(2-14-01)

DATA EVALUATION REPORT

TRITICONAZOLE

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY - RAT [OPPTS 870.6200 (§82-7)]

MRID 44933603

Prepared for

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

Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity – Rat; [OPPTS 870.6200 (§82-7)]

DP BARCODE: D261924
P.C. CODE: 125620

SUBMISSION CODE: S568827
TOX. CHEM. NO.: not given

TEST MATERIAL: Triticonazole

SYNONYMS: (IRS)-E-2-(4-chlorobenzylidine)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentan-1-ol

CITATION: Weiler, M.S. 1997. 13-Week dietary neurotoxicity study with triticonazole in rats. Corning Hazleton Inc., 3301 Kinsman Boulevard, Madison, WI 53704 Laboratory Project Identification No. CHW 6224-228. July 14, 1997. MRID 44933603. Unpublished.

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

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 44933603), groups of 10 Crl:CD(SD)BR VAF/Plus rats/sex/group were administered Triticonazole (97.2%, Lot No. 9550347) at concentrations of 0, 500, 2500, or 10,000 ppm in the diet for 13 weeks. (Average daily intake was 0, 32.5, 169.9, or 695.1 mg/kg for males and 0, 38.5, 199.4, or 820.3 mg/kg, for females). Functional observational battery (FOB) and motor activity testing were performed prior to administration and during weeks 4, 8, and 13 of the study. Body weights and food consumption were determined weekly. Clinical signs were recorded twice daily. At study termination all animals were sacrificed and examined for gross lesions. Designated tissues of the nervous system from 6 rats/sex from the control and high-dose groups were processed for microscopic neuropathological evaluation.

There were no treatment-related clinical signs or deaths. Body weight was decreased ($p<0.05$) 7.6% in high-dose males and 8.7% in high-dose females compared to controls at week 2 (Males: 291, 298, 289, and 268*; Females: 196, 199, 199, and 179* for control, low-, mid-, and high-dose groups, respectively), and body weight gain was decreased ($p<0.05$) 42% in high-dose males (55, 58, 53, and 32* for control, low-, mid-, and high-dose groups, respectively) and 72% in high-dose females compared to controls for the 1-2 week interval (wk1-2: 25, 24, 22, and 7*; wk 1-4: 68, 73, 72, and 54* for control, low-, mid-, and high-dose groups, respectively). The body weight effects were accompanied by decreased ($p<0.05$) food consumption for high-dose males (13%, 152 vs. control 175) and females (19%, 110 vs. control 135) during week 1. There

was a dose-related increase in females not responding to touch at week 13 (1/10 control, 4/10 low, 5/10 mid, and 7/10 high dose). The biological relevance of this observation is unclear but it is related to treatment. There were significant differences ($p<0.05$) in the number of rears for high-dose males at week 8 (3.5, 3.2, 4.6, and 7.5* corresponding to control, low-, mid-, and high dose groups) and for low- and high-dose females at week 13 (15.5, 7.6*, 14.3, and 8.9* respective of dosing groups) and in hindlimb grip strength of mid-dose females at week 8 (734, 827, 925*, and 829 corresponding to control, low-, mid-, and high-dose groups). Number of rears increased in the high-dose treated males at 8 weeks correlated with the finding of the acute study (MRID 44803036) at 8 weeks, where there were dose-related increased in the number of rears in males. There were no histological lesions associated with treatment, brain size was not affected, and there were no microscopic observations indicative of a neurotoxic effect at any dose.

The NOAEL is 170 mg/kg/day for males and less than 38.5 mg/kg/day for females. The LOAEL was 695 mg/kg/day for males and not established for females based on increased number of rears for males at 8 weeks and for females decreased number of rears at 8 weeks and decreased in sensitivity to touch at 13 weeks.

This study is classified **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a subchronic neurotoxicity study [OPPTS 870.6200 (§82-7)] in rats.

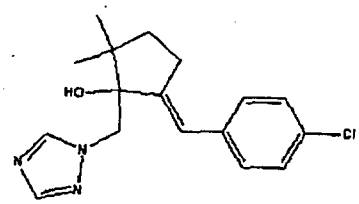
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Compliance statements were provided. No Flagging statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Triticonazole

Description: white powder
CAS No.: 131983-72-7
Lot/Batch No.: Lot No. 9550347
Purity: 97.2%
Contaminants: none given
Structure:



2. Vehicle

The test material was administered in the diet, Certified Rodent Diet #5002 meal (PMI® Feeds, Inc.).

3. Test animals

Species: rat
Strain: Crl:CD®(SD)BR VAF/Plus®
Age and mean weight at study initiation: approx. 7 weeks old, males 210 - 254 g and females 155 - 196 g.
Source: Charles River Laboratories, Inc., Portage, MI.
Housing: individually, in suspended wire mesh stainless steel cages
Food: Certified Rodent Diet #5002 meal (PMI® Feeds, Inc.), *ad libitum.*
Water: water was available *ad libitum*
Environmental conditions:
Temperature: 19 - 25 °C
Humidity: 50%±20%
Air changes: not given
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In life dates

Start: October 14, 1996
End: January 16, 1997

2. Animal assignment

The number of animals assigned to the exposure groups is listed in Table 1. Animals were randomly assigned to the dietary groups as indicated in the table below such that body weights were homogenous within each group as determined by the Bartlett's Test for homogeneity. Each animal was assigned a permanent number and a transponder encoded with this number was implanted into each animal.

TABLE 1. Study design

Dietary Conc. (ppm)	Dose (mg/kg)		Number of animals	
	Males	Females	Males	Females
0	0.0	0.0	10	10
500	32.5	38.5	10	10
2500	169.9	199.4	10	10
10000	695.1	820.3	10	10

Data taken from pp. 15 and 59, MRID 44933603.

3. Validation of test methods

Studies were conducted with acrylamide and paraoxon to establish the sensitivity, reliability, and validity of the neurotoxicity assessment procedures in this study, the adequacy of training of technical personnel. (Acrylamide Study: HWI 2100-030. March 29, 1996 and Paraoxon Study HWI 2100-004. April 11, 1996. No MRID Nos. as of June 1998., see Appendix of Acute Neurotoxicitiy DER, MRID 44933602).

4. Rationale for dose selection

The range-finding study (benchmark and time-to -peak effect neurotoxicity study, MRID 44933602) with triticonazole in rats was used to establish dose range for subsequent studies (see Appendix of Acute Neurotoxicitiy DER, MRID 44933602). The selection of doses for this study was based on the anticipation that the high dose would produce some toxicity (decreased body weight gains), the lowest dose would serve as a NOAEL, and the middle dose was selected for the purpose of evaluating any potential neurotoxicological effects.

5. Preparation, administration, and analysis of test diets

Test diets were prepared for week 1, biweekly during weeks 2-5 and monthly thereafter. A specified amount of basal diet was weighed into a mixing bowl from which a portion was removed and placed in a Waring blender. The appropriate amount of triticonazole was weighed, added to the blender, overlaid with basal diet from the mixing bowl, blended, and this premix transferred to the mixing bowl. A portion of diet from the mixing bowl was then transferred to the blender, blended to recover residual test material, returned to the bowl, and the contents thoroughly mixed. Concentration analyses were done on duplicate samples (approx. 100 g each) of all dose preparations from the first, third, and fourth mixes. Homogeneity analyses were taken from the low- and high-dose levels mixed for week 1; duplicate samples were taken from the top, middle, and bottom of the preparations used for concentration analyses. Additional homogeneity analyses were done at week 6 when the batch size increased by >30%. For stability analysis, four sets of duplicate samples were taken from the low- and high-dose diets mixed for week 1. One set was analyzed on the day of mixing, and the remaining sets were stored at room temperature for 12, 19, and 36 days and analyzed.

Results –

Concentration: Actual concentrations of the test article ranged from 94.2% to 108% of target concentrations.

Homogeneity: Mean values of the homogeneity analyses ranged from 94.2% to 103% of theoretical concentrations for the 500 ppm diet and 103% to 108% of theoretical concentrations for the 10,000 ppm diet.

Stability: The mean concentrations of 500 and 10,000 ppm diets were within 3% and 8% of initial concentrations when stored at room temperature for 12, 19, or 36 days.

Conclusion: Concentration, stability, and homogeneity are acceptable for the purposes of this study.

6. Statistical analysis

One-way ANOVA was used to analyze body weight, body weight gain, food consumption, FOB continuous data, MA counts, and brain measurements. Levene's test was used to test for variance homogeneity. In the case of heterogeneity of variance, at $p \leq 0.05$, transformations were used to stabilize the variance. ANOVA was used on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's multiple comparison t-test was used for pairwise comparisons between control and treated groups.

All data were tested at the $p \leq 0.05$.

C. METHODS

1. Observations

Cage-side observations for gross signs of substance-related effects and mortality were conducted for animals in all groups daily. Detailed physical examinations for signs of toxicity were carried out once each week.

2. Body weight

Animals were weighed on the first day of treatment, weekly thereafter, and on each day that the FOB was done.

3. Food consumption

Individual food consumption was determined weekly.

4. Functional observational battery (FOB)

Rats were subjected to a FOB prior to exposure and during weeks 4, 8, and 13 of treatment. The FOB tests were performed by technicians blind to animal treatment.

a. Home Cage Observations

Position, activity, body tone, tremor, convulsions, vocalization, and arousal upon opening the cage.

b. Handling observations

Ease of removal and handling, vocalization, palpebral closure, exophthalmus, excessive lacrimation, excessive salivation, respiration, appearance of fur, piloerection, and writhing.

c. Open arena observations

Latency time to first step, number of grooms, rears, fecal boli, and urine pools; position, gait abnormalities, activity, stereotypy, body tone, tremor, convulsions, and other observations.

d. Sensorimotor Tests/Reflexes

Startle response, righting reflex, analgesic reflex, body temperature, body weight, grip strength (forelimb and hindlimb), and landing foot splay.

5. Motor and locomotor activity (MA)

Motor activity measurements were assessed for each animal following the FOB observations (prior to exposure and during weeks 4, 8, and 13 of treatment). Individual activity was monitored by testing animals in a stainless steel, covered 18 inch diameter circular open field enclosure. MA was based on the number of photobeam breaks occurring over the 40-minute session and was reported every 2 minutes.

6. Ophthalomology

Opthalomological evaluation was not required and was not performed.

7. Sacrifice/necropsy/neurohistopathology

At study termination all rats were anesthetized with sodium pentobarbital (after an overnight fast) and tissues perfused *in situ* with 10% buffered formalin. All animals were examined grossly (external surfaces, orifices, brain, spinal cord, organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck) for lesions when sacrificed. The (X) tissues were collected from each animal. Brain dimensions (length, width, heith) were measured; however, no organ weights were recorded. The (XX) tissues from 6 control and 6 high-dose animals/sex were embedded in paraffin and examined utilizing hematoxylin and eosin (H&E).

The (*) tissues from 6 control and 6 high-dose animals/sex were embedded in epoxy resin and sections were stained with toluidine blue.

X	BRAIN*	X	SPINAL CORD	X	PERIPHERAL NERVES
XX	Eight coronal sections	XX	Cervical	XX*	Sciatic nerve, cross- longitudinal-sections
XX*	Optic nerves	XX*	–Dorsal root ganglia – Dorsal and ventral root fiber –Cervical Swelling	XX*	Tibial nerve, cross-sections
		XX	Thoracic	XX*	Sural nerve, cross-sections
		XX	Lumbar	XX*	Trigeminal nerve
		XX*	– Dorsal root ganglion –Dorsal and ventral root fiber –Lumbar Swelling Cauda Equina		**OTHER**
				XX*	Gastrocnemius muscle Gasserian ganglion
				XX	Eyes
				XX*	Anterior tibialis muscles
				XX	Pituitary

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

There were no deaths or clinical signs attributed to administration of the test substance in this study.

B. BODY WEIGHT AND BODY WEIGHT GAINS

Mean body weight for selected weeks and cumulative mean body weight for control and treated groups are presented in Table 2. Body weight was decreased ($p<0.05$) 7.6% in high-dose males and 8.7% in high-dose females compared to controls at week 2, and body weight gain was decreased ($p<0.05$) 42% in high-dose males and 72% in high-dose females compared to controls for the 1-2 week interval.

TABLE 2. Mean body weights (g) and body weight gains (g) for male and female rats treated with Triticonazole in the diet for 13 weeks.

Week	Dietary concentration (ppm) Triticonazole							
	0	500	2500	10,000	0	500	2500	10,000
	Males				Females			
1	237	240	236	236	172	176	177	172
2	291	298	289	269*	196	199	199	179*
3	336	346	336	317	214	220	217	201
4	373	383	375	356	228	234	235	216
6	417	428	423	398	245	256	256	236
8	465	480	471	441	262	274	280	253
9	462	484	475	450	262	276	282	257
10	478	497	490	462	270	282	289	261
11	500	520	505	481	280	288	298	270
12	521	540	523	488	286	297	308	277
14	536	553	537	505	295	303	311	284
Body weight gain								
1-2	55	58	53	32*	25	24	22	7*
1-4	159	167	161	142	68	73	72	54*
12-13	9	13	7	13	5	4	2	4
1-14	299	313	301	269	124	128	134	112

Body weight data taken from pp. 52-55, MRID 44933603.
* Significantly different compared to control at $p < 0.05$

D. FOOD CONSUMPTION

The body weight effects described above were accompanied by decreased (*=p<0.05) food consumption for high-dose males (13%) and females (19%) during week 1 (Males: 174±12.2, 178±9.9, 176±10.9, 152±6.5; Females: 135±6.8, 129±11.3, 132±12.8, 110±15.4* for control, low-, mid-, high-dose groups, respectively). No other effects on food consumption were noted.

E. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Grip Strength

There was a significant (*=p<0.05) decrease of 11% compared to controls in hindlimb grip strength of mid-dose females at week 8 (Trial 1: Males: 901±92, 979±181, 916±145, 960±171; Females: 734±103, 827±167, 925±155*, 829±123 for control, low-, mid-, high-dose groups, respectively). This statistical difference was not considered treatment-related or biologically significant since no dose-response was present and the effect was not noted at week 13.

Foot Splay

Landing foot splay was not significantly affected by administration of the test material.

Other FOB Endpoints

There was a dose-related increase in females not responding to touch at week 13 (incidence was 1/10 controls, 4/10 low-dose, 5/10 mid-dose, and 7/10 high-dose). The biological relevance of this observation is unclear, but it is related to treatment. There was a significant increase (*=p<0.05, Table 3) in the number of rears for high-dose males at week 8 (114% compared to control) and a significant decrease for low- (51% compared to control) and high-dose (43% compared to control) females at week 13.

Table 3. FOB - Number of rears (counts ± SD) for male and female rats treated with Triticonazole in the diet for 13 weeks.

	Dietary concentration (ppm) Triticonazole			
	0	500	2500	10,000
	Males			
Predose	9.3 ± 6.58	7.5 ± 5.52	8.1 ± 2.73	10.6 ± 3.89
Week 4	4.2 ± 4.71	3.7 ± 1.77	5.1 ±3.14	3.9 ± 1.10
Week 8	3.5 ± 3.17	3.2 ± 3.26	4.6 ± 4.14	7.5 ± 3.84*
Week 13	0.9 ± 1.20	2.2 ± 1.81	3.2 ± 4.10	3.9 ± 3.63

Table 3. FOB - Number of rears (counts ± SD) for male and female rats treated with Triticonazole in the diet for 13 weeks.				
	Females			
Predose	11.8 ± 5.31	11.3 ± 4.50	11.1 ± 3.70	9.2 ± 3.49
Week 4	11.4 ± 5.50	8.3 ± 4.37	13.2 ± 4.21	9.0 ± 3.83
Week 8	13.6 ± 6.33	9.2 ± 6.43	11.4 ± 6.83	8.5 ± 4.43
Week 13	15.5 ± 4.50	7.6 ± 6.60*	14.3 ± 5.21	8.9 ± 5.76*

Data taken from p. 74, MRID 44933603.
*Significantly different compared to control at $p<0.05$.

F. MOTOR ACTIVITY

No differences in activity were observed at any time interval during any of the test sessions among males or females at any test concentration. Data are summarized in Table 4.

Table 4. Overall (0-40 minute) motor activity (counts ± SD) for male and female rats treated with Triticonazole in the diet for 13 weeks.				
	Dietary concentration (ppm) Triticonazole			
	0	500	2500	10,000
	Males			
Predose	1429 ± 271.2	1478 ± 252.2	1633 ± 401.8	1583 ± 319.0
Week 4	1559 ± 337.0	1465 ± 343.9	1724 ±567.4	1654 ± 499.9
Week 8	1813 ± 643.8	1917 ± 424.4	1976 ± 686.9	2142 ± 686.2
Week 13	1760 ± 440.5	1601 ± 430.4	1670 ± 392.2	1737 ± 478.7
	Females			
Predose	1444 ± 546.0	1391 ± 328.5	1549 ± 301.9	1412 ± 335.3
Week 4	1612 ± 587.0	1852 ± 394.9	1540 ± 325.6	1522 ± 323.4
Week 8	1945 ± 631.3	2084 ± 379.4	1636 ± 513.7	1829 ± 569.3
Week 13	1739 ± 789.3	2063 ± 505.9	1546 ± 410.4	1835 ± 347.5

Data taken from pp. 85-88, MRID 44933603.
* Significantly different compared to control at $p < 0.05$

G. OPHTHAMOLOGY

Ophthalmology was not required and was not performed.

H. NEUROPATHOLOGY

There were no observations of treatment-related neuropathological findings in any of the exposure groups for either sex. No treatment related differences were observed in terminal body weights, brain sizes, or brain size to body weight ratios (Table 5).

Table 5. Terminal body weight (g), brain size (mm), and brain size /body weight ratio for male and female rats treated with Triticonazole in the diet for 13 weeks (mean±SD).

Sex	Measurement	Dietary concentration (ppm) Triticonazole			
		0	500	2500	10,000
Males	Body Weight Brain Size BS/BW	502±52.2 74.62±0.99 0.1486	521±25.4 75.63±1.29 0.1451	497±27.9 75.30±0.85 0.1514	471±19.5 7.24±1.54 0.1597
Females	Body Weight Brain Size BS/BW	271±17.3 73.14±1.18 0.2699	282±22.7 73.06±0.69 0.2595	290±22.7 73.20±0.98 0.2525	263±25.7 73.05±1.14 0.2776

III. DISCUSSION

A. DISCUSSION

There were no deaths attributable to the administration of the test substance in this study. Daily cage-side observations revealed no substance-related clinical signs observed among males and females treated with any concentration of Triticonazole.

Body weight and body weight gain were transiently decreased in high-dose males and females compared to controls at weeks 1-2; body weight effects were accompanied by food consumption decreases for high-dose males and females during week 1.

The FOB and motor activity assessments revealed mild treatment-related abnormalities or signs of neurotoxicity. There was a dose-related increase in females not responding to touch at week 13, the biological relevance of this observation is unclear but it is related to treatment. There were increases in the number of rears for high-dose males at week 8 and decreases for low- and high-dose females at week 13 and decreases in hindlimb grip strength of mid-dose females at week 8. No treatment-related gross or microscopic lesions were evident in any of the treatment groups compared to the control group for males or females treated with Triciconazole.

Note for the neurotoxicity studies in rats treated with Triticonazole: in the range-finding study (gavage administration of 0, 50, 1000, 2000 mg/kg) motor activity was increased in both sexes; in the acute study (gavage administration of 0, 80, 400, 2000 mg/kg) the

number of rears was increased in a dose related manner for males at the end of the study (8 weeks); in the subchronic study (dietary administration of 0, 32/38, 170/199, 695/820 for males/females) the number of rears was increased in the high-dose males at 8 weeks and decreased for the low. The range-finding study (MRID 44933602) and the acute study (MRID 44802036) suggested possible treatment/dose-related effects on activity, although the subchronic study does not show a dose-related effect on activity even at 8 weeks, the high dose males are affected. In addition however, with longer testing regime of the subchronic study, males do not show statistical significant increases in rears at 13 weeks (variability increases with dose at week 13 and thus may mask any effect) and females in a non-linear manner show decrease rearing at the low and high doses at 13 weeks. Thus, increased activity seen in these studies may be related to some nuisances in the route of administration, are temporary in display, and related to high dosage.

The NOAEL is 170 mg/kg/day for males and less than 38.5 mg/kg/day for females. The LOAEL was 695 mg/kg/day for males and not established for females based on increased number of rears for males at 8 weeks and for females decreased number of rears at 8 weeks and decreased sensitivity to touch at 13 weeks.

This study is classified **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a subchronic neurotoxicity study [OPPTS 870.6200 (§82-7)] in rats.

B. STUDY DEFICIENCIES

The positive control data referred to in the study report did not illustrate any chemicals that increase activity thus, it is more difficult to appraise the study findings.