DATA EVALUATION RECORD

12/3/02

THIACLOPRID (YRC 2894)/014019

STUDY TYPE: SUBCHRONIC NEUROTOXICITY [FEEDING] - RAT; OPPTS 870.6200b [§82-7]; (No OECD guideline) MRID 44927645

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Work Assignment No. 02-27

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity [Feeding] - Rat; OPPTS 870.6200b [§82-7]; (No OECD guideline).

PC CODE: 014019

DP BARCODE: D279817
SUBMISSION NO.: S604757

TEST MATERIAL (PURITY): YRC 2894 Technical (Thiacloprid; 96.6-97.5% a.i.)

SYNONYMS: [3-[(6-Chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]-cyanamide

CITATION: Sheets, L. and B. Hamilton (1997) A subchronic dietary neurotoxicity screening study with technical grade YRC 2894 in Fischer 344 rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, Kansas. Laboratory report number 107619, June 3, 1997. MRID 44927645. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, Missouri.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (1997, MRID 44927645) YRC 2894 Technical (Thiacloprid; 96.6-97.5% a.i., batch #290894) was administered to 12 Fischer 344 CDF(F-344)/BR rats/sex/dose at dietary concentrations of 0, 50, 400, or 1600 ppm (equivalent to 0, 2.94, 24.2, or 101 mg/kg bw/day for males and 0, 3.41, 27.9, or 115 mg/kg bw/day for females) for 13 weeks. All animals were subjected to ophthalmological examinations prior to treatment and at termination. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group prior to treatment and during weeks 4, 8, and 13 of the study. At study termination, 6 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination and all control and high-dose animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no treatment-related deaths or clinical signs. At 1600 ppm, body weight gain was decreased during the first week (↓83% for males and ↓62% for females) and for the last month of the study (↓22% for males and 19% for females), with absolute body weights decreased on day 7 (↓12% for males; $p<0.05$). Mean food consumption was decreased for both sexes during the first week (↓63 and 66% for males and females, respectively; $p<0.05$) and remained decreased for males during weeks 2-4 (85-88%; $p<0.05$). There were no treatment related effects on ophthalmology, brain weight or gross and histologic pathology or neuropathology, and on

motor/locomotor activity. The only treatment-related FOB finding was decreased hindlimb grip strength in high-dose males during week 13 (↓21%s; $p<0.05$). **The LOAEL for is 1600 ppm (101 and 115 mg/kg bw/day for males and females, respectively), based on decreased body weight gains and food consumption in both sexes and decreased hindlimb grip strength in males. The NOAEL is 400 ppm (24.2 and 27.9 mg/kg bw/day for males and females, respectively).**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (OPPTS 870.6200b; [§82-7]; no OECD guideline).

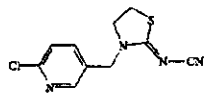
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical Grade YRC 2894

Description:	Pale-yellow powder
Batch #:	290894
Purity:	96.6 - 97.5 % a.i.
Compound Stability:	Stable for the duration of the study under freezer conditions.
CAS # of TGAI:	111988-49-9



2. **Vehicle and/or positive control:** Corn oil was used as a vehicle at 1% of the diet by weight. In addition, a small amount of acetone was used as a solvent in diet preparation and was allowed to evaporate. A positive control was not used.

3. Test animals:

Species:	Rat	
Strain:	Fischer 344 CDF(F-344)/BR	
Age/weight at study initiation:	Approximately 8 weeks/ males: 173.1-224.5 g.; females: 117.9-145.2 g.	
Source:	Sasco, Inc., Madison, Wisconsin	
Housing:	Individually in suspended, stainless-steel wire-mesh cages.	
Diet:	Purina Mills Rodent Lab Chow 5001-4 in "etts"form *ad libitum* except during neurobehavioral testing.	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	17.8-25.6 °C
	Humidity:	40-70%
	Air changes:	not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days	

B. STUDY DESIGN:

1. **In life dates:** Start: August 28, 1995; End: November 30, 1995

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 using a computerized randomization procedure so that the body weight of each individual animal was within ±20% of the mean body weight for its sex, and the mean body weights of all groups were comparable at the initiation of testing. Test substance was administered in the diet at nominal concentrations of 0, 50, 400, or 1600 ppm for 13 weeks.

   Dose levels were chosen based on the results of a 13-week feeding study with the test substance at dietary concentrations of 0, 25, 100, 400 and 1600 ppm.(MRID 44927714). At 100 ppm, males had minimal changes in thyroid parameters and increased hepatic microsomal enzyme activity. At 400 and 1600 ppm, there was further elevation of metabolic enzyme activities, increased liver weight and hepatocellular hypertrophy. At 1600 ppm, body weight gain was reduced by 14-17% in both sexes, and additional effects involving the thyroid (increased T3, TBC and organ weight) were also evident. Dietary concentrations of 0, 50, 400, and 1600 ppm were selected for the current study with the expectation that the 1600 ppm concentration would result in significant systemic toxicity, the 400 ppm dietary concentration would cause an intermediate level of toxicity, and the 50 ppm dietary concentration would be a NOAEL.

TABLE 1. Study design

Experimental parameter	Dietary concentration (ppm)			
	Control	50	400	1600
Total number of animals/sex/group	12	12	12	12
Behavioral Testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	6/sex	6/sex	6/sex	6/sex
Dosage to animals (mg/kg bw/day)[a]				
Males	0.00±0.00	2.94±0.04	24.2±0.3	101±1
Females	0.00±0.00	3.41±0.03	27.9±0.2	115±1

Data taken from text, p. 19, and Table 4, p. 37, MRID 44927645.
a Data are given as Mean ± Standard Error.

3. **Test substance preparation and analysis**: Diets were prepared weekly by mixing appropriate amounts of test material with Purina Mills Rodent Lab Chow 5001-4 and stored at freezer conditions until used, and the animals were given fresh food weekly. Diet preparation included the use of corn oil as a vehicle at 1% of the diet by weight, and "a small amount" of acetone was used as a solvent then allowed to evaporate, with no other details provided. Homogeneity and stability were tested using sample diets with nominal 20 and 2000 ppm concentrations of test material. Samples of the test diets used during weeks 1, 5, 10, and 14 of the study were analyzed for concentration.

Results:

Homogeneity analysis: The mean concentrations of the 20 and 2000 ppm diets were 97.5 and 90.7% of nominal, respectively, with concentrations of the individual subsamples ranging from 92.0-106% of nominal and from 81.4-94.4% of nominal, respectively.

Stability analysis: After 7, 14, 21, and 28 days of storage in the freezer, the measured concentrations of the 20 ppm diet were 114, 109, 102, and 95.9% of initial, respectively, and the measured concentrations of the 2000 ppm diet were 110, 94.9, 90.6, and 93.9% of initial, respectively. After 1, 3, 7, 10, and 14 days of storage at room temperature, the measured concentrations of the 20 ppm diet were 98.5, 85.2, 83.2, 74.5, and 76.5% of initial, respectively, and the measured concentrations of the 2000 ppm diet were 85.4, 97.6, 90.7, 81.4, and 87.5% of initial, respectively.

Concentration analysis: Absence of the test material was confirmed in the vehicle control diet. Measured concentrations of the test material in the 50, 400, and 1600 ppm diets were 82-95%, 84.5-96.8%, and 87.3-106% of nominal, respectively.

The analytical data from the homogeneity analysis and the increases in the concentration of the test material in the diets over time during the stability analyses indicate that there may have been a problem with either the analytical method or the mixing procedure. The results of the concentration analyses indicated that the mixing procedure may have been inadequate and that there may have been significant variance between nominal and actual dosage to the study animals, particularly at the lowest dietary concentration.

4. **Statistics**: Continuous data were initially analyzed using Bartlett's test for homogeneity of variances among groups, and non-homogeneous data were analyzed using the Kruskal-Wallis Test followed by a Mann-Whitney U Test for pairwise comparisons. Homogeneous continuous data were generally analyzed using Analysis of Variance (ANOVA), followed by Dunnett's test if significant. Motor and locomotor activity session data and continuous data collected during the Functional Observational Battery (FOB) were analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test week, and for weeks for which there was a significant treatment effect, Dunnett's test was used to determine which groups, if any, were significantly different from the control group. Motor and locomotor activity data from the 10-minute intervals were analyzed using 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 those weeks, the data for each interval were analyzed using a one-way

ANOVA followed by Dunnett's test, as appropriate. Categorical data collected during the FOB were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively. Ophthalmology and micropathology frequency data were analyzed using a Chi-square Test followed by a one-tailed Fisher's Exact Test in cases of significant variation by the Chi-square analysis. Micropathology severity grades were analyzed using a Kruskal-Wallis Test followed by a Mann-Whitney U Test for pairwise comparisons if significant.

A significance level of $p<0.05$ was used in all statistical tests except for Bartlett's Test, in which a significance level of $p<0.001$ was used.

The reviewer considers the statistical analyses used in the study to be appropriate.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed for mortality, moribundity, and clinical signs at least twice daily on week days and once daily on holidays and weekends. Detailed physical examinations were conducted weekly.

2. **Body weight:** Body weights were recorded weekly, and perfused animals were also weighed at termination.

3. **Food consumption:** Individual food consumption was determined weekly. Test substance intake of each group was calculated using mean food consumption data and the mean of 4-6 analytical determinations of diet concentration.

4. **Cholinesterase determination:** Cholinesterase activity was not measured.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** FOB testing was conducted on all animals prior to treatment and during weeks 4, 8, and 13. The same technicians were used throughout testing and were blind to the treatment status of the animals. Testing was done in a standard animal room other than the one in which the animals were normally maintained and followed an overnight acclimation to the new location, with feeders being removed at least 30 minutes prior to testing. Males and females were tested on separate days, and the open field was cleaned during the interim to reduce residual scent from the opposite sex. The noise level of the testing environment was not reported.

   The FOB included home cage observations, handling observations, open field observations, and sensorimotor/reflex tests conducted in the stated order. Positive observations were scored as to whether they were slight, barely perceptible, or infrequent (score = 1) or moderate to severe (score = 2). Open field observations were conducted for a duration of 2 minutes and included counts of incidences of rearing, urination, and defecation as well as qualitative descriptions of fecal consistency and urine volume. The equipment used to measure grip strength was not described in the study report.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Ease of removal from home cage	X	Piloerection
X	Piloerection*	X	Reactivity*	X	Respiratory abnormalities
X	Gait abnormalities*	X	Muscle tone*	X	Posture*
X	Abnormal movements*	X	Palpebral closure*	X	Gait abnormalities*
X	Convulsions*	X	Lacrimation* / chromodacryorrhea	X	Convulsions*
X	Tremors*	X	Salivation* / oral staining	X	Tremors*
X	Other clonic motor movements	X	Perianal, urine, or nasal staining	X	Other clonic motor movements
X	Tonic motor activity	X	Pupil size/ pupil response* a	X	Tonic motor activity
X	Spontaneous vocalizations	X	Eye prominence (exophthalmia)*	X	Bizarre / stereotypic behavior*
X	Activity level	X	Dehydration or emaciation	X	Arousal / general activity level*
X	Reactivity	X	Malocclusion / broken teeth	X	Vocalizations
X	Repetitive head bobbing	X	Alopecia/bite marks/missing toenails	X	Rearing+
			Respiratory rate+	X	Urination / defecation*
			Red/crusty deposits*		
	SENSORY OBSERVATIONS		PHYSIOLOGICAL OBSERVATIONS		NEUROMUSCULAR OBSERVATIONS
X	Approach response+	X	Body weight*	X	Forelimb grip strength*
X	Touch response+	X	Body temperature+	X	Hindlimb grip strength*
X	Auditory startle response*			X	Landing foot splay*
X	Pain response* (tail pinch)		OTHER OBSERVATIONS		
X	Air righting reflex+				

* Required parameters; + Recommended parameters
a Pupil response was tested only if dilated.

b. Locomotor activity: Sets of up to 8 animals were subjected to motor activity and locomotor activity evaluation approximately 1 to 2.5 hours after the last animal in the set completed FOB testing. Animals were tested individually in figure-eight mazes for 90-minute sessions, comprised of 10-minute intervals. Computerized data collection was used to record the numbers of interruptions of 8 photo beams positioned throughout the maze. Motor activity was measured by counting the number of beam interruptions, and locomotor activity was measured by elimination of consecutive interruptions of a given beam, i.e. multiple interruptions of a given beam only counted as one until the animal moved enough to interrupt one of the other beams at a different location in the maze. During testing, amplified "white noise" was used to minimize acoustical variations, and uniformity of the light intensity over the mazes was verified.

6. **Ophthalmology:** Eyes were examined prior to treatment and during week 12 of the study, and animals with pre-existing ophthalmological abnormalities were excluded from the study. The pupillary light reflex was tested, and pupils were dilated prior to examination by indirect ophthalmoscopy.

7. **Sacrifice and pathology:** The first 6 males and 6 females from each dose level were selected for neuropathology. These animals were deeply anesthetized by intraperitoneal injection of sodium pentobarbital, perfused with sodium nitrite in phosphate buffer via the left ventricle, and fixed *in situ* using 4% (w/v) glutaraldehyde and 4% (w/v) EM grade formaldehyde in phosphate buffer. If perfusion was considered inadequate, a replacement animal was used, and the remaining animals were sacrificed without perfusion by carbon dioxide asphyxiation.

All animals were subjected to gross necropsy that included examination of all organs, body cavities, cut surfaces, and external body orifices and surfaces. Brains from perfused animals were weighed after perfusion fixation, and the following tissues were collected from perfused animals and post-fixed in 10% phosphate buffered formalin: brain; spinal cord (including dorsal root ganglia and spinal nerves); both eyes with optic nerves; sciatic, tibial, and sural nerves; gasserian ganglia; gastrocnemius muscle; and the tail with physical identifier. A metal rodent brain matrix (RBM-4000C, Activational Systems, Inc., Warren, MI) was used to cut the brain into eight standard brain levels, and brain and spinal cord tissues were embedded in paraffin, sectioned at approximately 5 µm, and stained with hematoxylin and eosin (H & E), luxol fast blue-cresyl violet, and Sevier-Munger silver stains. Spinal nerve roots and dorsal root ganglia, gasserian ganglia, the gastrocnemius muscle, the optic nerve, and the eyes were embedded in glycol methacrylate (GMA), sectioned at 2 to 3 µm, and stained with a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial, and sural nerves) were embedded in epoxy resin, sectioned at approximately 1 µm, and stained with toluidine blue.

The CHECKED (X) tissues were evaluated from all perfused animals of the control and high-dose groups.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Olfactory bulbs	X	Sciatic
X	Cerebral cortex	X	Tibial
X	Hippocampus	X	Sural
X	Basal ganglia (caudate-putamen/globus pallidus)		**OTHER**
X	Thalamus	X	Lumbar dorsal root ganglion
X	Hypothalamus	X	Lumbar dorsal root fibers
X	Midbrain (tectum, tegmentum, cerebral peduncles)	X	Lumbar ventral root fibers
X	Cerebellum	X	Cervical dorsal root ganglion
X	Medulla oblongata	X	Cervical dorsal root fibers
		X	Cervical ventral root fibers
	SPINAL CORD		
X	Cervical (transverse and longitudinal sections)	X	Gastrocnemius muscle
X	Thoracic (transverse and longitudinal sections)		
X	Lumbar (transverse and longitudinal sections)		
X	Cauda equina (longitudinal section)		
	OTHER		
X	Gasserian Ganglia		
	Trigeminal nerves		
X	Optic nerve		
X	Eyes		

8. **Positive** controls: The study report made the following assertions:

- The sensitivity, reliability, and validity of the FOB procedures used in the study and the adequacy of training of technical personnel were established in previously conducted studies with acrylamide, carbaryl, and untreated rats (MRID 42770301).
- The sensitivity, reliability and validity of the motor activity and locomotor activity test procedures were established in previously conducted studies with triadimefon, chlorpromazine, and untreated animals (MRIDs 42770301, 43656301).
- The sensitivity and reliability of the tissue processing, staining, and neurohistological examination methods used by the laboratory for detecting lesions in peripheral nerves and the central nervous system were established in previous studies with trimethyltin and acrylamide (MRID 42770301).

However, copies of MRIDs 42770301 and 43656301 were not included in the study report or as supplemental material. It was therefore not possible for the reviewer to verify the sensitivity of the test methods to detect changes in the evaluated parameters or that the methods used in the positive control studies (e.g. the equipment, duration of motor activity sessions and intervals, types of stains, tissue processing, and sections evaluated for neuropathology, the particular pathologist who read the slides, etc.) were the same as those used in the current study.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** There were no treatment-related clinical signs.

2. **Mortality:** There were no deaths during the study.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Selected body weight data are given in Table 2. The mean absolute body weight of the high-dose males and females was significantly decreased on day 7. Mean absolute body weights for high-dose males remained statistically significantly decreased throughout most of the remainder of the study, the decreases were of insufficient magnitude to be considered biologically relevant. The high-dose male group also had decreased body weight gain for days 0-7, followed by compensatory increased body weight gain for days 7-35 and decreased body weight gain for days 63-90. The high-dose female group had significantly decreased body weight gains for days 0-7 and 63-90. The increased body weight gains noted for all treated female groups during days 7-35, and for the mid- and high-dose female groups during days 35-63 were most likely incidental to treatment as they did not exhibit a clear dose-response pattern.

C. **FOOD CONSUMPTION**: The mean food consumption of the high-dose male group was decreased to 63% of that of controls during the first week of the study ($p<0.05$) and remained decreased during weeks 2-4 (85-88% of controls; $p<0.05$). The high-dose female group also had decreased mean food consumption during the first week of the study (66% of controls; $p<0.05$). Additional statistically significant decreases were noted sporadically for high-dose males and females but were of insufficient magnitude to be considered biologically relevant. There were no treatment-related effects on food consumption at the low- and mid-dose treatment levels. The calculated mean intake of the test substance for each group is given in Table 1.

TABLE 2. Selected body weight and body weight gain data (g) [a]				
Study day/Interval	Nominal dietary concentration (ppm)			
	Control	50	400	1600
Males				
0	194.0±10.5	194.2±10.8	193.4±14.7	196.0±15.8
7	229.2±9.8	231.0±12.2	226.4±13.8	201.9±15.3 (88) [b] *
35	290.8±12.1	291.3±14.8	287.9±16.1	272.6±19.4 (94) *
63	331.6±15.8	332.3±18.4	327.2±18.0	313.7±22.9
91	359.5±16.6	363.1±21.1	356.0±19.4	335.6±22.7 (93) *
0-7[c]	35.2	36.8	33.0	5.9 (↓83%)
7-35[c]	61.6	60.3	61.5	70.7 (↑15%)
35-63[c]	40.8	41.0	39.3	41.1
63-90[c]	27.9	30.8	28.8	21.9 (↓22%)
Females				
0	131.3±7.4	131.1±7.4	131.5±7.5	131.8±6.9
7	146.1±9.2	145.7±7.8	146.1±7.4	137.4±7.0 (94) [b] *
35	166.9±13.3	170.6±9.0	173.8±12.1	165.3±6.7
63	182.6±15.7	187.1±8.1	191.1±12.7	183.2±5.1
91	192.2±17.8	196.4±12.2	202.5±14.7	191.0±6.0
0-7[c]	14.8	14.6	14.6	5.6 (↓62%)
7-35[c]	20.8	24.9 (120)	27.7 (133)	27.9 (↑34%)
35-63[c]	15.7	16.5	17.3 (110)	17.9 (↑14%)
63-90[c]	9.6	9.3	11.4 (119)	7.8 (↓19%)

Data taken from Table 2, pp. 33-34, MRID 44927645.
a Given as Mean ± Standard Deviation; n = 12.
b Numbers in parentheses equal percent of control; calculated by reviewer.
c Calculated by reviewer from group mean body weight data and not subjected to statistical analysis.
Significantly different from control: * $p<0.05$.

D. NEUROBEHAVIORAL RESULTS:

1. **FOB findings:** Selected FOB results are given in Table 3. Statistically significant findings included decreased body weights in high-dose males during weeks 4, 8, and 13 (93-94% of controls; $p<0.05$) and decreased hindlimb grip strength in high-dose males during week 13 (79% of controls; $p<0.05$). There were no toxicologically relevant non-statistically significant findings.

11

TABLE 3. Functional observational battery results [a]

Observation	Nominal dietary concentration (ppm)			
	Control	50	400	1600
Males				
Body Weight (g)				
Pretest	164±12	162±11	163±16	164±14
Week 4	265±11	266±13	264±14	246±17 (93) [b] *
Week 8	317±15	318±18	316±17	299±21 (94) *
Week 13	354±18	356±18	349±19	329±22 (93) *
Forelimb grip strength (kg)				
Pretest	0.48±0.07	0.48±0.06	0.44±0.07	0.44±0.08
Week 4	0.80±0.11	0.80±0.07	0.78±0.09	0.79±0.11
Week 8	0.91±0.16	0.92±0.12	0.86±0.10	0.94±0.13
Week 13	0.96±0.15	0.94±0.12	0.99±0.19	0.95±0.09
Hindlimb grip strength (kg)				
Pretest	0.24±0.05	0.26±0.04	0.22±0.04	0.25±0.03
Week 4	0.31±0.04	0.30±0.06	0.28±0.04	0.31±0.05
Week 8	0.33±0.05	0.34±0.07	0.31±0.06	0.34±0.05
Week 13	0.39±0.07	0.38±0.03	0.36±0.04	0.31±0.08 (79) *
Females				
Body Weight (g)				
Pretest	125±6	125±6	126±6	126±7
Week 4	157±11	162±9	162±10	155±7
Week 8	176±14	179±8	184±13	176±7
Week 13	189±17	193±12	199±15	189±5
Forelimb grip strength (kg)				
Pretest	0.48±0.08	0.50±0.08	0.50±0.08	0.48±0.07
Week 4	0.73±0.08	0.69±0.12	0.75±0.09	0.72±0.09
Week 8	0.84±0.10	0.80±0.10	0.84±0.09	0.80±0.07
Week 13	0.85±0.15	0.79±0.13	0.85±0.10	0.82±0.14
Hindlimb grip strength (kg)				
Pretest	0.20±0.04	0.21±0.04	0.24±0.04	0.21±0.03
Week 4	0.27±0.03	0.25±0.05	0.33±0.07	0.29±0.06
Week 8	0.32±0.03	0.29±0.05	0.34±0.06	0.30±0.03
Week 13	0.31±0.07	0.31±0.06	0.32±0.05	0.31±0.06

Data taken from Table 6, pp. 62-69, MRID 44927645.
a Values given as Mean ± Standard Deviation; n=12.
b Numbers in parentheses equal percent of control; calculated by reviewer.
Significantly different from control: * $p<0.05$.

2. **Motor activity:** Total motor activity counts and locomotor activity counts are given in Tables 4 and 5, respectively. No significant differences were found between the total motor activity or locomotor activity of the treated and control groups of either sex on any testing day. Sessions were long enough for motor activity to approach asymptotic levels by the last 20% of the session for untreated animals, and habituation was demonstrated by all groups on all testing days. Occasional non-statistically significant differences were noted for individual blocks, but no dose- or time-related pattern was evident. Females generally had higher motor activity and locomotor activity than males.

TABLE 4. Session motor activity (total activity counts per session) [a]

Test Day	Nominal Dietary Concentration (ppm)			
	Control	50	400	1600
Males				
Pre-test	523±192	601±211	584±192	619±192
Week 4	505±123	552±135	528±159	522±149
Week 8	595±145	504±171	537±168	500±94
Week 13	534±148	486±156	491±220	482±86
Females				
Pre-test	795±192	953±214	907±181	880±293
Week 4	831±281	910±185	867±339	820±187
Week 8	834±204	988±319	971±385	928±236
Week 13	880±264	1147±585	1062±359	898±224

Data taken from Table 7, pp. 70-71, MRID 44927645.
a Values given as Mean ± Standard Deviation; n=12.

TABLE 5. Session locomotor activity (locomotor counts per session) [a]				
Test Day	Nominal Dietary Concentration (ppm)			
	Control	50	400	1600
Males				
Pre-test	197±76	230±89	202±59	236±78
Week 4	181±42	196±43	187±61	183±61
Week 8	223±68	185±77	207±71	179±36
Week 13	227±81	208±88	211±119	196±40
Females				
Pre-test	291±85	365±92	320±77	312±91
Week 4	267±111	339±88	278±111	270±70
Week 8	299±97	363±126	331±140	324±87
Week 13	298±91	411±221	365±124	319±87

Data taken from Table 8, pp. 72-73, MRID 44927645.
a Values given as Mean ± Standard Deviation; n=12.

E. OPHTHALMOLOGY: There were no treatment-related ophthalmological findings. Unilateral or bilateral corneal opacity was noted in 1/12, 1/12, 2/12, and 3/12 males and 0/12, 1/12, 1/12, and 0/12 females from the control, low-, mid-, and high-dose groups, respectively, and retinal degeneration was noted in 1/12 high-dose males and 1/12 mid-dose females, as compared to none in any other group.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related lesions noted at necropsy. Abnormal findings were limited to urine staining in 1/12 females from each of the control and low-dose groups, lacrimation in 1/12 and 2/12 control and low-dose females, and unilateral exophthalmos in 1/12 low-dose females.

2. **Brain weight:** Brain weight data are given in Table 6. The mean terminal body weights, brain weights, and brain-to-body weight ratios of all treated groups were similar to those of their respective controls.

TABLE 6. Absolute and relative brain weights [a]				
Parameter	Nominal Dietary Concentration (ppm)			
	Control	50	400	1600
Males				
Body weight (g)	358.9±13.9	361.3±31.3	355.3±26.7	331.3±29.1
Brain weight (g)	1.83±0.13	1.81±0.12	1.79±0.11	1.83±0.08
Brain/body weight (%)	0.511±0.041	0.505±0.060	0.506±0.030	0.555±0.055
Females				
Body weight (g)	195.8±16.6	199.9±12.7	205.3±12.1	193.1±7.5
Brain weight (g)	1.71±0.09	1.69±0.06	1.72±0.11	1.67±0.04
Brain/body weight (%)	0.879±0.081	0.847±0.054	0.838±0.042	0.867±0.024

Data taken from Table OW1K-SUM, pp. 361-362, MRID 44927645.
a Values given as Mean ± Standard Deviation; n = 6.

3. **Neuropathology:** No treatment-related neuropathological alterations were observed in animals from the high-dose group, and the mid- and low-dose groups were therefore not examined. The most commonly observed lesion was minimal nerve fiber degeneration in the trapezoid body of the medulla, which was noted in 5/6 males in the control and high-dose groups and 3/6 and 5/6 females in the control and high-dose groups respectively. Minimal axonal swelling was noted in scattered locations in the brain including the cerebellar peduncles (1/6 animals from the male and female control groups), the cerebellar white matter (1 male control, 2 high-dose males, and 2 high-dose females), the cerebellar roof nuclei (1 high-dose male and 1 high-dose female) and the medulla oblongata (1 high-dose male). One control and one high-dose male had unilateral retinal degeneration with nerve fiber degeneration of the optic nerve, optic chiasm, and optic tract. Minimal axonal swelling and/or nerve fiber degeneration were also noted in most animals at one or more locations in the spinal cord. Minimal gliosis was noted in the cervical spinal cord of one high-dose female.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the 1600 ppm dietary concentration represented a maximum-tolerated-dose (MTD) based on decreased absolute body weights but did not produce any neurobehavioral or micropathological evidence of neurotoxicity. The overall NOAEL for the study was 50 ppm, based on decreased food consumption at 400 ppm, and 1600 ppm was the NOAEL for neurotoxicity.

B. **REVIEWER COMMENTS:** Systemic toxicity was evident at 1600 ppm as decreased body weight gain and food consumption. The reviewer does not consider the 8-10% decrease in food consumption noted only during the first week of treatment at 400 ppm to be of sufficient magnitude to be considered toxicologically relevant and therefore disagrees with the study author's use of 400 ppm as an overall LOAEL for the study.

Possible neurotoxicity was evident in males at 1600 ppm as decreased mean hindlimb grip strength during week 13 of the study. The reviewer disagrees with the study author and considers a decrease of 21% compared to the respective control group to be biologically relevant, as both groups had similar standard deviations. Although there were no correlating neuropathological lesions, and no other neurofunctional changes were observed, the nominal dietary concentration of 1600 ppm may represent a threshold dose.

Therefore, the LOAEL for Thiacloprid (YRC 2894 Technical) in Fischer 344 CDF(F-344)/BR rats is 1600 ppm (101 and 115 mg/kg bw/day for males and females, respectively), based on decreased body weight gains and food consumption in both sexes and decreased hindlimb grip strength in males. The NOAEL is 400 ppm (24.2 and 27.9 mg/kg bw/day for males and females, respectively).

C. **STUDY DEFICIENCIES:** The following deficiencies were noted:

The results of the diet analyses indicated that the mixing procedure may have been inadequate and that there may have been significant variance between nominal and actual dosage to the study animals. This deficiency is mitigated by the fact that the analytical concentrations were used to calculate the average daily test article intake by the animals on study.

The strain gauge used for measuring grip strength in the FOB was not described in the study report.

- A few of the required FOB parameters were not assessed (palpebral closure during home cage observation; "red crusty deposits"; and "altered fur appearance").

DATA FOR ENTRY INTO ISIS

Subchronic Neurotoxicity Study - rats (OPPTS 870.6200b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
014019	44927645	subchr neurotox	rats	90 days	oral	diet	2.94-115	♂ 0, 2.94, 24.3, 101 ♀ 0, 3.41, 27.9, 115	♂ 24.3 ♀ 27.9	♂ 101 ♀ 115	body wt decr (both) food cons decr (both) decr grip strength (males)	Toxicity