serum alanine aminotransferase activity in 5000-ppm group male rats was decreased by 26–45% at all time points compared with control levels; statistical significance was achieved at weeks 24 and 97. In 5000-ppm group females, alanine aminotransferase activity was significantly decreased by 27–39% at all time points compared with controls. Total cholesterol was significantly decreased in high-dose male rats by 42% ($p<0.01$) at week 76 and 19% (N.S.) at week 97 and in female rats by 26% ($p<0.05$) and 32% ($p<0.05$) at weeks 76 and 97, respectively, compared with control levels. Other statistically significant changes in other parameters in 5000-ppm group rats were transient, not dose related, too small to be considered biologically significant.

F. URINALYSIS

Specific gravity was slightly, but significantly increased in high-dose male rats at 23 weeks and in high-dose female rats at 47 and 75 weeks. Urine pH was significantly increased (pH = 6.1) in the high-dose group females at 97 weeks compared with the pH of controls (pH = 5.8); however, the pH of the urine in the controls was below the range of the other time points (pH = 6.0 - 6.2). The significant increase in urine pH in 25- and 750-ppm group females at 75 weeks was not dose related.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

No treatment-related changes were observed on absolute organ weights in male or female rats receiving the test material after 26, 53, or 99/100 weeks. Relative organ weights for brain (113%), heart (111%), kidneys (112%), liver (123%), and spleen (122%) were significantly increased in high-dose females at 26 weeks. Other statistically significant changes in relative organ weights were not dose-related (i.e., statistical changes relative to controls were seen at lower doses but not at the high dose) and were not considered treatment related.

2. Gross pathology

No treatment-related gross lesions were observed in male or female rats receiving any dose of the test material for 26 weeks. At 53 weeks, the liver was swollen in 73% ($p<0.05$) of 5-ppm group males compared with 27% of controls and moderate hair loss was observed in 80 or 87% ($p<0.05$) of 25- and 5000-ppm group females compared with 40% of controls.

Notable gross findings in main study animals are summarized in Table 4. The adrenals appeared large in 28% ($p<0.05$) of 5- and 5000-ppm group males and 34% ($p<0.01$) of 25- and 750-ppm group males compared with 10% of controls. In 5000-ppm group female rats, cystic kidneys occurred in 10% ($p<0.05$) compared with 0% of controls, areas of change in the lungs occurred in 22% ($p<0.05$) compared with 6% of controls, and the ovaries appeared large in 18% ($p<0.01$) compared with 2% of controls. Other findings occurred with significantly increased incidences in animals dying before study termination, but the incidences were not significant when combined with animals that survived to study termination.

TABLE 4. Notable gross findings in male and female rats fed RPA400727 for 99/100 weeks – main study group

Organ/lesion	Dietary Concentration (ppm)				
	0	5	25	750	5000
Males					
Adrenals Appear large	5/50[a]	14/50*	17/50**	17/50**	14/50*
Females					
Kidneys Cyst	0/50	3/50	2/50	1/50	5/50*
Lungs Areas of change	3/50	12/50**	10/50*	8/50	11/50*
Ovaries Appear large	1/50	2/50	3/50	3/50	9/50**

Data taken from Tables 14b (pp. 159-172), 14E (pp. 182-188), and 14F (pp. 189-194), MRID 44802107.
[a]Number of animals with a lesion/number of animals examined.
*p<0.05, **p<0.01; statistically significant, treated group compared with controls, calculated by the reviewer.

3. Microscopic pathology

a. Non-neoplastic –

Table 5 summarizes the notable nonneoplastic lesions in male and female rats fed RPA400727 for 26, 53, or 99/100weeks. No treatment-related lesions were observed in male rats administered the test material for 26 or 53 weeks. Multinucleated cells were present in the zona fascicularis of the adrenal cortex in 60% ($p<0.01$) of high-dose females sacrificed at 26 weeks interim group, 21% (N.S.) sacrificed at 53 weeks, and in 6% (N.S.) sacrificed at 100 weeks (main study animals) compared with 0% of controls at all time points. Chronic inflammation of the zona fascicularis of the adrenal cortex was observed in 29% of high-dose females sacrificed at 53 weeks, in only 2% of high-dose females sacrificed at 100 weeks, and none of the controls sacrificed at any time point

Also in main study animals, the incidence of stromal fatty infiltration of the pancreas was significantly increased in males at all doses; there was no clear dose-related trend. In contrast, the incidence of this lesion was significantly decreased in high-dose group females (4%, $p<0.05$) compared with controls (20%). The incidence of chronic myocarditis of the ventricle (not listed in the Table 5) was significantly increased (90% vs 50% for controls, $p<0.05$) in high-dose male rats surviving to study termination, but was only marginally increased (82% vs 68% for controls, $p=0.08$) when survivors were combined with animals dying before study termination. No other lesions occurred with significantly increased incidences in high-dose male rats, but high-dose males had a significantly lower

incidence of liver congestion than did the control males. High-dose group females in the main study had significantly higher incidences of centriacinar fatty vacuolation in liver hepatocytes (66%) compared with controls (32%). The incidence of alveolar macrophage accumulation in the lungs was 14% ($p<0.05$) in high-dose group females compared with 0% in controls. High-dose females had significantly lower incidences of hemorrhagic degeneration of the adrenal cortex, chronic myocarditis in the heart ventricle, senile portal tract changes, and extra-medullary hematopoiesis in the spleen.

TABLE 5. Incidences of notable nonneoplastic lesions in male and female rats fed RPA400727 for 26 weeks, 53 weeks, or 99/100 weeks.

Organ/Lesion	Dietary concentration (ppm)				
	0	5	25	750	5000
Males – main study					
Pancreas stromal fatty infiltration	3/50	7/32**	11/39**	14/37**	10/50**
Females – 26 weeks					
Adrenal cortex zona fascicularis, multinucleated cells	0/15[a]	0/15	0/15	0/15	9/15**
Females – 53 weeks					
Adrenal cortex Zona fascicularis, multinucleated cells Zona fascicularis, chronic inflammation	0/15 0/15	0/15 0/15	0/14 0/14	0/14 0/14	3/14 4/14*
Females – main study					
Adrenal Cortex Zona fascicularis, multinucleated cells Zona fascicularis, chronic inflammation	0/50 0/50	0/46 0/46	0/48 0/48	0/46 0/46	3/50 1/50
Liver, hepatocytes Centriacinar fatty vacuolation	16/50	15/50	11/50	23/50	33/50**
Lungs Accumulation of alveolar macrophages	0/50	3/50	1/50	1/50	7/50*
Pancreas Stromal fatty infiltration	10/50	3/38	3/34	3/33	2/50*[N]

Data taken from Tables 15C (pp. 215-217), 15D (218-222), and 15G (pp. 239-255), MRID 44802107.
[a]Number of animals with the lesion/number of animals examined at that site.
N indicates negative trend.
*$p<0.05$, **$p<0.01$; statistically significant treated groups compared with the control group.

b. Neoplastic

Table 6 summarizes the notable neoplastic lesions in male rats receiving the test material for up to 99 weeks. High-dose males had a significantly higher incidence of pituitary adenomas than did the control rats. The incidence in the treated animals (58%) was within the upper range of historical controls (60%) and the incidence in the concurrent control (38%) was within the lower range of historical controls (36.7%). The incidence of keratoacanthoma of the skin was 23% ($p<0.05$) for 5-ppm group male rats and 22% ($p<0.05$) for 5000-ppm group male rats compared with 0% for the controls. There were no statistically significant increases in the incidences of neoplasms in female rats administered any dose of the test material. Fibroadenomas of the mammary gland and pituitary adenomas occurred at high incidences in all female groups including controls.

TABLE 6. Incidences of notable neoplastic lesions in male rats receiving RPA400727 for up to 99 weeks

Organ/Lesion	Dietary concentration (ppm)				
	0	5	25	750	5000
Pituitary					
Adenoma	19/50[a] (38%)	24/38	24/43	25/43	29/50*(58%)
Carcinoma	0/50	1/38	0/43	2/43	0/50
Historical Control					
Pituitary Adenoma: range: 18/49 (36.7%) – 30/50 (60.0%), total: 195/423 (46.10%)					
Pituitary carcinoma: range: 0/423					
Skin					
Keratoacanthoma	0/20	5/22*	2/21	2/24	6/27*

Data taken from Table 16G and 17, pages 267-273, MRID 44802107.
[a]Number of animals with the lesion/number of animals examined at that site.
*$p<0.05$, statistically significant, treated groups compared with the control group.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The investigator concluded that administration of 5000 ppm of the test material caused mild toxicity (adrenal cortex and liver were identified as target organs) including body weight depression. No clear treatment related effects occurred at 750 ppm; this concentration is considered the no-observed-effect-level (NOEL).

B. REVIEWER'S DISCUSSION/CONCLUSIONS

Administration of RPA400727 in the diet at concentrations up to 5000 ppm caused no effects on clinical signs or mortality after 26, 53, or 99/100 weeks. The study was terminated early because of excessive deaths particularly in control and 25-ppm group males and 5-ppm group females; only 28% of animals in these groups were alive at study termination. Females administered the 5000-ppm diet had statistically significant

decreases in weekly mean body weights throughout the study. Body weight gain was significantly decreased during the first week of the study in 5000-ppm group females and no compensatory growth occurred until the second year when treated females gained 39% more weight than the controls. Mean body weights of males were similar to those of controls throughout the entire study; body weight gain showed a statistically significant decrease during the first week of the study, but not for the first year, second year, or the entire study. Overall food consumption and weekly food conversion efficiency were not affected by treatment of either sex with any dose of the test material.

Statistically significant differences were observed for some hematologic parameters in treated animals compared with controls, but the differences were transient or too small to be considered biologically significant (±4% differences for erythrocyte parameters, lymphocyte counts at 76 weeks, platelet counts at 24 and 76 weeks, and prothrombin time at 76 and 97 weeks). None of these differences were considered treatment related. Statistically significant decreases were observed for serum alanine aminotransferase activity in 5000-ppm group males at some time points and in 5000-ppm group females at all time points. No pathologic correlates were associated with the decreased activity in males; liver lesions were observed in the females. Nevertheless, no disease state has been associated with decreased serum alanine aminotransferase activity. Total cholesterol levels were significantly decreased in high-dose male and female rats during the second year of the study compared with control levels. It is unlikely that the decreased levels were associated with body weight gain or diet, because high-dose male and female rats gained more weight during the second year of the study than did controls and no effect was observed on food consumption. The only statistically significant changes in urinalysis parameters were a slight increase in specific gravity of urine from high-dose rats of both sexes and an increase in the pH of urine from high-dose females; the increase in specific gravity was transient, and the increase in urine pH in the high-dose female was due to the low pH for the controls.

Postmortem examination of treated animals revealed no statistically significant changes in absolute organ weights in rats receiving the high-dose. Relative organ weights were increased at the high dose in females only at 26 weeks, probably because of decreases in body weight. These and other statistically significant changes in relative organ weights were not considered treatment related. In addition, no pathologic correlates were observed upon gross or microscopic examination.

Statistically significant increases in the incidences of gross findings in high-dose animals were limited to the adrenal gland (appeared large) at all doses in males in the main study group, kidney (cystic) and ovaries (appeared large) in high-dose females, and areas of changes in the lungs in 5-, 25-, and 5000-ppm group females. The changes in the lungs were associated microscopically with accumulation of alveolar macrophages at the high-dose in females. The only microscopic lesion that occurred at significantly increased incidence in high-dose males was stromal fatty infiltration of the pancreas; the incidence of this finding was significantly increased at all doses, but not in a clear dose-related manner. In contrast, high-dose females had a decreased incidence of stromal fatty

infiltration in the pancreas. Therefore, this lesion is not considered treatment related in male rats.

The incidences of adrenal cortical lesions (multinucleated cells and chronic inflammation in the zona fascicularis) were increased in high-dose females. The incidence of multinucleated cells was increased in both interim sacrifice groups and in the main study group. This lesion was observed only at the high-dose in females and is considered treatment related at all time points at the high-dose. High-dose female rats also had a significantly increased incidence of chronic inflammation of the zona fascicularis at 53 weeks. Except for one high-dose female in the main study group that died before study termination, this lesion did not occur in controls or the lower doses in females or at any dose in males. High-dose main study females had increased incidences of centriacinar fatty vacuolation of hepatocytes and accumulation of alveolar macrophages in the lungs. The etiology for the alveolar macrophages was unknown; the study author considered this finding to be of doubtful toxicologic significance. Nevertheless, the reviewer considers the accumulation of alveolar macrophages to be treatment related and may be related to test material inhaled as dust particles during feeding. The lesions are not considered to be adverse, because inflammatory or granulomatous lesions were not observed.

In conclusion, the lowest-observed-adverse-effect level (LOAEL) is 5000 ppm (286.6 mg/kg/day) based on decreased body weight and body weight gain and adrenal cortical and liver toxicity in females; no adverse effects were observed in male rats. The no-observed-adverse-effect level (NOAEL) was 750 ppm (38.3 mg/kg/day) for females and ≥5000 (203.6 mg/kg/day) ppm for males.

High-dose male rats had a statistically significant increase in the incidence of pituitary adenomas. The historical control incidence of pituitary adenomas was very high. The incidence in the concurrent male test groups was in the upper part of the range for historical controls, and the incidence in concurrent controls was in the lower part of range, resulting in a statistical difference between the two groups. Therefore, pituitary adenomas are not considered treatment related. The 5- and 5000-ppm group male rats also had a high incidence of keratoacanthoma of the skin. These animals were group housed, which often leads to fighting and, consequently, scratches and abrasions to the skin; these lesions could have resulted in the elevated incidence of keratoacanthomas. Therefore, at the doses tested, the RPA400727 showed no carcinogenic activity in this study. The animals were adequately dosed based on adverse effects on body weights and liver and adrenal cortical toxicity in females. The male rats, however, could have received a higher dose.

This chronic toxicity/oncogenicity study in the rat is **Acceptable/Guideline** and does satisfy the guideline requirement for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. The variance between the actual and measured concentration for the 5-ppm dietary group was much higher than acceptable, resulting in a higher dose than reported in the study. Because, the NOAEL was higher than 5 ppm, this deficiency had no impact on the conclusions of this study.

C. STUDY DEFICIENCIES

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Verification of the dietary mixtures showed that the concentration of test material in the 5-ppm diet was markedly higher than the target. Calculations of weight-normalized doses were based on nominal concentration; therefore, the intake of test material from the 5-ppm concentration was much higher than that reported by the study author. Because the NOAEL for this study was higher than 5 ppm, this deficiency had no impact on this study.

No other deficiencies were noted for this study.

ATTACHMENT-Neoplastic Incidence Tables

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
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