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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

18 July 2003
HED TXR No. 0052027

MEMORANDUM

SUBJECT: Tebuconazole
Acute, Subchronic and Developmental Neurotoxicity Study Reviews
EPA Pesticide Chemical Code 128997
EPA DP Barcodes: 257119, 264727

TO: Myrta Christian/Mary Waller, PM 21
Fungicide Branch
Registration Division (7505C)

FROM: Stephen C. Dapson, Ph.D.
Branch Senior Scientist
Registration Action Branch 3
Health Effects Division (7509C)

Stephen C. Dapson 07/18/2003

cc: Kathleen C. Raffaele, Ph.D./RAB3/HED (7509C)

ACTION REQUESTED: Review Submitted Acute, Subchronic and Developmental Neurotoxicity Studies with Tebuconazole.

CONCLUSIONS: The following are the Executive Summaries from the Acute, Subchronic and Developmental Neurotoxicity Studies with Tebuconazole.

Acute Neurotoxicity Study OPPTS 870.6200; OPP §81-8 (MRID# 44449301):

In an acute oral neurotoxicity study (MRID 44449301), technical grade tebuconazole (Batch # 603-0013, 96.5% purity) was administered by gavage to 12 male Fischer 344 rats at doses of 0, 100, 500, or 1000 mg/kg and to 12 female Fischer 344 rats at doses of 0, 100, 250, or 500 mg/kg. Because a NOAEL did not appear to be attained in this study, a second (supplemental) acute oral neurotoxicity study (MRID 44545701) using the same batch and doses of 0, 20, or 50 mg/kg to 12 Fischer 344 rats/sex/group was performed. Based on analytical results, actual doses were 0, 0, 21, 50, 103, 497, and 950 mg/kg for male rats and 0, 0, 21, 50, 103, 239, and 497 mg/kg for female rats. In the main study, functional observational battery (FOB) and motor activity tests were performed pretreatment, on the day of test material administration (day 0), and on days 7 and 14 posttreatment; in the supplemental study, an abbreviated FOB and motor activity tests were performed on day 0 only. At the completion of the main study (day 14), 6 rats/sex in the control and high-dose groups were subjected to perfusion, and brain and nervous tissues were examined microscopically. No histopathological evaluations were performed in the supplemental study.

The high dose of 1000 mg/kg resulted in mortality of 6/12 male rats within two days of treatment. One male rat in the 500 mg/kg dose group also died. There was no treatment-related mortality in females of any dose group. Clinical signs of incoordination, decreased activity, and nasal and perianal stains were observed on day 0, primarily in the two highest dose groups (males, 500 and 1000 mg/kg; females, 250 and 500 mg/kg). Effects on body weight were minimal; weights were 96-101% of control weights on day 14. There was no effect of treatment on brain weight and there were no histopathological findings in the brain or tissues of the nervous system that could be attributed to treatment.

FOB tests on the day of treatment showed treatment-related effects on gait (incoordination, ataxia); activity, arousal, and response to stimuli (all decreased); hindlimb grip strength (decreased); footsplay (decreased in females only); and body temperature (decreased) in one or both sexes in the two highest dose groups (males, 500 and 1000 mg/kg and females, 250 and 500 mg/kg). Most of these parameters were statistically significantly different from control values ($p<0.05$). Increased arousal in the open field and decreased footsplay were also noted in females at 100 mg/kg. All effects except decreased footsplay in 250 and 500 mg/kg females were resolved by post-test day 7. No changes in FOB parameters were observed in males in the 100 mg/kg dose group. No treatment-related FOB effects were observed in either sex in the 20 and 50 mg/kg dose groups.

Relative to concurrent controls, motor and locomotor activity were increased on day 0 in males and females in the 100 mg/kg dose group and decreased in the higher dose groups (males, 500 and 1000 mg/kg and females, 500 mg/kg; all $p<0.05$). There was no statistically significant change in 250 mg/kg females. No adverse effect of treatment on motor activity was observed in either sex in the 20 and 50 mg/kg dose groups, although there was a slight (non-statistically significant) increase in 50 mg/kg males that was considered compound-related. No effect of treatment was observed on motor or locomotor activity on days 7 and 14.

The LOAEL is 100 mg/kg based on increased motor activity in male and female rats and decreased footsplay in female rats. The NOAEL for both male and female rats is 50 mg/kg.

This study is considered **Unacceptable/Guideline** as an acute oral neurotoxicity study and does not fulfill FIFRA guideline requirements for an acute oral neurotoxicity study in rats [OPPTS 870.6200 (§81-8)]. This study can be upgraded by submission of (1) a report from the range-finding study, so that time of peak effect can be verified; (2) additional information regarding the test substance preparation and analysis, as described below.

Subchronic Neurotoxicity Study OPPTS 870.6200; OPP §82-1b (MRID# 4458801)

In a 90-day dietary neurotoxicity study (MRID 44588001), tebuconazole (Batch No.: 603-0013, 96.7%-98.2% purity) was administered to 12 Fischer 344 rats/sex/dose at dietary levels of 0, 100, 400, or 1600 ppm. Based on analytical measurements, doses were 0.00, 7.57, 29.2, and 107 mg/kg/day for males and 0.00, 8.81, 34.0, and 122 mg/kg/day for females. Functional observational battery (FOB) and motor activity tests were performed pretreatment and during weeks 4, 8, and 13. At the completion of the study, 6 rats/sex/dose group were subjected to perfusion; brain and nervous tissues were examined microscopically in high dose and control

groups only.

No deaths occurred and there were no clinical signs attributable to treatment. Relative to controls, body weights of male and female rats in the group receiving 1600 ppm in the diet were statistically significantly reduced after one week, by 7% in males and 5% in females (both $p<0.05$). Lower body weights continued throughout the remainder of the study with reductions of 8% for males and 7% for females at study termination (both $p<0.05$). Food consumption was reduced throughout the study in this group, with average daily food consumption for males and females of 94% and 92% relative to respective controls. Food efficiency was reduced by 11% and 13% in high-dose males and high-dose females, respectively. Relative to controls, there were no treatment-related effects on body weight or food consumption or efficiency in the other dietary groups.

No treatment-related effects were observed on FOB tests or motor activity tests. There were no gross or histopathological findings that could be attributed to treatment with tebuconazole.

A LOAEL was not attained in this study. The NOAEL is 1600 ppm in the diet (107 mg/kg/day in male rats and 122 mg/kg/day in female rats) based on treatment-related but toxicologically insignificant reductions in body weight, food intake, and food efficiency; the absence of effects in the FOB and motor activity tests; and the absence of histopathological lesions in the brain and nervous system.

This study is considered to be **Unacceptable/Guideline** and does not fulfill FIFRA guideline requirements for a subchronic neurotoxicity (90-day) study in rats [870.6200(§82-1b)] because a LOAEL was not identified, and the limit dose was not tested.

Developmental Neurotoxicity Study OPPTS 870.6300; OPP §83-6[a] (MRID# 45074301)

In a developmental neurotoxicity study (MRID 45074301), tebuconazole (96-96.9% a.i.; Lot/Batch #603-0013) in corn oil was administered via the diet to pregnant Crl:CD®BR VAF/Plus® (Sprague Dawley) rats (25/dose) from gestation day (GD) 6 to lactation day (LD) 11 at doses of 0, 100, 300 or 1000 ppm (equivalent to [GD/LD] 0/0, 8.8/16.3, 22.0/41.3, and 65.0/125.4 mg/kg/day). No analytical data were provided. P dams were allowed to deliver naturally. On day 5 postpartum, litters were standardized to a maximum of 10 pups/litter. Pups were assigned to one of 5 Subsets (20 pups/sex/dose in each subset). Physical development landmarks were evaluated for all subsets (including surface righting, eye opening, pinna unfolding, acoustic startle response, and pupil constriction); sexual maturation was evaluated in subsets 2-4. Subset 1 pups were sacrificed on postnatal day 12; brains were weighed for all Subset 1 pups, and histopathological evaluations were performed on 6/sex in control and high dose groups (morphometric analysis was performed on 6/sex in control, mid-dose, and high dose groups). Subset 2 pups were evaluated for learning and memory on day 23-25 (passive avoidance) and on day 59-63 (Water M-maze). Subset 3 pups were evaluated for motor activity (days 14, 18, 22, and 62) and for auditory startle habituation (days 23 and 63). Subset 4 pups received detailed weekly clinical evaluations. In addition, 6 animals/sex/group in Subset 4 were selected for neuropathological evaluations; brains were weighed and the high dose and control animals were evaluated histopathologically on day 83 (morphometric analysis was performed on

6/sex in control, mid dose, and high dose groups). Subset 5 pups were sacrificed and necropsied on day 22.

At 1000 ppm, two P females died as a result of prolonged gestation. Body weights were slightly decreased ($p \leq 0.01$) in the P females during gestation (↓4-8%) and early lactation (↓6-12%). Body weight gains were decreased ($p \leq 0.01$ or 0.05) during GDs 6-9 (↓400%) and 6-21 (↓22%), and during LDs 1-12 (↓55-164%). When compared to concurrent controls, absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$ or 0.01) in the dams throughout gestation (↓9-23%) except during the GD 0-6 interval, and during the LD intervals 4-7 (↓20%) and 7-12 (↓18%). Relative (g/kg/day) food consumption was reduced ($p \leq 0.05$ or 0.01) starting on GD 6 (6-20%) and during early lactation (up to day 12, (↓8-12%). There was also an increase in alopecia in high dose dams. The number of live fetuses/dam was decreased relative to concurrent controls (↓6%, $p \leq 0.01$); while the number of dead fetuses/dam was increased relative to concurrent controls (↑200%, $p \leq 0.01$).

No treatment-related findings were observed in dams at 300 or 100 ppm.

The LOAEL for maternal toxicity is 1000 ppm (equivalent to [GD/LD] 65.0/125.4 mg/kg/day) based on decreased body weights, body weight gains, and food consumption, prolonged gestation with mortality, and an increased number of dead fetuses. The NOAEL is 300 ppm (equivalent to [GD/LD] 22.0/41.3 mg/kg/day).

At 1000 ppm, the stillborn index was increased (↑200%, $p \leq 0.01$) and the number of pup deaths (calculated by reviewers) was increased during days 1-5 (↑229%). Body weights were decreased ($p \leq 0.01$) in the males from PND 5 to 86 (↓7-23%) and in the females from PND 5 to 72 (↓5-24%). Pinna unfolding was delayed (↑19%, $p \leq 0.01$) relative to concurrent controls. There were decreases in several morphometric measurements of the brain, including decreased ($p \leq 0.01$) thickness of the cerebellum in the males and females on day 12 (↓10-14%) and on day 83 (↓7-9%), and an increased thickness of the germinal layer of the cerebellar cortex in the Day 12 males (↑23%, $p \leq 0.01$). Absolute brain weights were decreased in the Day 12 and Day 83 animals (↓10-16%, $p \leq 0.01$ or 0.05). Relative (to body) brain weights were increased ($p \leq 0.01$ or 0.05) in the day 12 males and females (↑10-15%). There were also statistically significant changes in motor activity on days 14 (43% decrease in males [$p<0.01$], 24% decrease in females [n.s.]) and 22 (39% increase in males [$p<.05$], 19% increase in females [n.s.]), and changes in auditory startle amplitude at both time points (decreased in both sexes on day 23 [14-33%], decreased in females [20%] and increased in males [71%] on day 63).

At 300 ppm, there were also decreases in body weight (3-7%) and body weight gain (4-16%, PND5-23 and 72-86 in males, PND5-51 in females). Pinna unfolding was delayed (↑16%). There were changes in auditory startle amplitude in both sexes: a dose-related decrease in females on day 23 (decreased 26%), and a dose-related increase in males on day 63 (increased 18%). In addition, there was a decrease in absolute brain weight in both sexes (3-4%) on day 12 (statistically significant for females only), and in brain measurements (anterior/posterior cerebrum).

At 100 ppm, there were decreases in body weight (3-7%) and body weight gain (5-13%) (PND 5-37 in males, PND 5-51 in females). There were decreases in motor activity (on days 14 and 18 in

males [28-35%]) and changes in auditory startle amplitude (decreased 9% in day 14 females, increased 16% in day 63 males, n.s.). There was also a decrease in absolute brain weight in both sexes on day 12 (4%, statistically significant for both sexes), and in brain measurements (anterior/posterior cerebrum).

The LOAEL for offspring toxicity is 100 ppm based on decreases in body weights, decreases in absolute brain weights and measurements, and decreases in motor activity.

The NOAEL is not determined.

This study is classified as **acceptable/guideline (§83-6[a])** and satisfies the requirement for a developmental neurotoxicity study in rats, pending submission of additional information regarding analytical data and positive control studies, as described in the study review.

B

DATA EVALUATION REPORT

TEBUCONAZOLE

STUDY TYPE: ACUTE NEUROTOXICITY - RAT [OPPTS 870.6200 (§81-8)]
MRID 44449301 and MRID 44545701

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
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Task Order No. 00-26A

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Robert H. Ross, Group Leader	Signature: Date:	Robert H. Ross MAR 17 2000
Quality Assurance: LeeAnn Wilson, M.A.	Signature: Date:	L. A. Wilson MAR 17 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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Kathleen C. Raffaele Date 12/21/00
Ghazi A. Dannan Date 2/21/01
Stephen C. Dapue Date

DATA EVALUATION RECORD

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

DP BARCODE: D257119
P.C. CODE: 128997

SUBMISSION CODE: S529953
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Tebuconazole, technical grade (96.5, 97.3% a.i.)

SYNONYMS: Folicur®; α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol

CITATIONS: Sheets, L.P., Gilmore, R.G. (1997) An acute oral neurotoxicity screening study with technical grade tebuconazole (FOLICUR®) in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 96-412-JI, December 8, 1997. MRID 44449301. Unpublished.

Sheets, L.P. (1998) An acute oral neurotoxicity screening study with technical grade tebuconazole (FOLICUR®) in Fischer 344 rats. A special acute oral neurotoxicity study to establish a no-observed-effect level with technical grade Tebuconazole in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 97-912-LZ, April 9, 1998. MRID 44545701. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorn Road, Kansas City, MO 64120-0013

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 44449301), technical grade tebuconazole (Batch # 603-0013, 96.5% purity) was administered by gavage to 12 male Fischer 344 rats at doses of 0, 100, 500, or 1000 mg/kg and to 12 female Fischer 344 rats at doses of 0, 100, 250, or 500 mg/kg. Because a NOAEL did not appear to be attained in this study, a second (supplemental) acute oral neurotoxicity study (MRID 44545701) using the same batch and doses of 0, 20, or 50 mg/kg to 12 Fischer 344 rats/sex/group was performed. Based on analytical results, actual doses were 0, 0, 21, 50, 103, 497, and 950 mg/kg for male rats and 0, 0, 21, 50, 103, 239, and 497 mg/kg for female rats. In the main study, functional observational battery (FOB) and motor activity tests were performed pretreatment, on the day of test material administration (day 0), and on days 7 and 14 posttreatment; in the supplemental study, an

abbreviated FOB and motor activity tests were performed on day 0 only. At the completion of the main study (day 14), 6 rats/sex in the control and high-dose groups were subjected to perfusion, and brain and nervous tissues were examined microscopically. No histopathological evaluations were performed in the supplemental study.

The high dose of 1000 mg/kg resulted in mortality of 6/12 male rats within two days of treatment. One male rat in the 500 mg/kg dose group also died. There was no treatment-related mortality in females of any dose group. Clinical signs of incoordination, decreased activity, and nasal and perianal stains were observed on day 0, primarily in the two highest dose groups (males, 500 and 1000 mg/kg; females, 250 and 500 mg/kg). Effects on body weight were minimal; weights were 96-101% of control weights on day 14. There was no effect of treatment on brain weight and there were no histopathological findings in the brain or tissues of the nervous system that could be attributed to treatment.

FOB tests on the day of treatment showed treatment-related effects on gait (incoordination, ataxia); activity, arousal, and response to stimuli (all decreased); hindlimb grip strength (decreased); footsplay (decreased in females only); and body temperature (decreased) in one or both sexes in the two highest dose groups (males, 500 and 1000 mg/kg and females, 250 and 500 mg/kg). Most of these parameters were statistically significantly different from control values ($p<0.05$). Increased arousal in the open field and decreased footsplay were also noted in females at 100 mg/kg. All effects except decreased footsplay in 250 and 500 mg/kg females were resolved by post-test day 7. No changes in FOB parameters were observed in males in the 100 mg/kg dose group. No treatment-related FOB effects were observed in either sex in the 20 and 50 mg/kg dose groups.

Relative to concurrent controls, motor and locomotor activity were increased on day 0 in males and females in the 100 mg/kg dose group and decreased in the higher dose groups (males, 500 and 1000 mg/kg and females, 500 mg/kg; all $p<0.05$). There was no statistically significant change in 250 mg/kg females. No adverse effect of treatment on motor activity was observed in either sex in the 20 and 50 mg/kg dose groups, although there was a slight (non-statistically significant) increase in 50 mg/kg males that was considered compound-related. No effect of treatment was observed on motor or locomotor activity on days 7 and 14.

The LOAEL is 100 mg/kg based on increased motor activity in male and female rats and decreased footsplay in female rats. The NOAEL for both male and female rats is 50 mg/kg.

This study is considered **Unacceptable/Guideline** as an acute oral neurotoxicity study and does not fulfill FIFRA guideline requirements for an acute oral neurotoxicity study in rats [OPPTS 870.6200 (§81-8)]. This study can be upgraded by submission of (1) a report from the range-finding study, so that time of peak effect can be verified; (2) additional information regarding the test substance preparation and analysis, as described below.

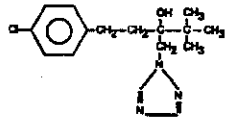
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided in both reports.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Tebuconazole, technical grade

Description: white powder
Batch No.: 603-0013
Purity [Certificate of analysis not provided]: 96.5% a.i. (October 1996)
97.3% a.i. (February 1997)
96.2% a.i. (January 1998)
Stability of compound: stable in tap water for 8 days under refrigeration.
CAS #: 80443-41-0
Structure:



2. Vehicle and/or positive control

The test material was dissolved in 0.5% methylcelllulose/0.4% Tween 80 in deionized water.

3. Test animals

Species: rat
Strain: Fischer 344 CDF(F-344)/BR
Age and mean weight at test substance administration: 9 weeks old;
mean body weight for main study: males: 159-164 g; females: 116-118 g
(weights were not provided for animals in supplemental study)
Source: Sasco, Inc., Madison, WI
Housing: individually in suspended stainless steel wire-mesh cages
Diet: Animals were fed Purina Mills Rodent Lab Chow 5001-4 ("etts"), *ad libitum*; food was withheld during neurotoxicity testing; animals were fasted overnight prior to dosing
Water: Drinking water (tap) was available *ad libitum*.
Environmental conditions:
Temperature: 17.8-25.6°C
Humidity: 40-70% (initial study); 30-70% (supplemental study)
Air changes: Not given
Photoperiod: 12 hour light/12 hour dark
Acclimation period: at least 6 days

B. STUDY DESIGN

1. In life dates

Start: October 21, 1996; end: November 8, 1996 (original study)
Start: August 12, 1997; end: August 14, 1997 (supplemental study)

2. Animal assignment

Animals were assigned to the test groups in Table 1 by means of a computerized randomization procedure (balanced by weight) following selection for adequate body weight (within 20% of the mean, by sex) and freedom from clinical signs.

TABLE 1: Study design
Original study

Test group	Nominal Dose (mg/kg/)		Actual Dose (mg/kg)		Number of animals	
	Males	Females	Males	Females	Males	Females
1 (control)	0	0	0	0	12	12
2 (low-dose)	100	100	103	103	12	12
3 (mid-dose)	500	250	497	239	12	12
4 (high dose)	1000	500	950	497	12	12
Supplemental study						
1 (control)	0	0	0	0	12	12
2	20	20	21	21	12	12
3	50	50	50	50	12	12

Data taken from pp. 19, 23, MRID 44449301 and pp. 16, 19, MRID 44545701.

3. Dose selection rationale

Doses in the original study were based on a previous acute oral (presumably gavage) range-finding study (cited study No. 96-912-HK). Male and female rats (group size not given) were administered nominal doses of 100, 500, or 1000 mg/kg. Doses and effects were stated to be: 100 mg/kg, no clinical signs; 500 mg/kg, undefined signs of toxicity; and 1000 mg/kg, more severe signs of toxicity and a 20% mortality incidence in females. Clinical signs were said to be consistently more severe in females; signs were apparent within 2-4 hours of dosing -- the peak effect time was estimated at 4 hours following treatment. Surviving animals recovered by the next day. Based on these observations, doses of 0, 100, 500, and 1000 mg/kg were chosen for male rats and doses of 0, 100, 250, and 500 mg/kg were chosen for female rats. The range-finding study should be submitted, for verification of peak effect time.

In the initial neurotoxicity screening study (MRID 44449301), rats exposed to the lowest dose of 100 mg/kg exhibited increased activity in the figure-eight maze (both sexes), decreased hind limb splay (females only), and increased arousal in the open field during the FOB (females only). Therefore, in order to attain a NOAEL, doses of

0, 20, and 50 mg/kg were chosen for both sexes for the supplemental study (MRID 44545701).

Based on the range-finding study, Functional Observation Battery testing was begun at least 4 h post-dosing. Motor Activity testing was concluded approximately 7 h post-dosing. The same peak effect time was used for both the main study and the supplemental study.

4. Test material preparation and analysis

The test substance was prepared in deionized water containing 0.5% methylcellulose/0.4% Tween 80; the dosing volume was 10 ml/kg. No further details on dose preparation were provided.

The percent active ingredient of the stock material was confirmed by nuclear magnetic resonance and mass spectroscopy at the time of dosing and within six months after each study (analytical reports were not provided). For homogeneity, three aliquots were taken from each of two test concentrations chosen to bracket the range of test concentrations, 2.0 and 200 mg/ml (original study). The stability of these two solutions was confirmed by analyzing samples on day 0 and following storage in the refrigerator at days 2, 4, and 8. Each dosing solution in both studies was analyzed to measure the concentration.

Results -

Homogeneity analysis: The concentrations of the three aliquots of the 2.0 mg/ml solution averaged 1.75±0.029 mg/ml and the mean concentration in the three aliquots of the 200 mg/ml solution was 188±7.5 mg/ml. The coefficients of variation were 1.6% and 4.0%, respectively.

Stability analysis: For the 2.0 mg/ml solutions, samples taken on study day 0 and following refrigeration for 2, 4, or 8 days were 1.98, 2.05, 1.96, and 2.07 mg/ml (99.0-105% of day 0 concentration). Respective values for the 200 mg/ml solution were 204, 199, 200, and 204 mg/ml (97.5-100% of day 0 concentration).

Concentration analysis: The measured concentrations of tebuconazole in the 10, 20, 50, and 100 mg/ml solutions were 10.3, 23.9, 49.7, and 95.0 mg/ml (95.0-103% of nominal). The analytically-confirmed doses were 0, 103, 497 and 950 mg/kg for males and 0, 103, 239, and 497 mg/kg for females. Measured concentrations of the 20 and 50 mg/kg doses were 105% and 101% of nominal (raw data not provided). Based on these results, the measured doses were 21 and 50 mg/kg.

The analytical data indicated that the mixing procedure was adequate, that the dosing solutions were stable for 8 days under refrigeration, and that the variance between targeted and actual dosage to the animals was acceptable. Based on the analytical results, the actual doses of tebuconazole were 0, 0, 21, 50, 103, 497, and 950 mg/kg for males and 0, 0, 21, 50, 103, 239, and 497 mg/kg for females.

Analytical data verifying the purity of test substance (i.e. certificates of analysis) were not provided. Information provided regarding test substance preparation was not sufficient (e.g. mixing procedures, frequency of preparation, and storage conditions were not included). This information should be provided.

5. Statistics

Statistical evaluations, which consisted primarily of analyses of variance (ANOVA), were performed with computerized programs. Continuous data in the FOB were analyzed by a repeated-measures ANOVA followed by a one-way ANOVA if there was a significant interaction between dose group and test week. For weeks in which there was a significant treatment effect, Dunnett's test was applied to determine which groups were significantly different from the control group. The categorical data from the FOB were analyzed using the General Linear Modeling (GLM) and Categorical Modeling (CATMOD) procedures followed by Dunnett's test and an analysis of contrasts, respectively.

ANOVAs were also applied to the motor and locomotor activity. Session activity data were analyzed with a repeated measures ANOVA followed by a one-way ANOVA if there was a significant interaction with test occasion. Dunnett's test was used to determine if there was a treatment-related effect between the control and a treatment group. Interval data were subjected to a two-way repeated-measures ANOVA, using both test interval and test occasion as the repeated measures, followed by a repeated measures ANOVA to determine on which weeks there was a significant treatment by interval interaction. For weeks with a significant treatment by interval interaction, the data for each interval were subjected to a one-way ANOVA to determine at which intervals there was a significant treatment effect. For those intervals, Dunnett's test was used to determine if there was a significant difference between the control and a treatment group. [Taken from study report, p. 22.]

C. METHODS

1. Observations

All animals were observed cage side once/day for clinical signs of toxicity and mortality; more detailed observations that were similar to those of the FOB were completed and recorded daily.

2. Body weight

All animals in the main study were weighed prior to study initiation and weekly thereafter as part of the FOB. Animals in the supplemental study were weighed prior to treatment (to determine dose volumes); body weights from the supplemental study were not reported.

3. Food consumption and compound intake

Food consumption was not determined. Compound intake was based on gavage doses.

4. Functional observational battery (FOB)

For the main study, all animals/sex/group were subjected to a baseline FOB one week prior to treatment. The FOB was repeated at approximately four hours following administration of the dose (day 0, time of peak effect), and again at seven and 14 days following treatment. The combined FOB and motor activity tests took approximately 3 hours.

For the supplemental study, abbreviated FOB evaluations were performed only at the time of peak effect (approximately 4 h post-dosing). A complete FOB evaluation was not performed (NOAELs had been determined for most parameters in the main study); grip strength, body weight, body temperature, and righting reflex were not measured, hind limb splay was measured in females only.

The following FOB observations were performed by trained technicians who were blind to the treatment of the animals (evaluations were performed by the same observer throughout the study):

a. Home cage observations

Posture, piloerection, gait abnormalities, involuntary motor movements, vocalizations, and any abnormal activity.

b. Observations during handling

Ease of removal from cage, reaction to handling, muscle tone, palpebral closure, pupil size, pupil response to light, lacrimation, salivation, stains, and other abnormal signs.

c. Open field observations

Piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behavior, gait abnormalities, vocalizations, arousal, rearing, defecation, and urination.

d. Sensorimotor and neuromuscular measurements

Approach response, auditory response, tail pinch response, righting reflex, grip strength, and landing foot splay.

e. Other

Body weight and body temperature.

5. Motor activity

Motor activity was measured following each FOB session. Motor activity was measured for 90 minutes in one of eight automated, computerized figure-eight mazes (white noise, approximately 70dB, was generated in the background during testing). Motor activity was measured as the total number of beam interruptions that occurred during each of nine consecutive 10-minute intervals (subsessions). Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test sessions. Animals were tested in a semi-random order, balanced across devices, and testing was staggered over two days for each sex. Males and females were tested on separate days.

6. Sacrifice and pathology

A complete gross necropsy was conducted on all animals that died or were sacrificed at study termination. Six animals/sex/group (the first six that had been randomly selected for FOB and motor activity) were anesthetized with pentobarbital and then perfused via the left ventricle with sodium nitrite in phosphate buffer followed by fixation with Universal fixative (4% w/v glutaraldehyde and 4% w/v EM-grade formaldehyde) in phosphate buffer. The entire brain and spinal cord, both eyes with optic nerves, and selected bilateral peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, and gastrocnemius muscle, were dissected from each animal and fixed. The brain was weighed and the brain:body weight ratio calculated.

The following tissues (and any gross lesions) from control and high-dose animals were examined microscopically:

1) eight coronal sections of the brain and four levels of the spinal cord (cervical, thoracic, lumbar, and cauda equina) were embedded in paraffin and stained with H&E, luxol fast blue/cresyl violet, and Sevier-Munger stain;

2) cervical and lumbar dorsal root ganglia (including dorsal and ventral root fibers), gasserian ganglion, eyes, optic nerves, and gastrocnemius muscle were embedded in glycol methacrylate, sectioned at 2-3 μm and stained using a modified Lee's stain;

3) sciatic, tibial, and sural nerves were embedded in epoxy resin, cross-sectioned at 1 μm, and stained with toluidine blue (sciatic nerve was also sectioned longitudinally [1 μm thickness] and similarly stained).

The remaining animals that survived to term were sacrificed by carbon dioxide asphyxiation; additional tissues were not collected.

7. Positive Control Data

Positive control studies were cited by the author (report references, p. 31, dated 1993 and 1994). Although we will not require additional data to be submitted in support of this study, several issues regarding the cited data will need to be resolved as additional studies are received from this laboratory (see discussion, Appendix A).

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

One male rat in the 500 mg/kg dose group and six male rats in the 1000 mg/kg dose group died within one or two days following treatment (all survived through day 0 FOB and motor activity evaluations). No males in the control or 100 mg/kg dose groups and no females in any group died during the 15-day observation period.

Treatment-related clinical signs, observed in males in the 500 and 1000 mg/kg dose groups and females in the 250 and 500 mg/kg dose groups, consisted primarily of uncoordinated gait and decreased activity (Table 2). Stains were observed around all orifices. Except for perianal stains (which were observed for 5 days), all signs resolved by day 3 following treatment. The bodies of animals were cool to touch prior to death.

TABLE 2. Clinical signs in male and female rats administered tebuconazole by gavage

Clinical sign	Dose (mg/kg)											
	Males[a]						Females					
	0	20	50	100	500	1000	0	20	50	100	250	500
Ataxia	0			0	9	8	0			0	6	12
Decreased activity	0			0	2	10	0			0	0	0
Body cool to touch	0			0	1	6	0			0	0	0
Salivation	0			0	1	1	0			0	0	0
Oral stain	0			0	2	4	0			0	2	1
Clear lacrimation	0			0	1	5	0			0	0	0
Red lacrimal stain	0			0	1	1	0			0	0	1
Red nasal stain	0			0	11	5	0			0	11	12
Urine stain	0			0	1	7	0			0	0	0
Perianal stain												
-original study	6	-	-	8	11	9	3	-	-	3	12	12
-supplemental study	1	2	1	-	-	-	0	0	0	-	-	-

Data taken from Table 1, pp. 34 and 35, MRID 44449301 and Table 1, p. 25, MRID 44545701; perianal stain was the only clinical sign reported in the supplemental study. Numbers represent incidence rates. [a]Observations were made prior to deaths and are based on 12 animals/sex/group.

B. BODY WEIGHT AND WEIGHT GAIN

Group mean body weights and total body weight changes are shown in Table 3. Body weights were not recorded in the 20 and 50 mg/kg groups except pretreatment in order to determine dose volumes. Weight gains were minimal (males) or negative (females) between pretreatment and day 0 due to the overnight fast prior to treatment on day 0. On day 7, mean body weight was significantly reduced (94% of control value, $p<0.05$) in the 11 males that survived the 500 mg/kg treatment. Mean body weights were not affected in the six males that survived the 1000 mg/kg dose or in females in any dose group.

TABLE 3. Group mean body weights (g) for selected days and total body weight gains (g) of rats administered an acute oral dose of tebuconazole[a]

Males				
Day of study	Dose (mg/kg)			
	0	100	500	1000
Pretreatment	156±7	159±10	155±8	156±4
0	163±6	164±9	159±10	162±4
7	209±9	208±13	197±12* (94)	200±8 (95)
14	232±10	231±15 (100)	223±16 (96)	234±6 (101)
Total weight gain[b]	76	72 (95)	68 (89)	78 (103)
Females				
Day of study	Dose (mg/kg)			
	0	100	250	500
Pretreatment	123	123	123	122
0	118	116	117	116
7	144	142	144	142
14	155	154 (99)	155 (100)	153 (99)
Total weight gain[b]	32	31 (97)	32 (100)	31 (97)

Data taken from Table 2, p. 36, MRID 4444930. n=12/sex/group except on days 7 and 14, n=11 for males at 500 mg/kg and n=6 for males at 1000 mg/kg. [a] Percent of control value in parenthesis. [b] Calculated by reviewer. *Statistically significant, ANOVA, $p<0.05$.

C. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

In the main study, treatment-related effects were observed in male rats in the 500 and 1000 mg/kg dose groups and in female rats in the 100, 250, and 500 mg/kg dose groups on day 0 (Tables 4a and 4b). Statistical significance in Tables 4a and 4b is based on comparisons for data collected in the original study only (comparing the original control group and the three highest dose groups for each sex).

In males, incidences of observations that were statistically significantly different from controls in either the 500 or 1000 mg/kg dose groups (or both) included: home cage - both slight and moderate/severe gait incoordination, decreased activity, and changes in posture; handling - the presence of slight clear lacrimation, the presence of perianal stains, and the presence of slight red nasal stains; open field - both slight and moderate/severe gait incoordination, changes in arousal; reflex/physiologic - decreased responses to approach, touch, and noise; decreased air righting ability (not statistically significant, but

similar to effect seen in females), reduced hindlimb grip strength, and lowered body temperature (Table 4a and 4c). All of these effects were resolved by test day 7.

Similar observations were recorded for females in the 250 and 500 mg/kg dose groups and included: home cage - slight and moderate/severe gait incoordination and change in posture; handling - slight yellow perianal stain; open field - slight and moderate/severe gait incoordination, increased arousal (at all doses - not statistically significant, but similar to the effect seen in males); and reflex/physiologic - diminished reactions to approach, touch, and noise, decreased air righting ability, lower hindlimb grip strength (at the high dose only), decreased footsplay (at all doses), and lowered body temperature (Tables 4b and 4c). All of these effects, except for landing foot splay (at 250 and 500 mg/kg, Table 4c), were resolved at test day 7.

In the supplemental study, no treatment-related effects were observed in either sex in the 20 and 50 mg/kg groups at the time of peak effect on day 0 (the only time point evaluated for this group). For comparison, landing foot splay in females was 37±4 mm, 38±5 mm, and 41±7 mm, in controls, 20, and 50 mg/kg groups, respectively.

TABLE 4a. Functional observation battery results for male rats administered an acute oral dose of tebuconazole by gavage (day of administration)[a]

Parameter	Males					
	Dose (mg/kg)					
	0	20	50	100	500	1000
Home cage						
incoordination (slight)	0, *0*[b]	*0*	*0*	0	6#	5#
incoordination (moderate/severe)	0, *0*	*0*	*0*	0	1#	6#
decreased activity	0, *0*	*0*	*0*	0	2*	9*
posture- standing normally	0, *1*	*3*	*1*	0	6@	0
rearing	1,—[c]	—	--	0	0@	1
sitting/lying normally	11, *11*	*9*	*11*	12	6@	11
Handling						
clear lacrimation (slight)	0, *0*	*0*	*0*	0	2	5#
red lacrimation (slight)	0, *0*	*0*	*0*	0	1	2
red lacrimation (moderate/severe)	0, *0*	*0*	*0*	0	0	1
clear salivation (slight)	0, *0*	*0*	*0*	0	0	2
stains						
brown perianal (slight)	0, *0*	*0*	*0*	1	4#	0
yellow perianal (slight)	0, *0*	*0*	*0*	0	0	1#
yellow perianal (moderate/severe)	0, *0*	*0*	*0*	0	0	3#
red nasal (slight)	0, *0*	*0*	*0*	0	4#	1
cool to touch	0, *0*	*0*	*0*	0	1	0

TABLE 4a. Functional observation battery results for male rats administered an acute oral dose of tebuconazole by gavage (day of administration)[a]

Males						
Parameter	Dose (mg/kg)					
	0	20	50	100	500	1000
Open field						
gait incoordination (slight)	0, *0*	*0*	*0*	0	8#	4#
gait incoordination (moderate/severe)	0, *0*	*0*	*0*	0	1#	8#
body dragging/flattened (moderate/severe)	0, *0*	*0*	*0*	0	1	0
posture - standing normally	12, *12*	*12*	*12*	12	11	8
sitting or lying normally	0, *0*	*0*	*0*	0	1	4
arousal - normal	0, *7*	*9*	*6*	3	6@	0
sluggish - minimal movement	1, *0*	*2*	*1*	3	1@	0
sluggish - some exploratory movement	11, *5*	*1*	*5*	6	5@	12
Reflex/physiologic						
approach response - no reaction	0, *0*	*0*	*0*	0	4@	10@
slight reaction	12, *12*	*12*	*12*	12	8@	2@
touch response - no reaction	0, *0*	*0*	*0*	0	4@	8@
slight reaction	12, *12*	*12*	*12*	12	8@	4@
auditory response - no reaction	0, *0*	*0*	*0*	0	4@	8@
slight reaction	12, *12*	*12*	*12*	12	8@	4@
tail pinch - no reaction	0, *0*	*0*	*0*	0	2	4
slight reaction	12, *12*	*12*	*12*	12	10	8
righting response - slightly uncoordinated	0, *NP*	*NP*	*NP*	0	1	2
landing on side	0, *NP*	*NP*	*NP*	0	1	4
landing on back	0, *NP*	*NP*	*NP*	0	1	0

Data taken from Table 3, pp. 37-61, Table 4, pp. 63 and 67, Table 5, pp. 70 and 71, MRID 44449301 and Table 2, pp. 26-45, MRID 44545701.

[a] 12 animals/sex/group.

[b] Two values in a row in the control column indicate results from the original study (MRID 44449301) and the supplementary study (MRID 44545701), respectively; values in italics were obtained from the supplementary study; comparisons should be made to concurrent study controls only (i.e. main study values [in regular type] should be compared to main study controls [in regular type]; supplementary study values [in italics] should be compared to supplementary study controls [in italics]).

[c] Behavior not reported.

NP = not performed.

* Statistically significant, ANOVA, $p<0.05$ (20 and 50 mg/kg dose groups not included in analyses);@response profile significantly from control ($p<0.05$); #significantly different from control ($p<0.05$) without regard to distribution of severity.

TABLE 4b. Functional observation battery results for female rats administered an acute oral dose of tebuconazole by gavage on the day of administration

Females						
Parameter	Dose (mg/kg)					
	0	20	50	100	250	500
Home cage						
incoordination (slight)	0, *0*	*0*	*0*	0	5#	6#
incoordination (moderate/severe)	0, *0*	*0*	*0*	0	0#	6#
decreased activity	0, *0*	*0*	*0*	0	0	1
posture- standing normally	0, *2*	*4*	*2*	1	5*	8*
rearing	1, *0*	*0*	*0*	0	0*	1*
sitting/lying normally	11, *10*	*8*	*10*	11	7*	3*
Handling						
clear lacrimation (slight)	0, *0*	*0*	*0*	0	0	0
red lacrimation (slight)	0, *0*	*0*	*0*	0	0	0
red lacrimation (moderate/severe)	0, *0*	*0*	*0*	0	0	1
clear salivation (slight)	0, *0*	*0*	*0*	0	0	0
stains						
brown perianal (slight)	0, *0*	*0*	*0*	0	0	0
yellow perianal (slight)	0, *0*	*0*	*0*	0	1	5*
yellow perianal (moderate/severe)	0, *0*	*0*	*0*	0	0	0
red nasal (slight)	0, *0*	*0*	*0*	0	0	0
cool to touch	0, *0*	*0*	*0*	0	0	0
Open field						
gait incoordination (slight)	0, *0*	*0*	*0*	0	9*	5*
gait incoordination (moderate/severe)	0, *0*	*0*	*0*	0	1*	7*
body dragging/flattened (moderate/severe)	0, *0*	*0*	*0*	0	0	0
posture - standing normally	12, *12*	*12*	*12*	12	12	12
sitting or lying normally	0, *0*	*0*	*0*	0	0	0
arousal - normal	3, *10*	*11*	*9*	9	8	6
sluggish - minimal movement	0, *0*	*1*	*0*	0	0	0
sluggish - some exploratory movement	9, *2*	*0*	*3*	3	4	6

TABLE 4b. Functional observation battery results for female rats administered an acute oral dose of tebuconazole by gavage on the day of administration

Females						
Parameter	Dose (mg/kg)					
	0	20	50	100	250	500
Reflex/physiologic						
approach response - no reaction	0, *0*	*0*	*0*	0	3*	5*
slight reaction	12, *12*	*12*	*12*	12	9*	7*
touch response - no reaction	0, *0*	*0*	*0*	0	2	4*
slight reaction	12, *12*	*12*	*12*	12	10	8*
auditory response - no reaction	0, *0*	*0*	*0*	0	2	1
slight reaction	12, *12*	*12*	*12*	12	10	11
tail pinch response - no reaction	0, *0*	*0*	*0*	0	0	1
slight reaction	12, *12*	*12*	*12*	12	12	11
righting response - slightly uncoordinated	0, *NP*	*NP*	*NP*	0	2	3*
landing on side	0, *NP*	*NP*	*NP*	0	0	5*
landing on back	0, *NP*	*NP*	*NP*	0	0	0

Data taken from Table 3, pp. 37-61, Table 4, pp. 63 and 67, Table 5, pp. 70 and 71, MRID 44449301 and Table 2, pp. 26-45, MRID 44545701.

[a] 12 animals/sex/group.

[b] Two values in a row in the control column indicate results from the original study (MRID 44449301) and the supplementary study (MRID 44545701), respectively; values in italics were obtained from the supplementary study; comparisons should be made to concurrent study controls only (i.e. main study values [in regular type] should be compared to main study controls [in regular type]; supplementary study values [in italics] should be compared to supplementary study controls [in italics]).

[c] Behavior not reported.

NP = not performed.

* Statistically significant, ANOVA, $p<0.05$ (20 and 50 mg/kg dose groups not included in analyses); @response profile significantly from control ($p<0.05$); #significantly different from control ($p<0.05$) without regard to distribution of severity.

Table 4c. Functional Observation Battery results.

	Dose (mg/kg/day)			
Observation	control	low dose	mid dose	high dose
Males	**0**	**100**	**500**	**1000**
Forelimb Grip Strength (kg)				
-Pretest	0.49±0.05	0.48±0.05	0.50±0.08	0.50±0.07
-Day 0	0.65±0.07	0.66±0.06	0.60±0.11	0.58±0.11
-Day 7	0.67±0.08	0.70±0.06	0.62±0.10	0.68±0.05
-Day 14	0.72±0.08	0.73±0.08	0.69±0.12	0.73±0.05
Hindlimb Grip Strength (kg)				
-Pretest	0.23±0.02	0.22±0.02	0.24±0.02	0.23±0.03
-Day 0	0.29±0.05	0.27±0.04	0.22±0.05*	0.18±0.05*
-Day 7	0.32±0.05	0.28±0.05	0.25±0.05	0.30±0.10
-Day 14	0.37±0.06	0.33±0.05	0.32±0.05	0.33±0.04

	Dose (mg/kg/day)			
Observation	control	low dose	mid dose	high dose
Landing Foot Splay (mm)				
-Pretest	69±11	65±8	66±10	67±11
-Day 0	61±12	55±8	54±8	59±8
-Day 7	65±13	64±8	60±7	63±11
-Day 14	61±10	56±7	56±9	64±6
Body Temperature (°C)				
-Pretest	38.2±0.4	38.1±0.4	38.0±0.5	38.2±0.4
-Day 0	36.7±0.4	36.8±0.5	34.8±1.8*	34.0±0.8*
-Day 7	38.0±0.5	38.0±0.5	37.9±0.5	38.0±0.5
-Day 14	37.7±0.5	37.9±0.6	37.7±0.4	37.8±0.5
Females	**0**	**100**	**250**	**500**
Forelimb Grip Strength (kg)				
-Pretest	0.50±0.08	0.46±0.06	0.51±0.07	0.51±0.07
-Day 0	0.61±0.06	0.64±0.06	0.62±0.06	0.61±0.07
-Day 7	0.63±0.07	0.64±0.07	0.66±0.05	0.63±0.06
-Day 14	0.65±0.07	0.67±0.07	0.65±0.09	0.63±0.05
Hindlimb Grip Strength (kg)				
-Pretest	0.24±0.03	0.23±0.04	0.23±0.02	0.23±0.02
-Day 0	0.29±0.03	0.30±0.04	0.27±0.04	0.23±0.04*
-Day 7	0.30±0.06	0.29±0.04	0.30±0.04	0.29±0.04
-Day 14	0.32±0.04	0.33±0.05	0.31±0.05	0.33±0.04
Landing Foot Splay (mm)				
-Pretest	52±7	54±9	54±6	54±7
-Day 0	55±10	46±4*	42±8*	41±8*
-Day 7	58±6	54±8	51±5*	49±6*
-Day 14	51±6	52±6	48±9	48±8
Body Temperature				
-Pretest	38.2±0.6	38.1±0.6	38.0±0.7	38.2±0.5
-Day 0	37.0±0.6	36.6±0.5	36.0±0.8*	35.0±0.8*
-Day 7	38.0±0.5	37.8±0.4	38.0±0.5	38.1±0.5
-Day 14	37.7±0.6	37.8±0.6	37.8±0.7	37.9±0.7

Data were extracted from MRID 44449301, pp. 37-69. Values represent mean ±s.d. N=12/sex/group, except n=11 for 500 mg/kg males on days 7 and 14 (also on day 0 for grip strength only), and n=6 for 1000 mg/kg males on days 7 and 14. *p<.05,** p<.01 compared with controls.

D. MOTOR AND LOCOMOTOR ACTIVITY

In the main study, motor activity for the total 90-minute session on the day of treatment (day 0) was statistically significantly different from control values for males in all treatment groups (increased activity in the 100 mg/kg dose group and decreased activity in the 500 and 1000 mg/kg dose groups) and for females in the 100 mg/kg dose group (increased activity) and 500 mg/kg dose group (decreased activity) (Table 5). Activity in the females receiving 250 mg/kg was not significantly different from control activity. Locomotor activity of treated groups followed trends similar to motor activity for both

sexes, but changes were less pronounced (in males, only the decrease in activity at the high dose remained statistically significant, in females; only the increased activity at the low dose remained statistically significant). The differences in activity at day 0 - increased activity at low doses and decreased activity at high doses - were considered treatment related by the study authors. The only statistically significant change in total activity at any other time point was a decrease in activity in 500 mg/kg males on day 7 (significant for both motor and locomotor activity); this finding was not dose-related, since no decrease was seen in surviving males at 1000 mg/kg.

In the supplemental study, no statistically significant differences were found for total motor activity in either males or females on day 0 (the only day on which these animals were tested). For males, there was an apparent dose-related increase in activity in both treatment groups (7% at 20 mg/kg, 24% at 50 mg/kg [see Table 5]). There was no apparent change in activity levels in females at either dose. As in the main study, effects on locomotor activity paralleled those in total motor activity, with slight increases in males (8% at 20 mg/kg, 19% at 50 mg/kg), and no changes in females (see Table 5). The increase in motor activity at 50 mg/kg in males is considered compound-related, but as this was the only effect seen at this dose (no similar increase was seen in females), the increase was not statistically significant, and the magnitude of the increase was only slightly greater than might be expected due to normal variability (the authors asserted, based on control data from their laboratory, that differences of <20% are within the range of normal variability for rats in their laboratory), the effect may not be toxicologically significant.

Data for individual 10-minute subsessions were presented in both studies. In the main study, subsession data for total activity showed that for both males and females the statistically significant decrease in activity in the two highest dose groups (relative to the respective control groups) was confined to the first two 10-minute intervals (Table 5a). Although overall activity was statistically significantly increased in the low dose group (100 mg/kg) for both sexes on day 0, the differences did not attain statistical significance for any single interval. On day 7, a single incidence of decreased activity in male rats in the 500 mg/kg dose group during the first interval paralleled the significant decrease in total activity for this group at that time point, but was not dose related (no decrease was seen in surviving males at 1000 mg/kg) and was therefore not considered to be treatment related.

As noted above for total activity, changes in locomotor activity for individual intervals paralleled those for motor activity. For male rats, locomotor activity was significantly depressed ($p<0.05$) on day 0 in the 500 and 1000 mg/kg groups during the first two intervals (counts were 55 ± 12, 64 ± 16, 31 ± 14*, and 20 ± 12* for the first interval; 26 ± 12, 29 ± 15, 9 ± 7*, and 5 ± 5* for the second interval, for the control, 100, 500, and 1000 mg/kg males, respectively); a significant increase in locomotor activity during interval 7 in the 500 mg/kg dose group was considered sporadic (counts were 0 ± 0, 0 ± 1, 4 ± 7*, and 1 ± 2 for

control, 100, 500, and 1000 mg/kg groups, respectively). There was no statistically significant change during any interval in the 100 mg/kg dose group.

For females in the 100 mg/kg dose group, locomotor activity was statistically significantly increased ($p<0.05$) relative to control values during intervals 2, 3, and 4 on day 0. Also on day 0, locomotor activity was significantly depressed ($p<0.05$) during intervals 1 and 2 in the 500 mg/kg dose group, but not during any interval in the 250 mg/kg dose group (counts were 71±26, 75±19, 55±14, 44±16* for interval 1; 34±22, 53±24*, 16±13, 14±6* for interval 2, 15±16, 33±24*, 9±6, 7±7 for interval 3, 4±10, 19±24*, 8±8, 6±6 for interval 4, for control, 100, 250, and 500 mg/kg females, respectively). Increased activity during interval 8 in the 250 mg/kg dose group was not treatment related (counts were 0±0, 3±6, 5±5*, and 4±5 for control, 100, 250, and 500 mg/kg females, respectively, during interval 8).

A decrease in locomotor activity during interval 1 on day 7 in the 500 mg/kg males (counts were 117±13, 116±19, 93±19*, and 104±14 for the control, 100, 500, and 1000 mg/kg males, respectively), and an increase in activity during interval 5 on day 14 in the 1000 mg/kg males (counts were 2±6, 0±1, 0±1, and 13±20 for control, 100, 500, and 1000 mg/kg males, respectively) were not considered treatment related. No statistically significant changes in activity relative to the controls occurred during any intervals in females on days 7 and 14.

No statistically significant changes were observed in individual subsession motor activity in male and female rats in the 20 and 50 mg/kg dose groups, although there was a trend toward increased activity in 50 mg/kg males. (Table 5b). The only statistically significant increase in locomotor activity of male rats was seen during interval 2 in the 50 mg/kg dose group (locomotor counts for that interval were 41±11, 43±13, and 55*±12 [*$p<0.05$] for control, 20 mg/kg, and 50 mg/kg groups, respectively).

In the main study, motor activity control values were considerably lower on day 0 than on other test days. This finding was attributed by the authors to the overnight fast prior to treatment and subsequent testing. When control values for total motor activity on day 0 in the two studies are compared, it is obvious that the values for both males and females in the main study are lower than in the supplemental study, attaining a 16% difference for males and a 32% difference for females (Table 5). The higher baseline level of activity in the supplemental study may be attributable to the lack of pre-testing in these animals; activity levels would be expected to be higher during the first exposure to new surroundings.

TABLE 5. Motor and locomotor activity of rats administered an acute oral dose of tebuconazole by gavage[a,b]

Males

Parameter	Dose (mg/kg)					
	0	20	50	100	500	1000
Motor activity						
pretreatment	612± 203			649± 164	566± 144	641± 106
day 0						
-main study	301± 92	-	-	420± 168*	150± 94*	112± 51*
-supplemental study[c]	357± 80	383± 85	441± 116			
day 7	563± 195			545± 120	415± 84*	685± 145
day 14	513± 114			550± 145	395± 156	613± 172
Locomotor activity						
pretreatment	263± 83			262±65	252±65	280±49
day 0						
-main study	89±20	-	-	114±50	72±41	40±31*
-supplemental study	144±23	156±33	171±30			
day 7	215±65			204±37	162±38*	254±48
day 14	184±34			194±51	147±66	214±59

Females

Parameter	Dose (mg/kg)					
	0	20	50	100	250	500
Motor activity						
pretreatment	950±180			914±245	1004±249	885±272
day 0						
-main study	435±236	-	-	672±343*	375±107	215±90*
-supplemental study	638±195	681±176	618±215			
day 7	752±230			684±170	809±120	780±114
day 14	1047±302			976±299	972±251	903±168
Locomotor activity						
pretreatment	380±73			338±112	382±114	340±122
day 0						
-main study	131±72	-	-	213±124*	125±35	100±40
-supplemental study	195±49	219±50	214±55			
day 7	274±80			247±69	314±68	291±47
day 14	368±106			351±118	346±104	347±77

Data taken from Tables 6 and 7, pp. 72-75, MRID 44449301 and Tables 4 and 5, pp. 46-49, MRID 44545701; values represent mean ± s.d.; [a] Summary of 90-minute sessions; [b] n=12 animals/group, except n=11 for 500 mg/kg/males at days 7 and 14, n=6 for 1000 mg/kg males at days 7 and 14; [c] animals in supplemental study (MRID 44545701) were tested on day 0 only (no data were collected at pre-test, day 7, or day 14); * Statistically significant, ANOVA, $p<0.05$ (20 and 50 mg/kg dose groups not included in analyses).

Table 5a. Total Motor Activity Results - Individual Interval Data.

Observation	Dose (mg/kg/day) control	low dose	mid dose	high dose
Males	**0**	**100**	**500**	**1000**
Pretest				
-Interval 1	234±44	260±40	238±61	274±41
-Interval 2	204±61	220±63	171±60	229±53
-Interval 3	106±79	116±66	102±65	118±44
-Interval 4	50±54	22±44	29±53	19±28
-Interval 5	9±18	2±7	0±0	0±0
-Interval 6	0±1	3±4*	0±0	0±0
-Interval 7	0±1	2±3	0±0	0±1
-Interval 8	1±5	13±23	6±18	0±0
-Interval 9	7±10	11±17	20±55	0±0
Day 0				
-Interval 1	162±38	192±42	60±29*	36±19*
-Interval 2	97±45	115±55	20±18*	16±12*
-Interval 3	21±27	41±52	9±10	14±10
-Interval 4	7±15	14±31	17±19	9±10
-Interval 5	1±2	10±20	11±14	12±9
-Interval 6	1±2	10±19	2±3	7±8
-Interval 7	0±0	14±27	9±12	8±6
-Interval 8	6±12	17±40	14±15	6±9
-Interval 9	7±22	7±15	9±13	5±12
Day 7				
-Interval 1	278±30	270±24	210±50*	279±29
-Interval 2	161±61	172±25	133±57	199±46
-Interval 3	69±57	60±52	37±36	126±82
-Interval 4	24±46	15±20	10±22	44±55
-Interval 5	18±36	9±22	2±7	10±25
-Interval 6	12±28	10±26	1±1	12±28
-Interval 7	0±0	1±2	3±8	15±37
-Interval 8	0±1	3±8	17±52	0±0
-Interval 9	1±3	5±11	1±3	0±0
Day 14				
-Interval 1	265±35	284±27	226±67	290±23
-Interval 2	155±36	171±53	110±64	174±49
-Interval 3	65±30	60±52	29±41	71±66
-Interval 4	15±26	24±48	19±27	37±49
-Interval 5	8±23	2±3	1±2	39±56*
-Interval 6	2±7	1±2	0±0	1±2
-Interval 7	0±0	6±13	0±0	0±1
-Interval 8	4±9	3±7	5±17	0±0
-Interval 9	0±0	1±1	4±12	1±2

Observation	Dose (mg/kg/day) control	low dose	mid dose	high dose
Females	**0**	**100**	**250**	**500**
Pretest				
-Interval 1	282±43	287±35	286±50	300±53
-Interval 2	259±39	247±52	246±51	253±76
-Interval 3	218±61	211±55	195±63	186±60
-Interval 4	113±56	110±87	159±82	97±90
-Interval 5	48±54	37±55	62±54	28±43
-Interval 6	13±29	5±13	14±33	6±11
-Interval 7	7±20	1±3	10±26	1±2
-Interval 8	6±19	2±4	18±46	3±7
-Interval 9	5±14	14±48	14±48	15±52
Day 0				
-Interval 1	213±71	234±51	140±42*	88±31*
-Interval 2	121±68	171±57	55±55*	29±13*
-Interval 3	56±60	98±68	32±25	13±13
-Interval 4	19±43	55±68	25±26	14±11
-Interval 5	7±24	45±76	26±34	18±16
-Interval 6	13±30	21±42	25±33	17±16
-Interval 7	5±11	13±28	32±20*	13±12
-Interval 8	1±2	15±30	22±24	10±11
-Interval 9	0±0	19±39	18±30	13±10
Day 7				
-Interval 1	266±45	280±34	284±50	266±42
-Interval 2	209±69	206±61	241±52	240±32
-Interval 3	151±57	130±47	171±51	174±41
-Interval 4	62±73	42±61	88±55	71±48
-Interval 5	38±57	11±25	10±16	8±14
-Interval 6	11±30	1±3	10±25	1±3
-Interval 7	3±5	3±6	5±16	3±6
-Interval 8	3±6	5±14	0±1	3±5
-Interval 9	9±27	6±19	0±1	15±38
Day 14				
-Interval 1	343±58	324±58	288±38	309±40
-Interval 2	241±49	233±49	220±38	208±47
-Interval 3	166±45	184±72	175±66	155±49
-Interval 4	132±79	135±76	124±60	114±72
-Interval 5	86±66	59±57	90±61	68±66
-Interval 6	44±48	36±48	61±62	32±49
-Interval 7	28±52	1±3	7±16	16±40
-Interval 8	6±14	1±2	1±3	1±3
-Interval 9	1±2	3±5	6±17	0±0

Data were extracted from MRID 44449301, pp. 76-83. Values represent mean ±s.d. N=12/sex/group, except n=11 for 500 mg/kg males on days 7 and 14, and n=6 for 1000 mg/kg males on days 7 and 14. *p<.05,** p<.01 compared with controls.

Table 5b. Total Motor Activity Results - Supplemental Study - Individual Interval Data.

	Dose (mg/kg/day)		
Observation	0	20	50
Males-Day 0			
-Interval 1	194±28	182±37	200±43
-Interval 2	121±38	111±36	157±49
-Interval 3	32±36	51±27	55±32
-Interval 4	2±4	13±16	9±14
-Interval 5	0±0	8±24	3±5
-Interval 6	0±0	5±9	5±10
-Interval 7	5±12	5±9	1±3
-Interval 8	0±1	7±19	6±20
-Interval 9	2±6	3±9	5±16
Females -Day 0			
-Interval 1	224±53	236±44	217±56
-Interval 2	154±52	157±39	147±47
-Interval 3	108±44	123±58	102±47
-Interval 4	70±34	69±42	49±36
-Interval 5	40±48	37±47	40±56
-Interval 6	24±39	21±31	30±42
-Interval 7	9±20	4±9	3±10
-Interval 8	5±10	18±34	12±19
-Interval 9	3±3	16±40	17±32

Data were extracted from MRID 4545701, pp. 50-51. Values represent mean ±s.d. N=12/sex/group.
*p<.05,** p<.01 compared with controls.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

Mean terminal body weights of treated males and females were not affected at any dose level. Mean absolute and relative (to body weight) brain weights did not differ between control and treatment groups. For males, brain weights in the 0, 100, 500, and 1000 mg/kg dose groups were 1.732±.047, 1.755±0.027, 1.719±0.053, and 1.751±0.062 g. Respective relative brain weights were 0.745%, 0.741, 0.745, and 0.735%. For females in the respective groups, absolute brain weights were 1.640±0.047, 1.669±0.038, 1.676±0.096, and 1.667±0.059 g. Respective relative brain weights were 1.005, 1.074, 1.078, and 1.044%.

2. Gross pathology

No gross lesions were observed in animals that survived to terminal sacrifice. The lungs of the male in the 500 mg/kg dose group that died on day 3 were discolored (red). Nasal discharge and wetness and staining of the ventral body surfaces were observed grossly in the males in the high-dose group that died prior to sacrifice.

3. Microscopic pathology

There were no compound-related microscopic lesions in males or females in the high-dose group. Animals from the lower dose groups were not examined microscopically. Background lesions found with similar incidence in both treated and control animals included minimal axonal swelling and nerve fiber degeneration at several levels of spinal cord, and single incidences of nerve fiber degeneration in several brain levels.

III. DISCUSSION

A. DISCUSSION

Technical grade tebuconazole was acutely toxic to male rats at the highest dose tested (1000 mg/kg), resulting in the deaths of 6 of 12 male rats at that dose and 1/12 males at 500 mg/kg. Females were not tested at 1000 mg/kg and no deaths occurred in female rats at 500 mg/kg.

Treatment-related transient clinical signs were observed in males in the 500 and 1000 mg/kg dose groups and females in the 250 and 500 mg/kg dose groups and consisted primarily of uncoordinated gait and decreased activity (males in the 1000 mg/kg group). Stains were observed around all orifices in many of these animals. With the exception of perianal stains which persisted for five days, these effects were resolved by the third day after treatment.

Animals were subjected to the FOB and motor activity tests within approximately four hours following administration of the dose, the time of peak activity as indicated in an earlier study. Results of the FOB clearly indicated functional/neurological effects in males in the 500 and 1000 mg/kg dose groups. These observations included decreased hindlimb grip strength, decreased body temperature, incoordination/ataxia, diminished response to a variety of stimuli, and lacrimation, with incidences being dose-related. No FOB parameters were statistically significantly different from the control group in the male 100 mg/kg dose group. Similar neurological effects were observed in females in the 500 mg/kg dose group and to a lesser extent in the 250 mg/kg dose group, again indicating a dose-response effect. In addition, decreased landing foot splay was seen only in females, and was also significantly decreased at 100 mg/kg. The decreased foot splay in females was the only effect which persisted, remaining statistically significant at 250 and 500 mg/kg in females on day 7. All parameters were comparable to controls on day 14 in both sexes. No effects on measured FOB parameters were seen in the supplemental study, at doses of 20 and 50 mg/kg, in males or females.

Motor activity was affected by tebuconazole in both sexes at all doses administered in the main study. For both male and female rats, motor activity increased at the lowest dose (100 mg/kg), and decreased at higher doses. Motor activity was clearly depressed in males at 500 and 1000 mg/kg and in females at 500 mg/kg. These differences relative to the respective controls were reflected by statistical differences in the early 10-minute intervals. These neurotoxic effects were transient as activity was generally within control

ranges at days 7 and 14 (the decreased activity of males in the 500 mg/kg dose group on day 7 is considered statistical variation; there was no change in activity in the 1000 mg/kg dose group in males, and no similar effects were seen in females).

In the supplemental study, no changes in motor activity were seen in females at either dose (20 or 50 mg/kg). In males, there was a slight dose-related increase, approximately 24% above control levels, at the higher dose (50 mg/kg). Although the increase was considered compound-related, it was neither statistically significant nor large enough in magnitude to be considered toxicologically relevant.

The study authors attribute the effects of the exposure to tebuconazole - increased activity at low doses and decreased activity at high doses - to the same mechanism of action as that of the structurally-related chemical triadimefon. The study authors report that administration of triadimefon also produces increased activity at low doses and decreased activity at high doses; triadimefon causes transient increased psychomotor stimulant effects by elevating synaptic concentrations of dopamine via inhibition of dopamine re-uptake.

Treatment with tebuconazole had no effect on body or absolute or relative brain weights. There were no compound-related gross or microscopic findings in the nervous system tissues evaluated.

In summary, based on differences relative to the concurrent control groups, the LOAEL is 100 mg/kg based on increased motor activity in male and female rats and decreased footsplay in female rats. The NOAEL for both male and female rats is 50 mg/kg.

This study is considered **Unacceptable/Guideline** as an acute oral neurotoxicity study and does not fulfill FIFRA guideline requirements for an acute oral neurotoxicity study in rats [OPPTS 870.6200 (§81-8)]. This study can be upgraded by submission of (1) a report from the range-finding study, for verification of time of peak effect; (2) additional information regarding the test substance preparation and analysis, as described above.

B. STUDY DEFICIENCIES

Range-finding/time of peak effect study was described, but not submitted.

Submitted information regarding test substance preparation and analysis were not complete.

Positive control studies were cited by the author (report references, p. 31, dated 1993 and 1994). Although we will not require additional data to be submitted in support of this study, several issues regarding the cited data will need to be resolved as additional studies are received from this laboratory (see discussion, Appendix A).

APPENDIX A - POSITIVE CONTROL DATA

In their study report, Bayer cited positive control data from MRID No. 43656301 and 42770301. We were unable to locate any previous review of these studies; we have summarized our current findings below. The submitted studies were complete and well-conducted, the reports were clear and well-written, individual data were included, and the doses chosen appear to have been appropriate. Although we will not require that additional data be submitted in support of the current studies, several outstanding issues regarding the submitted positive control data will need to be resolved as additional studies are received from this laboratory. These issues are discussed below, following the study summaries.

Study Summaries

MRID 42770301

L.P Sheets, B.P. Stuart, and S.G. Lake (1993) Historical Control and Method Validation Studies in Rats for the Acute and Subchronic Neurotoxicity Screening Battery (Study Nos. listed below). Miles Inc., Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Unpublished.

This MRID consisted of a series of studies, performed between Sept. 1990 and Feb. 1992. A brief summary of each study is given below. The reports for each individual study were complete, including methods, results, and individual animal data (except as noted below). Although there was a list of study personnel in the overall introduction, the specific personnel involved in the individual studies were not named (except for pathologists, see below). In addition, although an SOP was provided for the Functional Observation Battery evaluations (FOB), footnotes in each study noted that the procedure described in the SOP differed 'slightly' from the procedure used in the study; the specific differences were not described (c.f. p. 32)

1. Study No. 90-992-HN. Acute Motor Activity Study with Untreated Rats (p. 21).

Motor activity was evaluated in untreated adult Sprague-Dawley rats (Sas:CD(SD)BF; Sasco, Inc., Houston, TX), 12/sex/group (9 weeks old at start of study), at several different time points. Five groups were tested at the following time points: (1) days 0, 1, 8, 15; (2) days 1, 8, 15, 22; (3) day 8; (4) day 15; or (5) day 22 after start of study. The purpose was to evaluate changes in activity with repeated testing, controlling for the age of animals at first test occasion. This study design also provided information regarding reproducibility of test results across groups of animals. Motor activity was evaluated for 90 minutes using figure 8 mazes with infrared emitter/detector pairs; uniform background noise (70 dB (A)) and uniform lighting were provided. Changes in motor activity were evaluated over the entire 90-minute period and for 10-minute subsessions; measured parameters were defined as motor activity (all beam breaks) or locomotor activity (non-consecutive beam breaks). Study results indicated that the motor activity measurements appeared reproducible and reliable, although there was a large amount of inter-individual variability. There appear to be some differences in activity levels associated with repeated testing (activity decreased more rapidly within the session), as well as differences

based on age and sex of subjects (late session activity remained higher in rats first tested at older ages; activity levels were generally higher in females).

These data were collected in September-October, 1990.

2. Study No. 90-992-IF. Subchronic FOB and Motor Activity Study with Untreated Rats (p. 29).

Motor activity and FOB parameters were evaluated in untreated Fischer 344 CDF (F-344)/Br1 rats (Sasco, Inc., Madison, WI), 12/sex/group (approximately 6 weeks old at start of study), at several time points. Four groups were tested as follows: (1) weeks 0, 4, 8, and 13; (2) week 4; (3) week 8; or (4) week 13 of study. As above, this design allowed evaluation of changes in activity or FOB results with repeated testing, as well as effects due to age of the animal. The design also provided information regarding reproducibility and reliability of test results. Motor activity was evaluated as described above: FOB procedures were included in an Appendix (see note above). Study results were similar to those noted above, demonstrating reliability of the procedures; changes in results with age of the animal were noted (differences in habituation patterns), but there was no apparent effect of repeated testing on motor activity (intervals between test repetitions were considerably longer in this subchronic study than in the acute study above). As animals were untreated, sensitivity of the procedures was not evaluated.

These data were collected from Nov. 1990-Feb. 1991

3. Study No. 90-912-IL. Acute Motor Activity Study with Triadimefon and Chlorpromazine (p. 41).

Motor activity was evaluated in male Sprague-Dawley rats (Sas:CD(SD)BF, Sasco, Inc., St. Louis, MO), 10/group (approximately 9 weeks old at start of study), following treatment with triadimefon (184 mg/kg, by gavage in PEG 400, at 90 min after dosing), chlorpromazine (1.8 mg/kg, i.p., at 1 hour after dosing), untreated control, or vehicle control (5 ml/kg PEG 400). Doses and times were chosen based on a pilot study. [Although the report states on p. 46 that 10/sex/group were tested, data were reported for males only, and subjects were listed as males only; the stated inclusion of both sex groups may have been an error.] Motor activity was evaluated according to the procedure described above. Activity levels were increased (approximately doubled) following triadimefon, and decreased (by approximately one-half) following chlorpromazine. The decrease following chlorpromazine was not statistically significant, probably due to large variance in results (see pp. 168-170); examination of individual data revealed considerable overlap across treatment groups (e.g. see p. 419).

These data were collected during April, 1991.

4. Study No. 91-992-KT. FOB and Neuropathology Study with Acrylamide (p. 52).

FOB and neuropathology were evaluated in male Sprague-Dawley rats (Sas:CD(SD)BF, Sasco, Inc., Madison, WI), 8/group (approximately 9 weeks old at start of study), following treatment with acrylamide (25 or 50 mg/kg, i.p., in saline [or saline control], 3 times/week for 3 weeks).

Doses were chosen based on a range-finding study. FOB evaluations were conducted weekly, prior to that day's treatment, using the same observer on all occasions; on day 14, 5 observers were used to evaluate inter-observer reliability [these data were not included in the report]. At study termination, rats were deeply anesthetized and sacrificed via intravascular perfusion (4% EM-grade formaldehyde and 4% glutaraldehyde in phosphate buffer). Sections were evaluated from six brain areas (coronal sections), and 3 levels of spinal cord, cauda equina, gasserian ganglion, optic nerves, and eyes. Listed tissues were embedded in glycolmethacrylate and sectioned at 2-3 μm. All tissues were stained with Methylene Blue-Basic Fuchsin, Luxol Fast Blue/Cresyl Violet, and Bielschowsky Silver Stain. Peripheral nerves (bilateral sciatic, tibial, and sural) were embedded in Epon, sectioned at 1 μm and stained with Toluidine Blue. Tissues from all dose levels were evaluated.

Ataxia and decreased body weight gain were observed at the high dose; additional effects on motor parameters were observed during FOB evaluations, again mainly at the high dose. There was also an increase in landing foot splay in treated animals (more pronounced on day 14, with only slight effects at the low dose). No effects were seen on grip strength at either dose or time point. Treatment-related neuropathological effects were demonstrated at 50 mg/kg (axonal degeneration, primarily in sciatic, tibial, and sural nerves), with some borderline changes at 25 mg/kg (the histopathology report was prepared by B.P. Stuart).

It should be noted that embedding and staining procedures used in this study were more extensive than called for in the guideline (or than used in the current studies), especially in that all tissues were plastic embedded. The group size was also larger, consisting of 8 animals/dose as opposed to six required in the guideline and evaluated in most studies. Since no changes were seen in grip strength, the sensitivity of this procedure was not demonstrated.

These data were collected during July-August 1991.

5. Study No. 91-962-LO. Neuropathology study with Trimethyltin (p. 63).

Neuropathology was evaluated in male Sprague-Dawley rats (Sasco, Inc., St. Louis, MO), 6/group (not more than 8 weeks of age at test substance administration), following treatment with a single dose of trimethyltin (12 mg/kg in saline, i.p.) or saline (1 ml/kg). The dose was selected based on a range-finding study. Two weeks following treatment, animals were sacrificed by intravascular perfusion as described above. Brain, spinal cord, eyes, bilateral peripheral nerves (sciatic, tibial, and sural), and gasserian ganglion were preserved. Cross-sections from six levels of brain, and cross- and longitudinal- sections from 3 levels of spinal cord were embedded in paraffin and stained with Hematoxylin and Eosin. Additional brain tissue, cauda equina, eyes and optic nerves, dorsal root ganglia, gasserian ganglion, and gastrocnemius muscle were embedded in glycol methacrylate and stained with Lee's stain (2-3 μm sections). Additional brain and spinal cord sections were stained with Luxol Fast Blue/Holmes Silver Nitrate. Peripheral nerves were embedded in Epon, sectioned at 1 μm and stained with Toluidine Blue.

The TMT concentration was not verified for this study -- concurrent analysis of the dosing solution yielded results that were stated in the report to be excessively high (see study report, p. 67). Preparation of duplicate dosing solutions using the same procedure yielded expected results.

Neuropathological findings were consistent with those expected following trimethyltin exposure (including neuronal necrosis in several brain regions, as well as some peripheral nerve degeneration). The histopathology report was prepared by Stephen Lake.

It should again be noted that the histopathological procedures described here vary from those in the current study (additional types of staining and embedding were used). Slides made using both glycolmethacrylate embedding and paraffin embedding were evaluated (only paraffin embedding was used in the current study), but it was not possible to determine, based on the submitted information, whether the same lesions were detected in both types of sections.

These data were collected during September-October, 1991.

6. Study No. 91-962-LR. Functional Observation Battery Study with Carbaryl (p. 71).

FOB was evaluated in male Sprague-Dawley rats (Sasco, Inc., St. Louis, MO), 6/group (approximately 8 weeks old at start of study), following treatment with carbaryl (15 or 30 mg/kg, i.p., in 2% Cremophor EL in saline) or saline (1 ml/kg, i.p.). FOB evaluations were performed 30-90 minutes after dosing (foot splay, grip strength, and body temperature were not evaluated). Doses were chosen based on a range-finding study. The report stated that FOB evaluations were performed by 6 technicians, to document inter-observer reliability; these data were not submitted.

Reported FOB findings were consistent with expected cholinergic effects, including muscle fasciculations and tremors, and gait abnormalities including ataxia and uncoordinated righting reflex.

These data were collected during October 1991-February 1992; the report stated that the testing was repeated on several occasions, but only one set of results was included in the report.

MRID 43665301

L.P. Sheets. (1994) A Motor Activity Historical Control and Method Validation Study Using Triadimefon and Chlorpromazine in Fischer 344 Rats. Study No. 93-992-WA; Miles Inc. Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Unpublished.

Motor activity was evaluated in adult male Fischer 344 rats (12/group) following acute administration of 2 mg/kg chlorpromazine (i.p. in saline) or 196 mg/kg triadimefon (by oral gavage in PEG 400). Controls were untreated or received 5 ml/kg PEG 400. Animals were not fasted prior to test substance administration. Motor activity was evaluated for 90 minutes using figure 8 mazes with infrared emitter/detector pairs; uniform background noise (70 dB (A)) and uniform lighting were provided. Changes in motor activity were evaluated over the entire 90-minute period and for 10-minute subsessions; measured parameters were defined as motor activity (all beam breaks) or locomotor activity (non-consecutive beam breaks). As expected, motor activity was increased (approximately 3-fold, $p<0.05$) following triadimefon administration and decreased following chlorpromazine administration (approximately 40%, n.s.

for total; statistically significant decreases were seen in some 10-min. subsessions). There were no differences in activity between PEG controls and untreated controls.

The study was conducted between 11/17/93 and 11/19/93.

Outstanding Issues

Our current policy regarding acceptable positive control data is as follows:

> Appropriate, adequate positive control data from the laboratories that performed the neurotoxicity studies should be provided to the Agency at the time of study submission. These positive control data should demonstrate the sensitivity of the procedures used, including the ability to detect both increases and decreases in parameters measured, as appropriate. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies.

In comparing the currently submitted positive control data to these criteria, we note the following:

1) The currently submitted tebuconazole study was performed in 1996-97. The majority of the submitted positive control studies were performed between 1990-1992; the only exception is the motor activity study in Wistar rats, performed in 1993. Although most of these studies appear to be outside the time frame described above (i.e. within the past few years), we will not require more recent studies be submitted prior to accepting the current studies. However, more recent studies will likely be required in the future.

2) Although both males and females were evaluated in the motor activity studies in control rats, all studies involving detection of changes following treatment were performed with male rats only. In order to validate the sensitivity of procedures to detect behavioral changes in females, female rats should be evaluated following treatment with appropriate positive control substances.

3) Personnel used in positive control studies should be the same as those used in submitted studies, to the extent possible. With the exception of the pathologists, personnel were not listed for the submitted positive control studies.

4) Since the footnotes in the study reports state that FOB procedures used in the studies were different from those described in the SOP, it is unclear whether the procedures used in the tebuconazole studies were similar to those used in the submitted positive control studies.

5) Changes in grip strength were not seen in any of the submitted studies, therefore the sensitivity of the procedure used for measuring grip strength has not been demonstrated.

6) Neuropathology procedures in the submitted positive control studies (numbers 5 and 6, above) were more extensive than those used in the tebuconazole studies, as described in the summary

section (above). Because results of the various types of staining used in the positive control studies were not presented separately, it is unclear whether the histopathology procedures used in the current study, without the additional procedures used in the positive control studies, are sufficiently sensitive to detect test material-related lesions. Studies used for validation of laboratory procedures should use the same procedures used in studies submitted in support of chemical registration.

7) Most of the studies (all those included in MRID 42770301) were performed in Sprague-Dawley rats. MRID 4366503, which included only motor activity testing, was performed in Fischer 344 rats. Since the current studies were performed using Fischer 344 rats, FOB and neuropathology procedures were not validated using the strain used in the current study.

8) In the studies evaluating changes in motor activity following treatment with chlorpromazine, the decrease in total motor activity was not statistically significant. Therefore, sensitivity of the test procedure to detect decreases in motor activity was not demonstrated.

Bayer should address these issues regarding the adequacy of the submitted positive control data in their future submissions.

DATA EVALUATION REPORT

TEBUCONAZOLE

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY - RAT
[OPPTS 870.6200 (§82-7)]
MRID 44588001

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Study - Rat OPPTS Number:[870.6200 [(§82-7)]

DP BARCODE: D257119
P.C. CODE: 128997

SUBMISSION CODE: S529953
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Tebuconazole, technical grade (96.7, 98.2% a.i.)

SYNONYMS: Folicur®; α-[2-(4-Chlorophenyl)ethyl-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol

CITATION: Sheets, L.P., Gilmore, R.G. (1998) A subchronic dietary neurotoxicity screening study with technical grade tebuconazole in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 96-472-GX, May 4, 1998. MRID 44588001. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorn Road, Kansas City, MO 64120-0013

EXECUTIVE SUMMARY: In a 90-day dietary neurotoxicity study (MRID 44588001), tebuconazole (Batch No.: 603-0013, 96.7%-98.2% purity) was administered to 12 Fischer 344 rats/sex/dose at dietary levels of 0, 100, 400, or 1600 ppm. Based on analytical measurements, doses were 0.00, 7.57, 29.2, and 107 mg/kg/day for males and 0.00, 8.81, 34.0, and 122 mg/kg/day for females. Functional observational battery (FOB) and motor activity tests were performed pretreatment and during weeks 4, 8, and 13. At the completion of the study, 6 rats/sex/dose group were subjected to perfusion; brain and nervous tissues were examined microscopically in high dose and control groups only.

No deaths occurred and there were no clinical signs attributable to treatment. Relative to controls, body weights of male and female rats in the group receiving 1600 ppm in the diet were statistically significantly reduced after one week, by 7% in males and 5% in females (both $p<0.05$). Lower body weights continued throughout the remainder of the study with reductions of 8% for males and 7% for females at study termination (both $p<0.05$). Food consumption was reduced throughout the study in this group, with average daily food consumption for males and females of 94% and 92% relative to respective controls. Food efficiency was reduced by 11% and 13% in high-dose males and high-dose females, respectively. Relative to controls, there

were no treatment-related effects on body weight or food consumption or efficiency in the other dietary groups.

No treatment-related effects were observed on FOB tests or motor activity tests. There were no gross or histopathological findings that could be attributed to treatment with tebuconazole.

A LOAEL was not attained in this study. The NOAEL is 1600 ppm in the diet (107 mg/kg/day in male rats and 122 mg/kg/day in female rats) based on treatment-related but toxicologically insignificant reductions in body weight, food intake, and food efficiency; the absence of effects in the FOB and motor activity tests; and the absence of histopathological lesions in the brain and nervous system.

This study is considered to be **Unacceptable/Guideline** and does not fulfill FIFRA guideline requirements for a subchronic neurotoxicity (90-day) study in rats [870.6200(§82-1b)] because a LOAEL was not identified, and the limit dose was not tested.

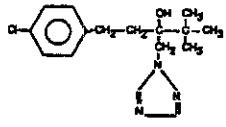
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Tebuconazole, technical grade (96.7%, 98.2%)

   Description: white powder
   Batch No.: 603-0013
   Purity: 96.7% a.i. (January 1996); 98.2% a.i. (September 1996)
   Stability of compound: stable at room temperature for 14 days; 36 days when frozen
   CAS #: 80443-41-0
   Structure:



2. Vehicle and/or positive control

   The vehicle was corn oil at 1% by weight of the diet. A small amount of acetone was used in the preparation process; the acetone was allowed to evaporate prior to exposure. The control diet was prepared in the same manner without the inclusion of the test substance.

3. Test animals

Species: rat
Strain: Fischer 344 CDF(F-344)
Age and weight at start of treatment: 8 weeks old; males: 143.5-183.8 g; females: 109.2-131.2 g
Source: Sasco, Inc., Madison, WI
Housing: individually in suspended stainless steel wire-mesh cages
Diet: Animals were fed Purina Mills Rodent Chow 5001-4 ("etts"), *ad libitum*; food was withheld during neurotoxicity testing
Water: Drinking water (tap) was available *ad libitum*.
Environmental conditions:
Temperature: 18-26°C
Humidity: 40-70%
Air changes: Not given
Photoperiod: 12 hour light/12 hour dark
Acclimation period: at least 6 days

B. STUDY DESIGN

1. In life dates

Start: May 13, 1996; end: August 14, 1996

2. Animal assignment

Animals were assigned to the test groups in Table 1 by means of a computerized randomization procedure (balanced by body weight) following selection for adequate body weight (20% of the mean within a sex) and freedom from clinical signs.

TABLE 1: Study design

Test group	Dietary Level (ppm)	Dose (mg/kg/day)		Number of animals	
		Male	Female	Male	Female
1 (Control)	0	0.00	0.00	12	12
2 (low-dose)	100	7.57	8.81	12	12
3 (mid-dose)	400	29.2	34.0	12	12
4 (high dose)	1600	107	122	12	12

Data taken from pp. 18 and 23, MRID 44588001.

3. Dose selection rationale

Doses were based on a previous 13-week oral toxicity study conducted with tebuconazole at concentrations of 0, 100, 400, and 1600 ppm in the diet and utilizing male and female rats (MRID 40700930). In that study, administration of 1600 ppm in the diet resulted in the death of one male and one female, reduced body weight gains of both sexes (8-10%), increased food consumption in both sexes (decreased in the

present study), and increased vacuolation in the adrenal cortex and a slight increase in hemosiderin accumulation of the spleen of females. Females in the 400 ppm dietary group had a lower body weight gain and increased vacuolation of the adrenal cortex. There were no clinical signs. The same dietary levels were chosen for the present study.

4. Test material preparation and analysis

Diets were prepared by dissolving the test material in a small amount of acetone and corn oil (1% by weight of the diet) and adding the mixture to the rodent chow. No further details on the mixing procedure/dietary preparation procedure were provided. The control diet was prepared in the same manner excluding the test material. The feed was available for *ad libitum* consumption for a period of one week. Fresh batches were prepared weekly. Samples to be analyzed for homogeneity were taken from the top, middle, and bottom of the mixing bowl for the 100 and 1600 ppm preparations (nine samples from each concentration). For stability analysis, a sample was taken from the 100 and 1600 ppm dietary preparations immediately after the mixing procedure. The sample was divided into two portions which were placed in the freezer for 7 days. After 7 days, one portion was taken from the freezer, stored at room temperature and sampled on days 7 (room temperature day 0), 8, 10, 14, 17, and 21. Samples were taken from the portion stored in the freezer on days 7, 14, 21, and 36. For concentration analyses, samples were taken from all concentrations at weeks 1, 2, 3, 7, 10, and 14. The active ingredient of the stock test material was measured in January and September, 1996.

Results -

Homogeneity analysis: The concentrations in the nine samples from the 100 ppm batch were top: 96.8, 81.3, and 90.7 ppm; middle: 86.9, 79.5, and 93.2 ppm; bottom: 81.5, 84.8, and 82.3 ppm. The mean value was 86.3±6.0 ppm with a CV of 7.0%. The concentrations in the nine samples from the 1600 ppm batch were top: 1450, 1485, and 1522 ppm; middle: 1534, 1464, and 1588 ppm; bottom: 1527, 1529, and 1505 ppm. The mean value was 1512±42 ppm; the CV was 2.7%.

Stability analysis: Samples from the 100 ppm concentration stored at room temperature (~22°C) and analyzed on days 0, 1, 3, 7, 10, and 14 contained 90.5, 84.4, 87.8, 79.9, 97.0, and 96.9 ppm, respectively. Based on a 106% method recovery, concentrations were 88.3-107% of the initial concentration. The lowest concentration was measured on sample day 7 and the highest on sample day 14. Samples from the 1600 ppm mixture, analyzed on the same respective days as above, contained 1765, 1734, 1709, 1373, 1538, and 1645 ppm. Recoveries were 77.8-98.2% of the initial concentration.

Following freezer storage, samples of the 100 ppm diet contained 95.6, 90.5, 87.2, 125, 112, and 113 ppm (91.2-131% of the initial corrected concentration). Samples of the 1600 ppm diet contained 1627, 1725, 1693, 1887, 1310, and 1817 ppm (80.5-116% of the initial concentration.

Concentration analysis: The concentrations in the 100, 400, and 1600 ppm dietary mixtures during weeks 1, 2, 3, 7, 10, and 14 ranged from 95.6-116 ppm, 332-441 ppm, and 1323-1595 ppm, respectively. Respective mean values were 104, 396, and 1459 ppm (104, 99.0, and 91.2% of nominal, respectively).

Based on the above results, the mixing procedure was adequate (CV of <10%), the test substance was stable for 14 days at room temperature and 36 days at -23 °C, and the mean concentrations in the 100, 400, and 1600 ppm test diet were 104, 369, and 1459 ppm, respectively.

5. Statistics

Statistical evaluations, which consisted primarily of analyses of variance (ANOVA), were performed with computerized programs. Continuous data in the FOB were analyzed by a repeated-measures ANOVA followed by a one-way ANOVA if there was a significant interaction between dose group and test week. For weeks in which there was a significant treatment effect, Dunnett's test was applied to determine which groups were significantly different from the control group. The categorical data from the FOB were analyzed using the General Linear Modeling (GLM) and Categorical Modeling (CATMOD) procedures followed by Dunnett's test and an analysis of contrasts, respectively.

ANOVAs were also applied to the motor and locomotor activity. Session activity data were analyzed with a repeated measures ANOVA followed by a one-way ANOVA if there was a significant interaction with test occasion. Dunnett's test was used to determine if there was a treatment-related effect between the control and a treatment group. Interval data were subjected to a two-way repeated-measures ANOVA, using both test interval and test occasion as the repeated measures, followed by a repeated measures ANOVA to determine on which weeks there was a significant treatment by interval interaction. For weeks with a significant treatment by interval interaction, the data for each interval were subjected to a one-way ANOVA to determine at which intervals there was a significant treatment effect. For those intervals, Dunnett's test was used to determine if there was a significant difference between the control and a treatment group.

C. METHODS

1. Observations

All animals were observed cage side twice daily (once daily on holidays and weekends) for clinical signs of toxicity and mortality. More detailed observations were carried out weekly.

2. Body weight

All animals were weighed prior to study initiation and weekly thereafter. Body weight changes were calculated from weekly weights.

3. Food consumption, food efficiency, and compound intake

Food consumption for each animal was determined weekly throughout the study period as g/animal/day. Mean feed consumption for each dose group was calculated as g/kg body weight/day (averaged over the duration of the study). Food efficiency was not calculated by the study authors. Food efficiency was calculated by the reviewer as [(g body weight gain/day)/(g food consumed/day)] x 100. Mean intake of tebuconazole (a.i.) for each dose group was calculated by the study authors as: actual dose level (ppm/1000) x mean feed consumption (g consumed/kg body weight/day).

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on all animals prior to treatment and during week 12. Following mydriasis, eyes were examined with a slit lamp microscope and with an indirect ophthalmoscope and a condensing lens. Animals with ophthalmological defects were not used.

5. Functional observational battery (FOB)

All animals/sex/group were subjected to a baseline FOB during the week prior to treatment and during weeks 4, 8, and 13. Animals were allowed to acclimate to the test room for one day. Sets of eight animals were evaluated individually; animals were selected in a semi-random manner. Testing was staggered over two days for each sex. Approximately 1 to 2.5 hours after conclusion of the FOB, all eight rats were tested in the figure-eight motor activity mazes. The following FOB observations were performed by a trained technician who was blind to the treatment of the animals (a second technician measured grip strength, foot splay, righting, and body temperature):

a. Home cage observations

Posture, piloerection, gait abnormalities, involuntary motor movements, vocalizations, and any abnormal activity.

b. Observations during handling

Ease of removal from cage, reaction to handling, muscle tone, palpebral closure, pupil size, pupil response to light, lacrimation, salivation, stains, and other abnormal signs.

c. Open field observations

Piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behavior, gait abnormalities, vocalizations, arousal, rearing, defecation, and urination.

d. Sensorimotor and neuromuscular measurements

Approach response, auditory response, tail pinch response, righting reflex, grip strength, and landing foot splay.

e. Other

Body weight and body temperature.

Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints and increases and decreases in activity were mentioned in the study report but were not provided.

6. Motor activity

Motor activity was measured following each FOB session. Motor activity was measured for 90 minutes in one of eight automated, computerized figure-eight mazes. Background noise was generated at approximately 70dB (broad spectrum) throughout testing, and uniform light intensity (100 ± 70 Lux) was also maintained. Motor activity was measured as the number of beam interruptions that occurred during each of nine 10-minute sessions. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test sessions. Data were presented on total activity over the 90-minute sessions and activity for each of the 10-minute intervals. Animals were tested in a semi-random order and testing was staggered over two days for each sex. Males and females were tested on separate days.

7. Sacrifice and pathology

All animals on study were given a complete gross necrospy. The first six animals/sex/dose were selected for perfusion and collection of tissues. These animals were anesthetized with an intraperitoneal dose of sodium pentobarbital and perfused via the left ventricle with a sodium nitrite in phosphate buffer flush followed by in situ fixation with Universal fixative in phosphate buffer. The entire brain and spinal cord; both eyes with optic nerves; peripheral nerves (bilateral) - sciatic, tibial, and sural; the gasserian ganglion; and gastrocnemius muscle were dissected and post-fixed (10% buffered formalin). The brain was weighed prior to post-fixing and the brain:body weight ratio calculated. The following tissues from control and high-dose animals were examined microscopically: 1) eight coronal sections of the brain, four levels of the spinal cord (cervical, thoracic, lumbar [cross and longitudinal sections], and cauda equina [longitudinal sections only]) [embedded in paraffin and stained with

hematoxylin and eosin; additional sections were stained with Luxol Fast Blue/Cresyl Violet and Sevier-Munger stains]; 2) dorsal root ganglia, gasserian ganglion, eyes, optic nerves, gastrocnemius muscle [embedded in glycol methacrylate, sectioned at 2-3μm and stained with a modified Lee's stain]; 3) peripheral nerves [embedded in epoxy resin, sectioned at 1 μm, and stained with toluidine blue], and any gross lesions. The remaining animals that survived to term were sacrificed by carbon dioxide asphyxiation; additional tissues were not collected.

8. Positive Control Data

Positive control studies were cited by the author (report references, pp. 28-29, dated 1993 and 1994). Although we will not require additional data to be submitted in support of this study, several issues regarding the cited data will need to be resolved as additional studies are received from this laboratory (see discussion, Appendix A).

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

Clinical signs consisted of bloody feces for one male in the 400 ppm treatment group (day 25, inferred from bedding material), red lacrimation in one female in the control group (#0108), red lacrimation and red lacrimation stain in one female in the 100 ppm group (#1112) and two females in the 400 ppm group (#2105 and 2111), and clear lacrimation (one occasion), red lacrimation and red lacrimation stain in two females in the 1600 ppm group (#3101 and 3104). The lacrimation signs appeared after 60 days of treatment and did not appear to be treatment related. No mortalities occurred in any test group.

B. BODY WEIGHT AND WEIGHT GAIN

Beginning with the first week of dietary administration, the group mean body weights of males and females in the 1600 ppm group were statistically significantly lower than the respective control values (7% in males and 5% in females; both $p<0.05$) (Table 2). This trend continued (with only a slight further reduction) for most weeks for males and all weeks for females. By day 91 the mean body weight of male rats in the 1600 ppm group was 92% of the control value ($p<0.05$) and the mean body weight of female rats in the 1600 ppm group was 93% of the control value ($p<0.05$). Body weight gains for males and females in this group were 84 and 80% of the respective control values. The body weights and body weight gains of the other groups were unaffected by treatment.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

Weekly food consumption (g/animal/day) was calculated for each dietary group by the study authors. By day 7, food consumption of males and females in the 1600 ppm group was reduced by 7% and 14% relative to the respective control values ($p<0.05$) (data not shown). During the last week of the study, the reductions were 7% for males and 6% for females. Average daily food consumption/animal for males and females was calculated by the reviewer as the mean of the 13 week daily means (Table 2). Average daily food

consumption was reduced for males and females in the 1600 ppm group compared with the respective control groups, by 6% for males and 8% for females. Food consumption was not affected in the other dose groups. Mean food consumption normalized to body weight was not affected in any group. Values for males in the control and 1600 ppm groups were 72.4 and 73.1 g/kg body weight. Respective values for females were 85.5 and 83.4 g/kg body weight. Food efficiency values were slightly reduced in a dose-dependent manner for both males and females (Table 2).

TABLE 2. Group mean body weights (g) for selected days, total body weight gains (g), average daily food consumption (g/animal/day), mean food consumption (g/kg body weight/day), and overall food efficiency (%) of rats administered tebuconazole for 90 days[a]

Day of study	Dietary administration (ppm)			
	0	100	400	1600
Males				
0	169.0±7.2	168.3±10.9	166.0±9.1	168.4±7.6
7	198.7±9.4	197.2±12.2	189.9±10.6	185.7±10.9*
42	284.6±16.3	284.3±21.2	273.0±13.0	266.4±13.8*
91	341.1±17.2	339.2±28.8 (99)	329.4±17.7 (97)	313.3±17.2 (92)*
Total weight gain, days 0-91[b]	172.1	170.9 (99)	163.4 (95)	144.9 (84)
Average daily food consumption, days 0-91[b]	19.46	19.47 (100)	19.07 (98)	18.38 (94)
Mean food consumption/ body weight, days 0-91	72.4±1.2	72.8±1.1	73.6±1.1	73.1±1.1
Food efficiency, days 0-91[c]	9.72	9.65	9.42	8.66
Females				
0	117.6±5.6	119.3±5.7	118.7±6.2	119.4±4.6
7	130.6±5.6	130.4±6.0	128.2±5.7	124.1±5.4*
42	170.5±4.9	168.6±10.8	169.5±7.5	159.1±6.5*
91	192.7±6.5	192.0±12.7 (100)	191.1±8.7 (99)	179.7±10.4 (93)*
Total weight gain, days 0-91[b]	75.1	72.7 (97)	72.4 (96)	60.3 (80)
Average daily food consumption, days 0-91[b]	14.02	13.81 (99)	14.00 (100)	12.90 (92)
Mean food consumption/ body weight, days 0-91	85.5±1.0	84.7±1.0	85.9±1.0	83.4±0.8
Food efficiency, days 0-91[c]	5.89	5.78	5.68	5.14

Data taken from Tables 2, 3, and 4, pp. 31-33, 35; MRID 44588001. Values represent mean±s.d. (s.e. for mean food consumption); n=12 for all groups and time points.

[a] Percent of control value in parenthesis.

[b] Calculated by the reviewer.

[c] Calculated by the reviewer: (g total body weight gain/91 days)/(g total food consumption) x 100.

*Statistically significant, ANOVA, $p<0.05$.

In the 1600 ppm group, food efficiency was reduced by 11% in males and 13% in females relative to respective control values.

Compound intake, based on dietary concentrations, was calculated by the study authors; values are presented in Table 1.

E. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

The clinical signs of lacrimation and lacrimation stains observed during the clinical observations (section A) were also recorded during the FOB. Single occurrences of a slightly impaired righting response were observed in a small number of animals, both males and females, in the control and treatment groups (one low-, mid-, and high-dose male and one control and low-dose female). This effect was considered unrelated to treatment. Treatment with tebuconazole had no affect on hindlimb or forelimb grip strength or on footsplay for either sex.

F. MOTOR AND LOCOMOTOR ACTIVITY

Motor activity in males ranged from 19% greater than the control value (week 8, 1600 ppm) to -14% (week 4, 400 ppm and week 13, 100 ppm). Data for females were even more variable, ranging from 27% greater than controls (week 4, 100 ppm) to -23% (week 8, 1600 ppm). None of the values were statistically significantly increased or decreased relative to control values. These results (Table 3) do not show trends for either sex, either dose-associated or across time, and reflect the inherent variability of these types of tests. The authors cite changes of <20% as within the range of normal variability for groups of 12 rats/sex/group. It should be noted that motor activity for the treatment groups during the pretreatment week ranged from 3 to 13% greater than the control value for males and from 9% less to 3% greater than the control value for females. Data for female rats showed greater motor activity and greater variability than for males. Examination of the data also shows that motor activity was similar across time for each group. Examination of the interval data, presented graphically, indicated a similar decline in activity with succeeding intervals for all groups at each test week. Activity was generally minimal by the fifth interval for males and the sixth interval for females, indicating similar habituation. Therefore, administration of tebuconazole in the diet did not affect motor activity, either positively or negatively.

Locomotor activity followed similar trends with comparable activity among control and treated males; females showed more variability but differences from control values were in both directions and statistical significance was not attained during any test. Interval activity did not differ among groups for any week.

G. OPHTHALMOSCOPIC EXAMINATION (after FOB and MA)

No treatment-related ophthalmologic findings were observed; corneal opacity was seen in two control males and one 100 ppm male, a corneal scar was seen in one control male, and retinal degeneration was seen in one 400 ppm female.

TABLE 3. Motor and locomotor activity of rats administered tebuconazole in the diet for 90 days

Parameter	Dietary administration (ppm)			
	0	100	400	1600
Males				
Motor activity				
pretreatment	550 (125)	602 (203)	620 (191)	564 (177)
week 4	601 (154)	623 (171)	514 (127)	556 (215)
week 8	596 (139)	578 (112)	651 (161)	711 (220)
week 13	486 (113)	420 (117)	529 (154)	546 (203)
Locomotor activity				
pretreatment	225 (50)	230 (55)	245 (72)	212 (59)
week 4	200 (47)	225 (71)	182 (60)	197 (75)
week 8	235 (63)	215 (52)	237 (64)	243 (76)
week 13	195 (47)	174 (63)	208 (70)	212 (78)
Females				
Motor activity				
pretreatment	835 (288)	860 (187)	884 (145)	759 (166)
week 4	863 (489)	1094 (534)	959 (445)	683 (311)
week 8	959 (319)	1003 (338)	916 (201)	741 (151)
week 13	778 (347)	962 (264)	916 (258)	779 (292)
Locomotor activity				
pretreatment	313 (131)	344 (98)	342 (62)	292 (62)
week 4	303 (203)	395 (203)	350 (173)	230 (115)
week 8	335 (111)	363 (140)	332 (80)	253 (53)
week 13	273 (122)	356 (104)	310 (92)	267 (125)

Data taken from Tables 7 and 8, pages 68-71, MRID 44588001. Values represent mean (s.d.); n=12 for all groups and time points.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

Mean terminal body weights of treated males and females in the high-dose group were 9 and 3% below control weights, respectively (Table 4). Absolute brain weights in this group were not affected by treatment, but relative brain weights were increased, by 110% ($p<0.05$) and 105% (not statistically significant) in males and females, respectively. The significant increase in relative brain weight of males is attributable to the lower body weight relative to controls.

2. Gross pathology

Gross examination revealed no treatment-related lesions.

TABLE 4. Terminal body weights and brain weights of rats administered tebuconazole in the diet for 90 days[a]

Terminal body and organ weights	Dietary administration (ppm)			
	0	100	400	1600
Males				
Body weight (g)	337.2±16.7	346.4±22.6	339.3±19.9	308.3±14.8 (91)*
Brain weight (g)	1.758±0.064	1.774±0.127	1.791±0.098	1.763±0.056 (100)
Relative brain weight (%)	0.523±0.038	0.513±0.034	0.529±0.034	0.573±0.022 (110)*
Females				
Body weight (g)	194.1±7.4	195.9±16.7	195.9±11.9	187.4±12.4 (97)
Brain weight (g)	1.736±0.054	1.694±0.083	1.792±0.089	1.757±0.102 (101)
Relative brain weight (%)	0.895±0.042	0.870±0.081	0.916±0.030	0.941±0.085 (105)

Data taken from Table OWIK SUM, pages 343 and 344, MRID 44588001. Values represent mean±s.d.; n=6 for all groups and time points.
[a] Values in parentheses are percent of control value.
*Statistically significant, ANOVA, $p<0.05$

3. Microscopic pathology

There were no microscopic lesions in the high-dose animals that were attributable to treatment. The lower dose animals were not examined. Background lesions occurring at similar frequency and severity in controls and high dose animals included axonal swelling or nerve degeneration in the brain (primarily at levels 4, 5, and 7), retinal degeneration in two control males and one high dose female, and axonal swelling and nerve fiber degeneration in the spinal cord.

III. DISCUSSION

A. DISCUSSION

Male and female Fischer 344 rats were administered tebuconazole in the diet for 90 days at concentrations of 0, 100, 400, or 1600 ppm. Based on analytical measurements, the actual doses were 0.00, 7.57, 29.2, and 107 mg/kg/day for males and 0.00, 8.81, 34.0, and 122 mg/kg/day for females. Although lacrimation was observed in small numbers of rats late in the study, the small number of animals affected and the lack of dose-response indicates that this sign is probably not treatment related. Relative to controls, decreased food consumption and related lower body weight gains occurred for both males and females in the 1600 ppm dietary group and were evident by the end of the first week of treatment. At study termination, reductions in body weight of 8% and 7% for males and females, respectively, in this group were statistically significant ($p<0.05$). Food efficiency in this group was slightly lowered in both sexes. In the absence of other signs, the decreased food intake and resulting lower body weights may be attributable to the lack of palatability of the feed. There was no effect on body weight, food consumption, or food efficiency in the lower dose groups. The body weight decreases in male and females rats of 8% and 7%, respectively, are treatment related but not toxicologically significant.

There were no compound-related effects on any parameters in the FOB at any dietary level. Likewise, there were no compound related effects on motor or locomotor activity at any dietary level. Brain weight was not affected by treatment and there were no gross or microscopic lesions that could be attributed to treatment.

Because the body weight reductions at the highest dietary concentration were not biologically significant and there were no effects on neurotoxicity tests or nervous tissues or organs at the highest tested concentration, a LOAEL was not attained in this study. The NOAEL is 1600 ppm in the diet (107 mg/kg/day in male rats and 122 mg/kg/day in female rats), the highest concentration tested.

In the dose selection rationale section, the study authors refer to increased food consumption in males and females in the 1600 ppm group in a similar subchronic study (MRID 40700930). The dietary concentrations in the present study were the same as in the earlier subchronic study in which the deaths of one male and one female rat in the 1600 ppm group were attributed to compound-related toxicity (effects on the adrenal glands were also observed at the 1600 ppm dietary level in that study). Similar effects did not occur at 1600 ppm in the current study, and in fact the achieved doses at 1600 ppm in the current study (107 and 122 mg/kg/day for males and females, respectively) were considerably lower than those achieved in the previous study (171.7 and 235.2 mg/kg/day for males and females, respectively). The reasons for this difference in intake cannot be specifically identified, but it should be noted that the previous study was performed more than 10 years ago (report dated 1986), using a different testing facility (Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, FRG), and a different strain of rats (Wistar [BOR:WISW(SPF Cpb)), that were younger at study initiation (6 weeks). Given these differences between the design of the previous and current study, we find that dosing for the current study should have been higher. However, since adverse effects have been seen at lower doses in other studies (including a developmental neurotoxicity study), the information that could be obtained from repeating this study at higher doses does not appear critical at this time. If the information is determined to be critical for future data needs, this study may need to be repeated at a later date, using higher dose levels.

A LOAEL was not attained in this study. The NOAEL is 1600 ppm in the diet (107 mg/kg/day in male rats and 122 mg/kg/day in female rats) based on treatment-related but toxicologically insignificant reductions in body weight, food intake, and food efficiency; the absence of effects in the FOB and motor activity tests; and the absence of histopathological lesions in the brain and nervous system.

This study is considered to be **Unacceptable/Guideline** and does not fulfill FIFRA guideline requirements for a subchronic neurotoxicity (90-day) study in rats [870.6200(§82-7)] because a LOAEL was not identified, and the limit dose was not tested.

B. STUDY DEFICIENCIES

Positive control studies were cited by the author (report references, pp. 28-29, dated 1993 and 1994). Although we will not require additional data to be submitted in support of this study, several issues regarding the cited data will need to be resolved as additional studies are received from this laboratory (see discussion, Appendix A).

APPENDIX A - POSITIVE CONTROL DATA

In their study report, Bayer cited positive control data from MRID No. 43656301 and 42770301. We were unable to locate any previous review of these studies; we have summarized our current findings below. The submitted studies were complete and well-conducted, the reports were clear and well-written, individual data were included, and the doses chosen appear to have been appropriate. Although we will not require that additional data be submitted in support of the current studies, several outstanding issues regarding the submitted positive control data will need to be resolved as additional studies are received from this laboratory. These issues are discussed below, following the study summaries.

Study Summaries

MRID 42770301

L.P Sheets, B.P. Stuart, and S.G. Lake (1993) Historical Control and Method Validation Studies in Rats for the Acute and Subchronic Neurotoxicity Screening Battery (Study Nos. listed below). Miles Inc., Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Unpublished.

This MRID consisted of a series of studies, performed between Sept. 1990 and Feb. 1992. A brief summary of each study is given below. The reports for each individual study were complete, including methods, results, and individual animal data (except as noted below). Although there was a list of study personnel in the overall introduction, the specific personnel involved in the individual studies were not named (except for pathologists, see below). In addition, although an SOP was provided for the Functional Observation Battery evaluations (FOB), footnotes in each study noted that the procedure described in the SOP differed 'slightly' from the procedure used in the study; the specific differences were not described (c.f. p. 32)

1. Study No. 90-992-HN. Acute Motor Activity Study with Untreated Rats (p. 21).

Motor activity was evaluated in untreated adult Sprague-Dawley rats (Sas:CD(SD)BF; Sasco, Inc., Houston, TX), 12/sex/group (9 weeks old at start of study), at several different time points. Five groups were tested at the following time points: (1) days 0, 1, 8, 15; (2) days 1, 8, 15, 22; (3) day 8; (4) day 15; or (5) day 22 after start of study. The purpose was to evaluate changes in activity with repeated testing, controlling for the age of animals at first test occasion. This study design also provided information regarding reproducibility of test results across groups of animals. Motor activity was evaluated for 90 minutes using figure 8 mazes with infrared emitter/detector pairs; uniform background noise (70 dB (A)) and uniform lighting were provided. Changes in motor activity were evaluated over the entire 90-minute period and for 10-minute subsessions; measured parameters were defined as motor activity (all beam breaks) or locomotor activity (non-consecutive beam breaks). Study results indicated that the motor activity measurements appeared reproducible and reliable, although there was a large amount of inter-individual variability. There appear to be some differences in activity levels associated with repeated testing (activity decreased more rapidly within the session), as well as differences based on age and sex of subjects (late session activity remained higher in rats first tested at older ages; activity levels were generally higher in females).

These data were collected in September-October, 1990.

2. Study No. 90-992-IF. Subchronic FOB and Motor Activity Study with Untreated Rats (p. 29).

Motor activity and FOB parameters were evaluated in untreated Fischer 344 CDF (F-344)/Br1 rats (Sasco, Inc., Madison, WI), 12/sex/group (approximately 6 weeks old at start of study), at several time points. Four groups were tested as follows: (1) weeks 0, 4, 8, and 13; (2) week 4; (3) week 8; or (4) week 13 of study. As above, this design allowed evaluation of changes in activity or FOB results with repeated testing, as well as effects due to age of the animal. The design also provided information regarding reproducibility and reliability of test results. Motor activity was evaluated as described above: FOB procedures were included in an Appendix (see note above). Study results were similar to those noted above, demonstrating reliability of the procedures; changes in results with age of the animal were noted (differences in habituation patterns), but there was no apparent effect of repeated testing on motor activity (intervals between test repetitions were considerably longer in this subchronic study than in the acute study above). As animals were untreated, sensitivity of the procedures was not be evaluated.

These data were collected from Nov. 1990-Feb. 1991

3. Study No. 90-912-IL. Acute Motor Activity Study with Triadimefon and Chlorpromazine (p. 41).

Motor activity was evaluated in male Sprague-Dawley rats (Sas:CD(SD)BF, Sasco, Inc., St. Louis, MO), 10/group (approximately 9 weeks old at start of study), following treatment with triadimefon (184 mg/kg, by gavage in PEG 400, at 90 min after dosing), chlorpromazine (1.8 mg/kg, i.p., at 1 hour after dosing), untreated control, or vehicle control (5 ml/kg PEG 400). Doses and times were chosen based on a pilot study. [Although the report states on p. 46 that 10/sex/group were tested, data were reported for males only, and subjects were listed as males only; the stated inclusion of both sex groups may have been an error.] Motor activity was evaluated according to the procedure described above. Activity levels were increased (approximately doubled) following triadimefon, and decreased (by approximately one-half) following chlorpromazine. The decrease following chlorpromazine was not statistically significant, probably due to large variance in results (see pp. 168-170); examination of individual data revealed considerable overlap across treatment groups (e.g. see p. 419).

These data were collected during April, 1991.

4. Study No. 91-992-KT. FOB and Neuropathology Study with Acrylamide (p. 52).

FOB and neuropathology were evaluated in male Sprague-Dawley rats (Sas:CD(SD)BF, Sasco, Inc., Madison, WI), 8/group (approximately 9 weeks old at start of study), following treatment with acrylamide (25 or 50 mg/kg, i.p., in saline [or saline control], 3 times/week for 3 weeks). Doses were chosen based on a range-finding study. FOB evaluations were conducted weekly, prior to that day's treatment, using the same observer on all occasions; on day 14, 5 observers were used to evaluate inter-observer reliability [these data were not included in the report]. At study termination, rats were deeply anesthetized and sacrificed via intravascular perfusion (4% EM-grade formaldehyde and 4% glutaraldehyde in phosphate buffer). Sections were evaluated from six brain areas (coronal sections), and 3 levels of spinal cord, cauda equina, gasserian ganglion, optic nerves, and eyes. Listed tissues were embedded in glycolmethacrylate and sectioned at 2-3 μm. All tissues were stained with Methylene Blue-Basic Fuchsin, Luxol Fast Blue/Cresyl Violet, and Bielschowsky Silver Stain. Peripheral nerves (bilateral sciatic, tibial,

and sural) were embedded in Epon, sectioned at 1 μm and stained with Toluidine Blue. Tissues from all dose levels were evaluated.

Ataxia and decreased body weight gain were observed at the high dose; additional effects on motor parameters were observed during FOB evaluations, again mainly at the high dose. There was also an increase in landing foot splay in treated animals (more pronounced on day 14, with only slight effects at the low dose). No effects were seen on grip strength at either dose or time point. Treatment-related neuropathological effects were demonstrated at 50 mg/kg (axonal degeneration, primarily in sciatic, tibial, and sural nerves), with some borderline changes at 25 mg/kg (the histopathology report was prepared by B.P. Stuart).

It should be noted that embedding and staining procedures used in this study were more extensive than called for in the guideline (or than used in the current studies), especially in that all tissues were plastic embedded. The group size was also larger, consisting of 8 animals/dose as opposed to six required in the guideline and evaluated in most studies. Since no changes were seen in grip strength, the sensitivity of this procedure was not demonstrated.

These data were collected during July-August 1991.

5. Study No. 91-962-LO. Neuropathology study with Trimethyltin (p. 63).

Neuropathology was evaluated in male Sprague-Dawley rats (Sasco, Inc., St. Louis, MO), 6/group (not more than 8 weeks of age at test substance administration), following treatment with a single dose of trimethyltin (12 mg/kg in saline, i.p.) or saline (1 ml/kg). The dose was selected based on a range-finding study. Two weeks following treatment, animals were sacrificed by intravascular perfusion as described above. Brain, spinal cord, eyes, bilateral peripheral nerves (sciatic, tibial, and sural), and gasserian ganglion were preserved. Cross-sections from six levels of brain, and cross- and longitudinal- sections from 3 levels of spinal cord were embedded in paraffin and stained with Hematoxylin and Eosin. Additional brain tissue, cauda equina, eyes and optic nerves, dorsal root ganglia, gasserian ganglion, and gastrocnemius muscle were embedded in glycol methacrylate and stained with Lee's stain (2-3 μm sections). Additional brain and spinal cord sections were stained with Luxol Fast Blue/Holmes Silver Nitrate. Peripheral nerves were embedded in Epon, sectioned at 1 μm and stained with Toluidine Blue.

The TMT concentration was not verified for this study -- concurrent analysis of the dosing solution yielded results that were stated in the report to be excessively high (see study report, p. 67). Preparation of duplicate dosing solutions using the same procedure yielded expected results.

Neuropathological findings were consistent with those expected following trimethyltin exposure (including neuronal necrosis in several brain regions, as well as some peripheral nerve degeneration). The histopathology report was prepared by Stephen Lake.

It should again be noted that the histopathological procedures described here vary from those in the current study (additional types of staining and embedding were used). Slides made using both glycolmethacrylate embedding and paraffin embedding were evaluated (only paraffin embedding was used in the current study), but it was not possible to determine, based on the submitted information, whether the same lesions were detected in both types of sections.

These data were collected during September-October, 1991.

6. Study No. 91-962-LR. Functional Observation Battery Study with Carbaryl (p. 71).

FOB was evaluated in male Sprague-Dawley rats (Sasco, Inc., St. Louis, MO), 6/group (approximately 8 weeks old at start of study), following treatment with carbaryl (15 or 30 mg/kg, i.p., in 2% Cremophor EL in saline) or saline (1 ml/kg, i.p.). FOB evaluations were performed 30-90 minutes after dosing (foot splay, grip strength, and body temperature were not evaluated). Doses were chosen based on a range-finding study. The report stated that FOB evaluations were performed by 6 technicians, to document inter-observer reliability; these data were not submitted.

Reported FOB findings were consistent with expected cholinergic effects, including muscle fasciculations and tremors, and gait abnormalities including ataxia and uncoordinated righting reflex.

These data were collected during October 1991-February 1992; the report stated that the testing was repeated on several occasions, but only one set of results was included in the report.

MRID 43665301

L.P. Sheets. (1994) A Motor Activity Historical Control and Method Validation Study Using Triadimefon and Chlorpromazine in Fischer 344 Rats. Study No. 93-992-WA; Miles Inc. Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Unpublished.

Motor activity was evaluated in adult male Fischer 344 rats (12/group) following acute administration of 2 mg/kg chlorpromazine (i.p. in saline) or 196 mg/kg triadimefon (by oral gavage in PEG 400). Controls were untreated or received 5 ml/kg PEG 400. Animals were not fasted prior to test substance administration. Motor activity was evaluated for 90 minutes using figure 8 mazes with infrared emitter/detector pairs; uniform background noise (70 dB (A)) and uniform lighting were provided. Changes in motor activity were evaluated over the entire 90-minute period and for 10-minute subsessions; measured parameters were defined as motor activity (all beam breaks) or locomotor activity (non-consecutive beam breaks). As expected, motor activity was increased (approximately 3-fold, $p<0.05$) following triadimefon administration and decreased following chlorpromazine administration (approximately 40%, n.s. for total; statistically significant decreases were seen in some 10-min. subsessions). There were no differences in activity between PEG controls and untreated controls.

The study was conducted between were 11/17/93 and 11/19/93.

Outstanding Issues

Our current policy regarding acceptable positive control data is as follows:

> Appropriate, adequate positive control data from the laboratories that performed the neurotoxicity studies should be provided to the Agency at the time of study submission. These positive control data should demonstrate the sensitivity of the procedures used, including the ability to detect both increases and decreases in parameters measured, as appropriate. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies.

In comparing the currently submitted positive control data to these criteria, we note the following:

1) The currently submitted tebuconazole study was performed in 1996-97. The majority of the submitted positive control studies were performed between 1990-1992; the only exception is the motor activity study in Wistar rats, performed in 1993. Although most of these studies appear to be outside the time frame described above (i.e. within the past few years), we will not require more recent studies be submitted prior to accepting the current studies. However, more recent studies will likely be required in the future.

2) Although both males and females were evaluated in the motor activity studies in control rats, all studies involving detection of changes following treatment were performed with male rats only. In order to validate the sensitivity of procedures to detect behavioral changes in females, female rats should be evaluated following treatment with appropriate positive control substances.

3) Personnel used in positive control studies should be the same as those used in submitted studies, to the extent possible. With the exception of the pathologists, personnel were not listed for the submitted positive control studies.

4) Since the footnotes in the study reports state that FOB procedures used in the studies were different from those described in the SOP, it is unclear whether the procedures used in the tebuconazole studies were similar to those used in the submitted positive control studies.

5) Changes in grip strength were not seen in any of the submitted studies, therefore the sensitivity of the procedure used for measuring grip strength has not been demonstrated.

6) Neuropathology procedures in the submitted positive control studies (numbers 5 and 6, above) were more extensive than those used in the tebuconazole studies, as described in the summary section (above). Because results of the various types of staining used in the positive control studies were not presented separately, it is unclear whether the histopathology procedures used in the current study, without the additional procedures used in the positive control studies, are sufficiently sensitive to detect test material-related lesions. Studies used for validation of laboratory procedures should use the same procedures used in studies submitted in support of chemical registration.

7) Most of the studies (all those included in MRID 42770301) were performed in Sprague-Dawley rats. MRID 4366503, which included only motor activity testing, was performed in Fischer 344 rats. Since the current studies were performed using Fischer 344 rats, FOB and neuropathology procedures were not validated using the strain used in the current study.

8) In the studies evaluating changes in motor activity following treatment with chlorpromazine, the decrease in total motor activity was not statistically significant. Therefore, sensitivity of the test procedure to detect decreases in motor activity was not demonstrated.

Bayer should address these issues regarding the adequacy of the submitted positive control data in their future submissions.

DATA EVALUATION RECORD

TEBUCONAZOLE

Study Type (§83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 2-01-72 A (MRID 45074301)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

TEBUCONAZOLE

Developmental Neurotoxicity (83-6[a])

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Stephen C. Dapson for

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study-rats

OPPTS Number: 870.6300

OPP Guideline Number: §83-6a

DP BARCODE: D264727
P.C. CODE: 128997

SUBMISSION CODE: S577006
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Tebuconzaole (96-96.9% a.i.)

SYNONYMS: H-1,2,4-triazole-1-ethanol *a*-[2-(4-chlorophenyl)-ethyl]-*a*-(1,1-dimethylethyl)-±(CAS)

CITATION: Parker, R.M., (2000) Developmental Neurotoxicity Study of Technical Grade Tebuconazole Administered Orally via Diet to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats. Primedica Argus Research Laboratories, Inc., Horsham, PA. Lab. Study No. 1702-004; Sponsor's Study Number 98-C612-QU, March 1, 2000. MRID 45074301. Unpublished.

SPONSOR: Bayer Corporation, Kansas City, MO

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45074301), tebuconazole (96-96.9% a.i.; Lot/Batch #603-0013) in corn oil was administered via the diet to pregnant Crl:CD®BR VAF/Plus® (Sprague Dawley) rats (25/dose) from gestation day (GD) 6 to lactation day (LD) 11 at doses of 0, 100, 300 or 1000 ppm (equivalent to [GD/LD] 0/0, 8.8/16.3, 22.0/41.3, and 65.0/125.4 mg/kg/day). No analytical data were provided. P dams were allowed to deliver naturally. On day 5 postpartum, litters were standardized to a maximum of 10 pups/litter. Pups were assigned to one of 5 Subsets (20 pups/sex/dose in each subset). Physical development landmarks were evaluated for all subsets (including surface righting, eye opening, pinna unfolding, acoustic startle response, and pupil constriction); sexual maturation was evaluated in subsets 2-4. Subset 1 pups were sacrificed on postnatal day 12; brains were weighed for all Subset 1 pups, and histopathological evaluations were performed on 6/sex in control and high dose groups (morphometric analysis was performed on 6/sex in control, mid-dose, and high

dose groups). Subset 2 pups were evaluated for learning and memory on day 23-25 (passive avoidance) and on day 59-63 (Water M-maze). Subset 3 pups were evaluated for motor activity (days 14, 18, 22, and 62) and for auditory startle habituation (days 23 and 63). Subset 4 pups received detailed weekly clinical evaluations. In addition, 6 animals/sex/group in Subset 4 were selected for neuropathological evaluations; brains were weighed and the high dose and control animals were evaluated histopathologically on day 83 (morphometric analysis was performed on 6/sex in control, mid dose, and high dose groups). Subset 5 pups were sacrificed and necropsied on day 22.

At 1000 ppm, two P females died as a result of prolonged gestation. Body weights were slightly decreased ($p \leq 0.01$) in the P females during gestation (↓4-8%) and early lactation (↓6-12%). Body weight gains were decreased ($p \leq 0.01$ or 0.05) during GDs 6-9 (↓400%) and 6-21 (↓22%), and during LDs 1-12 (↓55-164%). When compared to concurrent controls, absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$ or 0.01) in the dams throughout gestation (↓9-23%) except during the GD 0-6 interval, and during the LD intervals 4-7 (↓20%) and 7-12 (↓18%). Relative (g/kg/day) food consumption was reduced ($p \leq 0.05$ or 0.01) starting on GD 6 (6-20%) and during early lactation (up to day 12, (↓8-12%). There was also an increase in alopecia in high dose dams. The number of live fetuses/dam was decreased relative to concurrent controls (↓6%, $p \leq 0.01$); while the number of dead fetuses/dam was increased relative to concurrent controls (↑200%, $p \leq 0.01$).

No treatment-related findings were observed in dams at 300 or 100 ppm.

The LOAEL for maternal toxicity is 1000 ppm (equivalent to [GD/LD] 65.0/125.4 mg/kg/day) based on decreased body weights, body weight gains, and food consumption, prolonged gestation with mortality, and an increased number of dead fetuses. The NOAEL is 300 ppm (equivalent to [GD/LD] 22.0/41.3 mg/kg/day).

At 1000 ppm, the stillborn index was increased (↑200%, $p \leq 0.01$) and the number of pup deaths (calculated by reviewers) was increased during days 1-5 (↑229%). Body weights were decreased ($p \leq 0.01$) in the males from PND 5 to 86 (↓7-23%) and in the females from PND 5 to 72 (↓5-24%). Pinna unfolding was delayed (↑19%, $p \leq 0.01$) relative to concurrent controls. There were decreases in several morphometric measurements of the brain, including decreased ($p \leq 0.01$) thickness of the cerebellum in the males and females on day 12 (↓10-14%) and on day 83 (↓7-9%), and an increased thickness of the germinal layer of the cerebellar cortex in the Day 12 males (↑23%, $p \leq 0.01$). Absolute brain weights were decreased in the Day 12 and Day 83 animals (↓10-16%, $p \leq 0.01$ or 0.05). Relative (to body) brain weights were increased ($p \leq 0.01$ or 0.05) in the day 12 males and females (↑10-15%). There were also statistically significant changes in motor activity on days 14 (43% decrease in males [$p<0.01$], 24% decrease in females [n.s.]) and 22 (39% increase in males [$p<.05$], 19% increase in females [n.s.]), and changes in auditory startle amplitude at both time points (decreased in both sexes on day 23 [14-33%], decreased in females [20%] and increased in males [71%] on day 63).

At 300 ppm, there were also decreases in body weight (3-7%) and body weight gain (4-16%, PND5-23 and 72-86 in males, PND5-51 in females). Pinna unfolding was delayed (↑16%). There were changes in auditory startle amplitude in both sexes: a dose-related decrease in females on day 23 (decreased 26%), and a dose-related increase in males on day 63 (increased 18%). In addition, there was a decrease in absolute brain weight in both sexes (3-4%) on day 12 (statistically significant for females only), and in brain measurements (anterior/posterior cerebrum).

At 100 ppm, there were decreases in body weight (3-7%) and body weight gain (5-13%) (PND 5-37 in males, PND 5-51 in females). There were decreases in motor activity (on days 14 and 18 in males [28-35%]) and changes in auditory startle amplitude (decreased 9% in day 14 females, increased 16% in day 63 males, n.s.). There was also a decrease in absolute brain weight in both sexes on day 12 (4%, statistically significant for both sexes), and in brain measurements (anterior/posterior cerebrum).

The LOAEL for offspring toxicity is 100 ppm based on decreases in body weights, decreases in absolute brain weights and measurements, and decreases in motor activity.

The NOAEL is not determined.

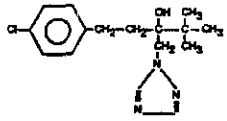
This study is classified as **acceptable/guideline (§83-6[a])** and satisfies the requirement for a developmental neurotoxicity study in rats, pending submission of additional information regarding analytical data and positive control studies, as described below.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Tebuconazole Technical
   Description: White powder
   Lot/Batch #: 603-0013
   Purity: 96-96.9% a.i.
   Stability: Stable in the diet for up to 14 days at room temperature and 36 days at -23°C
   CAS #: 107534-96-3
   Structure:



2. Vehicle: Corn Oil/Diet

3. Test animals: Species: Rat
   Strain: Crl:CD®BR VAF/Plus® (Sprague Dawley)
   Age at start of dosing: Approximately 11 weeks
   Weight at start of dosing: 272-275 g
   Source: Charles River Laboratories, Inc. Raleigh, NC
   Housing: Animals were housed individually in stainless steel wire-bottomed cages except during the cohabitation and postpartum periods. During cohabitation, animals (1 male, 1 female) were housed in the male's cage; starting no later than gestation day 20, dams were housed in nesting boxes (with Bed-o'cobs bedding [The Andersons Industrial Products Group, Maumee, OH]). After weaning, the F1 generation pups were individually housed in stainless steel wire-bottomed cages.
   Diet: Certified Rodent Diet #5002 during acclimation, cohabitation, prior to gestation day 6, and after lactation day 11; dietary admixtures using Purina Mills Rodent Lab Chow #5001-4 , "etts," from gestation day 6 through lactation day 11 (PMI Nutrition International, St. Louis, MO), ad libitum.
   Water: Reverse-osmosis filtered tap water, ad libitum
   Environmental conditions:
      Temperature: 65.9-73.1 °F
      Humidity: 42.4-74.2%
      Air changes: At least 10/hour of HEPA-filtered fresh air
      Photoperiod: 12 h dark/12 h light
   Acclimation period: 6 days
   Study duration: Approximately 4 months
   In-life dates: 5/5/98-9/3/98

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Following acclimation, males and females (1 male/1 female) were cohabited for a maximum of seven days and copulation was confirmed by the presence of a vaginal plug or sperm in a vaginal smear. The day copulation was confirmed was designated as gestation day (GD) 0.

2. Study schedule: P females that delivered a litter were administered the test substance continuously in the diet from GD 6 until lactation day (LD) 11. P females that did not deliver a litter were administered the test substance from GD 6 through GD 24.

3. Animal assignment: The animals were randomly assigned (stratified by body weight) to test groups as shown in Table 1.

Table 1. Animal assignment [a]

Test Group	Nominal Dose (ppm)	Actual Dose (ppm)	Achieved Dose (mg/kg/day) GDs 6-21	Achieved Dose (mg/kg/day) LDs 1-12	# of Females
Control	0	0	0	0	25
Low	100	107	8.8	16.3	25
Mid	300	266	22.0	41.3	25
High	1000	876	65.0	125.4	25

a Data were obtained from the study report Table B1, pages 64, 122-123.

4. Dose selection rationale: Dose levels were selected based on the results of a two-generation reproduction study in Wistar rats. Animals (25/sex/dose) were administered the test substance in the diet at concentrations of 0, 100, 300, or 1000 ppm. At 1000 ppm, hair loss was observed (P females) as well as decreased food consumption (P males), body weight gains (P and F1 males and females), litter size, birth weight, and pup survival to day 5. No treatment-related effects were observed at 100 or 300 ppm.

   Based on the results of this study, the dose levels shown in Table 1 were selected for this developmental neurotoxicity study in rats.

5. Dosage preparation and analysis: Corn oil was used as the vehicle for the test substance at 1% (by weight) of the diet. No further information concerning the method or frequency of preparation of the test diets was provided; however, it was stated that the dietary admixtures were provided to the testing lab by the sponsor and were stored frozen (-20°C). Aliquots for weekly use were stored at room temperature. Homogeneity and stability were determined using 100 and 1600 ppm samples stored at room temperature for 14 days or in the freezer for 36 days (assumed based on the availability of results

following a 36-day interval). Concentration analyses were performed on all dose preparations.

Results: It was stated that the test substance was homogeneous and stable in the diet for up to 14 days at room temperature and 36 days at -23°C. Measured concentrations of the dose formulations ranged from 87.6-107% of nominal (only a range was stated in the report). No analytical data were provided. Analytical data (including stability, homogeneity, and concentration verification) and information regarding diet preparation should be provided by sponsor to verify the statements in the study report.

6. Dosage administration: Doses were administered continuously in the diet from GD 6 through LD 11 (dams that delivered a litter) or GD 24 (rats that did not deliver a litter).

C. OBSERVATIONS

1. Parental animals: The P animals were checked for mortality and clinical signs of toxicity at least twice daily. Body weight and food consumption were measured daily during the exposure and post exposure periods beginning on GD 0. During the exposure and post-exposure periods, at approximately the same time each day, rats were examined for signs of autonomic dysfunction (lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, and defecation) and abnormal posture, movements, and behavior. Observers were unaware of the rat's treatment group. In addition, rats were observed for maternal behavior on lactation days 1, 5, 8, 14, and 22; variations from normal behavior were recorded. P females that did not deliver a litter were sacrificed on presumed GD 25, necropsied, and examined for gross lesions and evidence of pregnancy (uteri were stained with 10% ammonium sulfide and examined for implantation sites). All other P dams were sacrificed on LD 22 and subjected to a gross pathological examination.

2. Litter observations: Pups were evaluated for viability at birth and at least twice daily during the preweaning and postweaning periods. On post-natal day 5, litters were standardized to 10 pups each; when possible 5 pups/sex were retained for each litter. Because of the large number of pup deaths in litters from high dose dams, only 18 litters with at least 9 pups remained at that dose on post-natal day 12. In order to retain 20 litters with at least 9 pups each, two pups from other dams receiving 1000 ppm and two additional pups from control animals were transferred to 1000 ppm dams with smaller surviving litters. The transferred pups were uniquely identified and used for the purpose of balancing litter size only; on postnatal day 22, they were sacrificed and examined by gross necropsy.

Clinical observations were noted daily during the preweaning period and weekly during the postweaning period. Body weights were recorded on PNDs 1, 5, 8, 12, 14, 18, 22, weekly during the postweaning period, and at sacrifice. Food consumption was measured weekly during postweaning. In addition, the following litter observations (X) were made (Table 2a):

Table 2. Litter observations[a]

Observation	Time of observation (lactation day)						
	1	5[b]	8	12	14	18	22
Number of live pups	X	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X	X
External alterations	X						
Number of dead pups	X	X	X	X	X	X	X
Sex of each pup	X	X	X	X	X	X	X

a Data obtained from the study report Table B12, pages 137-141.
b Before and after standardization (culling).

On post-natal day 12, 20 litters/dose were randomly selected for continuation on study. F1 pups (1/sex/litter, where possible) were assigned to one of 5 subsets for further evaluation (See Table 2b). Physical development was measured by surface righting reflex (evaluated beginning on post-natal day [PND] 1, Subsets 1-5), pinna unfolding (evaluated beginning on PND 2, Subsets 1-5), eye opening (evaluated beginning on PND 12, Subsets 2-5), acoustic startle response (evaluated beginning on PND 13, Subsets 2-5), and pupil constriction (evaluated on PND 21, Subsets 2-5). Evaluations were continued until all pups in the litter met the criterion. Sexual maturation was evaluated in Subsets 2-4 (beginning PNDs 28-39) and was indicated by the age of vaginal patency or preputial separation.

All pups in Subset 1 (78 males and 76 females) were sacrificed on PND 12, examined for gross lesions, and brain weights were recorded. Six pups/sex/dose were processed for neurohistological examinations. All pups in Subset 2 (80 rats/sex) were subjected to a passive avoidance test, beginning on PND 23 to 25. Beginning on PND 59 to 63, the same pups were evaluated using a water-filled M-maze (see below for procedural information for passive avoidance and M-maze). All pups assigned to Subset 3 (80 rats/sex) were subjected to motor activity measurements on PNDs 14, 18, 22, and 62. The same animals were tested for auditory startle habituation on PNDs 23 and 63. All Subset 4 rats (79 males and 80 females) were examined weekly for signs of autonomic dysfunction and abnormal posture, movements, or behavior patterns and were sacrificed on PND 83. Six pups/sex from Subset 4 were randomly selected for fixed brain weights and neurohistological examination (see below). All remaining pups were subjected to a gross necropsy. Subset 5 pups (70 males and 67 females) were used to standardize litter size to 8 pups/litter from PNDs 12 to 22.

Table 2b. Pup Assignment for evaluations.*

Parameter	Subset 1	Subset 2	Subset 3	Subset 4	Subset 5
No. of animals initially assigned (M/F)	78/76	80/80	80/80	79/80	70/67
Physical Developmental Landmarks	X†	X	X	X	X
Sexual Maturation		X	X	X	
Neuropathology (Day 12) Brain weights in all pups Neuropathology in 6/sex/group	X				
Passive Avoidance (Day 23-25)		X			
Watermaze (Day 59-63)		X			
Motor Activity (Days 14, 18, 22, 62)			X		
Auditory Startle Habituation (Days 23, 63)			X		
Weekly detailed clinical observations				X	
Necropsy/Histopathology (Day 83) [6/sex/group]				X	
Necropsy (Day 22)					X

*Approximately 20 animals/sex/group were assigned to each subset. †Surface righting and pinna unfolding only.

Passive Avoidance Testing: Passive avoidance was evaluated in a two-compartment plexiglas chamber. One compartment was brightly lit, while the other was 'dark' and had an electrified grid floor. Training was conducted by placing the rat in the bright compartment; when the rat entered the dark compartment, the door between the compartments was closed and a brief shock was delivered (1 mA for 1 sec). The rat was removed to a holding cage for 30 sec, and the trial was repeated. Training was continued until the rat successfully avoided the dark compartment for 60 sec on two consecutive trials or for a maximum of 15 trials. Latency to enter the dark compartment was recorded for each trial. One week after initial training, each rat was retested as above to evaluate long term retention. Learning and retention were evaluated by comparing the number of trials to criterion and the latency to enter the dark compartment on trials one and two for each session.

Water Maze Testing: Water maze testing was conducted in a water-filled stainless steel modified M-maze. The maze was filled to approximately 9" deep, and water temperature was approximately 21° C. Rats were placed in the base of the maze and required to swim to a goal in one of the two arms (the initial arm chosen on trial 1 was designated as the incorrect goal). Upon reaching the correct goal, the rat was removed from the maze. After a 15-sec interval, the rat was returned to the maze for the next trial. Training continued until rat had made the correct choice in 5 consecutive trials, or for a maximum of 15 trials. Latency to reach the correct goal and the number of errors and trials to criterion were recorded. Testing was repeated one week later, to evaluate long term retention.

Motor Activity Testing: Motor activity was evaluated using a passive infrared sensor, mounted on a stainless steel wire-bottomed cage (solid flooring was used during the pre-weaning period). The number of movements and time spent in movement were recorded for a 90-minute period, during each of 18 5-minute subsessions. Groups were counterbalanced across cage position and sex, with each rat tested in the same cage position for all test days. Equipment was capable of monitoring a rack containing 32 cages simultaneously. Calibration was carried out at least semi-annually.

Auditory Startle Habituation: Auditory startle habituation was evaluated in a sound-attenuated chamber, with 4 testing units. Each rat was placed on a platform with a force-transducer. After a five minute adaptation period, rats were given 10 blank trials (with no sound stimulus), followed by 50 trials with sound stimulus (30 msec, 120 dBA bursts of noise) at 10 sec intervals, followed by an additional 10 blank trials. Peak response amplitude was calculated by subtracting the average response on blank trials from the response on stimulus trials. Peak response amplitude was averaged over each block of 10 consecutive trials.

Necropsy/Histopathology: All rats were necropsied upon sacrifice. Rats from Subset 1 were sacrificed on lactation day 12; the calvaria was removed after sacrifice and the head was immersed in buffered 10% formalin. Brains were removed and weighed after approximately 48 h in fixative. Brains from 6 pups/sex/group were processed for additional histopathological evaluation.

From Subset 4, six rats/sex/group were selected for neuropathological evaluation on day 83. Selected rats were administered heparin and sodium pentobarbital, and perfused in situ with buffered 10% formalin. Selected tissues were dissected and immersed in neutral buffered 10% formalin (head, vertebral column, and hindlimbs), and later processed for histopathological evaluation.

For day 12 brains (Subset 1), two linear measures were taken (anterior to posterior cerebrum and anterior to posterior cerebellum). Brains were embedded in paraffin, and 6 standardized sections were evaluated. Sections were 7 micrometers thick, and stained with hematoxylin and eosin. Seven additional morphometric measurements were taken (as listed in Table 17); all morphometric measurements were performed with observer unaware of treatment status of tissue. Initially, only high dose and control brains were evaluated (except for linear measurements, which were performed on all brains); because of differences seen in findings from high dose and control animals, mid dose brains were also evaluated morphometrically. Qualitative histopathological evaluations were performed on high dose and control animals only.

For day 83 brains (Subset 4), linear measures were taken as described above. Brains were embedded in paraffin, and 11 standardized sections were evaluated. Spinal cord,

Gasserian ganglia, nerve roots, and dorsal root ganglia were also embedded in paraffin. Sections were 5 micrometers thick, and were stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and the Bielschowsky's technique. Peripheral nerve tissues (sciatic nerve, tibial nerve [cross and longitudinal sections]; peroneal and sural nerves [longitudinal sections]) were embedded in glycol methacrylate, sectioned at 2 micrometers, and stained with hematoxylin and eosin, toluidine blue, and the Bielschowsky's technique.

3. Positive controls - A large number of studies and poster summaries were submitted as positive control data. Many of these studies were performed in adult rats, and may not be relevant to procedures used in young rats in the current study. Some of the submitted data were published studies performed in other facilities, with different principal investigators from the current study. The chemicals used in the submitted studies included: acrylamide (30 mg/kg, administered intraperitoneally for 17 days); trimethyltin chloride (8 mg/kg, administered as a single i.p. injection); MK-801 (0.3 mg/kg as a single i.p. injection); carbaryl (100 mg/kg, once by gavage); and DDT (100 mg/kg, once by gavage). These studies are described further in Appendix B.

   The submitted studies and information are not sufficient to validate the testing procedures used in the submitted study.

D. DATA ANALYSIS

1. Statistical analyses: All measured values were subjected to analysis of variance (ANOVA), repeated measures ANOVA, the Kruskal-Wallis Test, or Fisher's Exact test. Details of statistical analyses are provided in the study report, pp. 61-63.

2. Indices:

   Reproductive indices - The following reproductive indices as presented in the study report were calculated for the adults:

   delivering rate (%) = # of females producing at least one live pup/# of pregnant females x 100%

   Offspring viability indices - The following viability indices as presented in the study report were calculated for the litter:

   day 5 viability index (%) = # of live pups at day 5/ # of pups born alive x 100%
   lactation index (%) = # of live pups on day 22/total # of live pups at day 5 (postcull) x 100%

3. Historical control data: Historical control data were provided for the FOB, developmental landmarks, motor activity, passive avoidance, auditory startle, sexual maturation, watermaze, and brain weights and morphometrics.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: Two high-dose P females died following prolonged gestation, a result of treatment with the test substance. One low-dose female was found dead on lactation day (LD) 17; this death was considered not to be treatment-related because it was not dose-dependent. All other P females survived until scheduled sacrifice.

   Localized alopecia (Table 3) was observed ($p \leq 0.01$) on the undersides of the high-dose P females during gestation (3/25 treated vs. 0/25 controls) and lactation (3/21 treated vs. 0/25 controls). In addition, localized alopecia was observed (p=not statistically significant) on the limbs of high-dose P females during gestation (2/25 treated vs. 0/25 controls) and lactation (5/21 vs. 2/25 controls).

Table 3. Incidence of clinical signs in P females administered tebuconazole from GD 6 to LD 11[a]

Observation	Dose Group (ppm)			
	0	100	300	1000
Presumed Gestation				
Maximum possible incidence (# animals examined)	398[25]	400[25]	403[25]	411[25]
Localized alopecia				
Total	0[0]	0[0]	0[0]	60[5]**
Underside	0[0]	0[0]	0[0]	33[3]**
Limbs	0[0]	0[0]	0[0]	27[2]
Lactation				
Maximum possible incidence (# animals examined)	500[25]	483[24]	488[24]	452[21]
Localized alopecia				
Total	34[2]	0[0]	14[2]	133[7]**[b]
Underside	0[0]	0[0]	0[0]	66[3]**
Limbs	34[2]	0[0]	14[2]	74[5]

a Data extracted from the study report Table B2, pages 124-125. Number of affected animals is presented in brackets.

b Data represents those provided in the study report; however, it should be noted that the sum of alopecia on the underside and limbs does not equal what is presented as total alopecia.

** Statistically significant at $p \leq 0.01$

2. Body weight - Body weights and body weight gains for the P females are presented in Tables 4a and 4b. Body weights were slightly decreased ($p \leq 0.01$) in the high-dose P females throughout gestation (↓4-8%) and from LD1-LD13 (↓6-12%). Body weight

decreases were no longer significant on LD22. Body weight gains were decreased ($p \leq 0.01$ or 0.05) in the high-dose P females during GDs 6-9 (↓400%), 6-21 (↓22%), and 0-21 (↓16%) and during LDs 1-4 (↓164%), 4-7 (↓55%). Body weight gain was increased during days 7-12 (68%), perhaps due to removal of test material from diets starting on LD 11. In addition, body weight gains were decreased in the low-dose P females during LDs 1-4 (↓154%, $p \leq 0.05$); however, this difference was not dose-dependent and is not considered treatment-related.

Table 4a. Selected mean body weights (g) for P females administered tebuconazole from GD 6 to LD11[a]

Treatment interval (days)	Dose (ppm)			
	0	100	300	1000
Gestation				
0	237.9±10.1	237.8±9.9	239.2±9.5	238.6±10.3
7	274.7±14.0	277.4±11.9	273.3±11.6	262.6±12.5** (↓4)
16	330.7±19.0	330.2±17.2	327.7±14.9	305.3±15.7** (↓8)
21	390.1±28.5	400.8±23.4	396.6±21.9	366.5±23.1** (↓6)
Lactation				
1	287.3±18.8	291.4±16.8	285.1±17.4	269.2±15.2** (↓6)
6	302.8±17.5	298.3±24.9	298.4±17.7	274.1±16.9** (↓9)
10	327.5±22.7	327.7±13.6	325.2±22.1	289.2±19.8** (↓12)
22	339.2±20.8	337.6±22.4	334.6±24.7	329.6±23.4

a Data were obtained from the study report Tables B3 and B5, pages 126-127 and 129-130. Percent difference from controls is listed parenthetically; n= 25 (0 ppm), 24 (100, 300 ppm [0 ppm during day 1 of lactation only]), 23 (1000 ppm during gestation), or 21 (1000 ppm during lactation); for gestation day 21, n=23 (0 ppm), 20 (100 ppm), 22 (300 ppm); for lactation day 22, n=20 for all groups, except that n=19 for 100 ppm group starting day 18.

** Significantly different from controls at $p \leq 0.01$

Table 4b. Selected mean body weight gains (g) for P females administered tebuconazole from GD 6 to LD 11[a]

Treatment interval (days)	Dose (ppm)			
	0	100	300	1000
Gestation				
0-6	35.7±8.1	35.4±7.8	32.8±6.4	36.4±7.6
6-9	3.1±10.1	9.2±9.7*	5.1±8.0	-9.3±8.8** (↓400)
18-21	30.3±15.6	38.0±13.2	37.0±8.7	30.9±13.0
6-21	117.6±23.3	127.6±19.9	126.0±15.9	91.5±21.9** (↓22)
0-21	152.6±24.3	162.9±20.2	158.0±17.1	127.9±23.3** (↓16)
Lactation				
1-4	5.6±13.1	-3.0±14.0* (↓154)	1.7±11.6	-3.6±11.4* (↓164)
4-7	17.2±9.0	16.3±11.6	16.0±13.3	7.7±7.6** (↓55)
7-12	25.9±12.1	25.8±19.0	24.5±21.2	43.5±16.7** (↑68)
1-12	48.0±18.7	39.1±14.8	42.2±20.3	47.6±18.1
1-22	49.9±19.2	46.5±22.9	51.2±18.2	60.3± 25.2 (↑21)

a Data were extracted from the study report, Tables B4 and B6, pages 128 and 131. Percent difference from controls is presented parenthetically; n=25 (0 ppm), 24 (100, 300 ppm [0 ppm during day 1 of lactation only]), 23 (1000 ppm during gestation), or 21 (1000 ppm during lactation); for gestation day 21, n=23 (0 ppm), 20 (100 ppm), 22 (300 ppm); for lactation days 1-22, n=20 for all groups except that n=19 for 100 ppm group starting day 18.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. Food consumption - Selected food consumption data are presented in Table 5. When compared to concurrent controls, absolute (g/animal/day) food consumption was reduced ($p \leq 0.05$ or 0.01) in the high-dose dams throughout gestation (↓9-23%) except during the GD 0-6 interval, and during the LD intervals 4-7 (↓20%), 7-12 (↓18%), 1-12 (↓18%), and 1-22 (↓9%). Relative (g/kg/day) food consumption (Table 5) was reduced ($p \leq 0.05$ or 0.01) in the high-dose dams during the GD intervals 6-9 (↓20%), 9-12 (↓10%), 12-15 (↓8%), 15-18 (↓6%), 6-21 (↓9%), and 0-21 (↓5%). In addition, relative food consumption was decreased ($p \leq 0.01$) at the high-dose during the LD intervals 4-7 (↓12%), 7-12 (↓8%), and 1-12 (↓9%).

Table 5. Selected mean relative food consumption (g/kg/day) for P females administered tebuconazole from GD 6 to LD 11[a]

Treatment interval (days)	Dose (ppm)			
	0	100	300	1000
Gestation				
0-6	89.0±9.1	90.5±8.2	88.6±6.6	89.1±8.0
6-9	80.2±12.0	84.5±8.9	82.1±11.2	64.0±13.8** (↓20)
9-12	87.1±11.7	88.9±8.7	89.0±9.3	78.3±18.1* (↓10)
12-15	88.6±8.2	86.6±8.2	88.8±9.4	81.8±6.6** (↓8)
15-18	87.6±5.6	87.0±7.3	88.6±5.3	82.4±5.6** (↓6)
6-21	81.3±5.7	82.8±5.4	82.6±5.3	74.2±6.7** (↓9)
0-21	79.4±5.5	80.8±4.6	80.4±4.2	75.7±5.7* (↓5)
Lactation				
1-4	104.2±18.6	96.2±19.6	101.2±12.7	96.9±20.0
4-7	160.2±19.8	148.9±17.5	157.1±15.8	140.5±20.9** (↓12)
7-12	186.5±11.8	184.5±8.9	184.0±12.1	171.2±18.9** (↓8)
1-12	158.2±12.9	152.1±9.8	155.3±8.6	143.2±17.2** (↓9)
1-22	196.2±11.9	190.6±9.3	194.8±8.0	191.7±14.2

a Data were extracted from the study report Tables B8 and B10, pages 133 and 135. Percent difference from controls is listed parenthetically; n=25 (0 ppm), 24 (100, 300 ppm [0 ppm during day 1-4, 4-7, and 1-12 of lactation]), 23 (1000 ppm during gestation, except during day 9-12 interval [n=22]), or 21 (1000 ppm during lactation); for gestation day 21, n=22 (0 ppm), 20 (100 ppm), 22 (300 ppm), 23 (1000 ppm); starting lactation day 11, n=20 for all groups except that n=19 for 100 ppm group starting on day 17 and n=19 for 0 ppm group for the days 1-22 interval.

* *Significantly different from controls at p≤0.05*

** Significantly different from controls at p≤0.01

4. Parental pathology

   a) Macroscopic examination: No treatment-related pathological abnormalities were observed in any treated group at necropsy.

   b) Microscopic examination: Not done.

5. Reproductive performance: There were two maternal deaths at the high dose, apparently related to prolonged gestation and dystocia (see above). Mean gestation length was slightly increased at the high dose (23.0 days treated vs. 22.5 days controls [↑2%], p≤0.01). There were no other differences of toxicological concern observed in the reproductive performance of the P females (Table 6).

Table 6. Reproductive performance of P females [a]

Observation	Dose (ppm)			
	0	100	300	1000
Delivering rate	100	100	100	91.3
Gestation length (days)	22.5±0.5	22.6±0.5	22.7±0.5	23.0±0.2**
Number of litters	25	24	24	21

a Data extracted from the study report, Table B11, page 136
** Significantly different from controls at p≤0.01

6. Natural delivery data - Natural delivery findings for the reproductive performance of P dams are shown in Table 7. At the high dose, the number of live fetuses/dam was decreased relative to concurrent controls (↓6%, p≤0.01); while the number of dead fetuses/dam was increased relative to concurrent controls (↑200%, p≤0.01). There were no other treatment-related differences in natural delivery observations.

Table 7. Natural delivery observations of the P females administered tebuconazole from GD 6 to LD 11 [a]

Observation	Dose (ppm)			
	0.00	100	300	1000
# Animals Assigned	25	25	25	25
# Animals Pregnant Pregnancy Rate (%) [b]	25 (100)	24 (96)	24 (96)	23 (92)
# Nonpregnant [b]	0	1	1	2
Total # Implantations Implantations/Dam	379 15.2±1.5	365 15.2±1.6	359 15.0±2.1	312 14.8±2.4
Total # Litters Examined	25	24	24	21
Total # Live Fetuses Live Fetuses/Dam	333 13.9±2.4	340 14.2±1.7	340 14.2±2.4	276** 13.1±2.7 (↓6)
Total # Dead Fetuses (Stillborn) Dead Fetuses/Dam Dams with Stillborn pups (%)	2 0.1±0.3 2 (8.0)	2 0.1±0.4 1 (4.2)	2 0.1±0.3 2 (8.3)	7** 0.3±0.7 (↑200) 5 (23.8)
Mean Pup Weight, Day 1 (g)	6.5±0.5	6.6±0.5	6.4±0.4	6.2±0.4
Sex Ratio (% Male)	50.4±13.4	55.2±15.1	49.1±15.7	48.6±14.5

a Data extracted from the study report Tables B11-12, pages 136-141. Percent difference from controls is presented parenthetically.
b Calculated by reviewers.
** Significantly different from controls at p≤0.01.

B. OFFSPRING

1. Viability and clinical signs: There were fewer pups born alive, more stillborn pups, and more pup deaths during the first week after birth in the high dose group than in controls (Table 8). When compared to concurrent controls, changes (p≤0.01) in viability indices

were observed at 1000 ppm as follows: increased stillborn index (↑200%); decreased livebirth index (↓6%); and decreased day 5 viability (precull) (↓6%). The number of pup deaths (calculated by reviewers) were increased during days 1-5 (↑229%) and 1-21 (↑243%). No clinical signs of toxicological concern were observed.

Table 8. F_1 generation mean litter size and viability.[a]

Observation	Dose (ppm)			
	0	100	300	1000
Number of litters	25	24	24	21
Mean litter size				
Day 1	13.9±2.4	14.2±1.7	14.2±2.4	13.1±2.7
Day 5[c]	13.6±2.3	14.0±1.6	13.9±2.4	12.0±2.6
Day 5[d]	9.8±0.8	10.0±0.0	10.0±0.0	9.7±0.8
Day 8	9.8±0.8	10.0±0.0	10.0±0.0	9.6±0.9
Day 14	8.0±0.2	8.0±0.0	8.0±0.0	7.8±0.5
Day 22	8.0±0.2	8.0±0.0	8.0±.0.	7.8±0.5
Number deaths[b]				
Days 1-5[c]	7	4	6	23**
Days 1-22	7	4	6	24**
Viability indices (%)				
Stillborn	0.6	0.6	0.6	2.5** (↑200)
Livebirth	99.4	99.4	99.4	97.2**(↓6)
Viability (Day 5)[c]	97.9	98.8	98.2	91.7** (↓6)

a Data extracted from the study report Table B12, pages 137-141.
b Calculated by the reviewers from data contained in this table.
c Before culling
d After culling
** Significantly different from controls at $p \leq 0.01$

2. Body weights and body weight gains: Selected body weights and body weight gains for F1 pups are presented in Tables 9a and 9b. Body weights were decreased ($p \leq 0.01$) in the high-dose males from PND 5 to 86 (↓7-23%) and in the high-dose females from PND 5 to 72 (↓5-24%). At the mid-dose, body weights were decreased ($p \leq 0.01$ or 0.05) in the males from PND 5 to 23 and 72-86 and in the females from PND 5 to 51 (↓3-7% each). At the low-dose, body weights were decreased ($p \leq 0.01$ or 0.05) in the males from PND 5 to 37 (↓3-6%) and in the females from PND 5 to 51 (↓4-7%).

In the males, differences from controls in body weight gains were observed sporadically throughout the study at the high- (↓39-↑8) and mid- (↓4-16%) dose and during only two intervals at the low-dose (↓6-9%). In the females, differences from controls in body weight gains were observed sporadically throughout the study at the high-dose (↓39-↑7%) and during 4 and 5 intervals at the mid- (↓7-19) and low- (↓4-13%) doses, respectively.

Table 9a. Mean F_1 pup body weights (g).[a]

Post-natal Day	Dose (ppm)			
	0	100	300	1000
Males				
5	10.4±0.9	9.9±1.3* (↓5)	10.0±0.9* (↓4)	9.0±1.1** (↓13)
8	16.1±1.3	15.1±1.8** (↓6)	15.0±1.2** (↓7)	12.5±1.6** (↓22)
12	24.7±2.0	23.4±2.2** (↓5)	23.2±1.9** (↓6)	19.1±2.2** (↓23)
22	55.2±5.0	53.4±4.6* (↓3)	53.1±4.4* (↓4)	48.1±4.6** (↓13)
37	172.1±13.9	165.7±12.3*(↓4)	168.1±10.2	154.8±16.1**(↓10)
51	295.1±26.0	291.4±20.3	291.8±17.7	272.7±21.4**(↓8)
58	350.9±29.7	347.5±26.3	345.9±20.4	325.2±25.5** (↓7)
72	434.9±38.2	429.8±32.9	420.5±30.9* (↓3)	397.4±35.9** (↓9)
86	494.0±45.6	491.4±38.3	473.4±38.9* (↓4)	453.9±39.6** (↓8)
Females				
5	10.0±0.9	9.5±1.4** (↓5)	9.5±1.0** (↓5)	8.5±1.2** (↓15)
8	15.4±1.3	14.3±1.8** (↓7)	14.3±1.4** (↓7)	11.8±1.8** (↓23)
12	23.9±2.1	22.2±2.2** (↓7)	22.1±1.8** (↓7)	18.1±2.3** (↓24)
22	52.8±4.8	50.7±4.4* (↓4)	50.9±4.5* (↓4)	46.0±4.7** (↓13)
37	145.1±11.3	139.5±9.1** (↓4)	140.8±9.7* (↓3)	130.8±11.6** (↓10)
52	206.5±17.4	199.0±16.0*(↓4)	198.9±18.4*(↓4)	191.6±16.7**(↓7)
58	229.2±18.6	222.5±18.0	225.8±19.0	214.5±19.1** (↓6)
72	260.5±22.4	252.7±22.2	254.9±22.8	246.6±24.5** (↓5)
86	281.1±23.2	269.3±23.4	277.3±26.7	267.1±30.8

a Data obtained from the study report Tables C3 and C5, pages 294-295 and 298-299; includes weights of pups selected for continuation on study only. Percent difference from controls is listed parenthetically; n = 76-80 (days 5-12), n=56-60 (days 14-79), n=38-40 (day 86). *Statistically significant at $p \leq 0.05$;**Statistically significant at $p \leq 0.01$

Table 9b. Selected F_1 pup mean body weight gains (g).[a]

Post-natal Day	Dose (ppm)			
	0	100	300	1000
Males				
5-8	5.7±1.2	5.2±1.1** (↓9)	5.0±0.7** (↓12)	3.5±0.8** (↓39)
8-12	8.6±1.2	8.3±0.9	8.2±1.3	6.6±0.9** (↓23)
5-12	14.3±1.9	13.4±1.6** (↓6)	13.2±1.5** (↓8)	10.2±1.4** (↓29)
37-44	64.7±8.2	65.2±7.2	63.9±5.8	60.7±9.3* (↓6)
65-72	38.2±9.0	38.0±8.5	32.2±10.5** (↓16)	30.2±15.0** (↓21)
79-86	30.4±7.0	29.7±7.6	25.7±6.8** (↓15)	24.9±11.8* (↓18)
5-86	483.7±45.4	481.6±38.0	463.5±38.6* (↓4)	444.9±39.1** (↓8)
Females				
5-8	5.4±0.9	4.7±1.1** (↓13)	4.8±0.8** (↓11)	3.3±0.9** (↓39)
8-12	8.4±1.1	8.0±0.8* (↓5)	7.8±1.2** (↓7)	6.4±1.0** (↓24)
23-30	43.1±4.3	41.2±3.7* (↓4)	41.7±4.0	39.0±5.1** (↓10)
30-37	46.3±5.1	45.2±4.3	46.0±4.3	43.9±5.2** (↓5)
51-58	22.7±6.6	23.5±7.1	26.9±9.8* (↑19)	22.9±7.0
5-86	271.2±22.8	259.8±23.3	267.8±26.6	258.6±30.5

a Data obtained from the study report Tables C4 and C6, pages 296-297 and 300-301. Percent difference from controls is listed parenthetically; n = 76-80 (days 5-12), n=56-60 (days 14-79), n=38-40 (days 80-86).
* Statistically significant at $p \leq 0.05$; **Statistically significant at $p \leq 0.01$

Food consumption: Food consumption, measured from days 23-86 for animals in subsets 2-4, (g/day) was consistently decreased at the high dose for both males and females, ranging from 3-9% below control levels for males, 4-10% below control levels for females. Relative food consumption (g/kg/day) was consistently increased for high dose males and females, ranging from 3-7% above controls for males, 4-8% above controls for females. No reliable effects on food consumption were seen in the low and mid dose groups.

3. Offspring developmental landmarks: Pinna unfolding was delayed ($p \leq 0.01$) in the mid- (↑16%) and high- (↑19%) dose pups relative to concurrent controls (Table 10). No other treatment-related differences in developmental landmarks were observed.

Table 10. Offspring developmental landmark data (days) [a]

Observation	Dose (ppm)			
	0	100	300	1000
Surface righting reflex	2.7±1.5	3.3±1.7	3.2±1.8	2.6±1.8
Pinna unfolding	3.2±0.5	3.3±0.5	3.7±0.5** (↑16)	3.8±0.5** (↑19)
Eye opening	14.6±0.7	14.8±0.8	14.6±0.5	15.0±0.4
Acoustic startle response	13.0±0.2	13.2±0.4	13.0±0.2	13.2±0.4
Pupil constriction	21±0.0	21±0.0	21±0.0	21±0.0

a Data obtained from the study report, Table B14, pages 143-145. Percent difference from controls is listed parenthetically; n=20-25.

** Significantly different from controls at $p \leq 0.01$.

Sexual maturation: A slight delay in vaginal patency (33.2 days treated vs. 31.6 days controls [↑5%], $p \leq 0.01$) was observed in the high dose F_1 females (Table 11). No differences in preputial separation were observed between treated and control F_1 males.

Table 11. Preputial separation or vaginal patency (days) in F_1 generation males or females.[a]

Parameter	Dose (ppm)			
	0	100	300	1000
Preputial separation - Males	45.4±1.3	45.8±1.3	45.4±1.6	45.7±1.7
Vaginal patency - Females	31.6±2.0	32.0±1.9	32.1±2.0	33.2±1.6** (↑5)

a Data extracted from the study report Table C11, page 306. Percent difference from controls is listed parenthetically; n=56-60.

** Significantly different from controls at $p \leq 0.01$

4. Offspring neurotoxicological tests:

a) Passive avoidance: No treatment-related differences were detected in performance on the passive avoidance test for learning, short-term retention, long-term retention, or response inhibition (see Table 12). Increased latency in trial 2 was observed in the 100 ppm males (↑94%, $p \leq 0.05$); however, this difference was not dose-dependent and considered not to be treatment-related.

Table 12. Passive Avoidance performance in F1 pups (mean±standard deviation).[a]

Parameter	Dose (ppm)			
	0	100	300	1000
Males				
Session 1				
Trials to Criterion	5.8±3.3	4.6±1.5	5.2±1.4	5.1±1.6
Latency (sec.)				
Trial 1	8.4±4.3	7.5±4.7	6.4±3.5	7.0±6.0
Trial 2	17.4±13.8	33.7±19.7*	13.7±10.7	22.5±18.6
Failed to Learn	2	0	0	0
Session 2				
Trials to Criterion	3.4±2.9	2.8±0.6	2.9±0.8	2.6±0.6
Latency Trial 1 (sec.)	29.8±21.6	31.8±21.6	28.1±23.4	36.9±22.8
Females				
Session 1				
Trials to Criterion	5.2±2.1	4.6±0.8	4.7±0.8	5.4±2.2
Latency (sec.)				
Trial 1	9.0±5.2	8.8±4.3	11.4±12.4	8.7±5.5
Trial 2	20.8±16.2	20.3±11.2	20.4±13.6	24.5±17.7
Failed to Learn	0	0	0	0
Session 2				
Trials to Criterion	2.7±0.6	3.6±2.8	2.8±0.7	3.6±3.0
Latency Trial 1 (sec.)	34.2±24.1	28.2±20.6	35.6±21.9	30.8±22.5

a Data obtained from the study report Table E1, pages 439; n=19-20. *=$p<0.05$. Values represent mean±s.d.

b) Watermaze: No differences in watermaze performance were observed in any treated group (see Table 13).

Table 13. Water maze performance in F1 adults (mean±standard deviation).[a]

Parameter	Dose (ppm)			
	0	100	300	1000
Males				
Session 1				
Trials to Criterion	8.9±3.1	9.2±3.1	8.2±2.1	10.0±2.7
Errors per Trial	0.38±0.24	0.40±0.20	0.37±0.22	0.42±0.18
Latency Trial 2 (sec.)	17.3±12.5	14.9±11.2	14.8±8.3	13.5±5.3
Failed to Learn	0	1	0	0
Session 2				
Trials to Criterion	7.0±2.9	6.3±2.5	7.9±3.6	6.9±2.9
Errors per Trial	0.13±0.17	0.07±0.11	0.13±0.16	0.13±0.19
Latency Trial 1 (sec.)	12.0±10.4	8.0±4.4	7.8±4.8	11.0±6.5
Females				
Session 1				
Trials to Criterion	9.2±2.4	9.7±3.2	9.0±2.2	9.2±2.7
Errors per Trial	0.38±0.18	0.40±0.22	0.49±0.24	0.38±0.15
Latency Trial 2 (sec.)	12.5±7.6	14.5±7.6	13.8±8.0	16.6±10.6
Failed to Learn	0	3	1	2
Session 2				
Trials to Criterion	6.6±2.4	5.4±0.9	9.2±4.4	6.6±1.8
Errors per Trial	0.12±0.12	0.06±0.14	0.16±0.18	0.12±0.12
Latency Trial 1 (sec.)	10.0±5.1	9.1±5.7	9.8±7.8	9.2±5.1

a Data obtained from the study report Table E2, page 440; n=19- 20. Values for rats who failed to learn during session 1 were not included in means for session 2. *=$p<0.05$. Values represent mean±s.d.

c) Motor activity: On day 14, both low and high dose males and females showed decreases in motor activity; mid-dose males showed smaller decreases (see Table 14). Decreases in motor activity in females at the low and high dose lacked statistical significance (14 and 24%, respectively), but were consistent with the significant decreases in males (35% and 43%, $p<0.01$). On day 18, low dose males still showed significantly less activity than control males (28%, $p<0.05$). On day 22, high dose males showed a significant increase (39%, $p<0.05$). No difference in motor activity among treatment groups was seen on day 62, for either sex.

Differences in motor activity during the five minute intervals were also observed on days 14, 18, and 22 (more commonly in males); most of these differences occurred in groups that showed significant differences in total activity on the same days. For example, on day 14, there were statistically significant decreases in total activity for low and high dose males; examination of individual subsession activity for those days revealed corresponding statistically significant decreases in activity during blocks 4, 5,

8, and 14 in low and high dose males, with additional blocks showing significant decreases at the high dose only (complete motor activity data are presented in Appendix A).

Although there was no reliable dose-response in the motor activity changes seen on day 14 in treated males (or in the decrease seen in low dose males on day 18), similar decreases in activity (of smaller magnitude) were seen in low and high dose females on day 14. Examination of the subsession data, in particular for low dose males, revealed that the decrease in activity was very consistent across the entire 90-minute session. Although the decrease in activity was not statistically significant in females, the magnitude of the difference was sufficiently large (in males) and consistent (across sex and time points) that these effects are considered treatment-related at the low dose in both sexes.

We also note that there was a large degree of variability in the motor activity data, both within the current study and in the submitted historical control data (see Table 14).

Table 14. Total motor activity (number of movements) in F1 pups (mean±standard deviation).[a]

Post-natal Day	Dose (ppm)				
	0	100	300	1000	Historical Controls
Males					
14	784.0±299.7	508.8**±293.7 (↓35)	685.6±269.8 (↓13)	447.6**±227.8 (↓43)	244.0-784.0
18	934.8±320.6	673.6*±380.0 (↓28)	1004.6±195.7	956.0±322.3	NA
22	610.2±357.3	646.2±222.0	645.0±258.2	850.5* ±280.3 (↑39)	314.2-636.4
62	982.8±207.0	877.0±216.5	859.1±209.4	952.2±273.9	563.0-982.8
Females					
14	559.2±337.0	483.2±263.3 (↓14)	610.8±263.6	423.4±183.3 (↓24)	228.4-737.9
18	798.9±318.6	860.2±308.5	867.7±266.0	918.2±349.8 (↑15)	343.3-932.2
22	618.6±284.5	608.0±332.4	617.8±214.6	738.3±277.1 (↑19)	228.9-684.8
62	909.9±185.2	910.5±240.3	899.4±191.6	926.9±211.2	634.6-961.8

a Data obtained from the study report Table F1, pages 468-483; n=19 or 20. Percent difference from controls is listed parenthetically; n=19-20.

* Statistically significant at $p \leq 0.05$

** Statistically significant at $p \leq 0.01$

NA Data not provided

d) Auditory startle response: Data for auditory startle habituation are presented in table 15 (below). Only data for amplitude were provided; latency data were not submitted. Although there were no statistically significant differences among groups, there was a dose-related decrease in the response amplitude in all treated females on day 23 (a similar decrease was seen in high dose males on this day), and a dose-related increase in response amplitude in all treated males on day 63 (no similar effect was seen in day 63 females; in contrast, a slight decrease in response amplitude was seen at the high dose). It is likely that the effects seen in males on day 63 and in females on day 23 are treatment-related.

Table 15. Auditory startle habituation in F1 rats (mean±standard deviation).[a]

Parameter	Dose (ppm)			
	0	100	300	1000
Males				
Day 23				
Block 1	15.1±6.8	16.0±11.5	14.3±7.4	12.2±7.4
Block 2	8.9±4.8	11.4±10.4	10.2±5.3	8.8±5.6
Block 3	10.5±8.6	11.5±12.8	10.8±7.2	7.3±4.2
Block 4	10.2±9.3	10.2±12.5	8.8±6.4	10.2±8.9
Block 5	13.0±10.1	9.9±9.0	9.5±5.9	11.0±9.3
Mean	11.5±6.4	11.8±10.7	10.7±5.0	9.9±6.0 (↓14%)
Day 63				
Block 1	95.0±62.3	105.1±69.6	110.1±89.0	130.4±118.1
Block 2	55.8±52.2	62.2±55.8	71.3±54.3	112.9±125.6
Block 3	39.4±30.9	48.7±53.0	45.2±39.1	77.8±106.6
Block 4	31.6±19.8	45.0±48.5	34.5±26.8	57.3±75.0
Block 5	32.0±21.3	33.6±33.5	38.0±24.3	55.2±66.0
Mean	50.8±33.1	58.9±49.2 (↑16%)	59.8±40.5 (↑18%)	86.7±94.8 (↑71%)
Females				
Day 23				
Block 1	17.4±9.5	15.4±11.4	12.4±6.9	10.8±6.3
Block 2	12.4±6.6	12.2±10.9	9.3±7.1	7.8±5.4
Block 3	11.7±7.5	12.6±12.4	9.3±7.3	7.2±5.4
Block 4	11.1±6.8	8.8±8.0	8.2±6.6	7.9±5.5
Block 5	9.7±6.7	8.2±6.9	7.0±5.6	8.1±7.0
Mean	12.5±6.2	11.4±9.2 (↓9%)	9.3±5.5 (↓26%)	8.4±4.5 (↓33%)
Day 63				
Block 1	56.4±38.8	54.4±32.9	54.2±31.9	46.2±24.3
Block 2	31.8±32.2	27.2±20.4	33.7±31.4	23.3±15.8
Block 3	25.2±20.4	22.1±20.6	23.1±22.0	17.7±14.5
Block 4	16.7±16.2	19.4±12.0	23.0±18.4	16.8±13.4
Block 5	17.9±15.0	19.6±12.2	20.4±19.0	14.1±12.7
Mean	29.6±20.4	28.5±17.0	30.9±20.2	23.6±12.7 (↓20%)

a Data obtained from the study report Table F2, pages 484-485; n=18-20. *=$p<0.05$. Values represent mean±s.d of response amplitude (g).

5. Offspring postmortem results:

   a) Necropsy: No treatment-related gross pathological findings were noted in Subsets 1, 2, 3, or 4. Only gross lesions were examined histopathologically; the most common finding was kidney changes, most notably dilation of the pelvis, which was seen most frequently in control males.

   b) Brain weights: Body weights (Table 16) were decreased ($p \leq 0.01$) in the day 12 (Subset 1) high-dose males and females (↓23-27%) and low- and mid-dose females (↓8-10%). Absolute brain weights were decreased ($p \leq 0.01$ or 0.05) in the high-dose males (↓10-15%) and females (↓10-16%) on days 12 and 83, the day 12 low-dose males and females (↓4% each), and the day 12 mid-dose females (↓4%).

   In spite of the lack of a dose relationship between the 100 and 300 ppm groups, the decrease in brain weight on day 12 (Subset 1) is found in both sexes, and is of similar magnitude across sexes. A dose-related decrease in brain weight persisted in both sexes at day 83, although the magnitude of the decrease was smaller than on day 12 (the low and mid-dose differences from control were no longer statistically significant at day 83). The differences in absolute brain weight are considered treatment-related at all dose levels.

Table 16. Absolute and relative brain weights in F1 pups.[a]

Weight (g)	Dose (ppm)			
	0	100	300	1000
Males				
Day 12 (Subset 1)				
Terminal body weight	24.8±2.3	23.8±2.1 (↓4)	23.7±2.2 (↓4)	19.0±2.2** (↓23)
Absolute brain weight	1.359±0.060	1.301±0.061* (↓4)	1.317±0.061 (↓3)	1.153±0.089** (↓15)
Relative brain weight	5.526±0.455	5.501±0.351	5.590±0.440	6.094±0.497** (↑10)
Day 83 (Subset 4)				
Terminal body weight	510.7±37.8	483.5±52.4 (↓5)	457.3±32.6 (↓10)	454.7±31.6 (↓11)
Absolute brain weight	2.412±0.082	2.340±0.086 (↓3)	2.300±0.120 (↓5)	2.172±0.094** (↓10)
Relative brain weight	0.475±0.036	0.488±0.042	0.505±0.062	0.477±0.025
Females				
Day 12 (Subset 1)				
Terminal body weight	24.4±2.3	22.4±2.4** (↓8)	21.9±1.7** (↓10)	17.8±2.3** (↓27)
Absolute brain weight	1.325±0.061	1.267±0.070* (↓4)	1.273±0.057* (↓4)	1.115±0.101** (↓16)
Relative brain weight	5.466±0.437	5.706±0.426	5.825±0.424* (↑7)	6.302±0.430** (↑15)
Day 83 (Subset 4)				
Terminal body weight	271.0±25.1	274.7±35.0	265.3±25.2 (↓2)	261.7±12.5 (↓3)
Absolute brain weight	2.115±0.068	2.072±0.087 (↓2)	2.071±0.098 (↓2)	1.970±0.086* (↓7)
Relative brain weight	0.782±0.051	0.763±0.102	0.783±0.050	0.752±0.042

a Data obtained from the study report Tables D3-D4 and G3-G4, pages 420-421 and 643-644; n=6 (day 83) or 19-20 (day 11). Percent difference from controls is listed parenthetically.
* Statistically different from controls at $p \leq 0.05$
** Statistically different from controls at $p \leq 0.01$

c) Neuropathology: No histopathological lesions were found in the brains of animals from the control or high dose group following sacrifice on day 12. The lesions found in animals examined following sacrifice on day 83 are listed in Table 17. There appears to be an increase in incidence of peripheral nerve fiber degeneration among high dose males (intermediate dose groups were not evaluated); similar effects were not seen in females. In addition, hydrocephalus was detected in one high dose male. This could be a spontaneous finding, unrelated to test material. However, it should be noted that treatment-related central nervous system malformations, including hydrocephalus, have been seen in developmental studies with this chemical, so it is also possible that the hydrocephalus may be related to treatment with tebuconazole.

Table 17. Incidence of neuropathological findings on day 83.

	Treatment Group			
	Male		Female	
Lesion	Control	High Dose	Control	High Dose
Sciatic Nerve -nerve fiber degeneration	0	1 (#9463, MF)	1 (#9853)	0
Peroneal Nerve -nerve fiber degeneration	0	1 (#9408)	0	0
Tibial Nerve -myelin sheath swelling -nerve fiber degeneration	1 0	0 2 (#9463, 9478)	0 0	0 0
Axonal degeneration -some peripheral nerve	0	3	1	0
Hydrocephalus	0	1 (#9438, mild)	0	0
Dilation of the aqueduct (midbrain)	0	0	1 (#9856)	1(#9810, mild)

N=6 for all groups. All lesions were minimal unless otherwise specified. MF=multifocal; all other lesions were focal. Numbers in parentheses represent animal number. Data were extracted from individual animal pathology data tables, pp. 827-843.

d) Morphometric measurements: Morphometric measurements are presented in Table 18, below. Statistically significant differences were found for several measurements between treated (mostly high dose) and control dose groups, including decreased ($p \leq 0.01$) thickness of the cerebellum in the high-dose males and females in Subsets 1 (day 12, ↓10-14%) and 4 (day 83, ↓7-9%). In addition, an increased thickness of the germinal layer of the cerebellar cortex was observed in the Subset 1 high-dose males (↑23%, $p \leq 0.01$). Other differences in brain morphometry included decreased ($p \leq 0.05$) thickness of the frontal cortex (↓8%) and hippocampal gyrus (↓9%). The anterior/posterior measurement of the cerebrum was significantly smaller than the corresponding control measurements for all treated groups of males on day 83, and for all treated females on day 12. Measurements were also smaller for all treated males on day 12, but the difference was statistically significant only at the high dose.

It is likely that the decrease in anterior/posterior cerebrum measurement at all doses, as well as some of the other changes in morphometric measurements at the high dose, are treatment-related. These differences are consistent with the decrease in brain weight seen at all doses (see above). Failure to demonstrate strict dose-relationships among treatment groups is likely related to the small number of animals evaluated (6/sex). Statistically significant changes in other morphometric measurements at the mid-dose are not considered treatment-related, since there is no apparent consistency in the findings compared across groups and/or sexes, and examination of individual data failed to indicate a clear difference in the range of values for a given measurement between mid dose and control groups.

It should be noted that the mean brain weights for day 12 males selected for morphometric evaluation (shown in Table 18) are not consistent with those for the entire group of Subset 1 (day 12) control males (shown in Table 16, above). Specifically, the mean brain weight for the six control males evaluated morphometrically (1.305 g), was smaller than the corresponding mean brain weight determined for the twenty control males in Subset 1 (1.359 g) [when individual brain weight data are examined, it can be seen that the 4 control males with the lowest brain weights were all selected for histopathological evaluation; if brain weights are ranked from lowest (1) to highest (20), the mean rank of the six selected brains is 4.75, compared to 13.0 for the remaining fourteen (unselected) brains]. Similarly, the mean brain weight for mid-dose males in the morphometric group (1.346 g) was higher than the mean brain weight for mid-dose males in Subset 1 (1.317 g). The brains used for morphometric evaluation represent a subset of the 20 brains/sex/group used for determination of the means presented in Table 16. Since a larger number of subjects are included in the Table 16 data, the values in Table 16 are considered more reliable. It appears that the brains evaluated for morphometrics were not entirely representative of the treatment group. If the control brains chosen for morphometric analysis had been more representative, it is possible that larger treatment-related differences in morphometric measures would have been seen.

Table 18. Selected brain morphometry data for F1 pups.[a]

Morphometric parameter, thickness (μm unless otherwise stated)	Dose (ppm)			
	0	100	300	1000
Males				
Day 12				
Brain weight[#] (g)	1.305±0.035	1.307±0.047	1.346±0.046	1.151±0.058**
Ant/Post Cerebrum (mm)	12.63±0.19	12.40±0.20	12.45±0.32	12.18±0.22**
Ant/Post Cerebellum (mm)	5.53±0.43	5.38±0.33	5.32±.025	5.05±0.31
Frontal Cortex	1488.0±106.3	NA	1484.0±66.9	1368.0±26.3*
Parietal Cortex	1520±65.6	NA	1608±85.9*	1380±54.2**
Caudate Putamen	2304±145.6	NA	2488±178.1	2048±178.7*
Corpus Callosum	286.3±26.8	NA	257.5±30.1	263.8±72.5
Hippocampal Gyrus	984±56.8	NA	1052±73.5	932.0±41.3
Cerebellum	3376±134.6	NA	3440±222.4	2896±49.6** (↓14)
External germinal layer of cerebellar cortex	30.3±3.9	NA	34.5±2.0	37.2±4.4** (↑23)
Day 83				
Ant/Post Cerebrum (mm)	16.73±0.36	16.22±0.26*	16.02±0.40**	16.30±0.33*
Ant/Post Cerebellum (mm)	7.60±0.55	7.88±0.17	7.35±0.48	7.12±0.37
Frontal Cortex	1836±86.9	NA	1812.0±89.5	1808.0±82.7
Parietal Cortex	1868±41.3	NA	1748±79.5*	1848±108.4
Caudate Putamen	3624±207.6	NA	3280.0±112.2**	3464.0±140.5
Corpus Collosum	285.0±13.2	NA	304.0±22.4	275.3±51.1
Hippocampal Gyrus	1640.0±69.0	NA	1624.0±117.2	1644.0±90.8
Cerebellum	5088±160.6	NA	5280.0±187.1	4736±165.4** (↓7)
Females				
Day 12				
Brain weight[#] (g)	1.326±0.068	1.248±0.061	1.301±0.040	1.140±0.082**
Ant/Post Cerebrum (mm)	12.98±0.17	12.15±0.34**	12.48±0.17**	12.32±0.35**
Ant/Post Cerebellum (mm)	5.50±0.33	5.4±0.42	5.28±0.21	4.97±0.39
Frontal Cortex	1616.0±170.8	NA	1480.0±85.4	1464±116.6
Parietal Cortex	1616.0±91.9	NA	1528.0±49.6	1504.0±101.4
Caudate Putamen	2384.0±170.8	NA	2256.0±52.6	2240.0±99.1
Corpus Collosum	320.2±50.6	NA	272.0±29.6	276.8±40.7
Hippocampal Gyrus	1024.0±69.0	NA	984.0±54.7	932.0±51.3*
Cerebellum	3472±170.8	NA	3320±93.2	3120±221.0** (↓10)
External germinal layer of cerebellar cortex	38.0±7.1	NA	36.8±2.4	40.5±6.7
Day 83				
Ant/Post Cerebrum (mm)	15.88±0.42	15.77±0.31	15.60±0.33	15.88±0.38
Ant/Post Cerebellum (mm)	7.38±0.17	7.20±0.35	7.43±0.31	7.10±0.24
Frontal Cortex	1752±50.3	NA	1764±58.3	1772±76.5
Parietal Cortex	1824±72.8	NA	1736±79.8	1780±55.6
Caudate Putamen	3264±151.8	NA	3240±26.3	3360±221.0
Corpus Collosum	267.2±26.9	NA	264.0±16.9	254.3±18.7
Hippocampal Gyrus	1568±69.0	NA	1556±124.6	1548±98.1
Cerebellum	4960±84.1	NA	5064±181.5	4536±131.4** (↓9)

a Data obtained from the study report Appendices L and M, pages 775-778 and 810-813; n=6. *=p<0.05, **=p<0.01 vs. controls. Percent difference from controls is listed parenthetically; #brain weight included here is for the six animals subjected to morphometric evaluation only (brain weight at day 11 in Table 14 includes 19-20 animals/sex/group, the six included here are a subset selected for morphometric and histopathological evaluation).

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Maternal toxicity at 1000 ppm was characterized by mortality, decreased body weight and food consumption, and prolonged gestation. Offspring toxicity at 1000 ppm was characterized by mortality, reduction in body weight and body weight gains, decreased absolute brain weight, decreased cerebellar thickness, and a delay in vaginal patency. The LOAEL for offspring and dams is 1000 ppm and the NOAEL in offspring and dams is 300 ppm.

B. REVIEWER'S DISCUSSION: Tebuconazole (96-96.9% a.i.) in corn oil was administered via the diet to pregnant Crl:CD®BR VAF/Plus® (Sprague Dawley) rats (25/dose) from GD 6 to 24 or LD 11 at doses of 0, 100, 300 or 1000 ppm (equivalent to [GD/LD] 0/0, 8.8/16.3, 22.0/41.3, and 65.0/125.4 mg/kg/day). No analytical data were provided. P dams were allowed to deliver naturally. On day 5 postpartum, litters were standardized to a maximum of 10 pups/litter with 5/sex/litter, as described in the methods section. Pups were assigned to one of 5 Subsets and physical development, sexual maturation, and neurotoxicity were evaluated.

1. Maternal toxicity: Maternal toxicity was seen only at the high dose. Maternal systemic/clinical effects included alopecia, decreases in body weight (4-12%), body weight gain, and food consumption. Body weight had recovered to control levels by LD22 (subsequent to the termination of dosing after LD10). There were also several changes in reproductive parameters at the high dose, including increased number of dead fetuses and prolonged gestation. Maternal mortality (2 high dose dams) was seen in association with the prolonged gestation and dystocia.

   The LOAEL for maternal toxicity is 1000 ppm (equivalent to [GD/LD] 65.0/125.4 mg/kg/day) based on decreased body weights, body weight gains, and food consumption, and an increased number of dead fetuses. The NOAEL is 300 ppm (equivalent to [GD/LD] 22.0/41.3 mg/kg/day).

   The NOAEL is 300 ppm (equivalent to 41.3 mg/kg/day).

2. Offspring toxicity: Offspring toxicity was seen at all doses. The most consistent effect was decreased body weight, most notably during early lactation. Although there was no difference in body weight among groups on day 1, body weight for all treated groups was lower than that of control groups starting on day 5; the lower body weight persisted to approximately day 51 in low and mid-dose groups, and throughout the study in the high dose groups. The difference in body weight was supported by decreases in body weight

gain; decreases in food consumption were seen in the high dose only (as there was no direct exposure to pups through the food, these decreases in food consumption can not be related to changes in feed palatability).

In addition to the decrease in body weight, there was a decrease in survival for high dose pups, during postnatal days 1-5. There were also delays in several physical/developmental landmarks; pinna unfolding was delayed at the high and mid-dose, and vaginal patency was delayed in the high dose only.

Behavioral evaluations also revealed differences from controls across all treated groups, although the differences were not always dose-related. Although there were no apparent effects on the learning/memory tasks evaluated, there were changes in auditory startle amplitude and motor activity levels. For auditory startle, a dose-related decrease in amplitude was seen at all doses in day 23 females, and in high dose females on day 63. Males also showed a decrease in amplitude in the mid and high dose groups on day 23, but on day 63, there was a dose-related increase in amplitude across all treated male groups. Motor activity findings were less clear-cut; there was a decrease in activity at the low and high doses for males on days 14 and 18, and for low and high dose females on day 14. There was a slight decrease in activity of the mid-dose males on day 14, but no effects were seen in mid dose males on day 18 or in mid dose females on either day. There was also an increase in activity in high dose males on day 22 and high dose females on days 18 and 22; all treated groups demonstrated levels of activity comparable to controls on day 62. Although there was not a consistent pattern of effects across doses, the magnitude of effects in low dose males (35% decrease on day 14, 28% decrease in day 18), and the consistency of the pattern across sexes, provide evidence that the changes seen at the low dose were treatment related.

Decreases in brain weight were also seen in all tebuconazole-treated groups. These differences were statistically significant at the low dose for both males and females on day 12, for the mid-dose females on day 12, and for high dose males and females on days 12 and 63. Similar differences were also found in some other brain measurements, most notably the decrease in anterior/posterior cerebrum measurements. These differences in brain measurements increase the strength of the finding of a decrease in brain weight at all doses. The magnitude of the change was larger in the high dose group (15-16% decrease in brain weight at day 12, compared to a 4% decrease at the low dose), and several differences in morphometric measurements were also seen at that dose.

Taken together, these findings provide strong support for treatment-related findings in the offspring at all doses. These findings were seen in several types of behavioral indices, in body weight, and in brain weight and measurements. Some of the findings appear to be persistent, especially at the high dose.

The LOAEL for offspring toxicity is 100 ppm (equivalent to [GD/LD] 8.8/16.3 mg/kg/day) based on decreases in body weights (3-7%) and body weight gain (4-

13%), decreases in brain weight (2-4%) and measurements (3-6%), and decreases in motor activity.

The NOAEL is not determined.

This study is classified as **acceptable/guideline (§83-6[a])** and satisfies the requirement for a developmental neurotoxicity study in rats.

C. STUDY DEFICIENCIES: The following deficiencies were noted, but will not affect the conclusions of the review (requested information should be submitted):

- Information regarding homogeneity, stability, or concentration of test substance in the diet were provided in the text of the study report; however, supporting data were not submitted. Information regarding diet preparation was also not included in the report. These data should be submitted.
- Additional positive control data need to be submitted, as discussed above and described in Appendix B.

APPENDIX A

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 1): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 14 Postpartum				
Male rats	20	20	20	20
Number of movements				
Block 1	40.9±27.3	29.4±23.7	48.4±24.1	43.0±20.2
Block 2	45.4±28.9	32.2±24.7	49.6±22.4	36.2±20.6
Block 3	52.2±26.1	36.0±23.4	46.2±17.4	33.5±20.1*
Block 4	51.6±22.7	27.0±19.6**	44.2±26.2	30.2±23.8*
Block 5	47.4±24.8	27.8±24.2*	42.6±23.3	27.2±21.6*
Block 6	39.2±31.6	27.0±25.0	37.8±23.9	27.2±22.7
Block 7	41.6±28.8	34.0±27.4	38.9±29.8	24.4±21.0
Block 8	54.8±28.9	32.1±27.1*	35.1±29.9	21.2±18.7**
Block 9	50.6±24.1	32.2±29.9	31.7±26.9	20.9±17.4**
Block 10	39.6±32.3	31.8±25.6	27.9±24.1	24.5±19.0
Block 11	46.1±30.7	30.3±28.6	29.4±26.2	24.8±18.9*
Block 12	36.8±26.9	26.2±28.2	41.7±22.8	26.2±25.9
Block 13	34.8±31.1	20.0±24.1	40.2±25.9	25.0±23.4
Block 14	45.6±36.6	22.2±23.8*	40.5±26.1	17.3±19.3**
Block 15	38.8±30.3	21.9±20.4	33.8±26.1	21.2±22.7
Block 16	33.6±31.8	20.8±19.2	29.4±25.6	18.7±23.1
Block 17	41.4±29.1	28.5±20.4	33.4±26.9	10.6±15.3**
Block 18	43.6±32.2	29.2±27.6	35.0±26.6	15.4±18.2**
Total	784.0±299.7	508.8±298.7**	685.6±269.8	447.6±227.8**

Total = Sum of blocks; each Block consists of a 5 minute period.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 2): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 14 Postpartum				
Male rats	20	20	20	20
Time (seconds) spent in movement				
Block 1	38.8±33.8	24.8±28.8	43.7±25.8	46.2±34.9
Block 2	45.0±35.2	28.9±33.4	50.6±27.3	37.3±32.2
Block 3	55.4±37.0	36.4±33.3	50.4±24.5	35.0±32.1
Block 4	57.0±37.4	29.1±32.7	53.2±43.5	36.5±42.9
Block 5	55.0±38.2	28.1±35.8	44.8±35.7	31.6±33.4
Block 6	45.3±46.9	26.6±33.4	44.0±34.8	29.4±37.2
Block 7	50.8±44.0	36.3±37.2	53.2±52.0	29.4±39.7
Block 8	71.4±49.9	37.7±44.3*	45.8±45.5	20.8±31.9**
Block 9	67.0±42.1	37.0±44.8*	38.0±40.3	21.6±24.1**
Block 10	51.5±52.5	34.8±33.1	35.0±41.4	25.8±26.2
Block 11	59.8±47.5	34.8±40.4	32.4±36.2	24.8±24.4*
Block 12	50.8±53.6	29.2±34.7	50.6±39.0	35.3±49.2
Block 13	44.4±51.5	23.8±37.7	51.0±46.2	30.4±40.1
Block 14	60.8±57.4	25.8±36.4*	50.4±39.1	18.2±23.7**
Block 15	51.2±50.7	20.2±25.1*	49.8±46.9	23.2±34.0
Block 16	43.8±57.4	21.6±34.9	36.9±45.5	25.2±46.2
Block 17	55.0±50.9	30.5±31.0	42.3±47.9	10.1±20.0**
Block 18	62.2±58.9	32.6±41.4	49.4±52.0	13.1±22.2**
Total	965.2±510.9	538.1±452.0*	821.6±469.9	493.8±444.5**

Total = Sum of blocks; each Block consists of a 5 minute period.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 3): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 14 Postpartum				
Female rats	20	20	20	20
Number of movements				
Block 1	39.7±23.6	29.2±21.4	42.2±25.7	36.6±21.5
Block 2	36.9±21.9	34.2±20.3	40.1±20.4	26.8±20.9
Block 3	44.5±25.3	37.4±27.4	43.0±22.6	29.2±21.4
Block 4	41.1±22.8	37.4±20.8	45.1±20.1	30.6±18.6
Block 5	30.8±26.7	32.6±25.0	34.2±22.4	30.4±20.2
Block 6	35.8±29.0	31.6±22.8	31.8±27.9	29.4±22.8
Block 7	40.0±31.8	32.0±25.7	28.2±22.9	25.8±21.5
Block 8	28.4±27.4	28.6±27.4	30.6±24.0	17.4±18.8
Block 9	26.6±29.5	20.6±24.2	33.2±27.3	17.6±16.1
Block 10	29.0±28.7	22.8±22.6	32.8±22.4	21.1±24.6
Block 11	25.5±25.6	23.6±22.4	30.0±30.3	26.6±23.7
Block 12	28.4±27.1	26.9±25.2	30.4±25.3	23.0±24.9
Block 13	24.2±26.2	26.0±21.0	28.8±24.4	23.0±19.7
Block 14	21.8±25.3	26.3±27.1	27.1±24.7	23.2±23.1
Block 15	30.5±28.7	23.4±16.6	29.8±26.5	19.2±22.9
Block 16	29.8±30.6	17.3±16.5	30.3±26.0	15.4±17.8
Block 17	22.4±24.6	15.0±16.3	39.6±30.2*	13.5±15.9
Block 18	23.8±25.1	18.2±18.9	33.6±24.4	14.6±17.3
Total	559.2±337.0	483.2±263.3	610.8±263.6	423.4±183.3

Total = Sum of blocks; each Block consists of a 5 minute period.
* Significantly different from the carrier group value ($p \leq 0.05$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 4): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 14 Postpartum				
Female rats	20	20	20	20
Time (seconds) spent in movement				
Block 1	40.0±33.0	26.1±23.2	48.7±46.8	34.6±33.1
Block 2	37.2±35.3	32.6±28.1	50.8±41.6	25.7±28.3
Block 3	50.6±39.2	38.1±32.1	56.8±43.6	28.3±30.0
Block 4	49.4±37.3	40.0±31.9	61.2±50.2	27.6±26.4
Block 5	36.0±36.2	35.4±38.2	49.8±50.3	31.6±26.5
Block 6	45.5±47.7	31.8±31.9	48.0±57.3	32.8±32.3
Block 7	57.6±58.0	36.2±36.3	40.0±46.9	27.6±30.4
Block 8	41.9±50.8	32.4±35.2	41.1±43.7	18.6±29.1
Block 9	36.4±51.8	25.2±38.4	49.2±50.4	17.2±20.4
Block 10	39.4±51.0	28.9±36.5	43.4±41.9	20.6±30.2
Block 11	37.4±49.1	28.0±37.6	37.2±40.0	25.3±27.9
Block 12	44.9±53.9	31.9±40.2	38.2±41.2	24.0±30.7
Block 13	33.3±44.6	31.8±32.2	42.2±46.7	23.4±25.8
Block 14	33.6±56.6	34.4±43.3	40.9±57.0	25.6±36.8
Block 15	41.4±53.2	28.6±32.7	48.2±58.0	20.6±36.6
Block 16	42.7±53.5	21.3±32.8	44.8±49.4	14.2±23.4
Block 17	26.9±42.3	12.8±18.0	51.0±49.2	10.2±16.6
Block 18	35.3±52.4	20.2±29.2	49.4±49.4	12.4±20.0
Total	729.5±620.8	535.9±445.6	841.3±633.7	420.3±275.1

Total = Sum of blocks; each Block consists of a 5 minute period.

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 5): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 18 Postpartum				
Male rats	20	19a	20	20
Number of movements				
Block 1	65.0±20.3	50.9±24.0	61.6±13.9	50.5±24.3
Block 2	66.8±17.3	54.4±22.3	63.4±14.4	61.0±22.1
Block 3	60.6±15.2	44.4±24.5*	63.4±14.9	62.7±19.0
Block 4	60.8±18.6	44.3±24.8*	64.8±17.1	64.1±11.5
Block 5	60.4±12.1	44.5±25.0*	68.1±15.6	61.2±14.2
Block 6	58.0±20.1	41.0±29.2*	66.6±9.1	60.8±17.8
Block 7	52.6±28.4	43.2±30.2	58.1±16.2	59.8±22.9
Block 8	53.2±27.8	45.8±32.2	58.0±16.5	64.6±22.3
Block 9	50.3±30.8	39.8±36.7	56.6±23.9	51.4±28.6
Block 10	42.7±29.9	34.8±32.1	55.2±22.0	45.4±36.4
Block 11	41.7±28.3	35.0±32.2	55.7±18.2	47.6±32.9
Block 12	48.6±30.0	33.4±32.0	52.6±23.9	48.2±31.0
Block 13	48.4±31.7	36.0±32.5	47.0±27.8	52.6±29.1
Block 14	42.1±28.8	31.5±33.8	53.9±27.9	55.8±26.4
Block 15	48.8±29.5	24.4±31.2*	45.1±30.8	43.4±29.8
Block 16	49.6±30.5	30.3±32.0	48.6±27.1	45.4±31.0
Block 17	42.4±30.0	24.2±28.8	49.4±25.8	39.0±28.5
Block 18	42.6±29.9	15.8±25.5*	36.6±26.9	42.5±32.2
Total	934.8±320.6	673.6±380.0*	1004.6±195.7	956.0±322.3

Total = Sum of blocks; each Block consists of a 5 minute period.

a. Excludes values for rat 9317, which was not continued on study.

* Significantly different from the carrier group value ($p \leq 0.05$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 6): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 18 Postpartum				
Male rats	20	19a	20	20
Time (seconds) spent in movement				
Block 1	98.4±40.9	64.3±44.9	94.4±45.1	71.4±55.9
Block 2	107.0±39.8	70.7±41.5*	117.8±48.1	93.7±53.5
Block 3	105.0±46.5	62.7±52.7*	124.5±41.1	96.0±51.0
Block 4	104.6±48.8	61.3±51.1*	122.8±44.0	106.6±37.1
Block 5	100.8±44.8	66.6±50.8	118.3±45.3	95.6±47.2
Block 6	102.8±56.7	57.5±57.7*	123.1±51.6	100.4±43.8
Block 7	87.8±58.9	74.8±66.2	112.8±52.2	90.2±46.8
Block 8	89.4±58.6	74.9±65.7	108.2±42.8	103.4±54.0
Block 9	94.7±71.6	61.8±65.1	113.0±68.0	77.3±48.7
Block 10	80.4±72.3	60.4±66.0	101.5±54.1	74.2±68.0
Block 11	79.4±71.7	63.2±69.5	99.1±49.4	73.0±61.2
Block 12	90.2±70.7	56.3±59.8	95.1±60.9	72.5±55.3
Block 13	88.8±69.4	60.0±64.9	82.6±58.8	80.3±53.6
Block 14	68.5±56.7	64.0±75.1	92.7±57.0	93.0±57.8
Block 15	84.0±56.9	43.2±57.8	77.4±59.8	74.9±57.1
Block 16	91.1±69.2	51.5±63.6	77.7±55.6	76.4±60.6
Block 17	77.9±63.3	36.7±51.0	87.4±54.1	64.2±58.9
Block 18	72.7±62.9	28.7±56.8	68.4±61.0	70.4±67.7
Total	1623.6±844.3	1058.5±831.3	1817.0±643.6	1513.3±724.3

Total = Sum of blocks; each Block consists of a 5 minute period.

a. Excludes values for rat 9317, which was not continued on study.

* Significantly different from the carrier group value ($p \leq 0.05$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 7): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 18 Postpartum				
Female rats	20	19a	20	20
Number of movements				
Block 1	55.8±20.8	47.7±24.9	61.0±17.4	51.8±27.0
Block 2	59.8±19.0	57.2±26.0	63.0±13.6	64.4±20.1
Block 3	59.2±18.2	57.2±16.1	59.8±14.8	57.8±22.4
Block 4	54.4±18.0	54.5±22.1	63.6±12.0	63.8±20.2
Block 5	56.5±16.1	54.2±22.4	59.9±19.5	62.9±16.1
Block 6	52.6±23.9	59.2±24.0	54.0±21.6	56.4±25.7
Block 7	50.2±23.5	49.6±28.5	54.7±24.8	56.4±24.5
Block 8	52.6±22.2	56.6±23.2	52.9±21.2	56.5±23.4
Block 9	44.9±24.9	52.4±22.2	43.8±28.9	51.8±27.7
Block 10	40.7±31.6	46.4±29.5	39.6±29.0	50.5±28.4
Block 11	35.2±31.3	48.5±30.7	40.6±31.1	50.4±30.2
Block 12	32.8±27.5	41.2±29.8	33.8±25.8	49.1±29.1
Block 13	32.3±31.7	43.8±28.1	41.0±29.7	48.4±29.2
Block 14	31.0±28.5	37.9±29.6	37.1±29.0	46.6±31.2
Block 15	32.7±27.4	37.2±31.0	35.3±30.5	39.2±31.7
Block 16	32.2±29.4	35.9±31.1	39.8±26.9	36.7±30.1
Block 17	37.7±30.6	36.5±28.8	41.6±28.1	36.2±27.8
Block 18	38.1±35.4	44.3±29.8	46.2±31.3	39.3±28.8
Total	798.9±318.6	860.2±308.5	867.7±266.0	918.2±349.8

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 8): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 18 Postpartum				
Female rats	20	19a	20	20
Time (seconds) spent in movement				
Block 1	92.1±54.3	83.3±52.4	91.2±41.5	69.6±46.5
Block 2	98.2±40.3	86.5±53.6	101.6±31.4	96.6±41.2
Block 3	94.8±39.0	93.8±47.7	101.8±38.5	87.3±39.2
Block 4	90.8±47.3	101.0±54.3	106.8±26.9	107.3±44.8
Block 5	95.0±39.8	94.3±50.2	95.5±38.0	103.9±38.5
Block 6	84.2±42.4	95.5±46.7	84.8±48.3	95.5±49.5
Block 7	82.6±51.0	83.2±55.5	94.6±53.9	92.6±46.9
Block 8	94.3±46.2	96.9±56.3	88.3±48.0	94.9±48.3
Block 9	72.8±46.9	93.5±62.6	75.6±58.6	90.8±56.8
Block 10	71.3±61.0	86.4±67.6	67.0±60.6	92.4±59.4
Block 11	58.0±54.1	89.6±70.2	69.1±61.4	85.8±55.7
Block 12	53.9±50.3	69.3±60.3	57.8±53.3	93.0±58.4
Block 13	54.5±60.6	75.7±58.7	68.4±60.2	79.8±52.0
Block 14	49.8±51.4	66.8±60.0	61.6±52.4	80.5±60.6
Block 15	50.5±47.6	66.2±66.2	55.3±56.0	70.2±65.1
Block 16	56.8±58.2	57.0±53.2	68.5±51.2	71.9±65.2
Block 17	63.2±56.7	62.0±57.6	72.1±56.0	68.5±63.4
Block 18	60.4±59.5	79.0±65.3	76.6±57.1	71.8±63.1
Total	1323.3±650.4	1480.2±764.5	1436.8±565.0	1552.5±731.6

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

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TABLE F1 (page 9): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 22 Postpartum				
Male rats	20	19a	20	20
Number of movements				
Block 1	67.4±12.0	75.5±10.8	68.8±9.3	68.0±10.0
Block 2	53.3±21.8	57.8±21.6	58.7±15.2	60.6±17.1
Block 3	47.8±28.0	46.6±26.9	50.6±23.1	56.2±17.5
Block 4	46.5±25.7	48.8±25.0	46.1±23.5	56.6±17.0
Block 5	48.2±28.8	48.5±21.4	43.4±24.0	54.2±22.0
Block 6	31.5±27.5	39.6±26.6	43.0±24.2	47.0±22.3
Block 7	31.6±24.7	36.0±29.1	35.6±26.4	51.9±22.0*
Block 8	40.8±31.5	35.0±28.2	37.4±26.9	53.2±25.1
Block 9	35.0±28.8	34.9±26.5	33.6±26.6	46.3±24.2
Block 10	25.6±24.2	31.7±22.4	27.6±27.1	42.8±22.8
Block 11	26.6±26.5	31.0±25.6	32.2±25.5	47.4±27.2*
Block 12	21.4±24.3	27.4±24.1	29.3±27.0	41.2±26.8*
Block 13	23.6±29.9	24.3±24.9	30.4±26.9	41.6±24.2
Block 14	23.0±24.2	20.0±26.2	24.1±25.3	37.8±25.0
Block 15	22.7±26.5	20.2±23.8	18.2±20.1	43.5±30.2*
Block 16	19.6±25.1	25.0±28.6	22.0±24.0	32.5±27.4
Block 17	22.8±28.2	21.7±23.7	22.9±23.5	36.6±30.3
Block 18	22.6±25.9	22.1±23.4	21.2±21.7	33.2±28.2
Total	610.2±357.3	646.2±222.0	645.0±258.2	850.5±280.3*

Total = Sum of blocks; each Block consists of a 5 minute period.

a. Excludes values for rat 9317, which was not continued on study.

* Significantly different from the carrier group value ($p \leq 0.05$).

Protocol 1702-004: Developmental neurotoxicity study of technical grade Tebuconazole administered orally via diet to Crl:CD(R)BR VAF/Plus(R) presumed pregnant rats (Sponsor's study number: 98-C612-QU)

TABLE F1 (page 10): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 22 Postpartum				
Male rats	20	19a	20	20
Time (seconds) spent in movement				
Block 1	142.7±42.0	137.0±29.4	134.2±41.0	127.2±45.1
Block 2	94.8±54.0	85.7±40.9	98.8±49.2	98.3±42.6
Block 3	79.4±60.2	71.9±59.0	78.0±51.0	95.8±48.2
Block 4	76.0±52.1	67.4±51.4	78.6±56.5	94.8±45.7
Block 5	74.6±54.3	67.0±40.0	65.8±47.5	83.3±40.4
Block 6	42.4±51.6	56.7±55.2	60.6±47.5	81.8±57.6
Block 7	46.0±45.2	55.7±58.8	52.6±48.6	88.4±53.9*
Block 8	53.6±50.7	47.4±48.2	50.7±48.4	78.2±47.0
Block 9	63.9±72.2	51.8±55.6	42.8±41.8	65.8±43.0
Block 10	36.1±47.0	40.0±36.4	39.5±46.3	68.8±48.7
Block 11	46.4±61.3	35.7±33.6	46.7±42.6	66.8±46.9
Block 12	35.2±58.6	35.7±35.0	42.0±46.2	53.8±48.5
Block 13	36.0±54.2	33.7±40.0	42.6±43.1	54.8±39.5
Block 14	39.0±54.0	26.7±45.8	35.0±40.6	58.4±54.1
Block 15	34.4±53.7	24.7±35.2	27.0±36.4	58.1±50.0
Block 16	30.4±49.5	31.6±43.7	26.2±33.9	44.8±46.8
Block 17	30.3±42.5	28.7±41.3	29.7±43.8	49.8±46.3
Block 18	38.2±59.6	28.3±33.6	28.4±36.5	49.2±49.9
Total	999.6±791.9	925.8±429.5	979.6±524.4	1318.0±630.1

Total = Sum of blocks; each Block consists of a 5 minute period.

a. Excludes values for rat 9317, which was not continued on study.

* Significantly different from the carrier group value ($p \leq 0.05$).

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TABLE F1 (page 11): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 22 Postpartum				
Female rats	20	19a	20	20
Number of movements				
Block 1	61.9±18.1	57.8±14.4	62.4±13.1	60.0±13.6
Block 2	53.6±20.7	59.3±12.3	55.1±18.1	59.9±11.8
Block 3	48.4±19.2	46.9±21.8	46.1±19.4	52.4±17.7
Block 4	42.6±22.3	42.4±26.7	45.1±21.4	52.4±14.0
Block 5	48.0±22.1	39.1±26.1	43.8±23.2	47.5±19.1
Block 6	43.7±23.1	40.4±21.6	42.2±21.9	46.9±17.2
Block 7	37.6±24.6	37.7±26.9	44.6±23.5	45.8±21.3
Block 8	34.8±24.6	35.9±27.8	35.4±21.4	42.0±22.8
Block 9	35.3±25.8	34.5±27.0	39.2±21.4	45.2±25.1
Block 10	28.4±26.0	29.2±26.7	28.6±22.9	40.6±30.1
Block 11	34.2±28.6	33.2±28.2	21.2±24.8	34.8±26.8
Block 12	23.2±22.4	29.4±29.4	28.2±29.0	32.1±23.8
Block 13	19.4±24.8	25.5±27.1	25.4±26.2	35.1±30.0
Block 14	23.4±29.7	19.0±23.5	22.4±27.7	29.2±30.6
Block 15	23.4±22.6	22.4±29.3	23.2±27.5	34.4±30.3
Block 16	20.3±22.4	18.2±20.7	21.4±27.7	31.9±28.1
Block 17	17.0±21.8	19.9±26.8	20.2±22.7	27.0±31.7
Block 18	23.4±27.1	17.3±23.0	13.4±19.8	21.4±25.5
Total	618.6±284.5	608.0±332.4	617.8±214.6	738.3±277.1

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

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TABLE F1 (page 12): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 22 Postpartum				
Female rats	20	19a	20	20
Time (seconds) spent in movement				
Block 1	127.2±42.1	122.5±44.6	118.4±36.2	116.8±43.3
Block 2	92.4±49.4	112.1±41.0	90.5±40.4	101.3±20.1
Block 3	70.6±32.9	75.8±45.4	63.0±39.8	81.2±37.3
Block 4	53.4±34.8	71.0±56.8	63.0±38.9	83.2±37.0
Block 5	69.3±45.6	66.5±58.3	55.0±34.6	62.6±38.2
Block 6	64.6±45.0	61.9±45.8	62.8±43.3	65.1±33.8
Block 7	55.5±45.2	58.3±46.6	58.0±35.0	66.8±44.3
Block 8	44.3±36.5	52.9±49.4	46.2±40.6	59.4±37.9
Block 9	44.6±39.9	44.1±40.8	55.5±36.0	68.7±44.8
Block 10	33.3±33.3	45.7±49.9	35.8±31.8	64.1±48.9
Block 11	46.8±42.3	39.1±42.4	27.5±35.7	56.5±51.3
Block 12	29.2±32.3	44.0±47.7	43.2±53.6	42.5±39.9
Block 13	25.2±39.1	37.7±47.2	33.3±44.1	60.0±57.7
Block 14	33.0±47.8	32.3±49.3	27.6±38.8	43.5±47.8
Block 15	33.4±40.0	35.1±51.9	31.6±42.6	53.6±56.0
Block 16	27.9±32.4	20.6±26.8	28.2±42.3	49.6±52.5
Block 17	22.6±33.7	28.7±44.8	25.6±34.6	35.0±45.5
Block 18	28.2±35.4	21.6±31.5	15.0±29.3	30.3±42.5
Total	901.4±434.0	970.2±643.6	880.1±365.1	1140.3±477.0

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

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TABLE F1 (page 13): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 62 Postpartum				
Male Rats	20	19a	20	20
Number of movements				
Block 1	64.8±9.6	61.3±12.7	63.7±10.1	62.3±10.4
Block 2	67.1±10.2	65.8±8.1	65.5±9.0	66.8±9.0
Block 3	70.4±10.2	63.6±10.6	67.5±7.5	64.4±9.5
Block 4	67.0±9.8	67.3±10.5	67.8±10.6	69.6±10.1
Block 5	66.6±13.1	64.6±8.9	68.6±8.1	67.0±10.8
Block 6	68.1±11.0	60.8±17.4	67.6±12.2	66.8±12.9
Block 7	68.6±10.9	62.3±15.8	60.0±16.8	61.6±17.3
Block 8	66.0±14.7	58.6±21.8	59.6±20.9	63.2±17.2
Block 9	62.0±16.9	54.8±23.3	51.0±24.2	61.6±19.6
Block 10	60.8±17.3	55.5±27.8	49.9±21.9	55.0±25.6
Block 11	58.4±20.3	49.1±29.8	48.4±29.8	54.8±29.8
Block 12	52.9±23.1	48.4±29.4	34.4±31.7	48.1±26.0
Block 13	43.4±27.4	45.0±29.0	31.4±29.1	43.7±33.1
Block 14	37.4±30.0	35.0±31.8	28.6±31.4	44.1±33.3
Block 15	35.6±31.8	28.9±29.0	25.4±29.3	33.3±30.8
Block 16	35.7±33.7	20.7±26.2	23.0±26.2	30.4±31.6
Block 17	32.6±32.3	18.6±25.4	25.6±32.2	32.1±29.4
Block 18	25.4±33.1	16.6±23.1	21.2±27.1	27.7±32.2
Total	982.8±207.0	877.0±216.5	859.1±209.4	952.2±273.9

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

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TABLE F1 (page 14): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 62 Postpartum				
Male Rats	20	19a	20	20
Time (seconds) spent in movement				
Block 1	216.4±26.0	210.6±35.0	216.0±24.4	217.4±26.1
Block 2	179.3±37.6	195.6±33.5	188.2±26.2	192.2±30.0
Block 3	164.7±42.4	181.7±47.7	183.4±33.4	171.4±39.3
Block 4	155.5±38.0	161.0±40.7	158.3±31.1	159.2±44.0
Block 5	155.6±41.9	150.4±51.9	154.2±37.1	154.2±43.3
Block 6	131.1±34.7	134.6±55.9	144.4±42.2	141.6±43.2
Block 7	126.7±33.9	123.5±55.0	113.8±51.4	133.4±47.7
Block 8	124.3±38.4	98.6±52.0	109.3±53.7	126.2±54.6
Block 9	109.4±44.9	93.0±55.1	79.0±59.3	118.6±58.2
Block 10	118.0±50.4	90.9±53.2	79.8±53.3	101.8±64.2
Block 11	99.9±57.6	97.3±72.2	85.8±67.5	88.8±58.1
Block 12	94.0±63.0	80.2±63.4	53.6±63.0	76.0±49.4
Block 13	70.7±60.0	74.6±58.4	42.6±48.7	59.8±58.0
Block 14	66.1±66.3	61.3±68.5	50.2±68.4	72.6±70.2
Block 15	50.6±53.8	50.5±66.9	34.4±49.4	54.2±64.0
Block 16	49.6±57.1	31.8±50.2	31.4±47.8	56.6±75.8
Block 17	52.7±67.6	28.3±52.6	44.3±66.0	47.8±59.0
Block 18	41.7±64.8	24.9±44.0	37.6±57.9	44.4±65.7
Total	2006.2±465.9	1888.9±684.0	1806.4±588.9	2016.2±594.6

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.

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TABLE F1 (page 15): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 62 Postpartum				
Female Rats	20	19a	19b	20
Number of movements				
Block 1	62.8±6.3	62.7±10.2	59.6±9.1	60.6±7.6
Block 2	67.0±9.1	65.7±11.7	64.2±7.8	64.4±11.2
Block 3	65.4±10.0	67.3±11.7	63.4±8.2	64.2±7.3
Block 4	66.6±8.1	65.6±10.2	65.4±8.9	61.6±8.0
Block 5	67.4±7.4	68.7±8.6	66.7±8.8	66.8±8.0
Block 6	63.6±9.3	65.5±15.9	62.5±11.4	65.3±9.9
Block 7	66.7±12.9	61.7±17.9	62.5±16.6	63.6±8.5
Block 8	62.6±15.2	60.1±22.0	55.3±24.4	64.2±9.3
Block 9	56.8±22.7	59.5±24.8	58.5±22.4	58.9±19.8
Block 10	46.8±25.7	54.5±23.7	51.4±23.6	58.0±17.1
Block 11	43.2±29.0	50.1±28.1	47.2±27.5	58.4±20.8
Block 12	43.9±28.6	45.8±30.8	40.7±26.3	43.4±26.5
Block 13	36.6±30.0	41.7±32.7	37.7±35.1	36.8±30.9
Block 14	36.4±30.2	40.8±32.4	39.1±34.8	34.8±32.2
Block 15	35.4±30.6	25.8±32.2	26.5±30.4	38.6±33.0
Block 16	35.8±26.2	25.0±29.3	30.3±28.5	36.1±33.3
Block 17	28.8±27.9	25.7±30.7	33.8±31.1	27.9±29.9
Block 18	24.0±23.6	24.0±31.5	34.7±26.8	23.2±23.6
Total	909.9±185.2	910.5±240.3	899.4±191.6	926.9±211.2

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.
b. Excludes values for rat 9780, which was missing on day 23 of study; was found and sacrificed on day 33 or study.

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TABLE F1 (page 16): Motor Activity - Summary - F1 Generation Rats - Subset 3

Maternal dosage Group	I	II	III	IV
Maternal dietary concentration (ppm)	0(carrier)	100	300	1000
Day 62 Postpartum				
Female Rats	20	19a	19b	20
Time (seconds) spent in movement				
Block 1	217.2±20.5	216.0±24.0	227.3±26.6	228.4±18.8
Block 2	196.8±21.8	186.5±37.3	195.9±30.5	196.8±32.2
Block 3	175.4±34.8	196.2±38.9	187.5±35.7	188.0±29.3
Block 4	153.4±32.6	168.7±34.1	162.4±26.0	166.3±35.3
Block 5	147.5±27.4	158.6±39.8	159.5±35.9	158.5±37.9
Block 6	134.0±43.1	143.7±52.4	140.0±52.0	148.8±39.5
Block 7	122.0±31.0	118.0±53.0	117.6±47.7	146.5±41.9
Block 8	125.1±46.6	121.7±64.1	117.3±67.3	152.6±39.5
Block 9	93.3±49.2	104.5±59.3	126.0±57.7	121.0±53.4
Block 10	64.0±45.6	98.9±65.4	93.2±65.7	109.6±46.2*
Block 11	77.4±61.6	100.9±70.6	97.7±75.3	97.0±48.8
Block 12	67.7±54.8	85.6±67.1	82.6±76.0	70.8±56.1
Block 13	59.7±61.9	60.3±55.0	64.7±66.4	51.6±48.8
Block 14	59.2±61.4	61.0±58.2	63.2±66.1	57.7±62.2
Block 15	54.0±56.2	39.7±58.0	42.1±55.0	63.7±64.4
Block 16	50.0±49.6	38.7±56.8	54.7±60.8	55.0±56.1
Block 17	45.4±61.2	41.9±60.7	63.7±68.8	55.3±67.1
Block 18	33.0±46.9	36.2±54.3	51.4±54.8	32.0±40.5
Total	1875.0±401.8	1977.0±617.2	2046.9±616.5	2099.8±463.7

Total = Sum of blocks; each Block consists of a 5 minute period.
a. Excludes values for rat 9317, which was not continued on study.
b. Excludes values for rat 9780, which was missing on day 23 of study; was found and sacrificed on day 33 or study.

APPENDIX B

Submitted Positive Control Data

A volume of information was submitted as positive control data for the current study. Most of the information in this volume consisted of summaries of studies performed by scientists currently or formerly affiliated with the study laboratory. Some of the studies were performed at the study laboratory, others at institutions with which the scientists were previously affiliated.

The summarized information included a variety of studies (summarized briefly below), largely relevant to the development of the procedures used in the current developmental neurotoxicity and adult neurotoxicity study protocols at the study laboratory. However, apart from specific exceptions described below, the submitted information is not fully adequate to support the sensitivity of many of the procedures used in the current study.

Our current recommendations for positive control data are as follows:

> Appropriate, adequate positive control data from the laboratories that performed the Developmental Neurotoxicity studies should be provided to the Agency at the time of study submission. These positive control data should demonstrate the sensitivity of the procedures used, including the ability to detect both increases and decreases in parameters measured, as appropriate. While the positive control studies do not need to be performed using prenatal exposures, the laboratory must demonstrate competence in the evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for all critical endpoints, and for appropriate age groups. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies.

Based on our review of the submitted information, most of the submitted studies were performed outside of the recommendated time frame, and laboratory personnel varied from those involved in the current study. It is unclear whether test procedures were the same as those used in the current study, and not all procedures were evaluated following exposure to neurotoxic substances. Insufficient information was included for most studies; for example, individual animal data were rarely included and detailed procedural information was often not provided.

Since treatment-related effects were seen at all doses evaluated in the current study, we will not require submission of additional positive control data prior to acceptance of that study. We note, however, that insufficiently sensitive procedures could lead to a failure to detect effects on some parameters (for example, cognitive or motor activity testing) or a failure to detect effects at low doses.

Based on our evaluation of the submitted positive control data, according to current recommendations, additional data are needed to adequately validate the sensitivity and reliability of the procedures listed below, as currently used in the testing laboratory. The additional positive control data should be gathered using current personnel and equipment, with the same procedures as those used in the study, and using the same strain of rat. Complete study reports should be submitted, including individual animal data.

Procedures lacking appropriate positive control data:

1) Motor activity evaluation, all time points;
2) Learning and memory procedures, both time points;
3) Auditory startle habituation, both time points;
4) Neuropathology evaluations:
   -qualitative evaluations in treated pups
   -morphometric evaluations in adults (late time point).

As noted below, the submitted positive control data for pup morphometric evaluations was considered adequate (Study #12), but the submission for adult qualitative evaluations was incomplete, since the data tables were not provided. The adequacy of the data supporting adult qualitative evaluations cannot be determined in the absence of the data tables; those data should be submitted.

Summary of submitted studies

Each of the submitted studies, along with a brief description, is listed below. Studies are grouped according to the type of data included.

FOB validation studies

1. Parker, R.M. (1999) Neurotoxicity evaluation of positive control substances in Crl:CD®BR VAF/Plus Rats. Argus Research Laboratories, August 6, 1999, Unpublished study.

[Appendix O, Section 10, pp. 954-1131]

The study evaluated FOB performance using several known neurotoxic chemicals (acrylamide, Trimethyl tin, MK-801, Carbaryl, and DDT). Evaluations were made in 59-day old rats. This appears to be a GLP study, useful in validating FOB evaluations in adult rats. However, it was not possible to fully evaluate the adequacy of these data, because the portion of the report containing the individual animal data was excluded from the submission (study report pp. 143-229). Since full FOB evaluations were not performed in the current study, these data are not considered critical in support of this study.

2. Protocol 012-015: Neurotoxicity evaluation of DDT in Crl:CD®BR VAF/Plus Rats.

[Appendix O, Section 11, pp. 1133-1141]

This study was performed in 1992 at Argus Laboratories, and evaluated 50/51 day old rats on the FOB. As above, these data are relevant to FOB evaluations performed in adult rats, which were not performed in the current study.

FOB and motor activity in adult rats

3. Lochry, E.A., J.A. Foss, and M.S. Christian (1990). Validation of a functional observational battery and motor activity measure using positive test substances. Argus Research Laboratories,

Poster presented at the Annual Meeting of the American College of Toxicology, Orlando, Florida, October 1990.

[Appendix O, Section 12, pp. 1142-1174]

This information consists of a poster copy. The study described was performed on rats which were 65 days old at arrival in the laboratory (age at testing was not stated), and concerns performance of FOB and motor activity measures following administration of DDT, physostigmine, and acrylamide.

4. Protocol 012-014: Neurotoxicity evaluation of positive control substances in Crl:CD®BR VAF/Plus Rats.

[Appendix O, Section 13, pp. 1176-1242]

This submission consisted of summary data only, concerning FOB and motor activity testing using acrylamide and carbaryl.

Studies including assessments in developing animals

5. Foss, J.A. and E.A. Lochry. (1991) The assessment of motor activity in neonatal and adult rodents using passive infrared sensors. Argus Laboratories, Poster present at the Annual Meeting of the American College of Toxicology, Savannah, Georgia, October, 1991.

[Appendix O, Section 14, pp. 1243-1250]

This section consists of a poster copy. Habituation (for motor activity) was evaluated in adult rats and mice, and in neonatal rats. Several test substances were evaluated for adult rats (including acrylamide, IDPN, DDT, and triadiamefon), but neonates were untreated, and evaluated on several different days (13, 17, 21, and 58/59). Test sessions were 60 minutes long, with 5 minute subsessions. Summary data only were presented, and it was not clear whether significant differences were detected between days for the neonates (the poster stated only that differences were detected across time within a session).

6. Untitled.

[Appendix O, Section 15, pp. 1251-1289]

The submitted study evaluated motor activity, auditory startle, and neuropathology following treatment with acrylamide, amphetamine, TMT, and MK-801; only the motor activity data for acrylamide and amphetamine were submitted. The date of the study and personnel involved were not listed. Age of tested animals was not stated, although the weights (approximately 430 g for males and 250 g for females, pre-dosing) would indicate that adult rats were used. Motor activity data were presented as means, following treatment with acrylamide [45 mg/kg, for a maximum of 10 days], or amphetamine [0.75 mg/kg]. Testing was conducted in stainless-steel wire-bottomed cages, using passive infrared sensors; testing sessions were 90-minutes in duration,

with data tabulated for each 5 minute interval. Activity levels were decreased following acrylamide treatment, and increased following amphetamine treatment.

7. Lochry, E.A. and E.P. Riley. (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehavioral Toxicology 2:107-115.

[Appendix O, Section 16, pp. 1291-1299]

This was a published study, performed at the State University of New York at Albany, evaluating performance in passive avoidance and T-maze following prenatal exposure to alcohol.

8. Lochry, E.A., J.A. Foss, and M.S. Christian. (1990). Learning and retention paradigms in developmental neurotoxicity test batteries: passive avoidance and watermaze. Argus Research Laboratories, Poster presented at the 18th European Teratology Society Conference, Edinburgh, Scotland, September 1990.

[Appendix O, Section 17, pp. 1300-1305]

The submitted information consists of a copy of a poster, presenting information on the performance of weanling rats in passive avoidance and adult rats in a watermaze. The submission includes several tables of control data (performance of untreated rats), with statistical information regarding the variance of results. Minimal procedural information was provided.

9. Foss, J.A., E.A. Lochry, and A.M. Hoberman. (1990) Automated monitoring systems for motor activity and auditory startle applicable for both developmental and adult neurotoxicity studies. Argus Research Laboratories, poster presented at the 8th Inernational Neurotoxicology Conference, Little Rock, AK, October 1990.

[Appendix O, Section 18, pp. 1307-1320]

The submitted information consists of a copy of a poster (summary data only). Motor activity was evaluated on days 13, 17, 21, and 60 using 90 minute sessions; only untreated animals were evaluated. Apparently statistical evaluation was used to confirm habituation within sessions, no evaluation was made for differences across days. Similarly, auditory startle habituation was evaluated in untreated animals, on days 22 and 60. Habituation was demonstrated on day 60 for females, on both days for males. Minimal procedural information was included.

10. Foss, J.A. and E.P. Riley. (1989) Elicitation and modification of the acoustic startle reflex in animals prenatally exposed to cocaine. Neurotoxicology and Teratology 13:541-546.

[Appendix O, Section 19, pp. 1321-1327]

The submitted information consists of a published article, reporting a study performed San Diego State University. The potential for changes in the acoustic startle reflex was evaluated in adult rats following prenatal exposure to cocaine; no effects from exposure to test substance were demonstrated.

11. Lochry, E.A., Hoberman, A.M., and Christian, M.S. (1985). Detection of prenatal effects on learning as a function of differential criteria. Neurobehavioral Toxicology and Teratology 7:697-701.

[Appendix O, Section 20, pp. 1328-1333]

The submitted information consists of a published article, from 1985, with different authors from the current study, although it was performed at Argus Research Laboratories. In the study, 20-day old pups were tested in a water maze that appears similar to that used in the current study. Several different learning criteria were evaluated, and it was determined that sensitivity of the test procedure for detecting changes in behavior following treatment with test substance depended on the specific learning criteria used (i.e. how many consecutive correct trials were required).

The age at testing was different from that used in the current study, and the procedure appears to have been slightly different. In particular, the authors were not able to demonstrate sensitivity of the test to prenatal alcohol exposure using a learning criteria of 5 consecutive correct trials (the criteria used in the current study). This study is not sufficient to document sensitivity of the procedure as performed in for the current study, and does raise concern about the sensitivity of the current procedures to detect test-material related effects.

Neuropathology, day 12 and adult

12. Neuropathology validation

[Appendix O, Section 21, pp. 1334-1391]

This section was provided by Dr. Robert Garman, of Consultants in Veterinary Pathology, who performed the neuropathological evaluations in the submitted study. The submission consisted of biographical and professional information regarding Dr. Garman, a validation study of adult neuropathological procedures following treatment with acrylamide, TMT, and MK-801, and a validation study comparing morphometric evaluations on untreated day 12 and day 10 neonatal rats. In addition, a copy of a poster was submitted (p. 1392, authors listed as Foss, Hoberman, and Christian, with neuropathological evaluations performed by Dr. Garman, dated 1992), evaluating neuropathology in adult rats following prenatal exposure to lead nitrate.

The validation study for adult neuropathology was missing the data tables, which show the number and type of lesions detected in evaluated rats (these data were listed in the report table of contents as starting on p. 34, but the submitted report ended on p. 33). Since these data are critical in documenting the sensitivity of the study procedures, the results of the study could not be fully evaluated. No validation was included for qualitative neuropathological evaluation of neonatal (day 12) rats, and no neuropathological alterations (or functional effects) were detected in adult rats following prenatal exposure to lead nitrate. The validation comparing day 10 and day 12 neonatal rats for morphometric measurements was performed on control rats only, and did not demonstrate ability to detect changes following neurotoxic insult. It did, however, demonstrate the ability of the laboratory to detect changes in morphometric measurements from day 10 to day 12.

This submission provides useful information regarding the sensitivity of the morphometric procedures in pups. In addition, submission of the missing data tables should provide data regarding the sensitivity of the qualitative neuropathological evaluation for adult animals.

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R062995

Chemical:	**Tebuconazole**
PC Code:	**128997**
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/18/2003**
File ID:	**TX0052027**
Accession Number:	**000-00-0048**

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