(1-9-01)

DATA EVALUATION REPORT

TRITICONAZOLE (RPA400727)

STUDY TYPE: ONCOGENICITY FEEDING - MOUSE [870.4200 (§83-2b)]
MRID 44802108

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2b)]

DP BARCODE: D261924
P.C. CODE: 125620

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

TEST MATERIAL (PURITY): RPA400727 (Triticonazole) (purity, 97% a.i.)

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

CITATION: Eddie, M. (1994) RPA400727: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. Pharmaco-LSR Ltd., Eye, Suffolk, IP23 7PX, England. Laboratory report no.: 93/RHA446/0778, May 13, 1994, MRID 44802108. Unpublished.

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

EXECUTIVE SUMMARY: In an oncogenicity study (MRID 44802108), RPA400727 (97% a.i., batch no. DA646) was administered to groups of 52 male and 52 female CD-1 mice in the diet at concentrations of 0, 15, 150, or 1500 ppm for up to 78 weeks. In addition, 16 males and 16 females at each dietary concentration were terminated after 26 weeks for interim evaluation. These concentrations resulted in a nominal compound intake for each concentration level of 1.8, 17.4, and 202.2 mg/kg/day for males; 2.1, 20.1 and 209.5 mg/kg/day for females for 15, 150, and 1500 ppm dietary mixtures, respectively.

Treatment with RPA400727 did not result in changes in clinical signs or survival. Mean absolute body weights and food consumption were not affected by treatment of males or females with any dose at anytime during the study. The group mean body weight gain of high-dose males and females was decreased by 25% and 31% ($p < 0.01$), respectively, during the first year of the study and by 14% (N.S.) and 23% ($p<0.01$), respectively, over the entire study. The overall food efficiency for the 78-week study was decreased by about 19% in both sexes at 1500 ppm compared to the control groups.

Ophthalmoscopic examinations and hematology parameter determinations did not reveal any treatment-related findings.

Group mean absolute liver weights were increased by 27% and 14% ($p < 0.01$) at 26 weeks and by 17% ($p < 0.05$) and 15% ($p < 0.01$) at 78 weeks in high-dose males and females, respectively,

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compared to the controls. The mean liver weights relative to body weight were increased by 26% and 29% ($p < 0.01$) at 78 weeks in high-dose males and females, respectively. Increased incidences of centriacinar hepatocytic large fatty vacuolation were seen in both sexes at 1500 ppm after 26 weeks (males: control, 0%; 1500 ppm, 40%; females: controls 0%, 1500 ppm, 56%) and 78 weeks of treatment (males: control, 2%; 1500 ppm, 23%; females: control, 0%; 1500 ppm, 15%, $p < 0.01$) compared to the controls. Decreases were observed in the incidences of thickened stomach wall (control, 29%; 1500 ppm, 10%, $p < 0.05$) and dysplasia in the glandular region of the stomach of high-dose males (control, 41%; 1500 ppm, 14%, $p < 0.01$) compared to the control at 78 weeks.

The LOAEL is 1500 ppm in the diet (202.2 mg/kg/day for males; 209.5 mg/kg/day for females), based on decreased body weight gain and liver toxicity. The NOEL was 150 ppm (17.4 mg/kg/day for males; 20.1 mg/kg/day for females).

Treatment for up to 78 weeks with RPA400727 did not result in a significant increase in the incidence of neoplastic lesions in this study. The dosages were adequate based on decreased body weight gain and increased absolute liver weight with microscopic lesions.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200, (§83-2b)] in mice.

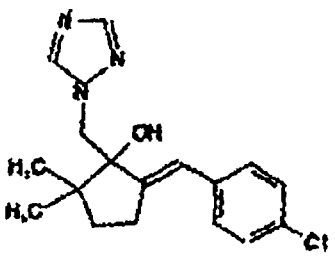
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: RPA400727

   Description: fine white powder
   Lot/Batch #: Batch no. DA646
   Purity: 97% a.i.
   Stability of compound: stable for the study duration, as stored in the laboratory
   CAS #: 131893-72-7



2. Vehicle and/or positive control: The test material was mixed with feed; a positive control was not included in this study.

3. Test animals: Species: mouse

Strain: CD-1
Age and weight at study initiation (week 0): age: 29-36 days; group mean weight: males: 23.4-30.1 g; females: 18.5-24.2 g
Source: Charles River (UK), Limited, Margate, Kent, England
Housing: animals were housed in a barrier facility, 4 mice (same sex) per cage. Cages were Type M2 (North Kent Plastics Ltd., Dartford, Kent, England) 33 x 15 x 13 cm. with stainless steel mesh lids. Sterilized wood shavings were used as bedding.
Diet: Laboratory Animal Diet No. 2 , ground to a powder by the manufacturer (Special Diets Services Ltd., Witham, Essex, England), *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 21 ± 2°C
Humidity: 55%, acceptable: 40-70%
Air changes: 15/hour
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 8 days

B. STUDY DESIGN:

1. In life dates - Start: June 13, 1991; end: December 23, 1992

2. Animal assignment

Animals were assigned to the test groups in Table 1 by computer generated random numbers. Animals with high or low body weights were replaced to insure maximum body weight variation within ±20% of the mean weight at study initiation. The interim phase animals were specifically numbered and identified at study initiation, and were sacrificed and examined after 26 weeks of treatment.

TABLE 1. Study design

Test group	Dietary concentration (ppm)	Dose to animals[a] (mg/kg/day)		Main study (78 weeks)		Interim sacrifice (26 weeks)	
		Male	Female	Male	Female	Male	Female
1 Control	0	0	0	52	52	16	16
2 Low	15	1.8	2.1	52	52	16	16
3 Mid	150	17.4	20.1	52	52	16	16
4 High	1500	202.2	209.5	52	52	16	16

Data taken from pp. 18 and 32, MRID 44802108.
[a]Daily dietary RPA400727 consumption was calculated from the mean weekly food consumption and body weight data and was based on nominal dietary levels of RPA400727.

3. Dose selection

The dose selections were based on previous studies (LSR Report Nos. 90/RHA360/0987, 90/RHA416/1345, and 91/RHA430/0735) in which dietary levels over 1500 ppm resulted in hepatic toxicity sufficiently severe to make higher levels unsuitable for long-term administration to mice.

4. Diet preparation and analysis

Test diets were prepared weekly by diluting an initially prepared premix with appropriate amounts of additional basal diet and mixing in a mixer. Diets were stored in sealed polyethylene bags, and the unused portions were discarded at the end of each week. Homogeneity of the lowest and highest dietary mixture concentrations was tested by analyzing samples taken from 6 different regularly spaced positions in the mix for each concentration. Stability of RPA400727 in the diet was tested on 5- and 50,000-ppm samples stored at 21 °C for 7 and 16 days. All dietary concentrations in the present study were analyzed for RPA400727 content during treatment weeks 1, 2, 3, 4, 12, 20, 28, 36, 44, 52, 60, 68, 74, and 78.

Results –
Homogeneity: The coefficient of variation of samples of the dietary mixture taken from 6 different positions within the mix for homogeneity testing ranged from 6.26% for the 15 ppm mixture to 3.98% for the 1500 ppm mixture. The mean analyzed concentrations were 96.0% and 102% of the target concentration for 15 and 1500 ppm, respectively.

Stability: Dietary mixtures containing 5 ppm RPA400727 stored for 7 days at 21 °C were shown to be about 85% of the initial assayed concentration of the freshly prepared mixture, and about 93% after 16 days storage. Small changes in the 5 ppm mixture were within the error of the assay procedure. The stability of a 50,000 ppm mixture was about 90% after 7 days and 87% after 19 days. The estimated shelf life of the 50,000 ppm mixture without significant loss was 10 days.

Concentration analysis: The routine concentration analyses of the 15 and 150 ppm dietary concentrations showed agreement within 15% of the target concentration in all cases. The overall means were 97.8 ± 7.03% and 94 ± 4.36% of the nominal concentration for 15 and 150 ppm, respectively. The 1500 ppm concentration agreed within 10% of the target concentration in all assays, and the overall mean was 94.8 ± 3.47 % of the target concentration.

The dietary mixture preparation procedures were shown to be acceptable.

5. Statistics

Group mean values and standard deviations were calculated. Cox's proportional hazards model and Tarone's partition of the Chi-square statistic into linear trend on

dose and deviation from linearity were used to compare inter-group differences in mortality. Differences in hematology parameters, except for morphological changes, were assessed by Students 't' test using a pooled error variance. Bartlett's test for homogeneity of variance was utilized for organ weights and body weight changes. If the differences were found to be statistically significant, a Behrens-Fisher test was used for pairwise comparisons. If not significant, a Dunnett's test was used.

Non-neoplastic macroscopic and microscopic findings were assayed using a two-tailed Fisher's Exact test. A one-tailed test was applied to analyze increases in neoplastic incidences.

Comparisons were considered significant at $p < 0.05$.

C. METHODS

1. Observations

Animals in the main and interim groups were inspected twice daily for signs of toxicity and mortality and were given a detailed examination once each week including palpation for masses.

2. Body weight

Animals in the main and interim groups were weighed at study initiation and weekly for the first 14 weeks of treatment followed by once every two weeks thereafter and prior to necropsy.

3. Food and water consumption and compound intake

The weekly food consumption for each cage was estimated throughout the study by the weight difference of the food minus an estimate of the amount spilled. The group mean achieved dosage (mg/kg/day) was calculated from the nominal test substance concentration, food consumption, and body weight. The group mean food efficiency (mean body weight gained/100 g food consumed) was calculated each week for the first 14 weeks of the study.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were done on all control and high-dose animals in the interim study groups after 24 weeks of treatment. The palpebrae and adjacent structures, conjunctiva, cornea, sclera, anterior chamber, iris, lens, vitreous, and ocular fundus were examined.

5. Blood was collected from the tail veins of all surviving animals in the main study after 50 and 76 weeks of treatment for differential leucocyte counts. Blood smears from all control and high-dose animals were examined. The mice were not

anesthetized or fasted prior to blood collection. Blood was also collected from the retro-orbital sinus of 10 non-fasted animals in each group at 77 weeks and from animals killed *in extremis* or for humane reasons; the animals were anesthesized with a mixture of oxygen, nitrous oxide, and halothane. EDTA was used as an anti-coagulant. Blood smears for the differential leukocyte counts were stained with Romanowsky stain. The CHECKED (X) parameters were examined in animals sampled after 77 weeks.

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		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for oncogenicity studies unless effects are observed, based on Subdivision F Guidelines.

b. Clinical chemistry

Clinical chemistry tests were not conducted and are not required for oncogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urinalysis was not conducted and is not required for oncogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and Pathology

Necropsies were done on all animals sacrificed at the scheduled periods for the main and interim groups and on all animals that died or were killed at unscheduled times during the treatment period. The mice were sacrificed by carbon dioxide inhalation. The CHECKED (X) tissues from all groups were collected for histopathological examination. All tissue samples were fixed in buffered 4% formaldehyde with or without saline except for eyes and optic nerves, which were placed in Davidson's fluid after which they were stored in 70% industrial methylated spirit. Preparations were primarily stained with hematoxylin and eosin. The right eyes and optic nerves, Harderian glands, cranial mammary glands, right submandibular salivary glands, right sciatic nerves, and tongues were retained in fixative, but not examined histologically unless found to be abnormal during gross necropsy. All other tissues from animals in the control and 1500 ppm main and interim groups and all animals that died or were killed at unscheduled times were examined by light microscopy. The kidneys, liver,

lungs, and gross lesions were examined in all animals. Smears were prepared from bone marrow, fixed in methanol, and stained with a May-Grunwald-Giemsa staining procedure. The (XX) organs from all animals were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
	Oral tissue	XX	Heart*	X	Periph. nerve*
X	Salivary glands, left*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	XX	Spleen*	X	Eye and optic nerve, left*
X	Duodenum*	X	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal/Harderian glands
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*+	X	Epididymides	X	Thyroids*
X	Gall bladder*	X	Prostate		Auditory sebaceous gland
X	Pancreas*	X	Seminal vesicle		(Zymbal's gland)
			Coagulating gland		
	RESPIRATORY		Preputial gland		OTHER
X	Trachea*	X	Ovaries*	X	Bone*
XX	Lung*	XX	Uterus*	X	Skeletal muscle*
	Nose	XX	Cervix	X	Skin* and subcutis
	Pharynx		Oviduct		Mediastinal tissue
	Larynx	X	Vagina		Mesenteric tissue
				X	All gross lesions and masses*

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

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No significant treatment-related clinical signs were seen during the daily or weekly examinations. There was a slight increase in the incidences of circling (control, 9/52; 1500 ppm, 14/52) and overactivity in females (control, 4/52; 1500 ppm, 10/52) in the main study, but the increases were not statistically significant, and there was no clear dose effect. The incidences of palpable swellings or masses were comparable in the control and high-dose groups in both sexes, and the mean times of onset of the first recorded palpable mass were longer in the high-dose groups (weeks 58 and 59) than in the controls (weeks 47 and 44 in males and females, respectively). The mean time of onset of the first palpable mass was not dose-related in either sex.

2. Mortality

The percent survival at selected times during the study is given in Table 2. There were no significant treatment-related trends or differences in survival of treated males or females compared to the control group. Ten of 52 male mice at 1500 ppm died during the first year of treatment compared to 5/52 in the control group; however the apparent increased mortality rate did not persist throughout the 78-79-week study. The lowest 79-week survival was seen in males at 150 ppm; females at 1500 ppm had higher survival than the other dose groups.

TABLE 2. Percent survival of male and female mice fed RPA400727 for 78 weeks

Weeks of study	Dietary concentration (ppm)			
	0	15	150	1500
Males (n = 52)				
Week 52	90	90	88	81
Week 79	63	62	50	58
Females (n = 52)				
Week 52	96	98	92	96
Week 79	73	73	73	88

Data taken from Table 3B pp. 51-54, MRID 44802108

B. BODY WEIGHT

The group mean body weights and the calculated weight gain in male and female mice over selected time periods during treatment are summarized in Table 3. The body weights of males at 1500 ppm were decreased by about 11% after 26 and 52 weeks of treatment compared to the control group, but only by about 6% at the end of the study. Female body weights were also decreased at 1500 ppm by about 11 to 15% over the same time intervals. The body weight differences between treated animals and controls never reached statistical significance. The group mean cumulative body weight gain was significantly and consistently decreased in males at 1500 ppm from week 2 to 76 of treatment and in high-dose females from week 26 to study termination compared to the control groups. The body weight gain for the first year of treatment was decreased in high-dose males by 25% and in high-dose females by 31% ($p < 0.01$). The overall group mean weight gain in high-dose males at week 78 was decreased by about 14% (NS) and in high-dose females by 23% ($p < 0.01$) compared to the control groups.

TABLE 3. Group mean body weights and body weight gains in male and female mice fed RPA400727 for up to 79 weeks (g)

Weeks of study	Dietary concentration (ppm)			
	0	15	150	1500
Males				
Body weight at wk. 0	25.8 ± 1.86[a]	26.2 ± 1.64	26.5 ± 1.57	26.2 ± 1.78
Body weight at wk. 26	48.8 ± 5.18	47.5 ± 5.35	47.3 ± 4.94	43.4 ± 3.94
Body weight at wk. 52	52.3 ± 5.70	49.9 ± 5.61	51.9 ± 5.35	46.7 ± 3.90
Body weight at wk. 78	48.9 ± 7.19	47.7 ± 6.14	51.1 ± 7.18	46.1 ± 4.18
Weight gain, wk. 0-26	23.0 ± 4.74	21.3 ± 4.94	20.8 ± 4.45*	17.1 ± 3.89**
Weight gain, wk. 0-52	26.5 ± 5.20	23.6 ± 5.09*	25.3 ± 5.27	20.0 ± 4.46**
Weight gain, wk. 0-78	22.9 ± 6.82	21.5 ± 5.95	24.6 ± 7.23	19.6 ± 4.75
Females				
Body weight at wk. 0	21.8 ± 1.11	21.4 ± 1.40	21.4 ± 1.26	21.7 ± 1.32
Body weight at wk. 26	35.4 ± 5.73	34.7 ± 5.45	36.7 ± 5.74	31.4 ± 5.24
Body weight at wk. 52	42.6 ± 8.72	40.0 ± 8.80	39.8 ± 7.27	36.0 ± 6.55
Body weight at wk. 78	42.3 ± 8.64	39.8 ± 7.55	40.9 ± 6.74	37.5 ± 7.06
Weight gain , wk. 0-26	13.6 ± 5.48	13.3 ± 4.90	15.3 ± 5.31	9.8 ± 4.75**
Weight gain, wk. 0-52	20.9 ± 8.46	18.6 ± 8.41	18.2 ± 7.14	14.4 ± 6.07**
Weight gain, wk. 0-78	20.6 ± 8.37	18.4 ± 7.27	19.4 ± 6.54	15.9 ± 6.70**

Data taken from Table 4A, pp. 55-59, and Table 4B, pp.60-65, MRID 44802108
[a]Mean ± standard deviation
* $p < 0.05$, ** $p < 0.01$, Significantly different from control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

There were no significant differences in the group mean weekly food consumption of treated and control groups. The overall group mean daily food consumption for males at 1500 ppm was slightly higher than the control by 5% and slightly lower by 5% in females (group mean total food consumption: males, 2987, 2961, 2966, and 3139 g; females, 2663, 2662, 2599, and 2541 g for control, 15, 150, and 1500 ppm, respectively. (See Table 5, pp.68-75, MRID 44802108).

2. Compound consumption

The compound consumption was calculated from the food consumption and body weight data. The results are given in Table 1.

3. Food efficiency

The group mean food conversion efficiency in males calculated over the first 14 weeks of the study was about 24% lower at the high dose compared to the control (control, 3.4%; 15 ppm, 3.3%; 150 ppm, 3.2%; 1500 ppm, 2.6%, NS). In high-dose females the mean food efficiency was about 10% lower than the controls (control, 2.0%; 15 ppm, 2.1%; 150 ppm, 2.1%; 1500 ppm, 1.8%. (See Table 6, p. 76, MRID 44802108). The total food efficiency over the 78-week study calculated by the reviewer from the overall weight gain and food consumption was about 19% lower in the high dose males and females than in the control groups (males: control, 0.767; 15 ppm, 0.726; 150 ppm, 0.829; 1500 ppm, 0.624; females: control, 0.774; 15 ppm, 0.691; 150 ppm, 0.746; 1500 ppm, 0.626).

4. Ophthalmoscopic examination

No significant differences were seen upon ophthalmoscopic examination of mice after 24 weeks of treatment compared to the control animals. Focal superficial opacity was found in 3/14 males and 3/16 females at 1500 ppm compared to 1/16 in both control groups. Focal superficial opacity is commonly seen in mice and was not believed to be treatment related.

D. BLOOD WORK

1. Hematology

No changes were seen in the differential white cell counts of rats in the 1500-ppm groups after 50 or 76 weeks of treatment compared to the control groups. Decreased total white blood cell (WBC) and neutrophil counts were seen in males at 15 and 150 ppm after 77 weeks of treatment (WBC count $10^3/mm^3$: control, 12.1 ± 6.3; 15 ppm, 8.1 ± 2.5; 150 ppm, 7.2 ± 3.1, $p < 0.05$; 1500 ppm, 10.6 ± 3.1; neutrophil count $10^3/mm^3$: control, 4.1 ± 3.6; 15 ppm 2.3 ± 1.2; 150 ppm, 1.7 ± 0.7, $p < 0.05$; 1500 ppm, 2.9 ± 1.1, NS). Though statistically significant, the differences are within the normal range for mice and show no clear dose effect. No significant changes were seen in treated females compared to the control group. Data were taken from Table 10, pp. 83-84, MRID 44802108.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

The absolute and relative (to body weight) organ weight changes after treatment with RPA400727 for 26 and 78 weeks are summarized in Table 4. The mean absolute liver weights were increased in high-dose males and females by 27% and 14% ($p < 0.01$), respectively at 26 weeks and by 17% ($p < 0.05$) and 15% ($p < 0.01$) at 78 weeks compared to the control groups. The relative liver weights were increased by 50% and 19% at 26 weeks and by 26% and 29% at 78 weeks in males and females,

respectively, at 1500 ppm compared to the controls ($p < 0.01$). The mean relative adrenal weights were increased by about 74% ($p < 0.05$) in high-dose males at 26 weeks compared to the control group. The absolute adrenal weight in males and the absolute and relative adrenal weights in females were slightly higher at 1500 ppm than the control groups after 26 weeks of treatment, but the differences were not statistically significant. The mean relative brain weight was significantly higher than the control group in high-dose males at 26 weeks by about 18% ($p < 0.01$) and in high-dose females at 78 weeks by 12% ($p < 0.05$). The mean terminal body weights were significantly decreased in high-dose males compared to the controls at both time points and in high-dose females at 78 weeks.

TABLE 4. Group mean organ and final body weights in male and female mice fed RPA400727 for 26 and 78 weeks (grams)

Organ	Dietary concentration (ppm)			
	0	15	150	1500
Males				
Final body wt., 26 wks.	48.4 ± 5.7[a]	49.0 ± 5.3	44.4 ± 4.8	40.6 ± 4.0**
Liver, abs. wt., 26 wks.	2.52 ± 0.33	2.55 ± 0.51	2.44 ± 0.35	3.19 ± 0.23** (27%)[b]
Liver, rel. wt.[c], 26 wks.	5.246 ± 0.741	5.189 ± 0.692	5.505 ± 0.682	7.892 ± 0.579** (50%)
Adrenal, abs. wt., 26 wks	0.005 ± 0.002	0.005 ± 0.002	0.005 ± 0.002	0.007 ± 0.003
Adrenal, rel. wt.[c], 26 wks.	0.0098 ± 0.0055	0.0095 ± 0.0036	0.0116 ± 0.0054	0.0171 ± 0.0097* (74%)
Brain, rel. wt., 26 wks.	1.0515 ± 0.1405	1.0247 ± 0.1168	1.1324 ± 0.1651	1.2394 ± 0.1494** (18%)
Final body wt., 78 wks.	47.6 ± 7.1	45.7 ± 6.5	49.1 ± 6.4	44.1 ± 3.6*
Liver, abs. wt., 78 wks.	2.88 ± 0.85	2.46 ± 0.52*	2.58 ± 0.55	3.36 ± 0.69* (17%)
Liver, rel. wt.[c], 78 wks.	6.051 ± 1.682	5.402 ± 0.996	5.312 ± 1.433	7.646 ± 1.631** (26%)
Brain, rel. wt.[c], 78 wks.	1.0937 ± 0.1725	1.1331 ± 0.1921	1.0440 ± 0.1333	1.1556 ± 0.0975
Females				
Final body wt., 26 wks.	33.1 ± 4.7[a]	32.2 ± 3.8	35.8 ± 6.4	31.7 ± 4.0
Liver, abs. wt., 26 wks.	1.82 ± 0.23	1.69 ± 0.27	1.75 ± 0.19	2.08 ± 0.27** (14%)
Liver, rel. wt.[c], 26 wks.	5.536 ± 0.605	5.279 ± 0.742	4.976 ± 0.769	6.582 ± 0.564** (19%)
Adrenal, abs. wt., 26 wks	0.007 ± 0.003	0.008 ± 0.003	0.007 ± 0.003	0.009 ± 0.006
Adrenal, rel. wt.[c], 26 wks.	0.0224 ± 0.0093	0.0261 ± 0.0129	0.0212 ± 0.0077	0.0292 ± 0.0172
Brain, rel. wt., 26 wks.	1.5883 ± 0.2329	1.5970 ± 0.1984	1.4574 ± 0.2879	1.6531 ± 0.2404
Final body wt., 78 wks.	40.6 ± 9.0	38.5 ± 7.2	39.6 ± 7.1	35.7 ± 6.7*
Liver, abs. wt., 78 wks.	1.90 ± 0.33	1.95 ± 0.48	1.95 ± 0.36	2.19 ± 0.55** (15%)
Liver, rel. wt.[c], 78 wks.	4.836 ± 1.009	5.122 ± 1.024	4.974 ± 0.696	6.230 ± 1.747** (29%)
Brain, rel. wt.[c], 78 wks.	1.3434 ± 0.3141	1.4196 ± 0.2769	1.3816 ± 0.2788	1.5062 ± 0.2786* (12%)

Data taken from Tables 11A-D, pp. 85-92, MRID 44802108
[a]Mean organ or body weight ± standard deviation.
[b]Percent increase from the control.
[c]Organ weighs were relative to the final bodyweight.
*$p < 0.05$, **$p < 0.01$, Significantly different from the control.

2. Gross pathology

There were no statistically significant macroscopic findings after 26 weeks of treatment with RPA400727. Selected macroscopic findings in animals in the main study are summarized in Table 5. Increased incidences of livers that appeared large were seen in high-dose males compared to the control group (control, 1/52; 1500 ppm, 8/52, $p < 0.05$). The mesenteric lymph node appeared large in 4/30 ($p < 0.05$) high-dose males surviving to 78 weeks and 5/52 (N.S.) overall compared with 0/33 controls surviving and 2/52 overall. An increased number of animals with a skin mass(es) was also seen in males at 1500 ppm (control, 1/52; 1500 ppm, 8/52, $p < 0.05$). There was a decreased incidence of thickened stomach wall in males treated with 1500 ppm (controls, 14/52; 1500 ppm, 6/52, $p < 0.05$). There were no treatment-related changes seen in females.

TABLE 5. Incidences of macroscopic findings in male and female mice fed RPA400727 for up to 78 weeks

Organ or tissue/finding	Dietary concentration (ppm)			
	0	15	150	1500
No. animals examined per group	52	52	52	52
Males				
Liver/ appear large	1 (2%)	1 (2%)	0	8* (15%)[a]
Mesenteric lymph node/ appear large	2 (4%)	3 (6%)	1 (2%)	5 (10%)
Skin/ masses	1 (2%)	4 (8%)	4 (8%)	8* (15%)
Stomach/ thickened wall	14 (27%)	14 (27%)	14 (14%)	6* (12%)
Females				
Liver/ appear large	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Skin/ masses	4 (8%)	6 (12%)	4 (8%)	3 (6%)
Stomach/ thickened wall	3 (6%)	7 (13%)	9 (17%)	7 (13%)

Data taken from Tables 12C and 12D, pp. 97-115, MRID 44802108
[a]Number of animals with lesion (% of animals examined with lesion)
*$p < 0.05$, significantly different from the control group.

3. Microscopic pathology

a) Non-neoplastic

Selected microscopic findings in the main study after treatment for up to 78 weeks are summarized in Table 6. The only notable microscopic finding that occurred with a significantly increased incidence in treated animals compared to the control was centriacinal hepatocytic large fatty vacuolation in both sexes at the high dose.

The incidence of this lesion was increased in the 26-week interim sacrifice animals (males: control, 0/16; 1500 ppm, 6/16, $p < 0.01$; females: control, 0/16; 1500 ppm, 9/16, $p < 0.01$) and in the main study groups compared to the controls groups (males: control, 1/52; 1500 ppm, 12/52, $p < 0.01$; females: control, 0/52; 1500 ppm, 8/52, $p < 0.01$). The increase in incidence of parafollicular hyperplasia of the mesenteric lymph nodes in high-dose males did not achieve statistical significance.

Significant decreases in the incidences of sinus histiocytosis in the mesenteric lymph nodes, increased colloid in the seminal vesicles, and dysplasia of the glandular region of the stomach were seen in high-dose males compared to the control group.

TABLE 6. Incidences of non-neoplastic microscopic findings in male and female mice fed RPA400727 for up to 78 weeks

Organ or tissue/finding	Dietary concentration (ppm)			
	0	15	150	1500
Males (n = 52)				
Liver/ centriacinar hepatocytic large fatty vacuolation	1 (2%)[a]	0	0	12** (23%)
Mesenteric lymph nodes/ parafollicular hyperplasia	1/49[b] (2%)	1/24 (4%)	1/28 (4%)	4/46 (9%)
Mesenteric lymph nodes/ sinus histiocytosis	12/49[b] (24%)	2/24 (8%)	3/28 (11%)	3/46* (7%)
Seminal vesicles/ increased colloid	15 (29%)	17/32 (53%)	14/40 (35%)	5* (10%)
Stomach/ glandular region dysplasia	21/51 (41%)	10/27 (37%)	16/32 (50%)	7/49** (14%)
Females (n = 52)				
Liver/ centriacinar hepatocytic large fatty vacuolation	0	1 (2%)	0	8** (15%)
Mesenteric lymph nodes/ sinus histiocytosis	3 (6%)	2/21 (10%)	2/16 (13%)	1/49 (2%)
Stomach/ glandular region dysplasia	5/51 (10%)	4/18 (22%)	4/19 (21%)	4 (8%)

Data taken from Table 13H, pp. 149-162, MRID 44802108
[a]Number of animals with lesion (% of animals examined with lesion)
[b]Incidence/number examined if less than 52
*$p < 0.05$; **$p < 0.01$, significantly different from the control group.

b) Neoplastic

A summary of common neoplasms seen in this study is given in Table 7. No significant treatment-related increases in neoplasms were found in the study. The most commonly found neoplasms were in the liver and lungs of males and lungs in females. A slight increase in the pulmonary adenoma incidence was observed in high dose females (control, 12%; 1500 ppm, 21%, NS), but the increased incidence was not statistically significant and did not show a clear dose relationship.

TABLE 7. Neoplastic findings in male and female mice fed RPA400727 for up to 78 weeks

Organ or tissue / neoplasm	Dietary concentration (ppm)			
	0	15	150	1500
Males (n = 52)				
Liver / hepatocellular adenoma	12 (23%)[a]	6 (12%)	8 (15%)	11 (21%)
Liver / hepatocellular carcinoma	4 (8%)	0	3 (6%)	5 (10%)
Lung / pulmonary adenoma	8 (15%)	3 (6%)	3 (6%)	3 (6%)
Lung / pulmonary carcinoma	3 (6%)	9 (17%)	6 (12%)	2 (4%)
Females (n = 52)				
Liver / hepatocellular adenoma	0	0	0	0
Liver / hepatocellular carcinoma	0	0	0	0
Lung / pulmonary adenoma	6 (12%)	10/51[b] (20%)	5 (10%)	11 (21%)
Lung / pulmonary carcinoma	4 (8%)	3/51 (6%)	0	2 (4%)

Data taken from Table 13G, pp. 147-148, MRID 44802108
[a]Number of animals with lesion (% of animals examined with lesion)
[b]Incidence/number examined if less than 52

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that the administration of up to 1500 ppm RPA400727 in the diet of mice for up to 78 weeks resulted in no significant increases in the incidences of any type of neoplastic lesions compared to control animals.

Treatment at 1500 ppm resulted in liver toxicity especially in males. The no-observed-effect level (NOEL) was 150 ppm.

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B. REVIEWER'S DISCUSSION

There were no treatment-related changes in clinical signs or survival seen in the study. The mean body weights of high-dose male and female mice were slightly lower than the controls during the first 52 weeks of treatment, but the differences were not statistically significant. The group mean body weight gain was decreased by 25% and 31% ($p < 0.01$) in high-dose males and females, respectively, compared to the controls during the first 52 weeks. The group mean food conversion efficiency calculated by the study authors over the first 14 weeks of the study was about 24% lower in males and 10% lower in females at 1500 ppm compared to the control groups. The overall mean food efficiency for the 78-week study calculated by the reviewer was about 19% lower in high-dose males and females compared to the controls. Decreased weight gain and food efficiency is indicative of a toxic effect of treatment in both sexes at 1500 ppm.

No treatment-related changes were seen during the ophthalmoscopic examinations, and no changes in hematology parameters were likely due to treatment.

Apparent treatment-related increases of 27% and 14% ($p < 0.01$) were seen in group mean absolute liver weights at 26 weeks, and increases of 17% ($p < 0.05$) and 15% ($p < 0.01$) were seen at 78 weeks in high-dose males and females, respectively, compared to the control groups. The group mean liver weights relative to body weights were increased by 26% and 29% in high-dose males and females, respectively, at 78 weeks. Significantly increased incidences of centriacinar hepatocytic large fatty vacuolation were seen in high dose males and females upon microscopic examination of 26-week interim sacrifice and main study animals. The increased liver weights with microscopic changes identifies the liver as a target organ for RPA400727 toxicity.

The mean adrenal weight relative to body weight was increased in high-dose males at 26 weeks by about 74% ($p < 0.05$). Since microscopic examination revealed no increase in possible treatment-related findings, the increase in adrenal relative weight is a likely result of the decreased weight gain and a normal response to increased stress levels in high-dose males compared to the control group. Increases in relative brain weights to body weights were seen in both sexes at 1500 ppm, which reflect the decreases in body weight gain seen in high-dose animals. Increased incidences of skin masses seen in high-dose males during gross necropsy were not confirmed upon microscopic examination. The increase in the incidence of mesenteric lymph nodes that appeared large in high-dose male survivors at 78 weeks was no longer apparent when combined with animals that died before study termination and the increased incidence of parafollicular hyperplasia of the mesenteric lymph node in high-dose males did not achieve statistical significance. A significant decrease in the incidence of sinus histiocytosis in the mesenteric lymph nodes was seen in high-dose males compared to the control (control, 24%; 1500 ppm, 7%, $p < 0.05$). The change seen in the mesenteric lymph nodes in high-dose males is of doubtful toxicological significance.

Additional findings that were significantly decreased in high-dose males compared to control animals include decreased incidences of increased colloid in the seminal vesicles

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(control, 29%; 1500 ppm, 10%, $p < 0.05$), thickened stomach wall (control, 29%; 1500 ppm, 10%, $p < 0.05$), and dysplasia in the glandular region of the stomach (control, 41%; 1500 ppm, 14%, $p < 0.01$). The stomach findings suggest beneficial effect of RPA400727 treatment in high-dose male mice, or as the study author noted they may reflect the intergroup disparity in growth, but are clearly of not toxicological significance.

The lowest-observed-adverse-effect-level (LOAEL) seen in this study was 1500 ppm (202.2 mg/kg/day for males, 209.5 mg/kg/day for females) based on decreased body weight gain and liver toxicity. A no-observed-adverse-effect-level (NOAEL) of 150 ppm (17.4 mg/kg/day for males and 20.1 mg/kg/day for females) was determined.

Treatment of CD-1 mice for up to 78 weeks did not result in a significant increase in the incidence of neoplastic lesions in this study. The animals were adequately dosed as evidenced by decreases in body weight gain and liver toxicity at the highest dose.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200, (§83-2b)] in mice.

C. STUDY DEFICIENCIES

No deficiencies were noted for this study.

ATTACHMENT-Neoplastic Incidence Tables

**THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
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