(1-9-2001)

DATA EVALUATION REPORT

TRITICONAZOLE

MRID 44933604

STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT [870.3800 (83-4)]

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 00-17P

Primary Reviewer:
Carol S. Forsyth, Ph.D., D.A.B.T.

Signature: Carol S Forsyth
Date: JUN 0 1 2000

Secondary Reviewers:
Kowetha A. Davidson, Ph.D., D.A.B.T.

Signature: K A Davidson
Date: JUN 0 1 2000

Robert H. Ross, M.S. Group Leader

Signature: Robert H. Ross
Date: JUN 0 1 2000

Quality Assurance:
Donna L. Fefee, D.V.M.

Signature: Robert H. Ross
Date: for D. L. Fefee
JUN 0 1 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

TRITICONAZOLE

Reproduction Study [870.3800 (§83-4)]

EPA Reviewer: William Greear, M.P.H., D.A.B.T.
Registration Action Branch 3/HED (7509C)
EPA Work Assignment Manager: M. Copley, D.V.M., D.A.B.T.
Registration Action Branch 1/HED (7509C)

William Greear, Date 11/29/00

for
[signature], Date 1/9/2001

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat [OPPTS 870.3800 (§83-4)]

DP BARCODE: D261924
P.C. CODE: 125620

SUBMISSION CODE: S568827
TOX. CHEM. NO.: N/A

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

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

CITATION: Henwood, S.M. (1993) Two-generation reproduction study with RPA400727 in rats. Hazleton Wisconsin, Inc., 3301 Kinsman Boulevard, Madison, WI 53704. Project No. HWI 6224-172. January 13, 1993. MRID 44933604. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a two-generation reproduction study, RPA400727 (97.1% a.i.; Lot No. DA646) was administered to groups of 28 male and 28 female Crl:CD®BR VAF/Plus® rats in the diet at concentrations of 0, 5, 25, 750, or 5000 ppm (MRID 44933604). One litter was produced by each generation. Test substance intake was not calculated by the study author. Based on a food factor of 0.05 for the adult rat, doses for the 5-, 25-, 750-, and 5000-ppm groups were 0.25, 1.25, 37.5, and 250 mg/kg/day, respectively. F_0 and F_1 male and female parental animals were administered test or control diet for at least 10 weeks prior to mating, throughout mating, gestation, and lactation, and until necropsy.

No treatment-related effects were observed for the 5-, 25-, and 750-ppm F_0 and F_1 males and females. No treatment-related clinical signs of toxicity were observed in males or females of either generation during premating. A total of four 5000-ppm F_0 females were sacrificed moribund or found dead during gestation and lactation. Deaths included one sacrificed with dystocia, one each found dead on GD 23 and lactation day 7 with no prior clinical signs, and one sacrificed on lactation day 9 following the observations of thin, hunched, languid, and labored breathing.

Absolute body weights of the F_0 males were similar between the treated and control groups throughout the study. The 5000-ppm F_0 females weighed between 92% and 89% ($p \leq 0.01$) of the controls during premating and gained 77% as much weight overall. The 5000-ppm F_1 males and females weighed 65-82% ($p \leq 0.01$) of the control levels during premating and the entire study (males only). The high-dose F_1 males gained 84% ($p \leq 0.01$) as much weight as controls while

2

weight gain by the high-dose F_1 females was similar to the control level. Food consumption by the 5000-ppm F_0 males and females was significantly ($p \leq 0.01$; 91% and 87%, respectively, of controls) less than the controls during premating week 0-1. Food consumption by the 5000-ppm F_1 males and females was significantly ($p \leq 0.05$ or 0.01; 80-94% of controls) less than the controls during the premating interval. Significantly ($p \leq 0.05$ or 0.01) lower body weights (72-91% of controls) and food consumption (61-89% of controls) of the 5000-ppm F_0 and F_1 dams during gestation and lactation were considered a continuation of premating effects.

No dose- or treatment-related findings were seen at gross necropsy of the parental animals. No differences in absolute or relative organ weights were observed between the treated and control males of either generation. Absolute and relative (to body weight) adrenal weights were significantly ($p \leq 0.05$ or 0.01) reduced for the 5000-ppm F_0 (left only) and F_1 (left and right) females as compared with their respective controls.

A significant ($p \leq 0.01$) increase in the incidence rate of microscopic lesions of the adrenal gland was seen in 5000-ppm males and females of both generations. At 0, 5, 25, 750, and 5000 ppm, cortical vacuolation occurred in 7/28, 6/28, 4/28, 6/27, and 27/28 F_0 males, respectively, and in 15/28, 9/28, 8/28, 9/28, and 27/27 F_1 males. In the high-dose females degeneration of the adrenal cortex, giant cell formation, and pigment deposition was observed in 22/24, 16/24, and 6/24 F_0 females, respectively, and in 11/28, 14/28, and 7/28 F_1 females, respectively. In addition, 6/24 high-dose F_0 females had collagen deposition in the adrenal cortex. None of these lesions in females were seen in the control or other treated animals.

Therefore, the LOAEL for systemic toxicity is 5000 ppm based on reduced body weights of the F_0 females and the F_1 males and females and microscopic lesions in the adrenal gland of F_0 and F_1 males and females. The systemic toxicity NOAEL is 750 ppm.

Mating performance and fertility were not affected by test article administration in the 5-, 25-, and 750-ppm groups of either generation. The duration of gestation was 22.6 days for the high-dose dams of both generations compared with 22.1 days for the control groups and attained statistical significance ($p \leq 0.05$) in the F_0 but not the F_1 group. In the 5000-ppm F_1 group, the number of females mated and the number pregnant was significantly ($p \leq 0.01$) reduced as compared with the controls. This correlated to a significantly ($p \leq 0.01$) lower fertility index for the high-dose group (64% vs 93% for the controls).

Live birth and viability indices in the high-dose groups were 82% and 82%, respectively, for the F_1 litters and 85% and 89%, respectively, for the F_2 litters compared with 92-99% for the controls. In addition, four high-dose F_0 dams had whole litter loss during lactation days 0-4. The number of live pups/litter on day 0 was slightly (n.s.) reduced in the high-dose F_1 litters (12.35 vs 13.70 for the controls), and was significantly ($p \leq 0.01$) reduced in the high-dose F_2 litters (11.13 vs 14.46 for the controls). Fetal sex ratios were not affected by treatment in either generation. No treatment-related clinical signs were observed during lactation in offspring of either generation.

Mean body weights of the high-dose male and female pups were significantly ($p \leq 0.01$) less than

the controls beginning on lactation day 7 for the F_1 pups and throughout lactation for the F_2 pups. Body weights of the F_1 and F_2 pups on lactation day 21 were 71% and 49-51%, respectively, of the control levels. Weight gains by the high-dose F_1 pups were 91-104% of the controls for lactation days 0-4 but were 65-66% of the controls for lactation days 14-21. Body weight gains of the high-dose F_2 pups were 39-50% of the control levels for lactation days 0-4, 7-14, and 14-21. Body weights of the F_1 and F_2 pups in the 5, 25, and 750 ppm groups were similar to, or slightly greater than, the controls throughout lactation.

Therefore, the reproductive toxicity LOAEL is 5000 ppm based on F_0 maternal mortality, decreased fertility of the F_1 animals, reduced F_1 and F_2 pup survival, and reduced F_1 and F_2 pup body weight. The reproductive toxicity NOAEL is 750 ppm.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a reproduction study (870.3800 [83-4]) in rats. No major deficiencies were identified in the conduct of this study.

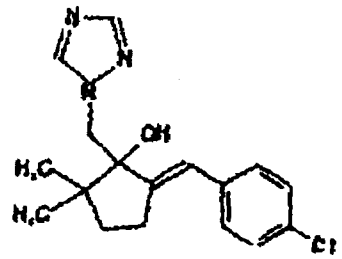
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: RPA400727

   Description: pale yellow powder
   Lot No.: DA646
   Purity: 97.1% a.i.
   Stability of compound: not stated
   CAS No.: not given
   Structure: [p. 227]



2. Vehicle and/or positive control

Certified Rodent Chow® #5002 meal was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: Rat
Strain: Crl:CD®BR VAF/Plus®
Age and weight at start of study: 7 weeks; males: 192-250 g; females: 171-221 g
Source: Charles River Laboratories, Inc., Portage, MI
Housing: Animals were housed individually in suspended stainless steel, screen-bottom cages except during mating; after day 15 of gestation and during lactation females were housed in polycarbonate cages with bedding.
Diet: Purina® Certified Rodent Chow® #5002 was available *ad libitum*.
Water: Water was available *ad libitum*.
Environmental conditions:
Temperature: 19-25°C
Humidity: 50±20%
Air Changes: not stated
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 14 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates

Start: September 16, 1991; end: June 18, 1992

2. Mating procedure

Each female was placed with one male from the same treatment group for a maximum of 21 days. The females were examined daily for evidence of mating by means of sperm in a vaginal smear or the presence of a copulatory plug. The day evidence of mating was observed was designated gestation day (GD) 0 and females were transferred to individual housing. Sibling mating was avoided. The day of birth was considered Day 0 of lactation. F_1 and F_2 generations were produced.

3. Study schedule

F_0 and F_1 male and female parental animals were administered test or control diet for at least 10 weeks prior to mating, throughout mating, gestation, and lactation, and until sacrifice. On lactation day 21, selected F_1 animals were weaned onto the same diets as their parents. Parental animals were sacrificed and necropsied after weaning of their litters.

4. Animal assignment

Animal assignment is given in Table 1. F_0 parental animals were assigned to groups using a computer-generated randomization based on body weight. For the F_1 generation, one male and one female pup (when possible) were randomly selected from each litter.

TABLE 1. Animal assignment

Dose Group	Dietary Concentration (ppm)	No. of Parental Animals per Group			
		F_0 Generation		F_1 Generation	
		Male	Female	Male	Female
Control	0	28	28	28	28
Low	5	28	28	28	28
Mid-1	25	28	28	28	28
Mid-2	750	28	28	28	28
High	5000	28	28	28	28

Data taken from text table p. 18 and text p. 22, MRID 44933604.

5. Dose selection rationale

A dose selection rationale was not given.

6. Dietary preparation and analysis

Dietary mixtures were prepared weekly. The 750- and 5000-ppm diets were prepared first. The 5- and 25-ppm diets were prepared by making dilutions of the 25- and 750-ppm diets, respectively. The required amount of test material was placed in a Waring® blender with approximately 250 g of diet and thoroughly mixed. This premix was transferred to a mixing bowl containing a specified amount of diet and the contents of the mixing bowl were thoroughly mixed. Samples for dietary analyses were taken directly from the mixing bowl. All diets were analyzed for concentration each week for the first 4 weeks of the study; thereafter, each week one dietary concentration was selected sequentially for analysis. Homogeneity was determined prior to study initiation and during weeks 4 and 17 for the low- and high-concentration diets from samples taken from the top, bottom, and two opposing sides of the mix. Stability of the low- and high-concentration diets was analyzed on samples stored in a refrigerator for one week followed by room temperature storage for 1 day, 8 days, and 2 weeks and on samples stored in the freezer for 5 weeks.

Results –

Homogeneity analysis: All samples taken for homogeneity analysis from the low- and high-concentration diets were within ±14% of nominal.

Stability analysis: After storage for 1 week in the refrigerator followed by up to 14 days at room temperature, concentrations of the low- and high-dose diets were 95.5-140.2% and 101.6-104.7%, respectively, of their initial measured concentrations. The high value for the low-dose diet occurred for the sample after 14 days at room temperature; reanalysis of this sample showed a concentration of 126.2% of initial. After 5 weeks in the freezer, the low- and high-concentration samples were 94.5% and 99.8%, respectively, of their initial measured concentrations.

Concentration analysis: Concentrations of the 5-, 25-, 750-, and 5000-ppm diets were 83.0-100%, 88.8-110.6%, 94.1-105%, and 94.8-116%, respectively, of nominal during the study.

Results of the dietary analyses show that the mixing procedure was adequate and the actual dosages to the animals were within an acceptable range.

C. OBSERVATIONS

1. Parental animals

All animals were observed twice daily for mortality and moribundity. Each animal was removed from its cage and examined for abnormal or unusual findings once weekly. Body weights and food consumption were recorded weekly during premating. Females were weighed on GD 0, 7, 14, and 20 and on lactation days 0, 4, 7, 14, and 21. Food consumption for mated females was measured on GD 0-4, 4-7, 7-14, and 14-20 and on lactation days 0-4, 4-7, 7-10, and 10-14. Food efficiency was not calculated.

2. Litter observations

Litter observations were made as shown in Table 2. All females were allowed to litter naturally. Pups were weighed and examined for gross deformities on lactation days 0, 4, 7, 14, and 21. On lactation day 4, litters were culled to 4 pups/sex, where possible. Pups were weaned on lactation day 21.

7

TABLE 2. Litter observations					
Observation	Lactation day				
	Day 0	Day 4	Day 7	Day 14	Day 21
No. pups	X	X	X	X	X
Pup weight	X	X*	X	X	X
Sex of each pup recorded	X	X			
External examination	X	X	X	X	X

Data taken from text p. 22, MRID 44933604.
*Pre- and post-cull.

4. Postmortem observations

a. Parental animals

Adults that died or were sacrificed moribund were necropsied. F_0 and F_1 parental animals were killed after weaning of their litters. All animals were sacrificed by sodium pentobarbital anesthesia and exsanguination. A gross necropsy was performed on all surviving adults and selected tissues were weighed [XX] and preserved [X]. Histopathological examination was conducted on the selected tissues from the control and high-dose groups. In addition, the adrenals from all animals were examined microscopically. Uteri from females that failed to mate or mated but failed to produce a litter were stained with 10% ammonium sulfide and examined microscopically.

b. Offspring

Pups found dead during lactation and culled pups were subjected to gross necropsy. F_1 weanlings not selected as parents were necropsied and tissues were collected and preserved from 20/sex/group as described for the adults. F_2 weanlings were subjected to gross necropsy.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta		Brain
	Teeth		Heart		Periph. nerve
	Oral cavity		Bone marrow		Spinal cord (cervical level)
	Salivary glands		Lymph nodes	XX	Pituitary
	Esophagus		Spleen		Eyes (optic n.)
	Stomach		Thymus		
	Duodenum				**GLANDULAR**
	Jejunum		**UROGENITAL**	XX	Adrenal gland
	Ileum		Kidneys		Harderian gland
	Cecum		Ureter		Lacrimal gland
	Colon		Urinary bladder		Mammary gland
	Rectum	XX	Testes		Parathyroids
XX	Liver	XX	Epididymides		Thyroids
	Gall bladder	XX	Prostate		
	Pancreas	X	Seminal vesicle		**OTHER**
			Coagulating gland		Bone
	RESPIRATORY	XX	Ovaries		Skeletal muscle
	Trachea		Oviduct		Skin
	Lung	XX	Uterus		Mediastinal tissue
	Nose/nasal cavity		Cervix		Mesenteric Tissue
	Pharynx	X	Vagina	X	All gross lesions and masses
	Larynx				

D. DATA ANALYSIS

1. Statistical analyses

Levene's test was used to analyze variance homogeneity and if significant, a rank transformation was used on the data. Analysis of variance (ANOVA) was used on the homogeneous or transformed data followed by Dunnett's test for pairwise comparisons between treated and control groups. When variance homogeneity was not established, nonparametric tests were used. The one-way ANOVA was used to analyze continuous data. Reproductive indices were analyzed by the Cochran-Armitage test and by a Fisher-Irwin exact test. Pup body weights were analyzed by a one-way analysis of covariance with the number of pups/litter as the covariate.

2. Indices

The following reproductive index was calculated.

Fertility index (%) = (No. animals pregnant/No. animals paired) × 100

Offspring viability indices: The following litter indices were calculated.

Live Birth index (%) = (No. pups born alive/No. pups born) × 100

Viability index (%) = (No. pups alive day 4 precull/No. pups born alive) × 100

Weaning index (%) = (No. pups alive day 21/No. pups alive day 4 postcull) × 100

3. Historical control data were not included for comparison with concurrent controls and test groups.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

One 750-ppm F_0 male was sacrificed moribund and one control F_0 female was found dead during week 5. Clinical signs in both of these animals prior to sacrifice included tremors, recumbent posture, cold to touch, and stained anogenital area; a cause of death was not determined. Four F_0 females in the 5000-ppm group were sacrificed or found dead during gestation or lactation. These deaths included one sacrificed with dystocia, one each found dead on GD 23 and lactation day 7 with no prior clinical signs, and one sacrificed on lactation day 9 following the observations of thin, hunched, languid, and labored breathing. One 5000-ppm F_1 male was sacrificed during week 15 and one 750-ppm F_1 female was sacrificed during week 8. Swollen hind limbs were observed prior to sacrifice in both F_1 animals. All remaining parental and adult animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in males or females of either generation during the study.

2. Body weight and food consumption

a. Premating

Selected body weight data for the F_0 adults are given in Table 3. Absolute body weights of the F_0 males were similar between the treated and control groups throughout the study. Cumulative body weight gains for the 5000-ppm F_0 males were significantly ($p \leq 0.05$; 82.9-92% of controls) less than the control group for premating weeks 0-1 and 0-2. Body weight gains by the treated males were similar to the controls during the remainder of the study. Absolute body weights of the 5000-ppm F_0 females were significantly ($p \leq 0.01$) less than the controls throughout premating beginning on week 1. The final premating body weight of the high-dose females was 89% of the control level. Mean cumulative body weight gains of the 5000-ppm F_0 females were significantly ($p \leq 0.01$) less than the controls beginning with week 1 and continuing throughout premating. Overall premating weight gain by the high-dose F_0 females was 77% of the control group level. Body weights and body weight gains for the 5-, 25-, and 750-ppm F_0 males and females were similar to the controls throughout premating. Food consumption by the 5000-ppm F_0 males and females was significantly ($p \leq 0.01$; 91% and 87%, respectively, of controls) less than the controls during premating week 0-1. No other treatment-related differences in food consumption were

observed during premating for males or females.

Selected body weight data for the F_1 adults are given in Table 4. Absolute body weights of the high-dose F_1 males and females were significantly ($p \leq 0.01$) less than their respective controls throughout premating. Final premating body weights of the 5000-ppm males and females were 79% and 82%, respectively, of the control group level. Absolute body weights of the 750-ppm F_1 males were also significantly ($p \leq 0.05$) less than the controls for premating weeks 0 and 1. Cumulative body weight gains by the high-dose F_1 males were significantly ($p \leq 0.01$) less than the controls throughout the study with premating and overall weight gains 86% and 84%, respectively, of the control levels. In contrast, body weight gain for the 5000-ppm F_1 females was significantly ($p \leq 0.05$) greater than the controls during week 0-1 and similar to control levels for the remainder of premating. Body weights and body weight gains for the 5- and 25-ppm F_1 males and the 5-, 25-, and 750-ppm F_1 females were similar to the controls throughout premating. Food consumption by the 5000-ppm F_1 males was significantly ($p \leq 0.01$) less than the controls throughout the study. Food consumption was occasionally significantly ($p \leq 0.05$ or 0.01) reduced for the 25- and 750-ppm F_1 males during premating and postmating as compared with the controls. Food consumption was also significantly ($p \leq 0.05$ or 0.01) less than the controls at most weekly intervals during premating by the 5000-ppm F_1 females and at week 0-1 for the 750-ppm F_1 females.

TABLE 3. Mean body weights (g) of the F_0 adults during the premating period					
Week	Treatment Group				
	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
Males					
Week 0	217.45	222.26	220.96	222.77	222.08
Week 2	307.56	312.47	310.01	312.91	304.96
Week 4	368.43	378.02	374.96	380.02	370.15
Week 6	414.95	423.04	421.96	428.90	414.09
Week 10 (end of premating)	476.82	487.96	479.81	497.41	471.94
Week 19 (termination)	549.78	558.00	557.68	577.07	541.21
Overall weight gain (0-19)	332.3	335.7	336.7	354.1	319.1
Females					
Week 0	200.11	198.57	195.74	192.64	194.89
Week 2	251.25	245.37	245.50	242.06	229.75** (91)[a]
Week 4	277.40	270.85	273.20	270.67	253.90** (92)
Week 6	300.16	289.83	294.36	291.13	271.10** (90)
Week 10 (end of premating)	331.62	322.48	325.81	323.44	296.57** (89)
Premating weight gain	131.5	123.9	130.1	130.8	101.7** (77)

Data taken from Tables 10 and 13, pp. 97-99 and 102-104, respectively, MRID 44933604.
[a]Numbers in parentheses are percent of control; calculated by reviewer.
Significantly different from control: **p≤0.01.

12

TABLE 4. Mean body weights (g) of the F_1 adults during the premating period

Week	Treatment Group				
	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
Males					
Week 0	193.29	187.30	189.96	179.21* (93)[a]	125.53** (65)
Week 2	314.48	309.49	310.28	298.41	220.48** (70)
Week 4	401.90	399.18	397.69	389.24	305.54** (76)
Week 6	461.54	463.00	456.90	450.00	357.64** (77)
Week 10 (end of premating)	546.35	551.49	537.86	536.80	429.60** (79)
Week 19 (termination)	658.36	665.25	635.88	641.13	517.02** (79)
Overall weight gain	465.1	478.0	445.9	461.9	391.9** (84)
Females					
Week 0	151.48	150.37	148.11	144.14	102.41** (68)
Week 2	203.78	202.94	200.02	194.93	155.51** (76)
Week 4	238.82	238.79	237.66	232.84	190.66** (80)
Week 6	261.13	263.67	263.05	259.62	213.17** (82)
Week 10 (end of premating)	290.71	293.56	294.09	291.13	239.07** (82)
Premating weight gain	139.2	143.2	146.0	147.0	136.7

Data taken from Tables 16 and 19, pp. 107-109 and 112-115, respectively, MRID 44933604.
[a]Numbers in parentheses are percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

b. Gestation and lactation

Absolute body weights of the 5000-ppm F_0 and F_1 dams were significantly ($p \leq 0.01$) less than the controls throughout gestation (88-91% and 78-81%, respectively, of controls) and lactation (84-89% and 72-81%, respectively, of controls). During gestation by the F_0 dams, body weight gains were significantly ($p \leq 0.01$) reduced in the high-dose group only for days 0-7. Body weight gains by the 5000-ppm F_1 dams were significantly ($p \leq 0.01$) less than the controls throughout gestation with overall weight gain 73% of the control level. Body weight changes of the F_0 and F_1 dams during lactation were not affected by treatment. Food consumption by the high-dose groups was significantly ($p \leq 0.05$ or 0.01) reduced on GD 0-4, 4-7, 7-14, and lactation days 7-10 for the F_0 dams (84-89% of controls) and throughout gestation and lactation for the F_1 dams (61-86% of controls) as compared with their respective control groups.

3. Test substance intake

Test substance intake was not calculated by the study author. Based on a food factor of 0.05 for the adult rat, doses for the 5-, 25-, 750-, and 5000-ppm groups were 0.25, 1.25, 37.5, and 250 mg/kg/day, respectively.

4. Reproductive function

Reproductive function tests for estrous cycle length or periodicity, sperm measures, or sexual maturation of the offspring were not conducted in this study.

5. Reproductive performance

The reproductive performances of the F_0 and F_1 animals are summarized in Table 5. Mating performance and fertility were not affected by test article administration in the F_0 generation. The duration of gestation was significantly ($p \leq 0.05$) longer in the 5000-ppm F_0 group as compared with controls due to two females with gestation lengths of 24 and 25 days.

In the 5000-ppm F_1 group, the number of females that mated and the number pregnant were significantly ($p \leq 0.01$) reduced as compared with the controls. This correlated to a significantly ($p \leq 0.01$) lower fertility index for the high-dose group. The duration of gestation was slightly (n.s.) longer in the 5000-ppm F_1 dams as compared with the controls. Mating performance and fertility were not affected by test article administration in the 5-, 25-, and 750-ppm F_1 groups.

TABLE 5. Reproductive performance of rats fed triticonazole for two generations					
Observation	**0 ppm**	**5 ppm**	**25 ppm**	**750 ppm**	**5000 ppm**
F_0 generation (F_1 litters)					
Number females paired	27	28	28	28	28
Number females inseminated	26	28	28	27	28
Number females pregnant (%)	23 (88)	27 (96)	22 (79)	25 (93)	28 (100)
Mean precoital interval (days)	2.12	2.81	3.32	2.81	2.61
Mean gestation length (days)	22.1	22.1	22.2	22.0	22.6*
Fertility index (%)	85	96	79	89	100
F_1 generation (F_2 litters)					
Number females paired	28	28	28	27	28
Number females inseminated	28	28	26	27	20**
Number females pregnant (%)	26 (93)	28 (100)	25 (96)	25 (93)	18** (90)
Mean precoital interval (days)	3.07	3.18	2.54	3.38	3.30
Mean gestation length (days)	22.1	22.2	22.1	22.1	22.6
Fertility index (%)	93	100	89	93	64**

Data taken from Tables 28-31, pp. 129-140, MRID 44933604.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

6. Parental postmortem results

a. Organ weights

Selected absolute organ weight data for the adult animals are given in Table 6. No treatment-related differences in absolute or relative organ weights occurred between the treated and control F_0 males. High-dose F_0 females had significantly ($p \leq 0.01$) reduced terminal body weights and absolute and relative-to-body left adrenal weights as compared with controls. F_0 females in the 5000-ppm group also had significantly ($p \leq 0.01$) increased absolute and relative-to-body liver weights as compared with the control group.

High-dose F_1 males and females had significantly ($p \leq 0.05$) lower terminal body weights as compared with the controls. Absolute and relative left and right adrenal weights of the high-dose F_1 females and right adrenal weights of the high-dose F_1 males were significantly ($p \leq 0.05$) less than the controls.

TABLE 6: Selected absolute organ weight data for parental animals (g)

	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
F_0 adults					
Terminal body weights					
Males	547.7	555.6	556.5	575.6	541.5
Females	362.9	355.7	359.2	360.5	334.0**
Left adrenal					
Males	0.0248	0.0308**	0.0264	0.0280	0.0261
Females	0.0422	0.0424	0.0420	0.0423	0.0314**
Right adrenal					
Males	0.0262	0.0272	0.0251	0.0260	0.0244
Females	0.0393	0.0414	0.0409	0.0387	0.0341
Liver					
Males	20.6711	20.1566	21.0414	21.5740	21.0929
females	14.5874	14.3021	14.4070	15.0075	16.5919**
F_1 adults					
Terminal body weights					
Males	650.7	658.7	632.9	636.2	513.1*
Females	325.5	332.6	332.9	329.5	276.8*
Left adrenal					
Males	0.0296	0.0299	0.0292	0.0262*	0.0267
Females	0.0390	0.0404	0.0366	0.0376	0.0268*
Right adrenal					
Males	0.0285	0.0293	0.0282	0.0251*	0.0255*
Females	0.0361	0.0382	0.0342	0.0345	0.0266*
Liver					
Males	23.2055	23.1730	22.6261	22.6247	18.8929*
females	12.4818	12.5511	12.7456	13.5258	13.3563

Data taken from Tables 34 and 40, pp. 146-149 and 170-173, MRID 44933604.
Significantly different from control: *$p \leq 0.05$, **$p \leq 0.01$.

b. Pathology

1) Macroscopic pathology - No dose- or treatment-related findings were observed at gross necropsy of the F_0 or F_1 adults. Lesions of the urinary tract were observed in one 750-ppm F_0 male and one control F_0 female which were sacrificed moribund or found dead, respectively.

2) Microscopic pathology - Lesions of the adrenal gland were observed in high-dose males and females of both generations (Table 7). In females the lesions were characterized by degeneration of the adrenal cortex, giant cell formation,

and pigment and collagen deposition. Degeneration of the adrenal cortex was not seen in males. However, in males, vacuolation of the cortical cells was observed. The four 5000-ppm F_0 females found dead or sacrificed early also had lesions in the adrenal cortex including degeneration (1), inflammation (1), and hemorrhage (2).

No treatment-related microscopic lesions were observed in the reproductive organs of adults that failed to mate or that mated but failed to produce a litter.

TABLE 7: Microscopic lesions of the adrenal gland in male and female rats fed Triticonazole for two generations

	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
F_0 males					
Cortical vacuolation	7/28	6/28	4/28	6/27	27**/28
F_0 females					
Degeneration	0/27	0/28	0/28	0/28	22**/24
Giant cells	0/27	0/28	0/28	0/28	16**/24
Pigment	0/27	0/28	0/28	0/28	6**/24
Collagen	0/27	0/28	0/28	0/28	6**/24
F_1 males					
Cortical vacuolation	15/28	9/28	8/28	9/28	27**/27
F_1 females					
Degeneration	0/28	0/27	0/28	0/27	11**/28
Giant cells	0/28	0/27	0/28	0/27	14**/28
Pigment	0/28	0/27	0/28	0/27	7**/28

Data taken from Tables 37 and 43, pp. 159-163 and 182-185, respectively, MRID 44933604.
**Incidence rate significantly different from control, $p \leq 0.01$; calculated by reviewer using Fisher's Exact test.

17

B. OFFSPRING

1. Viability and clinical signs

Viability data for the F_1 and F_2 litters are given in Table 8. High-dose pups of both generations had reduced survival during lactation days 0-4 as indicated by significantly ($p \leq 0.01$) lower live birth and viability indices as compared with the controls. In addition, four high-dose F_0 dams had whole litter loss during lactation days 0-4. The number of live pups/litter on day 0 was slightly (n.s.) reduced for the high-dose F_1 litters, and was significantly ($p \leq 0.01$) reduced in the high-dose F_2 litters as compared with their controls. Fetal sex ratios were not affected by treatment in either generation. No treatment-related clinical signs were observed during lactation in offspring of either generation.

TABLE 8. Viability of F_1 and F_2 litters during lactation

Observation/study time	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
F_1 litters					
Number of viable litters	23	27	22	25	26
Mean live litter size on day 0	13.70	15.22	15.32	16.12*	12.35
Gestation index (%)	100	100	100	100	93
Live birth index (%)	93	98**	99**	98**	82**
Viability index days 0-4 (%)	92	94	99**	97	82**
Weaning index days 4-21 (%)	95	100	100	100	94
Whole litter loss days 0-4 (n)	0	1	0	0	4
Sex ratio at day 0 (% male)	48	47	51	49	49
F_2 litters					
Number of viable litters	26	28	25	25	18**
Mean live litter size on day 0	14.46	14.32	13.52	15.20	11.13**
Gestation index (%)	100	100	100	100	89
Live birth index (%)	99	98	99	98	85**
Viability index days 0-4 (%)	98	100*	99	97	89**
Weaning index days 4-21 (%)	100	100	100	100	100
Whole litter loss days 0-4 (n)	0	0	0	0	1
Sex ratio at day 0 (% male)	51	49	54	54	53

Data taken from Tables 29 and 31, pp. 130-134 and 136-140, respectively, MRID 44933604.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

18

2. Body weight

Selected body weights of the F_1 and F_2 pups during lactation are given in Table 9. Mean body weights of the high-dose male and female F_1 pups were significantly ($p \leq 0.01$) less than the controls beginning on lactation day 7 and continuing until weaning. The differences between the high-dose and control pups became more pronounced later in lactation as indicated by body weight gains (calculated by reviewer). Weight gains by the high-dose male and female pups were 104% and 91%, respectively, of the controls for lactation days 0-4 but were 65% and 66%, respectively, of the controls for lactation days 14-21. At weaning, body weights of the high-dose male and female pups were only 71% of the control level. Body weights of the F_1 pups in the 5, 25, and 750 ppm groups were similar to, or slightly greater than, the controls throughout lactation.

Mean body weights of the high-dose male and female F_2 pups were significantly ($p \leq 0.01$) less than the controls throughout lactation. On lactation day 0, body weights of the high-dose pups were 90% of the controls. However by lactation day 21, body weights of the high-dose males and females at weaning were 51% and 49%, respectively, of the control level. Body weight gains (calculated by reviewer) of the high-dose pups were 39-50% of the control levels for lactation days 0-4, 7-14, and 14-21; during lactation days 4-7 weight gains by the high-dose pups were 61-63% of the control level. Body weights of the F_2 pups in the 5, 25, and 750 ppm groups were similar to the controls throughout lactation.

TABLE 9. Mean pup body weights (g) during lactation

Day of lactation	0 ppm	5 ppm	25 ppm	750 ppm	5000 ppm
F_1 pups					
Day 0					
male	6.46[a]	6.55	6.62	6.55	6.25
female	6.03	6.16	6.17	6.10	5.85
Day 4 (postcull)					
male	10.00	10.78	11.08*	10.63	9.96
female	9.45	10.32*	10.52*	10.10	9.00
Day 7					
male	17.33	17.98	18.43	17.65	15.21** (88)[b]
female	16.10	17.17	17.39	16.81	14.13** (88)
Day 14					
male	36.31	37.07	37.96	36.50	26.83** (74)
female	34.28	35.63	36.21	34.89	25.54** (75)
Day 21					
male	59.25	59.32	61.11	58.35	41.82** (71)
female	54.98	56.77	57.99	55.41	39.27** (71)
F_2 pups					
Day 0					
male	6.35	6.58	6.38	6.42	5.71** (90)
female	5.94	6.18	6.11	6.07	5.37** (90)
Day 4 (postcull)					
male	10.58	11.11	10.68	10.77	7.95** (75)
female	10.14	10.75	10.29	10.31	7.25** (71)
Day 7					
male	17.32	18.25	17.69	17.55	12.07** (70)
female	16.52	17.55	16.84	16.65	11.28** (68)
Day 14					
male	34.62	36.32*	35.95	35.36	19.81** (57)
female	33.60	35.04	34.45	34.17	18.67** (56)
Day 21					
male	56.21	58.57	58.43	57.31	28.49** (51)
female	53.96	56.10	55.38	54.76	26.66** (49)

Data taken from Tables 29 and 31, pp. 130-134 and 136-140, respectively, MRID 44933604.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.
[a]Covariate adjusted means.
[b]Number in parentheses is percent of control; calculated by reviewer.

3. Offspring developmental milestones

Pup developmental landmarks, such as auditory canal opening, pinna unfolding, and eye opening, were not monitored in this study.

4. Offspring postmortem results

a. Organ weights - Organ weights from weanlings were not obtained at necropsy.

b. Pathology

1) Macroscopic pathology - No treatment-related lesions were found in F_1 or F_2 weanlings at gross necropsy. High-dose pups of both generations that died during lactation had an increased incidence of no milk in the stomach indicating that these pups were either stillborn or died soon after birth.

2) Microscopic pathology - Tissues from the weanlings were not examined microscopically.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study author concluded that administration of RPA400727 to male and female rats for two generations resulted in systemic and reproductive toxicity at a dietary concentration of 5000 ppm. Deaths and/or premature sacrifices of four F_0 females were considered treatment-related. Mean body weights, body weight changes, and food consumption were decreased for the F_0 females and the F_1 males and females. The live birth and viability indices and pup body weights were significantly lower in both generations and the number of pups/litter was reduced in the F_2 generation. Lower adrenal weights in females and microscopic findings in the adrenals of males and females of both generations were considered treatment-related.

Therefore, the study author considered the NOAEL for reproductive toxicity and growth and development of offspring to be 750 ppm, based on increased maternal death, decreased pup body weights, pup mortality, and pathologic findings at 5000 ppm.

B. REVIEWER'S DISCUSSION

1. Systemic Toxicity

Intercurrent deaths of several parental animals during premating were considered incidental to treatment. No clinical signs of toxicity were observed at any time during the study in parental animals of either generation.

21

Body weights of the F_0 males were unaffected by treatment with the test article. On the other hand, body weights of the high-dose F_0 females and F_1 males and females were significantly less than their controls throughout premating. However, while weight gains for the F_0 females and the F_1 males were reduced, weight gain by the F_1 females was unaffected during premating. The lower body weights of the F_1 animals during premating were considered a continuation of the effects observed during lactation (see below).

Transient reductions in food consumption for the high-dose F_0 males and females during the first week of treatment correlated with an initial lower body weight gain in the males and was probably due to a lack of palatability of the test article to the animals. Since food consumption by the high-dose F_0 adults was similar to that of controls after the first week, the animals appeared to adjust to the taste. In contrast, food consumption by the high-dose F_1 males and females was reduced throughout the study. Reduced body weight gain by the F_0 females in the absence of an effect on food consumption, indicates a direct toxicity of the test article. Lower food consumption by the F_1 adults may have been due to the smaller size of the animals as well as a lack of palatability.

Gross necropsy of the F_0 and F_1 adults was unremarkable. The increased liver weights for the high-dose F_0 females were considered incidental to treatment since the magnitude of the increase was not biologically significant. The decrease in liver weight for the high-dose F_1 males may have been due to the smaller size of these animals at termination. No histopathological correlates were observed in the livers of either group.

The target organ of the test article appeared to be the adrenal cortex. Absolute and relative adrenal weights were consistently reduced in high-dose females of both generations although statistical significance was not reached for the right adrenal from the F_0 animals. Microscopic lesions of the adrenal cortex were observed in high-dose F_0 and F_1 males and females although the type of lesions differed between the sexes. In males the main finding was vacuolation while in females degeneration, giant cell formation, and pigment and collagen deposition were observed.

Therefore, the LOAEL for systemic toxicity is 5000 ppm based on reduced body weights of the F_0 females and the F_1 males and females and on microscopic lesions in the adrenal gland of F_0 and F_1 males and females. The systemic toxicity NOAEL is 750 ppm.

2. Reproductive Toxicity

Reduced absolute body weights of the high-dose F_0 and F_1 dams during gestation and lactation were considered a continuation of the premating effects. Decreased weight gain during gestation by the high-dose F_1 dams was probably a result of fewer pups/litter produced by this group as compared with the controls. 22

Deaths of four high-dose F_0 dams during gestation and lactation were considered treatment-related even though all F_1 dams survived. Because one female in this group was sacrificed with dystocia, the longer duration of gestation measured for the high-dose F_0 dams was considered treatment-related even though the increase was due to two females with gestation lengths of 24 and 25 days. The duration of gestation was also increased to the same extent in the F_1 females even though statistical significance was not attained.

Mating performance and fertility were not affected in the F_0 generation. However, in the high-dose F_1 group, fewer females mated and were pregnant resulting in a lower fertility index as compared with the control group.

The main reproductive toxicity effect of the test article was on pup survival and body weight gain. High-dose groups of both generations had significantly reduced pup survival during lactation days 0-4 with complete litter loss by four high-dose F_0 dams. The increased incidence of no milk in the stomach of the pups that died suggests that these pups were either stillborn or died soon after birth. Body weights of the high-dose pups were significantly less than the controls beginning on lactation day 7 for the F_1 pups and throughout lactation for the F_2 pups. Although body weight gains were most affected after day 14 in the F_1 pups, body weight gains by the F_2 pups were reduced throughout lactation. Therefore, both a lactational (prior to day 14) and a systemic effect were probably contributing to the reduced growth of the pups.

Therefore, the reproductive toxicity LOAEL is 5000 ppm based on F_0 maternal mortality, decreased fertility of the F_1 animals, reduced F_1 and F_2 pup survival, and reduced F_1 and F_2 pup body weight. The reproductive toxicity NOAEL is 750 ppm.

C. STUDY DEFICIENCIES

No major deficiencies were identified in the conduct of this study.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a reproduction study [OPPTS 870.3800 (§83-4)] in rats.

25