(2-15-2001)

DATA EVALUATION REPORT

TRITICONAZOLE

STUDY TYPE: ACUTE ORAL NEUROTOXICITY – RAT
[OPPTS 870.6200 (81-8)]
MRIDs 44802036, 44933602

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 37830
Task Order No. 00-17I

Primary Reviewer: Rosmarie A. Faust, Ph.D.	Signature: Date:	Robert N. Ross for R. A. Faust MAY 18 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast MAY 18 2000
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert N. Ross MAY 18 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson MAY 18 2000

Disclaimer

This Review may have been altered subsequent to signing by the contractor's signature above.

Oak Ridge National Laboratory is managed and operated by U.T. Battelle, L.L.C., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Meta Bonner, Ph.D.
Registration Action Branch III
EPA WAM: S. Williams, D.V.M.
Registration Action Branch I (7509C)

Meta Bonner, Date 2-14-2001
D.V.M. Joy, Date 2-15-2K1

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity- Rat [OPPTS 870.6200(§81-8)]

DP BARCODE: D261924
P.C. CODE: 125620

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

TEST MATERIAL (PURITY): Triticonazole (97.2%)

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) Acute neurotoxicity study with triticonazole in rats. Corning Hazleton Inc., 3301 Kinsman Boulevard, Madison, WI 53704. Laboratory Project Identification No. CHW 6224-227. July 14, 1997. MRID 44802036. Unpublished.

Weiler, M.S. (1997) Benchmark and time-to-peak effect neurotoxicity study with triticonazole in rats. Corning Hazleton Inc., 3301 Kinsman Boulevard, Madison, WI. Laboratory Project Identification No. CHW 6224-226. March 7, 1997. MRID 44933602. Unpublished.

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

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 44802036), groups of 10 male and 10 female Crl:CD®(SD)BR VAF/Plus® rats were administered triticonazole (97.2%; Lot No.9550347) as a single oral gavage dose of 0, 80, 400, or 2000 mg/kg. Doses were based on a range-finding study (MRID 44933602). The administration volume was 10 mL/kg and 0.5% methyl cellulose was the vehicle. Animals were observed for 14 days post-dosing. Body weights were determined on the day of dosing (day 1) and on days 8 and 15. Animals were checked twice daily for mortality and moribundity. Functional observational battery (FOB) and motor activity tests were conducted prior to dosing (week -1), on day 1 (within 2 hours after dosing), and on days 8 and 15. Six animals/sex/dose were fixed by *in situ* perfusion for neuropathological examinations.

There were no treatment-related clinical signs or deaths. FOB and motor activity assessments revealed minor biologically significant treatment-related abnormalities or signs of neurotoxicity. Statistically significant (*= $p \leq 0.05$) FOB findings were seen only in males and included decreased number of fecal boli at the low and high dose on day 1 (2.8, 0.7*, 2.3, and 1.1* for 0,

80, 400, and 2000 mg/kg-dose, respectively); dose-related increased number of rears at the high dose on day 8 (0.8, 1.7, 2.1, and 4.0* for 0, 80, 400, and 2000 mg/kg-dose, respectively); on day 15, although not significant (n.s.), dose-related increase in number of rears (1.2, 2.0, 1.9, and 3.9 for 0, 80, 400, and 2000 mg/kg-dose, respectively); and dose-related decreased body temperature at the high-dose on day 8 (38.7°C, 38.7°C, 38.5°C, and 38.4* for 0, 80, 400, and 2000 mg/kg-dose, respectively). These differences occurred in the limit dose group for males (2000 mg/kg) and were not seen by day 15. Although in the range-finding study, motor activity was increased at the 2 hours postdose in both sexes (males up to 254% and females up to 386% of controls), increased motor activity in this acute study was only observed in high-dose females at days 1 and 8 (121% and 126% of controls, respectively), and there was no significant dose-related differences in overall motor activity and no accompanying clinical signs.

There were no macroscopic or microscopic lesions in neural tissues indicative of a neurotoxic effect at any dose. Slightly smaller brain sizes of 400 and 2000 mg/kg-dose females ($p \leq 0.05$; 98.5% of controls) were observed, but differences were not as apparent with brain size/body weight ratios (0.345, 0.345, 0.349, and 0.343 for 0, 80, 400, and 2000 mg/kg-dose, repectively).

Under the conditions of this study, the NOAEL is 400 mg/kg for males and females. The LOAEL is 2000 mg/kg (limit dose) based on effects seen in males for dose-related increased number of rears at day 8 and in females for increased motor activity at days 1 and 8.

This acute oral neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats.

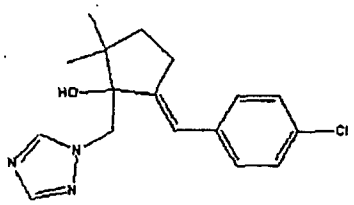
COMPLIANCE: Signed and dated Good Laboratory Practice Compliance, Quality Assurance, and Data Confidentiality statements were provided. No Flagging statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triticonazole

Description: white powder
Lot/Batch #: Lot No.9550347
Purity: 97.2%
CAS No. 131983-72-7
Structure:



2. Vehicle and/or positive control

0.5% Methylcellulose [MC (w/v); 4,000 cps, Supplier: Spectrum Gardena, California, Lot # LM0172] in reverse osmosis water

3. Test animals

Species: rat
Strain: Crl:CD®(SD)BR VAF/Plus®
Age and weight at study initiation: 49 days old, males, 227-272 g and females, 162-204 g
Source: Charles River Laboratories, Inc., Portage, MI
Housing: individually in stainless steel, screen-bottom cages
Diet: Certified Rodent Diet # 5002 (PMI® Feeds, Inc.), *ad libitum*
Water: provided *ad libitum*
Environmental conditions:
Temperature: 19-25°C
Humidity: 50%±20%
Air changes: not given
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates
Start: September 23, 1996 ; end: October 10, 1996

2. Animal assignment

The number of animals assigned to the dose groups is given in Table 1. The animals were randomly assigned to the four dose groups such that body weights were homogeneous 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

Dose group	Dose (mg/kg)*	Concentration (g/100 mL)	Number Assigned	
			Males	Females
1	0	0	10	10
2	80	8	10	10
3	400	40	10	10
4	2000	200	10	10

Data taken from MRID 44802036, p. 15. *The dose volume was 10 mL/kg of body weight. The control group received 0.5% MC only.

3. Validation of test methods

Positive control data were provided for Functional Observational Battery (FOB), motor activity (MA), and neuropathological endpoints. Positive control chemicals included acrylamide and paraoxon. (Acrylamide Study: HWI 2100-030. March 29, 1996 and Paraoxon Study HWI 2100-004. April 11, 1996. No MRID Nos. as of June 1998., Appendix of this DER).

4. Dose selection rationale

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 section of this DER). The limit dose for an acute neurotoxicity study, 2000 mg/kg, was chosen as the highest dose. The low dose was expected to produce no effects, and the mid dose was selected to provide an additional dose for evaluating potential neurotoxicity. Based on motor activity data in the range-finding study dose levels of 80, 400, and 2000 mg/kg were recommended for the acute study.

5. Dose preparation and analysis

For each dose level, the specified amount of test material was weighed and placed into a container with a portion of the vehicle. The preparation was mixed with a magnetic stirrer and stir bar and sufficient vehicle was added to achieve the desired volume. Dose preparations for the low- and mid-dose groups were prepared on the

day before dosing, refrigerated at 5°C±3°, and removed from the refrigerator at least 1 hour before dosing. The high-dose preparation was prepared on the day of administration and kept at room temperature until dosing. For concentration analysis, two samples (approximately 5 mL each) from each dose preparation were taken directly from the container and analyzed on the day of mixing. Because the results of the high-dose samples were outside the acceptable range, another high-dose preparation was mixed and used for dose administration. Homogeneity and stability of dose preparations were determined in an earlier study (MRID 44933602; see Appendix for details).

Results -
Concentration analysis: Analytical measurements indicated that the mean concentrations of the dose preparations were 94%-98.1% of the theoretical concentration. The theoretical concentrations of 8, 40, or 200 mg/ml corresponded to actual mean concentrations of 0, 7.85, 37.6, or 188 mg/mL, respectively.

Homogeneity analysis: Mean values ranged from 107% to 109% and 109% to 111% of the theoretical concentrations for 5 and 200 mg/ml preparations, respectively.

Stability analysis: After 1 week of refrigeration and 1 day at room temperature, the mean concentration for the 5 mg/mL dose preparation was 109% of the theoretical concentration. After 5 days of refrigeration and 1 day of room temperature, the mean concentration for the 200 mg/mL concentration was 102% of the theoretical concentration.

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

6. Estimated time-to-peak effect

The estimated time of peak effect was evaluated in MRID 44933602 (see Appendix for details). Based on increased motor activity in rats administered 2000 mg/kg of the test material, the estimated time-to-peak effect occurred between 2 and 4 hours after dosing.

7. Statistical analysis

Levene's test was used to test for variance homogeneity. In the case of heterogeneity of variance at ≤ 0.05, transformations were used to stabilize variance. One-way ANOVA was used to analyze body weights, body weight gains, FOB continuous data, MA counts, and brain measurements. ANOVA was used on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's 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

Animals were examined twice daily for mortality and moribundity. In addition, each animal was removed from its cage and examined on days 1, 8, and 15.

2. Body weight

Body weights were recorded on days 1, 8, and 15.

3. Food consumption and food efficiency

Food consumption and food efficiency were not determined.

4. Functional observational battery

Animals were tested one week prior to dosing (week -1), approximately 2 hours after dosing on day 1, and on days 8 and 15 approximately at the same time of day as on day 1. The FOB tests were performed by two technicians blind to animal treatment.

a. Home cage observations

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

b. Open field observations

Ease of removal from cage, ease of handling, vocalization, palpebral closure, exophthalmus, excessive lacrimation and salivation, respiration, fur appearance, piloerection, and writhing. In open field for 2 minutes: latency to first step, position, gait abnormalities (type/severity), activity, stereotypy, body tone, tremor, convulsions, and other unusual behavior.

c. Reflexes/Physiological Parameters

Approach response, touch response, catalepsy, number of grooms, number of urine pools, number of fecal boli, number of rears, pupillary status and response, corneal response to touch, startle response, air drop righting reflex, analgesic reflex, grip strength, foot splay, body temperature, and body weight.

5. Motor activity

After completion of the FOB, each animal was tested in a stainless steel, covered 18-inch diameter circular enclosure. Before starting the session, each animal was allowed to acclimate in the enclosure for 1 minute. Motor activity was measured as

the number of photobeam breaks (counts) over the 40-minute session and reported every 2 minutes.

6. Sacrifice/necropsy/neurohistopathology

At study termination, all rats were anesthetized with sodium pentobarbital (after an overnight fast), terminal body weights were determined, and tissues were perfused *in situ* with and preserved in 10% phosphate-buffered formalin. All animals were examined grossly (external surfaces; all orifices; cranial cavity; exposed external surfaces of brain and spinal cord; and thoracic, abdominal, and pelvic cavities and viscera).

Tissues from six control and six high-dose animals/sex were processed for neurohistopathological examination. The maximum length, width, and height (in mm) of each cerebrum and cerebellum were measured. The (XX) tissues from six control and six high-dose animals/sex were embedded in paraffin and examined using hematoxylin and eosin. Tissues marked (*) were embedded in epoxy resin and stained with toluidine blue.

X	BRAIN	X	SPINAL CORD	X	PERIPHERAL NERVES
XX	Olfactory bulb	XX	Cervical	XX*	Sciatic nerve, left
XX	Forebrain	XX*	Thoracic	XX*	Tibial nerve, left
XX	Caudate nucleus	XX	Lumbar	XX*	Sural nerve, left
XX	Hypothalamus/thalamus	XX*	Cervical dorsal root ganglia	XX*	Trigeminal ganglia
XX	Midbrain			XX*	Optic nerve, left
XX	Cerebellum	XX*	Lumbar dorsal root ganglia		
XX	Medulla				**OTHER**
				XX	Eye, left
				XX	Gastrocnemius muscle, left
				XX	Anterior tibialis muscle, left
				XX	Pituitary gland
				XX	Macroscopic lesions

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

No animals died during the study. No abnormal signs were noted during the general clinical observations.

B. BODY WEIGHT AND BODY WEIGHT GAIN

No effects on body weight were noted (see Table 2 below).

Table 2: Terminal Body Weights (gram)			
Sex	Dose Group	Number in Group	Mean and Stand. Dev
Male	1	10	305.5 ± 18.1
	2	10	315.9 ± 14.2
	3	10	296.0 ± 13.4
	4	10	314.1 ± 21.2
Female	1	10	211.6 ± 12.3
	2	10	208.7 ± 15.1
	3	10	205.9 ± 12.5
	4	10	209.9 ± 14.3

Data from Appendix C of MRID 44933602, pages 481 - 486.

C FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Home cage observations, open field observations, and sensorimotor/reflex tests did not show toxicologically relevant findings that were considered treatment-related.

Selected FOB results are shown in Table 3. Altered FOB parameters that were not statistically evaluated included resistance to being removed from the cage, activity in the open field, touch response (compared to predose evaluations, all groups including controls showed an unresponsiveness to touch), and air drop righting reflex (negative reflex in 1/10 control males on days 1, 8, and 15). Most of these alterations were seen at all test intervals and in control and treated animals. Statistically significant ($p \leq 0.05$) findings included decreased number of fecal boli for low- and high-dose males on day 1, and dose-related increased number of rears and dose-related decreased body temperature for high-dose males on day 8.

TABLE 3: Selected functional observational battery results

Observations	0 mg/kg		80 mg/kg		400 mg/kg		2000 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
No. of animals	10	10	10	10	10	10	10	10
Resistance for removal from cage, slight								
predose	6	6	5	6	8	7	4	7
day 1	9	10	10	10	10	10	9	10
day 8	9	10	10	10	10	10	9	10
day 15	9	10	10	10	10	10	9	10
Open field activity								
predose- moderate	9	10	10	10	10	10	10	9
low	1	0	0	0	0	0	0	0
day 1-moderate	8	9	8	10	9	10	9	9
low	2	1	2	0	1	0	1	1
day 8-moderate	8	8	8	10	9	9	9	5
low	2	1	2	0	1	0	1	2
high	0	1	0	0	0	1	0	3
day 15-moderate	7	9	8	10	7	9	8	6
-low	3	0	2	0	3	0	2	1
-high	0	1	0	0	0	1	0	3
Touch response (turns to stimulus)								
predose	10	7	10	7	10	7	10	10
day 1	2	3	2	5	1	3	4	3
day 8	0	2	2	2	0	3	1	3
day 15	0	3	2	1	0	3	0	1
Fecal boli								
predose	0.3	0.6	0.8	0.0	0.7	0.7	1.0	0.0
day 1	2.8	0.5	0.7*	0.0	2.3	0.4	1.1*	0.0
day 8	1.9	0.2	0.3	0.0	1.4	0.0	1.1	0.0
day 15	0.6	0.0	0.0	0.0	0.8	0.0	0.4	0.0
Number of rears								
predose	5.4	9.6	6.7	8.1	7.1	10.3	8.0	10.4
day 1	4.0	9.9	3.2	8.1	4.5	11.0	6.4	11.1
day 8	0.8	8.2	1.7	5.4	2.1	6.8	4.0*	12.7
day 15	1.2	10.8	2.0	9.3	1.9	10.3	3.9	11.4
Body temperature (°C)								
predose	38.4	38.9	38.3	38.7	38.3	38.7	38.4	38.9
day 1	38.9	39.0	38.9	39.0	38.8	39.1	38.9	39.2
day 8	38.7	38.9	38.7	38.9	38.5	38.9	38.4*	39.1
day 15	38.6	39.0	38.5	38.9	38.4	39.0	38.5	39.3

Data taken from Table 5, pp. 52-57; Table 6, pp. 58-63; Table 7, pp. 64-71; Table 8, pp. 72-79; Table 10, p. 81; Table 12, p. 83; and Table 16, p. 91, MRID 44802036.

*Significantly different from controls, $p \leq 0.05$.

D. MOTOR ACTIVITY

Overall motor activity data are summarized in Table 4. There were no statistically significant changes in total motor activity counts or motor activity by time interval during any of the test sessions. Compared to controls, increased motor activity was observed in high-dose males predosing (120%) and in high-dose females on the day of dosing (day 1) and day 8 (121% and 126%, respectively). These increases are likely due to increased activity during single 10-minute intervals. In the high-dose group, increased values were noted at interval 4 in males (239% of controls), and in females at interval 3 on day 1 (147% of controls) and at interval 4 on day 8 (194% of controls).

TABLE 4. Overall (0-40-minute) motor activity (counts ±SD) for male and female rats administered Triticonazole by gavage

Dose (mg/kg)	Males				Females			
	Predose	Day 1	Day 8	Day 15	Predose	Day 1	Day 8	Day 15
0	1152±311.5	1036±268.0	1370±321.3	1528±357.1	1283±335.0	1338±458.7	1151±390.1	1499±570.1
80	1349±393.3 (117)[a]	1055±305.3 (102)	1327±393.9 (97)	1487±413.5 (97)	1392±431.9 (108)	1191±352.3 (89)	1335±283.9 (116)	1385±341.4 (93)
400	1277±575.1 (111)	1162±529.5 (112)	1298±592.4 (95)	1747±452.1 (114)	1351±371.6 (105)	1291±384.2 (96)	1263±323.6 (110)	1447±343.3 (97)
2000	1386±335.5 (120)	1028±369.4 (99)	1309±316.3 (96)	1546±609.9 (101)	1365±439.8 (106)	1628±348.3 (121)	1450±368.3 (126)	1588±426.9 (106)

Data taken from pp. 92-95, MRID 44802036.
[a]Percent of control, calculated by reviewer.

E. SACRIFICE/NECROPSY/NEUROHISTOPATHOLOGY

No treatment-related gross lesions were observed within neural tissues. Microscopic findings included a low incidence of minimal axonal degeneration in sciatic nerves (one control and one high-dose animal/sex) and cervical spinal cord (one control and one high-dose male). Also noted were the following lesions: presence of a neurofilament bundle in the sciatic nerve of one control female; neuronal vacuolation in the lumbar dorsal root ganglion of one control male; axonal degeneration in the trigeminal ganglion of one control female; and axonal dilatation in the cervical dorsal root ganglion of one high-dose male. All microscopic findings were considered incidental and not related to test material administration.

Brain sizes of mid- and high-dose females were slightly ($p \leq 0.05$; 1.5%) smaller than those of the respective controls (Table 5). In the absence of notable macroscopic and microscopic lesions, the decreased brain size was attributed to slightly higher terminal body weights of control females (205.9±12.5 g and 209.9±14.3 g for mid- and high-dose females, respectively, vs. 211.6±12.3 for controls).

TABLE 5. Brain size (mm) of male and female rats administered Triticonazole [brain/body wt ratio]

Dose (mg/kg)	Males	Females
0	72.7950±0.9742 [b/bw: 0.238]	72.9900±1.0680 [b/bw: 0.345]
80	72.6900±1.2397 (99.9)[a] [b/bw: 0.230]	72.0700±0.8573 (98.7) [b/bw: 0.345]
400	72.3445±1.3093 (99.3) [b/bw: 0.244]	71.9450±1.1787* (98.5) [b/bw: 0.349]
2000	73.2200±1.1126 (100.6) [b/bw: 0.233]	71.9150±0.5332* (98.5) [b/bw: 0.343]

Data taken from MRID 44802036, pp. 96-97. [a]Percent of control and b/bw ratio calculated by reviewer.
*Significantly different from control, p≤0.05.

III. DISCUSSION

A. DISCUSSION

No animals died during the study. There were no clinical signs of toxicity or effects on body weight following administration of triticonazole at single gavage doses of up to 2000 mg/kg.

Scattered findings in the FOB are of questionable biological significance. Statistically significant (p≤0.05) alterations in the FOB were seen only in males. These included decreased fecal boli at the low- and high-dose on day 1, increased number of rears and decreased body temperature at the high-dose on day 8. Increased defecation is not considered treatment-related because of the high value for male controls and similarity to the predose value. Although the effects on body temperatures were dose-related, they are not considered treatment-related because they occurred only at day 8, were of low magnitude, were similar to predose values, or showed variability among controls. However, statistically significant dose-related increased rearing on day 8 was associated with non-statistically dose-related increased rearing on day 15 in males. Compared to the predose evaluation, unresponsiveness to touch was observed in all groups (including controls) on days 1, 8, and 15. Increased motor activity in high-dose females at days 1 and 8 (121% and 126% of controls, respectively) was accompanied by slightly increased activity in the open field; however, there was no dose-response and clinical signs or other FOB parameters indicating increased activity/alertness levels in females were not present. The range-finding study showed markedly increased motor activity for both sexes in the treated groups.

No treatment-related gross or microscopic lesions were evident within neural tissues in any of the treatment groups. The incidence of microscopic lesions was low and occurred in control and treated animals. Slightly (p≤0.05; 1.5%) smaller brain size of mid- and high-dose females was observed when compared to controls, however, when brain size

was compared to terminal body weights differences were not as apparent and no longer exhibited a linear dose-related effect.

Selection of dose levels based on the previously conducted range-finding study (MRID 44933602) appeared appropriate.

Under the conditions of this study, the NOAEL is 400 mg/kg for males and females. The LOAEL is 2000 mg/kg (limit dose) based on effects seen in males for dose-related increased number of rears at day 8 and in females for increased motor activity at days 1 and 8.

B. 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. Except for minor errors in reporting, no other deficiencies were identified.

C. CLASSIFICATION

This acute oral neurotoxicity study is classified as **Acceptable/ Guideline** and satisfies the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats.

APPENDIX

BENCHMARK AND TIME-TO-PEAK EFFECT STUDY

CITATION: Weiler, M.S. (1997) Benchmark and time-to-peak effect neurotoxicity study with triticonazole in rats. Corning Hazleton Inc., 3301 Kinsman Boulevard, Madison, WI. Laboratory Project Identification No. CHW 6224-226. March 7, 1997. MRID 44933602. Unpublished.

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

EXECUTIVE SUMMARY: In a range-finding study (MRID 44933602) five groups of Crl:CD® (SD)BR rats were administered triticonazole (97.2%; Lot No. 9550347) as a single oral gavage dose of 0, 50, 1000, or 2000 mg/kg body weight, in a vehicle of 0.5% methylcellulose. Groups 1, 2, 3, and 5 (4 ani/sex/gp) were treated on day 1 to establish a dose range for subsequent studies; Group 4 (8 males) was dosed on the following day to determine a time-to-peak effect. There were no significant dose-related differences in FOB measures. Overall motor activity data showed dose-related increases in percent of control activity for doses 50, 1000, and 2000 mg/kg of Groups 2, 3, and 5 (males:90%, 171%, and 254%; females: 247%, 267%, and 386%, respectively). The time-to-peak effect for motor activity as measured in Group 4 (all males, dose 2000 mg/kg) was graphically estimated as 3 hours after dosing using postdose timepoints 1 hour (224% of control) and 4 hours (209% of control). The NOEAL was not established in this study, the LOAEL is 50 mg/kg or lower. Based on motor activity data dose levels of 80, 400, and 2000 mg/kg were recommended for the acute study.

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

MATERIALS: Five groups of Crl:CD®(SD)BR VAF/Plus® rats (Supplier: Charles River Laboratories, Inc.), 5/sex/group(except Group 4, 8 males), were administered triticonazole (97.2%; Lot No. 9550347) as indicated in Table 1 for study design. Vehicle was 0.5% methylcellulose (Lot No. LM01702). Males weighed from 242 to 276 g and females from 147 to 178 g at the initiation of treatment. The animals were sacrificed on day 4 of the study (in life dates: start, September 4, 1996; end: September 8, 1996).

TABLE 1: Study design

Test group	Dose (mg/kg)[a]	Concentration (g/100 mL)	Number Assigned	
			Males	Females
1	0	0	4	4
2	50	5	4	4
3	1000	100	4	4
4[b]	2000	200	8	0
5	2000	200	4	4

Data taken from MRID 44933602, p. 14. [a]The dose volume was 10mL/kg of body weight. The control group received 0.5% MC only. [b]Group 4 was used to establish a time-to-peak effect.

Dose Preparation and Analysis: Each dose level was mixed separately. A specified amount of test material was weighed, mixed with the vehicle (0.05% methylcellulose) and stirred using a magnetic stir plate and stir bar. The dose preparations for Groups 1, 2, 3, and 5 were mixed within 48 hours of dosing, stored under refrigeration at 5°C±3°, and removed from the refrigerator at least 1 hour before dosing. The Group 4 dose preparation was mixed on the day of administration and held at room temperature until dosing.

For homogeneity analysis, one sample each (5 mL) was taken from the top, middle, and bottom of the low- and high-dose (Group 5) preparations and submitted for analysis. Stability of the dose preparations was evaluated in one set of samples collected from the low- and high-dose preparations mixed for day 1, stored for approximately 1 week in the refrigerator at 5°C±3° and at room temperature for approximately 1 day. Because the concentration of the high-dose preparation (in the stability test) was unacceptably high (140% of theoretical), a new high-dose preparation was mixed and kept for 5 days under refrigeration and 1 day at room temperature. Two sets of duplicate samples were collected and analyzed for test material content. For concentration analysis of preparations that were used in the study, samples were taken before the first day of dosing (Groups 1, 2, 3, and 5) and on the day of dosing (Group 4). The samples were kept at room temperature and analyzed on the same day.

Homogeneity, stability, and concentration were acceptable for the purpose of this study. For homogeneity, mean values ranged from 107% to 109% and 109% to 111% of the theoretical concentration of the 5 and 200 mg/ml preparations, respectively. Stability for the 5 mg/ml preparation after one week of refrigeration and 1 day of room temperature was 109% of the theoretical concentration, and stability of the 200 mg/ml preparation after 5 days under refrigeration and 1 day at room temperature was 102% of the theoretical concentration. The mean concentration of the dose preparations used in the study ranged from 102% to 111% of the theoretical concentration.

METHODS: Food [Certified Rodent Diet # 5002 (PMI® Feeds, Inc.)] and water were provided *ad libitum*. The animals were observed twice daily for mortality and moribundity. Body weights were determined on the day of dosing. Abbreviated FOB and motor activity assessments were performed for all animals before dosing (FOB only) and approximately 2 hours postdosing for all animals in Groups 1, 2, 3, and 5 and approximately 1 and 4 hours postdosing for males in Group 4 (4 males/interval). Testing was performed by two trained technicians (blind to animal treatment). In the FOB, each animal was evaluated upon removal from the cage and placement into a circular arena for 2 minutes. Observations included ease of removal, ease of handling, vocalization, palpebral closure, exophthalmus, excessive lacrimation and salivation, respiration, fur appearance, piloerection, and writhing. In the open field (for 2 minutes), observations included latency (time in seconds to first step), position, gait abnormalities, activity, stereotypy, body tone, tremor, convulsions, and other unusual behavior.

Following completion of the FOB, motor activity was assessed in a stainless steel, covered 18-inch diameter circular enclosure. Before starting the session, each animal was allowed to

acclimate in the enclosure for 1 minute. Motor activity was measured as the number of photobeam breaks (counts) over the 60-minute session and reported every 2 minutes.

RESULTS:

All animals survived treatment with the test material and no abnormal signs were noted during general clinical observations.

FOB results showed slightly excessive salivation in one high-dose female 2 hours postdosing and (observed only by one technician) in one control female. Except for three animals (one low-dose animal/sex and one mid-dose female), latency to first step was zero. In open field assessments, low activity was observed in one control animal/sex, one low-dose male, and in one high-dose male (Group 5) 2 hours after dosing; in two high-dose males (Group 4) 1 hour after dosing; and in two high-dose males (Group 4) 4 hours after dosing. The mean number of rears in low-, mid, and high-dose (50, 1000, and 2000 mg/kg) females were higher compared to the respective controls (11.8±6.13, 7.0± 2.45, and 7.8±2.63 compared to 3.8±2.36), no dose-response was noted and the number of rears in the predose assessment showed a similar pattern. It was concluded that FOB data do not lend themselves to determine a time-to-peak effect.

Overall motor activity data are shown in Table 2. Dose-related increased motor activity was seen in both sexes 2 hours after dosing, with high-dose (2000 mg/kg) animals displaying the most pronounced increases.

TABLE 2. Overall (0-60-minute) motor activity (counts ±SD) for male and female rats administered Triticonazole by gavage

Dose group	Males	Females
Group 1 (0 mg/kg) 2 hours postdose	1096±366.6 (100)[a]	705±583.5 (100)
Group 2 (50 mg/kg) 2 hours postdose	990±692.9 (90)	1739±872.5 (247)
Group 3 (1000 mg/kg) 2 hours postdose	1872±118.0 (171)	1884±387.6 (267)
Group 4 (2000 mg/kg) 1 hour postdose 4 hours postdose	2455±566.6 (224) 2293±817.7 (209)	no data
Group 5 (2000 mg/kg) 2 hours postdose	2781±713.2 (254)	2718±614.5 (386)

Data taken from Tables 12 and 13, pp. 48-49, MRID 44933602. [a]Percent of control.

The mean motor activity increases in high-dose (2000 mg/kg), Group 5, was 254% of controls for males and 386% of controls for females, seen 2 hours after dosing. Graphic presentation of motor activity data by 2-minute interval (data not shown) indicate that the time-to-peak effect is approximately 3 hours after dosing.

CONCLUSION: Based on motor activity data in Crl:CD®(SD)BR rats, dose levels of 80, 400, and 2000 mg/kg triticonazole and a time-to-peak effect for motor activity testing of approximately 3 hours are recommended for the acute study.

TAKEN FROM TXR 013439, dated 6/18/99

Appendix: Neurotoxicity Positive Control Data Base for the Covance (Madison, Wisconsin, formerly Hazleton) Laboratory as of July, 1998.

Test Chemical	Study No:, Date	Strain of rat and supplier, birth date and age at initial dosing.	Key Personnel	Dose Levels, vehicle and route	Results
Acrylamide	Study No.: HWI 2100-030, March 29, 1996 No MRID No. as of June 1998.	Crl:CD(SD)BR VAF/Plus strain, Charles River Portage Michigan, 5 weeks old at study initiation.	Molly Weiler, Janet Nokleby, Jacqueline Miller, Robert Young, Dixie Bushby, Cindy Cary, James Meehan, Deborah Pirkel, Jack Serfort, Richard Becker	0, 50 mg/kg administered intraperitoneally on days 1-9 for 6/sex and on days 1-21 for 6/sex. On every other day.	Clinical signs: Body weight decreased up to 15-19% at say 21 in males and 11% in females. Males only 5% decreased at day 8. FOB: No remarkable effects at day 8 (possible one female with gait abnormality). At day 21, numerous signs including flaccid muscle tone, gait abnormalities, females more affected than males, nearly paralytic conditions when handled. Motor activity: Not remarkable at day 8 but decreased at day 21. Pathology: Not considered remarkable on day 10 of sacrifice. On day 21, necrosis of the cortical purkinje cell (2/6 males and 5/6 females), axonal degeneration in dorsal, lateral and ventral funiculus (1-3/6) roots of thoracic, lumbar, sciatic nerve (females, 4/6), tibial nerve (all female and 4/6 males), sural nerve (all females and 3/6 males). **Conclusion (HED): Study is considered a good demonstration of the detection of acrylamide neurotoxicity and differentiated between effects at day 8 and at day 21 and multiple doses.**
Trimethyl tin	Not tested.				
Carbaryl	Not tested.				
Triadimefon	Not tested.				
Amphetamine	Not tested.				
Chlorpromazine	Not tested.				
DDT	Not tested.				

Test Chemical	Study No:, Date	Strain of rat and supplier, birth date and age at initial dosing.	Key Personnel	Dose Levels, vehicle and route	Results
Paraoxon. [Comparison of two technicians.]	Study No.: HWI 2100-004, Date April 11, 1996 No MRID No. as of June 1998.	Crl: CD(SD) BR VAF/Plus strain, Charles River Portage, Michigan. 6-7 weeks old at study initiation. Males only.	Molly Weiler, Anne Elliot, Jacqueline Miller, David Schuette, Dixie Bushee, Cindy Cary, Ron Markevitch, James Nold, Deborah Pirkel, Jack Serfort	0, 0.17 and 0.34 mg/kg in coin oil at 1 mL/kg subcutaneously, 20/sex.	Clinical signs: No effects on body weight. No signs reported. FOB: Numerous parameters affected: lip smacking, pinpoint pupils, salivation, tremors, unsteady gait, labored respiration, and hypothermia most at both doses. Motor activity: Decreased at 0.34 mg/kg. Pathology: Not assessed. Brain regional AChE: High standard deviations, but NOEAL < 0.17 mg/kg. **Conclusion (HED): Considered a good demonstration of effects of paraoxon and good correlation between technicians.**