DATA EVALUATION RECORD

12/2/02

THIACLOPRID (YRC 2894)/014019
[OPPTS 870.6200a (§ 81-8)]

STUDY TYPE: ACUTE NEUROTOXICITY - RAT
MRID 44927703 (Main Study), MRID 44927704 (Supplemental Study)

Prepared for

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

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-27

Primary Reviewer: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage AUG 16 2002
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast AUG 16 2002
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross AUG 16 2002
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson AUG 16 2002

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EPA Reviewer: John Doherty. Ph.D.
Reregistration Branch 3, Health Effects Division (7509C)
EPA Work Assignment Manager: Ghazi Dannan, Ph.D.
Registration Action Branch 3, Health Effects Division (7509C)

Signature: [signature]
Date 12/02/02
Signature:
Date

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DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rat [OPPTS 870.6200a (§81-8)] OECD 424.

PC CODE: 014019

DP BARCODE: D279817
SUBMISSION NO.: S604757

TEST MATERIAL (PURITY): YRC 2894 technical (Thiacloprid) (96.7-97.5 % a.i.)

SYNONYMS: (3-((6-chloro-3-pyridinyl)methyl)-2-thiazolidinylidene)-cyanamide

CITATIONS: Sheets, L.P., Gilmore, R.G. (1997) An acute oral neurotoxicity screening study with technical grade YRC 2894 in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 95-412-GI, May 12, 1997; Agriculture Division Report No. 107633. MRID 44927703. Unpublished.

Sheets, L.P. (1998) A special acute oral neurotoxicity study to establish a no-observed-effect level with technical grade YRC 2894 in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 97-912-MD, May 4, 1998; Agriculture Division Report No. 107633-1. MRID 44927704. Unpublished.

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

EXECUTIVE SUMMARY: In an acute neurotoxicity study (1997, MRID 44927703), groups of fasted, nine-week-old Fischer 344 rats (12/sex) were given a single oral dose of technical YRC 2894 (approximately 97% a.i., batch # 290894) in 0.5% methyl cellulose-0.4% Tween 80 in deionized water at doses of 20, 50, or 100 mg/kg bw and observed for 14 days. Functional observational battery [FOB] and motor activity testing was performed in 12 animals/sex/group pretreatment, at 4 hours post-dosing (the time of peak effect), and at days 7 and 14 post-treatment. Because a NOAEL for decreases in motor activity was not attained in female rats in this study, a second (supplemental) neurotoxicity study (1998, MRID 44927704) was performed. Doses in the supplemental study were 0, 2.5, or 10 mg/kg bw. Actual doses based on analytical results in the two studies were 0, 3.1, 11, 22, 53, and 109 mg/kg. Abbreviated FOB and motor activity tests were conducted in the supplemental study (Day 0, females only). At study termination, six animals/sex/group from the main study were euthanized and perfused *in situ* for neuropathological examination. Only the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system.

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There were no mortalities. A transient decrease in body weight was observed in males in the 100 mg/kg dose group. There was no affect of treatment on gross pathology, brain weights, or incidences of microscopic lesions of the brain, spinal cord, or peripheral nervous system.

The only clinical sign considered treatment-related in the lower dose groups occurred in the 50 mg/kg group and involved dilated pupils in 1 of 12 female rats. Clinical signs consisting of tremors, decreased activity, ataxia, dilated pupils, cool-to-touch body, urine stained fur, and partially closed eyelids were observed in the majority of male and female rats that received 100 mg/kg. Most of the above signs were observed only on the day of treatment, and all signs had resolved by day 5.

During the FOB, in addition to the clinical signs at higher doses, treatment-related signs of slight tremors and ptosis in males in the 20 mg/kg dose group (3/12 and 1/12, respectively) and slight incoordination of the righting reflex in 1 of 12 females in the 20 mg/kg dose group were observed on the day of treatment.

There was no effect of treatment on total motor or locomotor activity of male rats in the main study. However, subsession data indicated significantly reduced activity for early subsessions in the 100 mg/kg group. For females in the main study, total motor activity was significantly reduced at doses of 50 and 100 mg/kg ($p<0.05$) and total locomotor activity was significantly reduced at doses of 20, 50, and 100 mg/kg ($p<0.05$). Locomotor subsession data for males and females in the 100 mg/kg group indicated that rats did not move from where they were placed in the maze. In the supplemental study, non-significant reductions in total motor and total locomotor activity of 21 and 27%, respectively, in the 10 mg/kg group (females only) were considered treatment related and biologically significant. There were no effects of treatment in any dose group at the 7- and 14-day observation times. **The LOAEL in females was 11 mg/kg bw (based on reductions in motor and locomotor activity), with a NOAEL of 3.1 mg/kg bw. The LOAEL in males was 22 mg/kg bw (based on FOB observations of slight tremors and ptosis of the eyelids on the day of treatment), with a NOAEL of 11 mg/kg bw.**

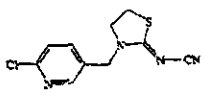
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424).

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** YRC 2894

Description:	Technical grade, pale-yellow powder, stored at freezer conditions
Lot/Batch #:	Batch No. 290894
Purity:	MRID 44927703: 97.5% a.i. (October 1995); 96.7% a.i. (April 1996) MRID 44927704: 97% a.i. (April 1997); 96.8% a.i. (October 1997)
CAS # of TGAI:	111988-49-9
Structure:	

2. **Vehicle and/or positive control:** the test material was dissolved in 0.5% methyl cellulose-0.4% Tween 80 in deionized water.

3. **Test animals:**

Species:	Rat	
Strain:	Fischer 344 CDF(F-344)/BR	
Age/weight at dosing:	Nine weeks old; males: 127-191g; females: 112-134 g	
Source:	SASCO, Inc., Madison, WI	
Housing:	Individually in suspended stainless steel wire-mesh cages	
Diet:	Purina Mills Rodent Lab Chow 5001-4 ("etts"), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	17.8-25.6°C
	Humidity:	40-70%
	Air changes:	not given
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	6 days	

B. STUDY DESIGN:

1. **In life dates:** MRID 44927703: Start: January 29, 1996; End: February 16, 1996
MRID 44927704: Start: August 4, 1997; End: August 6, 1997

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 by a computerized random sort program so that body weight means for each group were comparable. Following an overnight fast, rats were given a single dose of the test material by gavage in 0.5% methylcellulose-0.4% Tween 80 in deionized water at a dosing volume of 10 mL/kg, then observed once daily and weighed weekly for 14 days. Dose levels were chosen based on an acute oral range-finding study. In the range-finding study, doses of 27, 36, 85, 244, and 526 mg/kg were administered by gavage to male and female rats. Clinical signs - tremors, decreased activity, repetitive chewing movements, cool-to-touch body, dilated pupils, and clear lacrimation - preceded deaths (100% mortality) in the two highest dose groups. The NOEL for clinical signs was 27 mg/kg for males and 36 mg/kg for females. Based on these results, doses of 0, 20, 50, and 100 mg/kg were chosen for the acute neurotoxicity study. In the range-finding study, clinical signs were apparent within 2-4 hours following treatment and had resolved by the next day. Therefore, the estimated time of peak neurobehavioral effects for the FOB was initiated at approximately 4 hours after dosing. Administration of the test material was staggered over a 4-day interval to facilitate neurobehavioral observations. Males and females were treated on separate days. Survivors were sacrificed and a necropsy was performed.

In the initial neurotoxicity screening study (main study, MRID 44927703), male and female rats exposed to the lowest dose of 20 mg/kg showed evidence of compound-related effects in the FOB. These effects included eyelid ptosis and slight tremors in males and an impaired aerial righting response in one female. A compound-related decrease in motor activity was also evident in females that received 20 mg/kg. Therefore, in order to attain a NOAEL, doses of 0, 2.5, and 10 mg/kg were chosen for both sexes for the supplemental study (MRID 44927704).

TABLE 1: Study design Main 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)	20	20	22	22	12	12
3 (mid-dose)	50	50	53	53	12	12
4 (high-dose)	100	100	109	109	12	12
Supplemental study						
1 (control)	0	0	0	0	12	12
2	2.5	2.5	3.1	3.1	12	12
3	10	10	11	11	12	12

Data taken from pp. 19 and 24, MRID 44922703 and pp. 16 and 19, MRID 44927704.

3. **Test substance preparation and analysis:** The test substance was prepared in deionized water containing 0.5% methylcellulose and 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 freezer-stored stock material was confirmed by nuclear magnetic resonance and mass spectroscopy. Analyses were performed within six months prior to the study and following the study. The concentration of YRC 2894 in the vehicle was measured using liquid chromatography analysis. Homogeneity was tested by analyzing three samples each of nominal concentrations of the 2.0 and 100 mg/mL dosing solutions (equivalent to dose levels of 20 and 1000 mg/kg) in the main study. Stability was measured following storage of the 2.0 mg/mL stock solution at room temperature for 11 days and following storage of the 100 mg/mL solution at room temperature for eight days. Single samples of the 2, 5, and 10 mg/mL solutions were also tested for concentration. Homogeneity and stability (6-day) at room temperature were measured for a 0.25 mg/mL sample in the supplemental study. Concentration in the vehicle was measured for 0.25 and 1.0 mg/mL solutions.

Results:

Homogeneity analysis: The concentrations of the three aliquots of the 0.25 mg/mL solution were 0.3016, 0.3119, and 0.3208 mg/L; the mean value was 0.311±0.0096 mg/mL. The coefficient of variation was 3.1%. The concentrations of the three aliquots of the 2.0 mg/mL solution were 1.87, 1.78, and 1.83 mg/mL; the mean value was 1.82±0.045 mg/L. The coefficient of variation was 2.5%. The concentrations of the three aliquots of the 100 mg/mL solution were 102, 104, and 113 mg/mL; the mean value was 106±5.8 mg/mL. The coefficient of variation was 5.5%.

Stability Analysis: For the 0.25 mg/mL concentration, samples taken on days 0 and 6 (room temperature storage) were 0.311 and 0.309 mg/mL. The day 6 sample was 99.4% of the initial sample. For the 2.0 mg/mL dosing solution, the sample following storage at room temperature

was 95.1% (1.74 mg/mL) of the initial sample of 1.83 mg/mL. For the 100 mg/mL sample, the concentration following 11 days of storage at room temperature was 107% (113 mg/mL) of the initial measured concentration of 106 mg/mL.

Concentration analysis: Actual concentrations of 3.11, 1.06, 2.19, 5.28, and 10.9 mg/mL in the 0.25, 1.0, 2, 5, and 10 mg/mL nominal samples were 125, 106, 110, 105, and 109% of nominal.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The deviation of the 0.25 mg/mL sample by 125% is acceptable as effects are based on actual rather than nominal concentrations.

4. **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. Significance was determined at the 5% level. [Taken from MRID 44927703, pp. 22-23 and MRID 44927704, p. 18.] The Reviewer considers the analyses used to be appropriate.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed once daily for mortality and morbidity. Detailed clinical observations were also recorded daily.

2. **Body weight:** Animals in the main study were weighed weekly as part of the FOB. Animals in the supplementary study were weighed prior to treatment only, as body weight was not affected in main study animals at doses ≤50 mg/kg.

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

4. **Cholinesterase determination:** Cholinesterase activity was not determined. The study authors stated that YRC 2894 is a chloronicotinyl compound that has no inhibitory effect on acetylcholinesterase activity.

5. **Neurobehavioral assessment:**

   a. **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 test substance (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, FOB evaluations were performed only at the time of peak effect (approximately 4 hours post-dosing) as no effects were observed in males and females in the main study at the 7- and 14-day post-dosing observation times.

   The trained technicians who conducted the FOB were blind to the treatment of the animals. All observations were performed by a single technician, and a second technician performed the measurement procedures. For observations and tests other than the home cage observations, animals were transferred to a testing room where they were allowed to acclimate for 30 minutes. Environmental conditions in the testing room were the same as those of the home cage room. Sets of eight animals were evaluated individually during each FOB session. Where applicable, scoring criteria were provided for the measured parameters. The duration of the open field observations was two minutes. The strain gauges used for grip strength were not described.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity* (Ease of removal)		Mobility
X	Biting (chewing)	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection* (open field)	X	Convulsions*
X	Abnormal Movements*		Fur appearance	X	Tremors*
	Palpebral closure*		Palpebral closure*	X	Abnormal movements*
	Faeces consistency	X	Respiratory rate+ (open field)	X	Urination / defecation*
X	Piloerection, gait	X	Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*	X	Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*	X	Pupil size		Backing
X	Pain response*				Time to first step
X	Pupil response*			X	Vocalizations
	Eyeblink response		PHYSIOLOGICAL OBSERVATIONS		NEUROMUSCULAR OBSERVATIONS
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. **Motor activity:** Motor activity was evaluated approximately 30 minutes after the last animal was evaluated in the FOB. Motor activity was measured for 90 minutes (nine 10-minute subsessions) in one of eight automated figure-eight mazes. A Columbus Instruments (Columbus, OH) Universal Maze Monitoring System and a personal computer were used for automated data collection. Background noise of approximately 70 dB was provided by a white noise generator (Coulbourn Instruments, Model S81-02); the noise was amplified with a audio-mixer amplifier (Coulbourn Instruments, Model S82-24). A light intensity of 100±70 lux was maintained. Groups of rats were balanced across test times and test devices so that no animal was tested twice in the same maze. Testing was staggered over two days for each sex and males and females were tested on separate days. Both motor activity (all beam breaks) and locomotor activity (non-consecutive beam breaks) were measured. Habituation was noted.

6. **Sacrifice and pathology:** A complete gross necropsy was conducted on all main study animals sacrificed at 15 days post-treatment (no animals died). The first six males and six females at each dose level were anesthetized with an intraperitoneal dose of pentobarbital and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation with glutaraldehyde (4% w/v) and formaldehyde (4% w/v) in phosphate buffer. The brain, spinal cord, both eyes with optic nerves, selected nerves, and gastrocnemius muscle were dissected and post-fixed in 10% buffered formalin. The brain

was weighed. For microscopic evaluation, the brain was sectioned coronally at eight levels, and the spinal cord was sectioned at the cervical, thoracic, lumbar and cauda eqina levels. These tissues were embedded in paraffin and stained with hematoxylin and eosin, luxol fast blue/cresyl violet and Sevier-Munger stains. The dorsal root ganglia with root fibers from the cervical and lumbar swelling, gasserian ganglion, eyes, optic nerves, and gastrocnemius muscle were embedded in glycol methacrylate. These tissues were sectioned at 2-3 μm and stained with a modified Lee's stain. Peripheral nerves were embedded in epoxy resin, cross-sectioned (1 μm), and stained with toluidine blue. The sciatic nerve was also sectioned longitudinally. Tissues from the control and high-dose animals and any gross lesions were examined microscopically. The remaining animals were sacrificed by carbon dioxide asphyxiation; tissues of these animals were not collected.

The CHECKED (X) tissues were evaluated.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh
X	Center of cerebrum (not specifically stated)[a]		Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons (not specifically stated)[a]	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
X	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

[a] Although not specifically stated, it is assumed that the eight coronal sections of the brain included these sections.

7. **Positive controls:** Positive control data were not provided with this task. However, the study investigators have conducted studies "with acrylamide, carbaryl and untreated rats to establish the sensitivity, reliability, and validity of these test procedures." These studies, cited as MRID 42770301 (1993), and additional more recent studies using both male and female Fischer 344 rats should be provided in order to validate the present study.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** Treatment-related signs were observed in males and females in the 100 mg/kg group and consisted of tremors, decreased activity, ataxia, dilated pupils, cool-to-touch body, urine stained fur, and partially closed eyelids (Table 2). Additional signs in females

included red nasal stain, oral stain, clear nasal discharge, and clear lacrimation. Most of these signs were observed only on the day of treatment. All clinical signs had resolved by day 5. The only treatment-related sign in the 50 mg/kg group occurred in females and involved dilated pupils. Red lacrimal stain in a male in the 20 mg/kg group (days 0 and 1) and urine stain in one female in the 20 mg/kg group (day 4) were not considered treatment-related as these signs were not observed in the 50 mg/kg group. Treatment-related clinical signs were not observed in males or females in the 2.5 and 10 mg/kg groups in the supplemental study.

TABLE 2. Clinical observations in male and female rats administered YRC 2894 by gavage[a]

Observation	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
Males						
Tremors	0, *0*[a]	*0*	*0*	0	0	12 (0)
Decreased activity	0, *0*	*0*	*0*	0	0	12 (0)
Ataxia	0, *0*	*0*	*0*	0	0	8 (0)
Pupils dilated	0, *0*	*0*	*0*	0	0	6 (0)
Body cool to touch	0, *0*	*0*	*0*	0	0	12 (0)
Urine stain	0, *0*	*0*	*0*	0	0	6 (0)
Eyelids partially closed	0, *0*	*0*	*0*	0	0	6 (0)
Red lacrimal stain	0, *0*	*0*	*0*	1 (0-1)	0	0
Perianal stain	1 (0), *0*	*0*	*0*	0	0	0
Females						
Tremors	0, *0*	*0*	*0*	0	0	12 (0)
Decreased activity	0, *0*	*0*	*0*	0	0	11 (0)
Ataxia	0, *0*	*0*	*0*	0	0	8 (0)
Pupils dilated	0, *0*	*0*	*0*	0	1 (0)	11 (0)
Body cool to touch	0, *0*	*0*	*0*	0	0	12 (0)
Urine stain	0, *1 (1)*	*0*	*0*	1 (4)	0	7 (0-1)
Eyelids partially closed	0, *0*	*0*	*0*	0	0	11 (0)
Red nasal stain	0, *0*	*0*	*0*	0	0	8 (1-4)
Oral stain	0, *0*	*0*	*0*	0	0	7 (1)
Clear nasal discharge	0, *0*	*0*	*0*	0	0	5 (0)
Clear lacrimation	0, *0*	*0*	*0*	0	0	2 (0)
Perianal stain	0, *1 (1)*	*0*	*0*	0	0	0

Data were extracted from Table 1, pp. 33-34, MRID 44927703 and Table 1, p. 25, MRID 44927704.
[a]n=12 animals for all groups; numbers indicate the total number of animals with the indicated sign.
[b]Values represent incidents. Two values in a row in the control column indicate results from the original study (MRID 44927703) and the supplementary study (MRID 44927704), 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).

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2. **Mortality:** No deaths occurred in either study prior to scheduled sacrifice on day 15.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** The body weight of male rats in the 100 mg/kg group was transiently affected by treatment as indicated by a statistically significant lower body weight compared with the control group at day 7. The final body weight of males in all dose groups was unaffected by treatment. The body weight of females was not affected by treatment at any dose. Because body weights of rats were generally unaffected by treatment at these higher doses, rats were not weighed (except pretreatment) at lower doses in the supplemental study. Final body weight gains of male and female rats were likewise unaffected by treatment.

TABLE 3. Body weight and body weight gain (g)

Observation day	Dose level (mg/kg bw)			
	0	20	50	100
Body weight–Males				
Day 0	177±6	172±9	175±7	174±9
Day 7	216±10	209±14	214±8	202*±13
Day 14	235±13	227±17	237±9	229±15
Body weight–Females				
Day 0	121±6	123±6	123±4	124±4
Day 7	149±10	148±7	149±7	144±4
Day 14	158±11	156±8	158±8	157±6
Body weight gain–Males				
Day 14	58	55	62	55
Body weight gain–Females				
Day 14	37	33	35	33

Data were extracted from Table 2, p. 35, MRID 44927703.
Values represent mean ± s.d.
n=12 animals in each group.
*=$p<0.05$, when compared to control means.

C. **FOOD CONSUMPTION:** Food consumption was not measured.

D. **CHOLINESTERASE ACTIVITIES:** Cholinesterase activities were not measured.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings:** In the main study, treatment-related effects were observed at the time of peak effect in male and female rats (Tables 4a and 4b, respectively) in the 20, 50, and 100 mg/kg dose groups on the day of treatment (Day 0). Statistical significance designated with an asterisk (* $p<0.05$) 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; statistical significance in the supplementary study is designated by the symbol †).

In the 50 mg/kg groups, slight tremors were observed in male (6/12) and female (3/12) rats in the open field, and females had dilated pupils (4/12) and displayed a diminished reaction to approach (3/12). Incidences of all of these effects were statistically significant ($p<0.05$). Effects that appeared compound-related in the 20 mg/kg groups included slight tremors and ptosis of the eyelids in male rats (3/12 and 1/12, respectively; neither statistically significant) and an impaired righting reflex of 1 female rat. None of these effects were observed pretreatment (data not shown in Table 4a and 4b).

Treatment-related effects in male and female rats in the 100 mg/kg dose groups consisted of uncoordinated gait, tremors (both slight and moderate/severe), decreased activity, eyelid ptosis, dilated pupils, impaired righting reflex, lack of reaction to approach, and decreased body temperature. Females that received 100 mg/kg also had clear lacrimation (not statistically significant) and higher incidences of a prone posture with decreased arousal and fewer numbers of rearings in the open field. Touch and tail pinch reactions were also significantly lower in females in the 100 mg/kg group. All of these effects were resolved by the 7-day observation. Grip strength and hind-limb foot splay were not affected by treatment in any group.

No compound-related effects were observed in male and female rats that received doses of 10 or 2.5 mg/kg in the supplemental study. Observations were performed on Day 0 only, as no effects were observed in the higher dose groups at the 7- and 14-day observation times. Decreases in defecation in male rats and decreases in urine pools and body temperature in female rats in these lower dose groups were statistically significant, but NOAELs had been established for these effects at the higher doses. Furthermore, the body temperature values for female rats in the 2.5 and 10 mg/kg dose groups were similar to the values in the 20 and 50 mg/kg groups (Table 4b) which were considered NOAELs.

TABLE 4a. Functional observation battery results (Day 0)[a]

Observation	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
Males						
Home cage						
Gait incoordination (slight)	0, *0*[b]	*0*	*0*	0	0	6*
Tremors (slight)	0, *0*	*0*	*0*	0	0	3*
Tremors (moderate/severe)	0, *0*	*0*	*0*	0	0	7*
Decreased activity	0, *0*	*0*	*0*	0	0	10*
Ptosis of eyelids	0, *0*	*0*	*0*	0	0	7*
Handling						
Dilated pupils	0, *0*	*0*	*0*	0	0	9*
Open field						
Gait incoordination	0, *0*	*0*	*0*	0	0	9*
Tremors (slight)	0, *0*	*0*	*0*	3	6*	0
Tremors (moderate/severe)	0, *0*	*0*	*0*	0	0	12*
Ptosis of eyelids	0, *0*	*0*	*0*	1	1	7*
Reflex/physiologic						
Approach response (no reaction)	1, *0*	*0*	*0*	4	6*	10*
Auditory response (no reaction)	0, *0*	*0*	*0*	0	0	4
Righting reflex slight incoordination	0, *0*	*0*	*0*	0	1	5*
Body temperature (°C)	36.6±0.5 *36.6±0.3*	*36.6±0.5*	*36.5±0.5*	36.7	36.6	32.3*

Data were extracted from Table 5, p.70, MRID 44927703 and Table 2, pp. 26-43, MRID 44927704.

[a]n=12 animals for all groups.

[b]Values represent incidents. Two values in a row in the control column indicate results from the original study (MRID 44927703) and the supplementary study (MRID 44927704), 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).

*=$p<0.05$ compared with the concurrent control.

TABLE 4b. Functional observation battery results (Day 0)

Observation	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
Females						
Home cage						
Gait incoordination (slight)	0, *0*	*0*	*0*	0	0	2
Tremors (slight)	0, *0*	*0*	*0*	0	0	5*
Tremors (moderate/severe)	0, *0*	*0*	*0*	0	0	6*
Decreased activity	0, *0*	*0*	*0*	0	0	12*
Ptosis of eyelids	0, *0*	*0*	*0*	0	1	9*
Handling						
Dilated pupils	0, *0*	*0*	*0*	0	4*	11*
Lacrimation, clear, slight	0, *0*	*0*	*0*	0	0	2
Open field						
Posture, sitting/lying/flattened	1, *0*	*0*	*0*	0	3	12*
Gait incoordination, slight to severe	0, *0*	*0*	*0*	0	0	12*
Tremors (slight)	0, *0*	*0*	*0*	0	3*	0
Tremors (moderate/severe)	0, *0*	*0*	*0*	0	0	12*
Ptosis of eyelids	0, *0*	*0*	*0*	0	0	10*
Arousal, normal	10, *12*	*12*	*12*	11	10	0*
Rearing	3.3, *4.7*	*3.9*	*2.8*	2.4	3.0	0.0*
Reflex/physiologic						
Approach response (no reaction)	0, *0*	*0*	*0*	1	3*	11*
Touch response (no reaction)	0, *0*	*0*	*0*	0	0	8*
Auditory response (no reaction)	0, *0*	*0*	*0*	0	0	3
Tail pinch (no reaction)	0, *0*	*0*	*0*	0	0	10*
Righting reflex slight incoordination	0, *0*	*0*	*0*	1	1	9*
Body temperature (°C)	36.9 *37.0±0.5*	*36.3† ±0.6*	*36.4† ±0.6*	36.2	36.3	30.1*

Data were extracted from Table 5, p. 71, MRID 44927703 and Table 2, pp. 26-43, MRID 44927704.
[a]n=12 animals for all groups.
[b]Values represent incidences. Two values in a row in the control column indicate results from the original study (MRID 44927703) and the supplementary study (MRID 44927704), 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).
*=$p<0.05$ for the 20, 50, and 100 mg/kg dose groups compared with the concurrent control.
†=$p<0.05$ for the 2.5 and 10 mg/kg dose groups compared with the concurrent control.

2. **Motor activity:** Total motor and total locomotor activity are reported in Table 5. Subsession (interval) data are reported in Tables 5a for males and 5b for females. In the main study, decreases in motor activity in males in the 50 and 100 mg/kg groups (169 and 89 beam breaks, respectively compared with the control value of 205 beam breaks) did not attain statistical significance on the day of treatment (Day 0), but the value for the control group on this day was substantially lower than for other days (Table 5). For males in the 100 mg/kg group, activity was significantly decreased during the first two 10-minute subsessions of Day 0, but was similar to or greater than control activity during the remaining seven 10-minute subsessions (Table 5a). Reductions in activity during the first two 10-minute subsessions on Day 0 in the 50 mg/kg group did not attain statistical significance. For females, motor activity for the total 90-minute session on Day 0 was statistically significantly decreased (59 and 29% of the control value, respectively) in the 50 and 100 mg/kg groups (Table 5). Although not statistically significant, the value for females in the 20 mg/kg group on Day 0

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was 73% (375/514) of the control value. Decreased activity attained statistical significance during the second 10-minute subsession in the 20 mg/kg group and during the first three 10-minute subsessions in both the 50 and 100 mg/kg groups (Table 5b). Motor activity was unaffected for males and females in the 20, 50, and 100 mg/kg groups on days 7 and 14.

In the supplemental study, no significant differences were evident between the control value on Day 0 and activity counts for females in the 2.5 and 10 mg/kg groups, although the value for the 10 mg/kg group was 79% of the control value. Males were not tested in the supplemental study as a NOAEL had been attained in the main study, and females were not tested on days 7 and 14 as no effects were observed on these days in the main study. Subsession motor activity for females on Day 0 (Table 5b) also showed no significant differences between the control value and the subsession values for the 2.5 and 10 mg/kg groups, although subsession values in the 10 mg/kg group are lower than control values for the first 5 subsessions. The subsession data in Tables 5a and 5b show that habituation was attained over the 90-minute testing interval (usually by interval 6).

In the main study, locomotor activity followed trends similar to motor activity for both sexes, but the locomotor activity counts clearly indicated that on the day of treatment (Day 0) male and female rats in the 100 mg/kg group did not actively move from where they were placed in the maze (mean values of 3 and 2 different beam breaks for males and females, respectively, compared with 70 and 162 for the respective control groups) (Table 5). For males, the low locomotor value for the control group on Day 0 (compared with the other control values) is the apparent reason for a lack of significance for any, or all, of the treated male groups. Subsession data for locomotor activity are shown in Table 5c (only data for Day 0 are shown as activity for both motor and locomotor activity were not affected for either sex during pretreatment tests or on Days 7 and 14). For main-study females on Day 0, locomotor activity was significantly reduced during the first two or three intervals at all dose levels (20, 50, and 100 mg/kg).

Total locomotor activity of female rats in the 10 mg/kg group was reduced by 27% (not statistically significant) on Day 0 (Table 5). The total locomotor activity of females in the 2.5 mg/kg group on Day 0 was unaffected by treatment. Subsession data for Day 0 (Table 5c) indicate no significant differences between the respective interval values for the 2.5 and 10 mg/kg groups.

TABLE 5. Motor activity and locomotor activity of rats administered YRC 2894 by gavage

Parameter/ Test day	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
Males						
Motor activity						
Pre-test	463±72	NP	NP	462±143	543±146	515±131
Day 0	205±78	NP	NP	**249±84**	**169±89**	**89±51**
Day 7	539±218	NP	NP	587±179	499±166	416±173
Day 14	573±186	NP	NP	696±330	610±327	534±121
Locomotor activity						
Pre-test	178±46	NP	NP	173±47	206±68	191±41
Day 0	70±32	NP	NP	83±27	58±26	**3±3**
Day 7	192±87	NP	NP	218±73	187±64	156±53
Day 14	205±72	NP	NP	244±115	220±134	194±40
Females						
Motor activity						
Pre-test	762±151	NP	NP	677±189	811±234	902±313
Day 0	514±202			**375±115**	**302*±136**	**147*±111**
	447±111	*442±178*	*351±122*			
Day 7	772±220	NP	NP	686±317	773±385	611±242
Day 14	1016±168	NP	NP	840±230	1089±315	801±251
Locomotor activity						
Pre-test	278±83	NP	NP	244±91	295±97	329±121
Day 0	162±69			**103*±25**	**97*±38**	**2*±2**
	157±68	*129±39*	*115±39*			
Day 7	282±109	NP	NP	272±146	258±134	204±90
Day 14	346±83	NP	NP	295±104	365±118	266±110

Data were extracted from Tables 6 and 7, pp. 72-76, MRID 44927703 and Tables 4 and 5, pp. 46 and 47, MRID 44927704. Motor activity values represent mean beam breaks ± s.d. Locomotor activity values represent motor activity counts minus consecutive counts for a given beam. Two values in the control column for Day 0 indicate results from the original study (MRID 44927703) and the supplemental study (MRID 44927704), respectively; values in italics were obtained from the supplemental 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).

n=12 animals for all groups.

NP = not performed.

*=$p<0.05$, compared with controls.

TABLE 5a. Motor activity - individual subsession data

Day/ Interval	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
	Males					
Pre-test						
Interval 1	222±45, NP	NP	NP	239±65	239±50	235±49
Interval 2	152±46, NP	NP	NP	150±68	194±66	164±58
Interval 3	64±43, NP	NP	NP	68±39	74±60	73±50
Interval 4	18±23, NP	NP	NP	2±4	13±24	26±46
Interval 5	3±4, NP	NP	NP	0±1	1±2	9±31
Interval 6	1±4, NP	NP	NP	1±3	3±9	2±4
Interval 7	1±2, NP	NP	NP	0±1	6±9	2±6
Interval 8	2±8, NP	NP	NP	0±0	8±22	1±2
Interval 9	0±1, NP	NP	NP	1±3	4±13	3±7
Day 0						
Interval 1	140±40, NP	NP	NP	147±49	111±53	16*±13
Interval 2	39±31, NP	NP	NP	61±38	26±24	3*±4
Interval 3	8±15, NP	NP	NP	5±12	6±13	5±5
Interval 4	2±6, NP	NP	NP	0±0	2±7	11*±15
Interval 5	0±0, NP	NP	NP	3±9	2±4	21*±31
Interval 6	2±6, NP	NP	NP	2±4	7±19	10±12
Interval 7	3±8, NP	NP	NP	5±9	3±6	8±12
Interval 8	1±3, NP	NP	NP	8±15	7±13	8±9
Interval 9	10±26, NP	NP	NP	18±37	6±10	8±7
Day 7						
Interval 1	220±56, NP	NP	NP	245±53	212±33	215±51
Interval 2	163±67, NP	NP	NP	174±61	138±57	128±57
Interval 3	72±68, NP	NP	NP	97±59	74±57	38±47
Interval 4	51±51, NP	NP	NP	38±44	43±62	20±34
Interval 5	18±30, NP	NP	NP	24±36	8±14	7±19
Interval 6	5±8, NP	NP	NP	0±0	1±2	4±14
Interval 7	4±12, NP	NP	NP	1±2	3±9	2±7
Interval 8	5±17, NP	NP	NP	0±0	8±28	0±0
Interval 9	2±8, NP	NP	NP	10±22	14±26	1±3
Day 14						
Interval 1	254±72, NP	NP	NP	277±52	254±65	265±55
Interval 2	157±58, NP	NP	NP	197±72	159±81	156±44
Interval 3	58±50, NP	NP	NP	97±67	75±64	46±40
Interval 4	52±56, NP	NP	NP	70±92	63±82	21±39
Interval 5	11±21, NP	NP	NP	22±39	31±49	12±25
Interval 6	17±31, NP	NP	NP	18±34	12±33	2±4
Interval 7	4±9, NP	NP	NP	8±26	6±13	1±2
Interval 8	10±31, NP	NP	NP	7±19	6±9	14±26
Interval 9	11±25, NP	NP	NP	2±5	6±9	18±32

Data were extracted from Table 8, pp. 76-79, MRID 44927703 and Table 6, p. 48, MRID 44927704. Two values in the control column for Day 0 indicate results from the original study (MRID 44927703) and the supplemental study (MRID 44927704), respectively; values in italics were obtained from the supplemental 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).
NP = not performed.
n = 12 animals/group.
*$p<0.05$, compared with the concurrent control value.

TABLE 5b. Motor activity - individual subsession data

Day/ Interval	Dose level (mg/kg bw)					
	0	2.5	10	20	50	100
	Females					
Pre-test						
Interval 1	303±41, NP	NP	NP	271±38	315±38	312±55
Interval 2	202±49, NP	NP	NP	192±44	240±52	244±62
Interval 3	152±66, NP	NP	NP	97±55	158±81	171±91
Interval 4	70±67, NP	NP	NP	60±76	70±64	96±79
Interval 5	17±24, NP	NP	NP	25±58	10±18	40±62
Interval 6	4±10, NP	NP	NP	7±23	15±52	16±35
Interval 7	1±1, NP	NP	NP	4±10	3±8	7±21
Interval 8	2±4, NP	NP	NP	18±48	0±0	5±12
Interval 9	11±39, NP	NP	NP	3±10	0±1	12±31
Day 0						
Interval 1	213±48, *227±37*	*206±34*	*184±55*	205±40	146*±57	33*±22
Interval 2	147±38, *119±54*	*111±40*	*86±39*	90*±59	52*±24	11*±22
Interval 3	77±60, *58±33*	*51±44*	*37±28*	38±48	17*21±	22*±27
Interval 4	33±45, *20±24*	*33±54*	*17±28*	6±11	15±12	15±24
Interval 5	9±17, *6±14*	*28±49*	*5±14*	2±3	16±16	21±34
Interval 6	14±31, *4±10*	*4±9*	*2±4*	4±10	9±10	7±10
Interval 7	8±14, *5±14*	*5±7*	*9±22*	5±8	11±8	14±11
Interval 8	3±7, *5±13*	*3±6*	*6±17*	13±15	18±17	15±25
Interval 9	11±26, *4±9*	*2±4*	*5±7*	13±34	18±27	9±16
Day 7						
Interval 1	280±65, NP	NP	NP	251±46	267±43	275±59
Interval 2	219±58, NP	NP	NP	207±64	185±53	172±70
Interval 3	129±81, NP	NP	NP	109±80	121±71	96±81
Interval 4	68±69, NP	NP	NP	38±56	76±87	28±40
Interval 5	33±57, NP	NP	NP	35±60	48±68	14±30
Interval 6	20±38, NP	NP	NP	11±21'	26±51	3±7
Interval 7	23±36, NP	NP	NP	15±45	19±43	10±23
Interval 8	1±2, NP	NP	NP	11±31	20±54	10±18
Interval 9	0±1, NP	NP	NP	10±32	12±40	4±6
Day 14						
Interval 1	315±40, NP	NP	NP	274±72	292±41	307±57
Interval 2	239±35, NP	NP	NP	222±56	242±54	209±37
Interval 3	167±69, NP	NP	NP	150±53	168±65	144±74
Interval 4	131±66, NP	NP	NP	85±55	136±71	77±62
Interval 5	72±53, NP	NP	NP	47±57	81±75	40±58
Interval 6	42±46, NP	NP	NP	26±30	53±59	9±27
Interval 7	25±41, NP	NP	NP	16±33	45±62	4±7
Interval 8	8±14, NP	NP	NP	9±28	47*±57	10±34
Interval 9	18±50, NP	NP	NP	11±26	26±52	1±2

Data were extracted from Table 8, pp. 80-83, MRID 44927703 and Table 6, p. 48, MRID 44927704. Two values in the control column for Day 0 indicate results from the original study (MRID 44927703) and the supplemental study (MRID 44927704), respectively; values in italics were obtained from the supplemental 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).
NP = not performed.
n=12 rats/group.
*p<0.05, compared with the respective concurrent control value.

TABLE 5c. Locomotor activity - individual subsession data, Day 0

Interval	Dose level (mg/kg bw) 0	2.5	10	20	50	100
Males						
Day 0						
Interval 1	50±16, NP	NP	NP	55±15	42±17	2*±1
Interval 2	13±11, NP	NP	NP	18±13	9±9	0*±0
Interval 3	3±6, NP	NP	NP	2±6	3±7	0±0
Interval 4	0±1, NP	NP	NP	0±0	1±3	0±0
Interval 5	0±0, NP	NP	NP	0±0	1±1	0±0
Interval 6	0±0, NP	NP	NP	0±0	0±0	0±1
Interval 7	1±2, NP	NP	NP	1±3	0±0	0±1
Interval 8	0±1, NP	NP	NP	2±6	1±2	0±1
Interval 9	4±10, NP	NP	NP	6±15	2±3	0±1
Females						
Day 0						
Interval 1	74±19, *89±17*	*76±13*	*76±17*	62±14	46*±17	2*±2
Interval 2	44±14, *36±16*	*30±12*	*27±12*	23*±14	17*±9	0*±0
Interval 3	22±18, *17±33*	*11±10*	*7±5*	9*±10	6*±7	0*±0
Interval 4	9±13, *6±11*	*7±13*	*4±11*	2±5	5±4	0±0
Interval 5	4±7, *2±7*	*4±8*	*1±5*	0±1	5±6	0±0
Interval 6	5±10, *2±7*	*0±0*	*0±0*	0±1	4±5	0±0
Interval 7	2±5, *2±6*	*0±1*	*1±2*	1±2	4±4	0±0
Interval 8	1±2, *2±6*	*1±3*	*0±0*	3±7	6*±6	0±0
Interval 9	3±6, *0±1*	*0±1*	*1±2*	3±9	4±4	0±0

Data were extracted from Table 9, pp. 85 and 89, MRID 44927703 and Table 7, p. 49, MRID 44927704. Two values in the control column for Day 0 indicate results from the original study (MRID 44927703) and the supplemental study (MRID 44927704), respectively; values in italics were obtained from the supplemental 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] and supplementary study values [in italics] should be compared to supplementary study controls [in italics]).
NP = not performed.
n=12 rats/group.
*$p<0.05$, compared with the concurrent control value.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No gross lesions attributable to treatment were observed in male or female rats at any dose level.

2. **Brain weight:** There were no treatment-related effects on absolute or relative-to-body-weight brain weights. Mean absolute brain weights of males in the 0, 20, 50, and 150 mg/kg dose groups were 1.670, 1.726, 1.697, and 1.696 g, respectively. For females, the respective mean absolute brain weights were 1.647, 1.615, 1.672, and 1.618 g. Brains were not weighed in the supplementary study.

3. **Neuropathology:** No lesions attributable to treatment were observed. Occasional nerve fiber degeneration in the brain, mineralization of the cornea of the eye, and axonal swelling in different areas of the spinal cord were observed in both the control and high-dose groups. Because of a lack of significant findings in the high-dose groups, lower dose groups were not examined.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** In the main study, treatment-related clinical signs consisting of tremors, decreased activity, ataxia, dilated pupils, cool-to-touch body, urine stained fur, and partially closed eyelids were observed in males and females that received 100 mg/kg. The only sign considered treatment-related in the lower dose groups occurred in the 50 mg/kg group of females and involved dilated pupils. Most of the above signs were observed only on the day of treatment, and all signs had resolved by day 5. In the supplemental study, treatment-related signs were not observed in males or females in the 2.5 and 10 mg/kg groups. During the FOB, treatment-related clinical signs consistent with those observed during the daily observations were observed in all dose groups on the day of treatment. Observation of additional treatment-related signs including slight tremors and ptosis in males in the 20 mg/kg dose group and slight incoordination of the righting reflex in one female in the 20 mg/kg dose group was attributed by the study authors to the conduct of the FOB at the time of peak effect, whereas the clinical observations were conducted several hours later. **The study authors concluded that the no-effect level for the FOB was <20 mg/kg in both sexes.** In the supplementary study, there was no effect of treatment on FOB parameters in either the 2.5 or 10 mg/kg dose groups. No effects were observed at the 7 and 14 day post-treatment observations times.

In the main study, treatment-related decreases in motor and locomotor activity occurred in males in the 100 mg/kg group and in females in all treatment groups. For females in the supplemental study, the study authors concluded that an effect occurred in the 10 mg/kg dose group based on the non-statistically significant reductions in motor and locomotor activity of 21 and 27%, respectively (inherent variability of motor activity is generally ≤20%). These reductions were also reflected in the modest reductions in the first intervals of the motor and locomotor activity subsessions. **The study authors concluded that the no-effect level for motor activity was 50 mg/kg in males and 2.5 mg/kg in females.**

The only effect on body weight was a slight reduction in males compared with the control value (6%) in the 100 mg/kg group on day 7. This effect had resolved by day 14. There were no effects of treatment on absolute or relative brain weights, gross pathology, or microscopic pathology.

B. **REVIEWER COMMENTS:** The Reviewer agrees with the conclusions of the investigators that technical grade YRC 2894 was neurotoxic to male and female rats at all doses tested in the main study. Male and female rats administered 100 mg/kg showed clinical signs of tremors, decreased activity, ataxia, dilated pupils, cool-to-touch body, urine stained fur, and partially closed eyelids. Tremors, decreased activity, and a cool-to-touch body were observed in all 12 male rats and 11-12 female rats. Additional signs in females included red nasal stain, oral stain, clear nasal discharge, and clear lacrimation. Most of these signs were observed only on the day of treatment and had resolved by day 5. The only treatment-related sign in the 50 mg/kg group occurred in females and involved dilated pupils. Red lacrimal stain in a male in the 20 mg/kg group (days 0 and 1) and urine stain in one female in the 20 mg/kg group (day 4) were not considered treatment-related as these signs were not observed in the 50 mg/kg group. However, during the FOB, conducted approximately 4 hours after treatment (the time of peak effect), signs were also observed in the 20 mg/kg group. These

consisted of **slight tremors** and ptosis of the eyelids in male rats (3/12 and 1/12, respectively) and an impaired righting reflex in one of twelve female rats. According to the investigators, the regular clinical examinations occurred several hours after the FOB. Therefore, it is reasonable that some of the observed signs had resolved by the time of the regular clinical examinations. Treatment-related signs were not observed in males or females in the 2.5 and 10 mg/kg groups, either during the regular clinical examination or during the FOB.

Motor and/or locomotor activity were significantly decreased in female rats at doses of 20, 50, and 100 mg/kg on Day 0 in the main study. Decreases in motor and locomotor activity for male rats in the 50 and 100 mg/kg groups were not statistically significant. The lack of significance for males is most probably attributable to the low activity of the control group on this day which, in turn, may be attributable to the overnight fast (the value for the female control group was also lower on Day 0 compared with activity for this group on other days). In the supplemental study, motor and locomotor activity in the 2.5 and 10 mg/kg groups, tested in females only, were not significantly reduced. However, reductions in both total motor activity and total locomotor activity for the 10 mg/kg group were both >20% (21 and 27%, respectively). Values >20% are outside of the inherent intergroup variability of this test. Although these reductions in motor and locomotor activity in the 10 mg/kg group are equivocal, the reviewer agrees with the study investigators that they should be considered an effect of treatment. Strengthening this argument is the fact that the control values for females on Day 0 were similar in the main and supplemental studies and the motor and locomotor activity values show a good dose-response relationship across all doses.

Treatment with YRC 2894 had only a transient effect on body weight of male rats in the 100 mg/kg group. There were no effects of treatment on absolute or relative brain weights, gross pathology, or microscopic pathology.

Based on the effects seen in this study, the LOAEL in females was 11 mg/kg (based on reductions in motor and locomotor activity of 21 and 27%, respectively) with a NOAEL of 3.1 mg/kg. The LOAEL males was 22 mg/kg (based on FOB observations of slight tremors and ptosis of the eyelids on the day of treatment), with a NOAEL of 11 mg/kg.

C. **STUDY DEFICIENCIES:** If recent positive control data are provided, there are no major deficiencies in this study. The main and supplemental studies were conducted at two different times. Given the equivocal reductions in motor and locomotor activity in female rats in the 10 mg/kg group, it is possible that, if all doses had been administered at the same time, reductions in this dose group would not have been observed.