UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: December 29, 2015

SUBJECT: Fluridone: Review of acute neurotoxicity study

PC Code: 112900
Decision No.: 494948
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0057317
MRID No.: 48939603

DP Barcode: D429841
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 59756-60-4
40 CFR: N/A

FROM: Austin Wray, Ph.D., Toxicologist
Risk Assessment Branch IV,
Health Effects Division (7509P)

THROUGH: Michael S. Metzger, Branch Chief
Risk Assessment Branch V/VII
Health Effects Division (7509P)

TO: Sarah Meadows, Risk Manager Reviewer
Kathryn Montague, Risk Manager
Herbicide Branch
Registration Division (7505P)

I. CONCLUSIONS:

The acute neurotoxicity study in rats (MRID 48939603) is classified as **Acceptable/Guideline**. The LOAEL was 650 mg/kg/day based on decreased ambulatory counts and the prevalence of functional observational battery anomalies in males and females. The NOAEL was 125 mg/kg/day.

II. ACTION REQUESTED:

Review acute neurotoxicity study to support the fluridone registration review and registration for new use on cotton.

III. BACKGROUND:

The acute neurotoxicity study was submitted to fulfill a data deficiency requirement for fluridone registration review and registration for new use on cotton.

IV. RESULTS/DISCUSSION:

In an acute neurotoxicity study (MRID 48939603), groups of 6-week-old Crl:CD(SD) rats (10/sex/dose) were given a single oral gavage dose of fluridone (98.02% a.i.; Lot no. MAR11BE011) in 5% Acacia in deionized water at doses of 0 (vehicle control), 125, 650, or 2000 mg/kg bw and observed for 14 days. Functional observation battery (FOB) and locomotor activity data were recorded for all animals prior to the initiation of dose administration, on Day 0 at the time of peak effect (approximately 4 hours following dose administration), and on Days 7 and 14. Animals were sacrificed on Day 15 and perfused *in situ*. Brain weights and brain dimensions were recorded. A neuropathological evaluation of selected tissues from the central (CNS) and peripheral nervous systems was performed on 5 animals/sex in the control and 2000 mg/kg bw groups.

Treatment with fluridone did not affect clinical signs, mortality, gross necropsy findings, neuropathology, brain weight, or brain dimensions.

Dose-related changes in FOB parameters were observed in males and females at 125, 650 and 2000 mg/kg bw of fluridone. These changes were transient, occurring only on Day 0, and often disappeared by Day 7 or 14.

Anomalies in a multitude of FOB parameters was observed in the 2000 mg/kg bw group. Home cage observations revealed clonic convulsions (repetitive movement of mouth and jaws) (2 males; 5 females) and cage biting (2 males; 3 females). Open-field observations included gait abnormality (hunched body: 2 males; 5 females) and one male also exhibiting bizarre/stereotypic behavior (repetitive turning of head from side to side). Sensory observations revealed a number of animals had no reaction or response to approach (3 rats each), touch (3 rats each), startle (4 males; 2 females), and/or tail pinch (4 males; 3 females). Air righting reflex was also impaired (slightly uncoordinated: 2 males; 2 females; landing on their side: 1 male; 2 females). One high-dose female exhibited no hindlimb extension. Physiological observations found a longer catalepsy time in males only (2.4 vs. 0.4 seconds for controls), mainly due to 2 rats with long times. Females had reduced body temperature compared to controls (35.8 vs. 37.1°C for controls). Lastly, motor activity was reduced. Although mean cumulative total activity counts were not affected, mean cumulative ambulatory activity counts were decreased ($p<0.05$) in males (325 vs. 500 counts for controls) and females (521 vs. 828 counts for controls).

Similar effects were observed in male and female rats at 650 mg/kg bw on Day 0 but generally occurred at a much lower incidence and were mostly limited to minor FOB anomalies. These effects included: clonic convulsions (1 female), cage biting (1 female), gait abnormality (hunched body; 1 male), no response/reaction to approach (4 males; 1 female), touch (4 males; 1 female), startle (3 males), and/or tail pinch (3 males), and abnormal righting reflex (landing on its side; 1 female). Males from the 650 mg/kg bw dose group also exhibited longer catalepsy time outside the historical control range, which was considered a more serious neurotoxic response relative to the other FOB anomalies. Both males and females experienced a reduction in mean ambulatory counts concurrent with the behavioral anomalies.

Changes in FOB parameters on Day 0 were also noted in males and females at 125 mg/kg bw although many of the changes, similar to the 650 mg/kg bw dose group, occurred at a low incidence and were relatively minor anomalies. These effects included gait abnormality (hunched body) and uncoordinated air righting reflex (both in same female), and no reaction/response to approach (4 males; 1 female), touch (3 males; 2 females), startle (2 males), and/or tail pinch (1 male). At the 125 mg/kg bw dose, cumulative total and ambulatory motor activity counts were not affected.

Male and female rats from all dose groups exhibited depressed body weight gain (5-25% less than controls) during the two weeks after treatment. The impact of depressed body weight gain on absolute body weight was negligible (all dose groups were comparable to controls on Day 0, 7 and 14); therefore, the body weight gain depression was not considered adverse. Food consumption was not recorded.

The changes in FOB parameters in combination with significant depression of cumulative ambulatory activity counts was considered evidence of an adverse response at the mid and high doses. In the absence of a corroborative effect on motor activity, the FOB effects at the low dose were not considered adverse because the effects were limited to minor behavior aberrations not anticipated to impact quality of life and were of low incidence.

The LOAEL is 650 mg/kg bw based on decreased ambulatory counts and the prevalence of FOB anomalies in males and females. The NOAEL is 125 mg/kg bw.

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

DATA EVALUATION RECORD

FLURIDONE
STUDY TYPE: ACUTE NEUROTOXICITY - RATS
OCSPP 870.6200a [§81-8]; OECD 424

MRID 48939603

Prepared for

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U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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Summitec Corp. for the U.S. Environmental Protection Agency under Contract No. EP-W-11-014

EPA Reviewer: Austin Wray, Ph.D. **Signature:** Austin Wray
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Risk Assessment Branch I, Health Effects Division (7509P) **Date:** 12/15/15

Template version 09/11

TXR#: 0057317

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats OCSPP 870.6200 [§81-8]; OECD 424.

PC CODE: 112900 **DP BARCODE:** D429841

TEST MATERIAL (PURITY): Fluridone, 98.02% a.i.

SYNONYMS: None provided

CITATION: Herberth, M.T. (2012). An oral (gavage) acute neurotoxicity study of fluridone in rats. WIL Research, 1407 George Rd., Ashland OH and WIL Research, 310 Millstone Dr., Hillsborough, NC. Laboratory Project ID: WIL-867005, September 6, 2012. MRID 48939603. Unpublished.

SPONSOR: SePRO Corporation, 11550 North Meridian Ave., Suite 300, Carmel, IN

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 48939603), groups of 6-week-old Crl:CD(SD) rats (10/sex/dose) were given a single oral gavage dose of fluridone (98.02% a.i.; Lot no. MAR11BE011) in 5% Acacia in deionized water at doses of 0 (vehicle control), 125, 650, or 2000 mg/kg bw and observed for 14 days. Functional observation battery (FOB) and locomotor activity data were recorded for all animals prior to the initiation of dose administration, on Day 0 at the time of peak effect (approximately 4 hours following dose administration), and on Days 7 and 14. Animals were sacrificed on Day 15 and perfused *in situ*. Brain weights and brain dimensions were recorded. A neuropathological evaluation of selected tissues from the central (CNS) and peripheral nervous systems was performed on 5 animals/sex in the control and 2000 mg/kg bw groups.

Treatment with fluridone did not affect clinical signs, mortality, gross necropsy findings, neuropathology, brain weight, or brain dimensions.

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Anomalies in a multitude of FOB parameters was observed in the 2000 mg/kg bw group. Home cage observations revealed clonic convulsions (repetitive movement of mouth and jaws) (2 males; 5 females) and cage biting (2 males; 3 females). Open-field observations included gait abnormality (hunched body: 2 males; 5 females) and one male also exhibiting bizarre/stereotypic behavior (repetitive turning of head from side to side). Sensory observations revealed a number of animals had no reaction or response to approach (3 rats each), touch (3 rats each), startle (4 males; 2 females), and/or tail pinch (4 males; 3 females). Air righting reflex was also impaired (slightly uncoordinated: 2 males; 2 females; landing on their side: 1 male; 2 females). One high-dose female exhibited no hindlimb extension. Physiological observations found a longer catalepsy time in males only (2.4 vs. 0.4 seconds for controls), mainly due to 2 rats with long times. Females had reduced body temperature compared to controls (35.8 vs. 37.1°C for controls). Lastly, motor activity was reduced. Although mean cumulative total activity counts were not affected, mean cumulative ambulatory activity counts were decreased ($p<0.05$) in males (325 vs. 500 counts for controls) and females (521 vs. 828 counts for controls).

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Changes in FOB parameters on Day 0 were also noted in males and females at 125 mg/kg bw although many of the changes, similar to the 650 mg/kg bw dose group, occurred at a low incidence and were relatively minor anomalies. These effects included gait abnormality (hunched body) and uncoordinated air righting reflex (both in same female), and no reaction/response to approach (4 males; 1 female), touch (3 males; 2 females), startle (2 males), and/or tail pinch (1 male). At the 125 mg/kg bw dose, cumulative total and ambulatory motor activity counts were not affected.

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The changes in FOB parameters in combination with significant depression of cumulative ambulatory activity counts was considered evidence of an adverse response at the mid and high doses. In the absence of a corroborative effect on motor activity, the FOB effects at the low dose

were not considered adverse because the effects were limited to minor behavior aberrations not anticipated to impact quality of life and were of low incidence.

The LOAEL is 650 mg/kg bw based on decreased ambulatory counts and the prevalence of FOB anomalies in males and females. The NOAEL is 125 mg/kg bw.

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

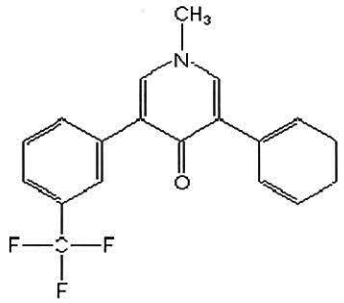
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Fluridone

Description: Off-white powder
Lot/batch #: MAR11BE011
Purity: 98.02%
CAS # of TGAI: 59756-60-4
Structure:



2. Vehicle and/or positive control: 5% Acacia (Lot no. 2AC0916, exp. date: 15 December 2012, manufactured by Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ; stored at room temperature) in deionized water

3. Test animals:

Species:	Rat	
Strain:	Crl:CD(SD)	
Age/weight at dosing:	~6 weeks old; males: 199-254 g; females: 138-197 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC.	
Housing:	All animals were housed 2-3 per cage by sex for approximately 6 days; then housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board	
Diet:	PMI Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*	
Water:	Reverse osmosis-treated drinking water *ad libitum*	
Environmental conditions:	**Temperature:**	71°F±5°F (22°C ± 3°C)
	Humidity:	50 ± 20%,
	Air changes:	10 fresh air changes per hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Minimum of 13 days	

B. STUDY DESIGN:

1. In life dates: Start: January 31, 2012; End: March 2, 2012

2. **Animal assignment and treatment:** Animals were randomly assigned by computer to the test groups noted in Table 1 based on body weight stratification in a block design. The animals were randomized into 4 study replicates to facilitate neurobehavioral observations. Each dose group and sex was approximately equally represented within each study replicate. The vehicle and test substance formulations were administered as a single oral gavage dose in a dose volume of 5 mL/kg. Animals were not fasted prior to dose administration.

   Dose levels were selected based on the results of a previous dose range-finding study in which the test substance was administered as a single oral dose to 3 groups of 3 Crl:CD(SD) rats/sex/group at dose levels of 550, 1750, and 2000 mg/kg. There were no significant clinical observations noted at any dose level. As a result, a high dose of 2000 mg/kg bw (limit dose for acute neurotoxicity studies) was chosen for the current study.

TABLE 1. Study design

Experimental parameter	Dose group (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Total number of animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Necropsy	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	-	-	5/sex

3. **Test Substance preparation and analysis**:

   The vehicle suspension was prepared once for administration to the control group (Group 1) and for preparation of the test substance formulations; aliquots were prepared for dispensation to the control group and stored refrigerated. The vehicle was mixed throughout the preparation, sampling, and dose administration procedures. The test substance formulations used for dose administration were prepared once as single formulations for each dose level, divided into aliquots for dispensation, and stored refrigerated (2°C to 8°C). The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures.

   Homogeneity, resuspension homogeneity, and stability (following 4 days of refrigerated storage) of the test substance in the vehicle at concentrations of 30 and 400 mg/mL were previously established. However, additional homogeneity and stability assessments were performed as part of the current study at 25 mg/mL. Prior to the initiation of dose administration, duplicate samples for homogeneity determination were collected from the top, middle, and bottom of a 25 mg/mL non-dosing formulation. In addition, a sufficient volume of the same 25 mg/mL non-dosing formulation, similar in size to the amount needed for 1 day of dosing, was stored refrigerated (2°C to 8°C) for 4 days. Following the 4-day storage period, the formulation was mixed using a magnetic stirrer for a minimum of 30 minutes and samples were collected from the top and bottom strata and assessed for stability. Samples for concentration analyses were collected from the middle stratum of each dosing formulation (including the control group). All analyses were conducted using a validated high performance liquid chromatography method using ultraviolet absorbance detection.

Results:

Homogeneity analysis: Homogeneity analyses of duplicate samples taken from the top, middle, and bottom of the 25 mg/mL test formulation revealed a mean concentration of 24.3 mg/mL (97.2% of target) and a % RSD of 0.89%.

Stability analysis: Stability analysis of duplicate samples taken from the top and bottom of the 25 mg/mL test substance formulation and stored refrigerated for 4 days revealed mean concentrations of 25.7, 26.1, 25.5, and 25.8 mg/mL. The mean of these values was 106% of the initial concentration of 25 mg/mL.

Concentration analysis: Concentration analyses of duplicate samples taken from the 25, 130, and 400 mg/mL test formulations revealed mean concentrations of 24.0, 113, and 403 mg/mL, respectively, resulting in concentrations that were 95.9, 87.1, and 101% of nominal, respectively. The test substance was not detected in the vehicle formulation that was administered to the control group.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. In addition, standard error (S.E.) was presented for body weight, brain measurements, and locomotor activity data.

   Statistics Conducted by WIL Research: All statistical tests were performed using WTDMS™ unless otherwise noted. Analyses were done using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group by sex. Body weight, body weight change, continuous FOB, and brain weight and measurement data were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test substance-treated groups to the control group. FOB parameters that yielded scalar or descriptive data, necropsy findings, and non-graded histopathologic findings were analyzed using Fisher's Exact Test. Statistical analysis of graded histopathologic findings was not conducted on this study due to the absence of multiple severities (i.e., those findings with more than 2 distinct severities including none/not remarkable).

   Statistics Conducted by BioSTAT Consultants, Inc.: All statistical analyses were done using SAS® version 9.2 or higher. All repeated measures analysis of variance (RANOVA) statistical analyses for total and ambulatory locomotor activity counts recorded during pretest and after dosing were conducted as follows. Each analysis endpoint was analyzed, by sex and session, with a RANOVA. Factors in the model included treatment group (TRT), time interval (TIME), and the interaction of time interval and treatment group (TRT*TIME). The SAS® procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC).

The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels. The linear dose by time interaction (LinTrt*Time) was evaluated and, if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals for the entire session only.

Nonmonotonic dose responses were evaluated whenever no significant linear trends were detected but TRT and/or TRTTIME interaction was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual test substance-treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted for each time interval. If only the TRT effect was significant, the comparisons were conducted across the pooled time intervals for the entire session. These nonmonotonic dose-response comparisons were conducted at the 0.01 significance level.

The reviewer considered the statistical analyses to be adequate.

C. METHODS:

1. **Mortality and clinical observations:** All animals were observed twice daily for mortality and moribundity.

   Clinical examinations were performed once daily on all animals. On the days of the FOB, no additional clinical findings were recorded.

2. **Body weight:** Individual body weights were recorded weekly, beginning 1 week prior to test substance administration (Day -7).

3. **Food consumption:** Food consumption was not measured.

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

5. **Neurobehavioral assessment:**

   a. **Functional Observational Battery (FOB):** FOB findings were recorded for all animals prior to the initiation of dose administration (Day -6), at the time of peak effect (approximately 4 hours post-dosing) on Day 0, and on Days 7 and 14. Testing was performed by the same biologists, to the extent possible, without knowledge of the animal's group assignment. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB.

   Scoring criteria were included for the measured parameters and are provided in Appendix G of the study report. For the open-field observations, the animal was placed in a standard arena (24" x 24" x 6"; constructed from black Plexiglas®) and observed for a

two-minute recording period. Forelimb and hindlimb grip strength were measured using a T-shaped grip bar and a Mark-10 series EG digital force gauge. For the rotarod test, animals were tested for up to 2 minutes on a 7.0-cm-diameter rod rotating at 12 rpm.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	Mobility
X	Biting	X	Lacrimation*/chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	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 behavior*
X	Startle response*			X	Backing
X	Pain response*(tail pinch)		**PHYSIOLOGICAL OBSERVATIONS**	X	Time to first step
X	Pupil response*	X	Body weight*		**NEUROMUSCULAR OBSERVATIONS**
X	Eyeblink response	X	Body temperature+	X	Hindlimb extensor strength
X	Forelimb extension			X	Forelimb grip strength*
X	Hindlimb extension		**OTHER OBSERVATIONS**	X	Hindlimb grip strength*
X	Air righting reflex+	X	Catalepsy	X	Landing foot splay*
X	Olfactory orientation			X	Rotarod performance

*Required parameters; +Recommended parameters
Note: Open field observations were evaluated over a 2-minute observation period.

b. <u>Locomotor activity</u>: Locomotor activity was assessed after completion of the FOB on all animals prior to the initiation of dose administration (Day -6), at the time of peak affect (approximately 4 hours post-dosing) on Day 0, and on Days 7 and 14. Locomotor activity was measured automatically using a computer-controlled system that utilized a series of infrared photobeams surrounding an amber plastic, rectangular cage to quantify the motor activity of each animal. Four-sided black plastic enclosures were used to surround the transparent plastic boxes and decrease the potential for distraction. The locomotor activity assessment was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB. The testing of treatment groups was conducted according to replicate sequence. Each animal was tested separately. Data were collected in 5-minute epochs, and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation.

Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of 1 photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams).

6. **Sacrifice and pathology:** On Day 15, all animals were anesthetized by an intraperitoneal injection of sodium pentobarbital and then perfused *in situ* with a 4.0% paraformaldehyde in a 0.1 M phosphate buffered solution. The central and peripheral nervous system tissues were dissected and preserved. Fixed brain weight and brain dimensions (length [excluding olfactory bulbs] and width) were recorded. Any observable gross changes and abnormal coloration or lesions of the brain and spinal cord were recorded.

   Additionally, the CHECKED (X) tissues from five males and five females from the control and 2000 mg/kg bw groups were processed and evaluated histologically. The tissues were prepared for a histopathological examination by embedding in paraffin (central nervous system tissues) or plastic (peripheral nervous system tissues), sectioning, and staining with hematoxylin and eosin.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh
X	Center of cerebrum	X	At Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerves
X	Medulla oblongata	X	Tibial Nerves
	SPINAL CORD	X	Peroneal Nerves
X	Cervical swelling (C_3-C_7)	X	Lumbar dorsal root gangla (T_{13}-L_4)
X	Lumbar swelling (T_{13}-L_4)	X	Lumbar dorsal root fibers (T_{13}-L_4)
	Thoracic swelling	X	Lumbar ventral root fibers (T_{13}-L_4)
	OTHER	X	Cervical dorsal root gangla (C_3-C_7)
	Gasserian Ganglion	X	Cervical dorsal root fibers (C_3-C_7)
X	Trigeminal nerves plus ganglia	X	Cervical ventral root fibers (C_3-C_7)
X	Optic nerves	X	Olfactory bulbs
X	Eyes	X	Hippocampus/dentate gyrus
X	Gastrocnemius muscle	X	Basal ganglia
X	Cervical spinal nerve	X	Thalamus
X	Lumbar spinal nerve	X	Hypothalamus

7. **Positive controls:** Positive control data were provided in Appendix F of MRID 48939603.

   Studies WIL-99435 (MRID 48939603, pp. 352-375) and WIL-99441 (MRID 48939603, pp. 376-404) were positive control validation studies of locomotor activity in Crl:CD (SD) rats. Locomotor activity was recorded using a Kinder Scientific MotorMonitor (Kinder Scientific, LLC, Poway, CA USA). The test sessions were conducted in a sound-attenuated room and were 60 minutes in duration, with 6 subintervals lasting 10 minutes each. Locomotor activity was evaluated as total and ambulatory activity counts obtained over each subinterval and the

entire 60-minutes sessions. In study WIL-99435, groups of 20 rats/sex were administered Haloperidol at dose levels of 0, 0.05, 0.1, and 0.5 mg/kg bw by a single IP injection 30 minutes prior to behavioral testing on postnatal day (PND) 13, 17, 21, or 61. Overall, haloperidol caused a dose-dependent decrease in locomotor activity (total activity counts and ambulatory counts) for both male and female rats at PND 13, 17, 21 and 61. Habituation was noted on PND 17, 21, and 61, but not on PND 13, males and females. In study WIL-99441, groups of 20 rats/sex were administered nicotine at dose levels of 0, 0.1 or 0.5 mg/kg bw by a single IP injection 3 minutes prior to behavioral testing on PND 13, 17, and 21. Also, groups of 20 young adult rats were administered amphetamine by a single IP injection 15 minutes prior to behavioral testing at dose levels of 0, 1 or 3 mg/kg bw (males and females) on PND 61 and at doses of 0, 0.2 and 1 mg/kg (females only) on PND 61. Nicotine caused an increase in locomotor activity (mean total and ambulatory activity) in male and female neonatal rats at PND 13, 17, and 21. In a similar manner, amphetamine stimulated locomotor behavior in male and female rats at PND 61, causing both total and ambulatory activity to increase. The subsequent female-only groups (0.2 and 1 mg/kg bw amphetamine) exhibited a dose-response with increased total and ambulatory activity compared to controls. In the PND 61 males and females (given 1 or 3 mg/kg bw amphetamine), the habituation profile was only present in the control and 3 mg/kg bw amphetamine treatment groups, whereas the habituation profile was present in the female-only PND 61 control and 0.2 mg/kg bw amphetamine groups. The experimental design used in these studies (WIL-99435 and WIL-99441) demonstrated that WIL Research can detect locomotor effects of haloperidol (decrease in motor activity) and nicotine and amphetamine (increases in motor activity) using the Kinder Scientific MotorMonitor System. It was noted that these validation studies used 20 rats/sex/group for evaluation, while the acute neurotoxicity study used only 10 rats/sex/group. Also, the acute neurotoxicity study did not specify that the Kinder Scientific MotorMonitor System was used.

In WIL-99443 (MRID 48939603, pp. 405-447), groups of 12 Crl:CD (SD) rats/sex/group were administered chlorpyrifos at a single dose of 0, 20 or 100 mg/kg bw by oral gavage 3.5 hours before FOB assessment and locomotor activity measurement. The methodology for the FOB assessment and locomotor activity determination appeared to be the same as that used in the main acute neurotoxicity study (MRID 48939603). The FOB detected a general decrease in the initiation of movement (time to first step in the open field), number of rears, body temperature and rotarod performance, along with impaired gait (hindlimbs splayed or dragging, walks on tip toes, hunched body), and increased catalepsy time. Motor activity (both total and ambulatory activity) measured by the Kinder Scientific MotorMonitor System showed a consistent decrease in mean activity by both males and females treated with 100 mg/kg bw chlorpyrifos. The experimental design used in this study demonstrated that WIL Research can detect behavioral changes in an FOB, as well as locomotor effects of chlorpyrifos using the Kinder Scientific MotorMonitor System.

The other two studies submitted for validation of FOB methodology, WIL-99263 (MRID 48939603, pp. 448-461) and WIL-99310 (MRID 48939603, pp. 462- 497), were conducted to train personnel on the conduct the FOB and to assess inter-observer reliability of the same technicians. There were three phases of testing: Phase I consisted of FOB assessments on naïve stock animals in order to ensure consistent and accurate use of all equipment and gain

knowledge of stereotypic behavior of naïve animals; Phase II consisted of an open discussion of each animal's observations conducted in a group setting (2 rats/group dosed with positive control or vehicle); and Phase III consisted of three animals/group administered a positive control or vehicle and observed at a time designated by the Study Director; each technician individually recorded observations of the animals and the resulting inter-observer reliability was evaluated. Substances evaluated in study WIL-99263 included Parathion, 3,3'-Iminodipropionitrile (IDPN) or deionized water, and study WIL-99310 included IDPN, Parathion, d-Amphetamine, and corn oil. After reviewing the responses of the technicians, the Study Director concluded that all but two of the technicians fulfilled the criteria for reliably observing FOB parameters to assess behavioral changes in rats. One of the two technicians was to receive additional training and demonstrated proficiency in the evaluation of the pupillary response, while the other observer was not considered to have met acceptable criteria for performing FOB testing. The technicians in the other study (WIL-99263) were considered to have met the acceptable criteria for evaluation.

No positive control studies demonstrating the ability to detect central and peripheral nervous system pathology were included.

Acute FOB historical control data and acute motor activity historical control data for Crl:CD(SD) rats were provided for a variety of vehicles (MRID 48939603; Appendix J and Appendix K). The FOB studies that were cited ranged over a period of time of 4/24/01 to 7/5/06, while the locomotor activity studies ranged over a period from 11/12/07 to 10/22/10. A description of testing methods was not included.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** No clinical signs related to treatment were observed. Findings were limited to low incidences of alopecia in males and females that were not related to dose.

2. **Mortality:** All rats survived to study termination.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Mean absolute body weight and body weight gain data are summarized in Table 2. No statistically significant, treatment-related changes in mean absolute body weight or body weight gain were noted in male or female rats at any dose. Male and female body weight gain was depressed (5-25% less than controls) in the two weeks after treatment, but did not have a biologically significant impact on absolute body weight indicating the effect was not adverse.

TABLE 2. Body weight and body weight gain (g ± SD) [a, b]				
Observation	**Dose level (mg/kg bw)**			
	Control (0)	**Low (125)**	**Mid (650)**	**High (2000)**
Body weight-males				
Day -7	170 ± 15.4	169 ± 14.8	169 ± 11.1	170 ± 14.5
Day 0	232 ± 17.8	232 ± 15.1	227 ± 14.1	230 ± 16.8
Day 7	286 ± 22.2	280 ± 15.5	274 ± 20.5	275 ± 17.4
Day 14	329 ± 29.3	322 ± 24.5	315 ± 29.3	319 ± 23.9
Body weight-females				
Day -7	141 ± 9.7	139 ± 11.7	140 ± 7.4	138 ± 12.4
Day 0	171 ± 12.9	172 ± 11.1	172 ± 9.8	169 ± 14.0
Day 7	195 ± 17.0	192 ± 13.5	191 ± 11.7	187 ± 21.3
Day 14	214 ± 17.0	213 ± 16.7	211 ± 13.9	206 ± 26.0
Body weight gain-males				
Day 0-7	54 ± 6.9	48 ± 7.5 (↓11%)	47 ± 11.0 (↓13%)	45 ± 8.2 (↓17%)
Day 0-14	97 ± 14.8	90 ± 19.3 (↓7%)	88 ± 19.7 (↓9%)	89 ± 19.0 (↓8%)
Body weight gain-females				
Day 0-7	24 ± 7.8	21 ± 7.2 (↓12%)	19 ± 5.7 (↓21%)	18 ± 9.1 (↓25%)
Day 0-14	43 ± 10.7	41 ± 9.5 (↓5%)	38 ± 6.4 (↓12%)	37 ± 13.7 (↓14%)

[a] Data extracted from MRID 48939603, pp. 84-87.
[b] n = 10
Number in parenthesis is the percent difference from controls.

C. FOOD CONSUMPTION: Food consumption was not measured.

D. NEUROBEHAVIORAL RESULTS:

1. FOB findings:

Home cage observations:
Home cage observations revealed a treatment-related effect in males and females in the high-dose group on Day 0 (Table 3). Two males at 2000 mg/kg bw exhibited clonic convulsions (repetitive movement of mouth and jaws) and cage biting. In females, 5 animals at 2000 mg/kg bw developed clonic convulsions, with 3 of the 5 additionally biting the cage.

One male at 125 mg/kg bw exhibited clonic convulsions and one female at 650 mg/kg bw exhibited clonic convulsions and cage biting; however both were considered spontaneous and not treatment related based on similar behavior reported in several animals from the historical controls and the lack of dose response between the low and mid doses.

Single incidences of repetitive mouth and jaw movement at 125 and 650 mg/kg bw were not considered an adverse response to treatment based on the nature of the effect and low incidence. This finding in animals from the 2000 mg/kg bw dose group was attributed to treatment due to higher frequency, particularly in the females (5/10 animals).

TABLE 3. Functional observational battery results: Home cage observations [a,b,c]				
Home cage observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Males				
Convulsions - Clonic				
-Pretest: Absent	10	10	10	10
-Day 0				
Absent	10	9	10	8
Repetitive movement of mouth/jaws	0	1	0	2
-Day 7 and Day 14: Absent	10	10	10	10
Biting				
-Pretest: None	10	10	10	10
-Day 0				
None	10	10	10	8
Biting of cage	0	0	0	2
-Day 7 and Day 14: None	10	10	10	10
Females				
Convulsions - Clonic				
-Pretest: Absent	10	10	10	10
-Day 0				
Absent	10	10	9	5*
Repetitive movement of mouth/jaws	0	0	1	5*
-Day 7 and Day 14: Absent	10	10	10	10
Biting				
-Pretest: None	10	10	10	10
-Day 0				
Absent	10	10	9	7
Biting of cage	0	0	1	3
-Day 7				
Absent	10	9	10	10
Biting of cage	0	1	0	0
-Day 14: None	10	10	10	10

[a] Data were extracted from 48939603, pp. 88-96.
[b] Values represent number of animals affected
[c] n=10
*=p<.05 compared with controls

Handling observations:

There were no dose-related differences in the handling observations of treated male or female rats compared to controls.

Open field observations:

A summary of selected open field observations is presented in Table 4. In males, findings on Day 0 included 1, 1, and 2 animals at the low-, mid-, and high-dose with a hunched posture, and 1 male at the high-dose with bizarre/stereotypic behavior (repetitive turning of head from side to side).

Open-field observations of females on Day 0 revealed a treatment-related effect on gait with 5 females exhibiting a hunched body at the high dose. Additionally, one female at the low dose had a hunched body. No other significant observations were noted.

TABLE 4. Functional observational battery results: Open field observations [a,b,c]

Home cage observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Males				
Gait:				
-Pretest: Normal	10	10	10	10
-Day 0				
Normal	10	9	9	8
Hunched body	0	1	1	2
-Day 7 and Day 14: Normal	10	10	10	10
Bizarre/Stereotypic behavior				
-Pretest: None	10	10	10	10
-Day 0				
None	10	10	10	9
Head search/repetitive turning side to side	0	0	0	1
-Day 7 and Day 14: Absent	10	10	10	10
Females				
Gait:				
-Pretest: Normal	10	10	10	10
-Day 0				
Normal	10	9	10	5*
Hunched body	0	1	0	5*
-Day 7 and Day 14: Normal	10	10	10	10

[a] Data were extracted from 48939603, pp. 121-136.
[b] Values represent number of animals affected
[c] n=10
*=p<.05 compared with controls

Sensory observations:

Sensory observations of male and female rats on Day 0 revealed a general decrease in response to outside stimuli (Tables 5 and 6). Although not always following a clear dose-response, the lack of response to stimuli in the groups of treated rats are generally considered an effect of treatment because animals from the control groups always exhibited a reaction.

Male rats in the low-, mid-, and high-dose groups exhibited no reaction to approach response (4, 4, and 3 rats, respectively), touch response (4, 4, and 3 rats, respectively), startle response (2, 3, and 4 rats, respectively), and/or tail pinch response (1, 3, and 4 rats, respectively). Air righting reflex was also impaired in high-dose males, with 2 rats being slightly uncoordinated and 1 rat landing on its side.

Females in the low-, mid-, and high-dose groups exhibited no reaction to approach response (1, 1, and 3 rats, respectively), touch response (2, 1, and 3 rats, respectively), with high-dose females additionally showing no startle response (2 rats), and/or tail pinch response (3 rats). Air righting reflex was impaired in mid- and high-dose females, with 2 high-dose rats being slightly uncoordinated and 1 mid-dose and 2 high-dose female rats landing on their side. Another high-dose female had no hind limb extension.

TABLE 5. Functional observational battery results: Sensory observations in males [a,b,c]

Observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Approach response				
-Pretest: Slow approach, sniffing or turning away	10	10	10	9
More energetic response, with/without vocalization	0	0	0	1
-Day 0				
No reaction	0	4	4	3
Slow approach, sniffing or turning away	10	4*	6	7
More energetic response, with/without vocalization	0	2	0	0
-Day 7				
Slow approach, sniffing or turning away	10	9	10	10
More energetic response, with/without vocalization	0	1	0	0
-Day 14				
Slow approach, sniffing or turning away	10	10	10	10
Touch response				
-Pretest: Animal may slowly turn/walk away	10	10	9	10
More energetic response	0	0	1	0
-Day 0				
No reaction	0	4	4	3
Animal may slowly turn/walk away	10	4*	6	7
More energetic response	0	2	0	0
-Day 7 and Day 14				
Animal may slowly turn/walk away	10	10	10	10
Startle response				
-Pretest: Slight reaction, ear flick/evidence snap heard	10	10	10	10
-Day 0				
No reaction	0	2	3	4
Slight reaction, ear flick/evidence snap heard	10	7	7	6
More energetic response, with/without vocalization	0	1	0	0
-Day 7				
Slight reaction, ear flick/evidence snap heard	10	10	10	10
-Day 14				
No reaction	0	0	0	1
Slight reaction, ear flick/evidence snap heard	10	10	10	9
Tail pinch response				
-Pretest: Animal may slowly turn, walk away	10	9	10	10
More energetic, with/without vocalization	0	1	0	0
-Day 0:				
No reaction	0	1	3	4
Animal may slowly turn, walk away	7	6	7	6
More energetic response, with/without vocalization	3	3	0	0
-Day 7:				
Animal may slowly turn, walk away	8	9	9	9
More energetic response, with/without vocalization	2	1	1	1
-Day 14:				
Animal may slowly turn, walk away	8	8	10	8
More energetic response, with/without vocalization	2	2	0	2

Air righting reflex				
-Pretest: Normal	10	10	10	10
-Day 0				
Normal	10	9	10	7
Slightly uncoordinated	0	0	0	2
Lands on side	0	1	0	1
-Day 7 and 14:				
Normal	10	10	10	10

[a] Data were extracted from 48939603, pp. 137-152.
[b] Values represent number of animals affected
[c] n=10
*=p<.05 compared with controls

TABLE 6. Functional observational battery results: Sensory observations in females [a,b,c]

Observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Approach response				
-Pretest: Slow approach, sniffing or turning away	10	9	10	10
More energetic response, with/without vocalization	0	1	0	0
-Day 0				
No reaction	0	1	1	3
Slow approach, sniffing or turning away	8	8	9	7
More energetic response, with/without vocalization	2	1	0	0
-Day 7				
Slow approach, sniffing or turning away	9	10	10	9
More energetic response, with/without vocalization	1	0	0	1
-Day 14				
Slow approach, sniffing or turning away	9	8	9	8
More energetic response, with/without vocalization	1	2	1	2
Touch response				
-Pretest: Animal may slowly turn/walk away	10	10	10	10
-Day 0				
No reaction	0	2	1	3
Animal may slowly turn/walk away	10	8	9	7
-Day 7 and Day 14				
Animal may slowly turn/walk away	10	10	10	10
Startle response				
-Pretest: Slight reaction, ear flick/evidence snap heard	10	10	10	10
-Day 0				
No reaction	0	0	0	2
Slight reaction, ear flick/evidence snap heard	10	10	10	8
-Day 7				
Slight reaction, ear flick/evidence snap heard	10	10	10	10
-Day 14				
No reaction	0	0	1	0
Slight reaction, ear flick/evidence snap heard	10	10	9	10

Tail pinch response				
-Pretest: Animal may slowly turn, walk away	9	8	8	10
More energetic, with/without vocalization	1	2	2	0
-Day 0:				
No reaction	0	0	0	3
Animal may slowly turn, walk away	8	8	8	6
More energetic response, with/without vocalization	2	2	2	1
-Day 7:				
Animal may slowly turn, walk away	9	6	5	6
More energetic response, with/without vocalization	1	4	5	4
-Day 14:				
Animal may slowly turn, walk away	9	6	7	6
More energetic response, with/without vocalization	1	4	3	4
Air righting reflex				
-Pretest: Normal	10	10	10	10
-Day 0				
Normal	10	9	9	6
Slightly uncoordinated	0	1	0	2
Lands on side	0	0	1	2
-Day 7 and 14:				
Normal	10	10	10	10

[a] Data were extracted from 48939603, pp. 137-152.
[b] Values represent number of animals affected
[c] n=10

Neuromuscular observations:
The neuromuscular parameters were unaffected by treatment.

Physiological observations:
A transient, statistically significant decrease in body temperature was observed in all groups of treated male and female rats compared to controls (Table 7). It was unclear if the reductions in body temperature were an adverse effect of treatment at all dose levels. The historical control data of 329 males reported a mean body temperature of 38.1 ± 0.85°C [minimum: 36.0°C; maximum: 39.2°C], while the historical control data of 249 females reported a mean of 38.3 ± 0.70°C [minimum: 36.5°C; maximum: 39.4°C] (MRID 48939603, p. 562 and 577, respectively). Compared to the historical control data and the pre-test values, the mean body temperatures of the treated males and females were not indicative of an adverse effect with the exception of the high-dose females.

Body temperature was not affected during the pretest or on Day 7 or 14.

Catalepsy in the mid- and high-dose males was increased on Day 0 compared to controls. An examination of the individual data revealed the increases were due to a few individual animals per group. In the mid-dose group, one male each had a time of 9.3 and 1.6 seconds, while in the high-dose group, one male each had a time of 19.3 seconds and 1.3 seconds and one female had a time of 1.5 seconds. Other catalepsy times were comparable to controls.

TABLE 7. Functional observational battery results: Physiological observations in males and females [a,b]

Home cage observation	Dose level (mg/kg bw)			
	Control (0)	Low dose (125)	Mid dose (650)	High dose (2000)
Males				
Body temperature (°C):				
-Pretest:	36.9 ± 0.24	36.7 ± 0.37	36.8 ± 0.31	36.8 ± 0.27
-Day 0:	37.4 ± 0.34	36.7** ± 0.42	36.5** ± 0.34	36.4** ± 0.42
-Day 7:	37.0 ± 0.40	37.0 ± 0.49	37.0 ± 0.33	37.1 ± 0.20
-Day 14:	37.5 ± 0.69	37.5 ± 0.73	37.3 ± 0.50	37.4 ± 0.79
Catalepsy (sec):				
-Pretest:	0.5 ± 0.10	0.4* ± 0.07	0.4 ± 0.11	0.5 ± 0.08
-Day 0:	0.4 ± 0.14	0.4 ± 0.05	1.4 ± 2.82	2.4 ± 5.95
-Day 7:	0.4 ± 0.09	0.5 ± 0.15	0.6 ± 0.38	0.5 ± 0.13
-Day 14:	0.5 ± 0.10	0.4 ± 0.11	0.5 ± 0.11	0.5 ± 0.20
Females				
Body temperature (°C):				
-Pretest:	36.6 ± 0.12	36.6 ± 0.46	36.7 ± 0.26	36.7 ± 0.39
-Day 0:	37.1 ± 0.35	36.6** ± 0.49	36.3** ± 0.26	35.8** ± 0.40
-Day 7:	37.9 ± 0.29	37.7 ± 0.34	37.6 ± 0.33	37.6 ± 0.41
-Day 14:	38.0 ± 0.43	38.3 ± 0.42	37.8 ± 0.46	38.0 ± 0.27

[a] Data were extracted from 48939603, pp. 161-168.
[b] n=10
*=p<0.01 compared with controls

All relevant FOB observations for individual animals have also been summarized in Tables 8-10 to display frequency of effects in each animal. These tables show that the incidence of treatment-related effects on sensory and open field parameters were similar in the low and mid dose groups. At the high dose, similar FOB signs were noted at a higher frequency along with additional effects that were not observed at lower doses.

Table 8. Summary of Day 0 FOB Observations for Individual Animals in 125 mg/kg bw dose group [a]

Animal Number	Rearing	Repetitive mouth and jaw movement	Biting Cage	Hunched Body	No Reaction/Response to:				Air Righting Reflex		Other Observations
					Approach	Touch	Startle	Tail Pinch	Uncoordinated	Lands on side	
Males											
40581					X	X				X	vocalization and energetic response to tail pinch
40590					X	X	X				
40600					X			X			
40613					X	X	X				
40614	X										
40595		X		X							
Females											
40641				X		X			X		
40648					X	X					

[a] Data were extracted from 48939603, pp. 647-993.
n= 10 males and 10 females

Table 9. Summary of Day 0 FOB Observations for Individual Animals in 650 mg/kg bw Dose Group [a]

Animal Number	Rearing	Repetitive mouth and jaw movement	Biting Cage	Hunched Body	No Reaction/Response to:				Air Righting Reflex		Other Observations
					Approach	Touch	Startle	Tail Pinch	Uncoordinated	Lands on side	
Males											
40575					X	X					↑ catalepsy (1.6 secs)
40580				X	X	X					
40584							X	X			
40588					X		X	X			
40597					X	X					
40610							X	X			↑ catalepsy (9.3 secs)
40611						X					
Females											
40618					X	X					
40631		X	X							X	

[a] Data were extracted from 48939603, pp. 647-993.
n= 10 males and 10 females

Table 10. Summary of Day 0 FOB Observations for Individual Animals in 2000 mg/kg bw Dose Group [a]

Animal Number	Rearing	Repetitive mouth and jaw movement	Biting Cage	Hunched Body	No Reaction/Response to:				Air Righting Reflex		Other Observations
					Approach	Touch	Startle	Tail Pinch	Uncoordinated	Lands on side	
Males											
40585								X			
40586					X	X	X				
40589				X			X	X			
40602		X	X		X	X		X			head search behavior
40604						X	X		X		
40605				X	X	X	X	X			
40582		X	X							X	
40591	X								X		↑ catalepsy (1.3 secs)
40608											↑ catalepsy (19.3 secs)
Females											
40620					X	X					energetic tail pinch response
40638		X	X				X	X			
40643		X		X	X	X		X			↑ catalepsy (1.5 secs)
40649				X	X	X				X	
40655		X		X			X	X		X	
40633		X	X	X							no hindlimb extension
40636									X		
40653				X					X		
40646		X	X								

[a] Data were extracted from 48939603, pp. 647-993
n= 10 males and 10 females

2. **Motor activity:** Statistically significant differences were observed in total and ambulatory motor activity of male and female rats on the day of dosing (Day 0). A summary of the motor activity data is presented in Tables 11 and 12.

   In male rats, the linear dose by time interaction (LinTrt*Time) was significant for both total and ambulatory counts indicating at least one time interval in both motor activity measures exhibited a monotonic dose response relationship. A within-session linear trend analysis revealed significantly decreased total activity counts at the mid-and high-dose over intervals 0-10 and 11-20 minutes, and statistically increased at 41-50 minutes and 51-60 minutes (high-dose only) compared to controls. A similar pattern was observed for within-session cumulative ambulatory activity counts; activity counts were statistically significantly decreased at the mid-and high-dose over intervals 0-10 and 11-20 minutes, and statistically increased in high-dose males at 41-50 and 51-60 minutes. Low dose males also exhibited significantly decreased ambulatory activity counts over the first interval of 0-10 minutes whereas total activity counts in the low dose males were comparable to controls at all time intervals. Despite significant differences from control in the within session analysis, treatment did not have a significant impact on cumulative total activity counts. Meanwhile, cumulative ambulatory activity counts were statistically significantly decreased at the mid- and high-dose (281 and 325 counts, respectively, vs. 500 counts for controls). The low dose cumulative ambulatory counts were comparable to controls indicating depressed activity in the 0-10 minute interval was not significant to the cumulative counts.

   As in male rats, the linear dose by time interaction (LinTrt*Time) was significant for both total and ambulatory counts. When conducting within-session analyses, mean cumulative total activity counts and ambulatory activity counts were statistically significantly decreased in females at the mid-and high-dose over intervals 0-10 and 11-20 minutes and in low-dose females at 11-20 minutes. Similar to the males, mean cumulative total activity counts in female rats were not affected by treatment, while the mean cumulative ambulatory activity counts were statistically significant decreased at the mid- and high-dose (432 and 521 counts, respectively, vs. 828 counts for controls) but not in the low dose.

   The cumulative mean values for total or ambulatory motor activity in males or female rats were not affected at the pre-test evaluation or on Days 7 or 14. No significant shifts in the pattern of habituation occurred in male or female dose groups on Days 7 and 14.

   Overall, within-session total and ambulatory counts were significantly different from controls for at least one time interval in all dose groups; however, the number of sessions affected and magnitude of the effect was greater at mid and high doses compared to the low dose. Furthermore, cumulative ambulatory counts for the entire observation period were significantly depressed at the mid dose and above.

TABLE 11. Motor activity (cumulative total activity counts for session) [a,b,c]

Test day	Dose level (mg/kg bw)			
	Control (0)	Low (125)	Mid (650)	High (2000)
Males				
Pre-test	2105 ± 597.3	2472 ± 909.5	2690 ± 1153.9	2407 ± 549.7
Day 0 - mean	2216 ± 811.1	1994 ± 708.5	1961 ± 738.6	2071 ± 555.8
Interval (min)				
0-10	1069 ± 202.3	959 ± 291.2	**823* ± 239.7**	**775* ± 135.8**
11-20	580 ± 332.5	436 ± 274.3	**270* ± 249.1**	**218* ± 175.8**
21-30	328 ± 369.3	152 ± 225.9	207 ± 266.7	222 ± 194.3
31-40	120 ± 105.3	120 ± 132.8	164 ± 166.7	**300* ± 218.7**
41-50	51 ± 50.4	207 ± 258.1	**275* ± 233.2**	**264* ± 227.4**
51-60	69 ± 87.5	121 ± 128.2	222 ± 262.1	**291* ± 291.0**
Day 7	2575 ± 588.7	3084 ± 998.5	2589 ± 523.0	2791 ± 1154.0
Day 14	2496 ± 711.1	2905 ± 464.7	2369 ± 729.0	2536 ± 286.5
Females				
Pre-test	2240 ± 478.2	2904 ± 695.7	2028 ± 427.7	2764 ± 955.2
Day 0- mean	2856 ± 847.9	2661 ± 975.8	2120 ± 724.3	2675 ± 837.7
Interval (min)				
0-10	1496 ± 170.9	1376 ± 339.8	**1005* ± 293.6**	**1201* ± 293.1**
11-20	761 ± 342.7	**504* ± 330.8**	**293* ± 260.9**	**309* ± 230.2**
21-30	284 ± 350.3	248 ± 298.0	168 ± 152.5	240 ± 272.4
31-40	119 ± 221.4	151 ± 221.7	220 ± 373.3	314 ± 292.2
41-50	45 ± 29.6	143 ± 227.4	215 ± 282.5	281 ± 256.9
51-60	152 ± 360.3	239 ± 329.2	220 ± 245.9	331 ± 295.2
Day 7	3774 ± 979.2	3518 ± 916.6	3053 ± 1071.2	3507 ± 1089.7
Day 14	3467 ± 1130.3	3059 ± 1083.0	2596 ± 485.3	3466 ± 897.3

[a] Data were extracted from MRID 48939603, pp. pp. 169-200
[b] Values represent mean ± SD
[c] n= 10
*Statistically different from controls; $p<0.05$

TABLE 12. Motor activity (cumulative ambulatory activity counts for session) [a,b,c]

Test day	Dose level (mg/kg bw)			
	Control (0)	Low (125)	Mid (650)	High (2000)
Males				
Pre-test	489 ± 175.9	584 ± 329.5	631 ± 344.8	537 ± 213.4
Day 0- mean	500 ± 268.8	365 ± 178.3	**281* ± 117.0**	**325* ± 127.8**
Interval (min)				
0-10	312 ± 96.6	**235* ± 98.7**	**184* ± 65.6**	**162* ± 55.5**
11-20	112 ± 111.3	57 ± 71.0	**20* ± 24.3**	**29* ± 31.0**
21-30	65 ± 97.1	21 ± 36.2	20 ± 37.0	19 ± 20.2
31-40	10 ± 21.9	16 ± 35.9	8 ± 11.9	35 ± 35.2
41-50	1 ± 2.8	27 ± 43.6	28 ± 32.3	**38* ± 36.5**
51-60	0 ± 0.3	10 ± 20.7	22 ± 36.8	**42* ± 57.6**
Day 7	566 ± 173.3	657 ± 245.2	568 ± 139.0	631 ± 391.0
Day 14	544 ± 206.4	630 ± 155.3	514 ± 148.9	574 ± 324.6
Females				
Pre-test	596 ± 125.9	853 ± 303.2	535 ± 114.8	753 ± 327.1
Day 0- mean	828 ± 265.1	638 ± 332.8	**432* ± 183.4**	**521* ± 212.4**
Interval (min)				
0-10	501 ± 71.7	417 ± 159.4	**253* ± 82.6**	**341* ± 121.9**
11-20	218 ± 117.8	**109* ± 90.4**	**44* ± 46.2**	**44* ± 47.3**
21-30	51 ± 113.4	42 ± 84.8	19 ± 27.1	21 ± 28.9
31-40	16 ± 46.0	10 ± 18.1	50 ± 104.2	31 ± 34.0
41-50	0 ± 0.4	16 ± 43.1	42 ± 60.7	46 ± 63.8
51-60	41 ± 130.0	45 ± 124.0	25 ± 50.8	38 ± 29.2
Day 7	1103 ± 348.9	1002 ± 369.4	845 ± 325.4	918 ± 303.0
Day 14	922 ± 320.4	846 ± 389.3	702 ± 163.5	913 ± 270.3

[a] Data were extracted from MRID 48939603, pp. pp. 169-200
[b] Values represent mean ± SD
[c] n= 10
*Statistically different from controls; $p<0.05$

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Gross necropsy findings were limited to one control male with a dark red discoloration of the brain.

2. **Brain weight, length, and width:** No statistically significant, treatment-related changes were observed in brain weight, length, or width in male or female rats treated with fluridone.

3. **Neuropathology:** No treatment-related changes were observed during microscopic examination. Findings in males were limited to a control male with minimal congestion of the cerebral cortex and one high-dose male with minimal congestion of the cerebellum and olfactory bulbs. In females, changes were limited to one control animal with minimal degeneration of the gastrocnemius muscle, and one high-dose female with mild congestion of the olfactory bulbs, another female with mild angiectasis of the cerebellum, cerebral cortex, and cervical and lumbar spinal cord.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Based on the results of the FOB evaluations at the time of peak effect (approximately 4 hours following dose administration) on study day 0, CNS activity, neuromuscular, sensorimotor, and/or physiological functional domains were affected at dose levels of 650 and 2000 mg/kg bw. FOB findings included repetitive movement of mouth and jaws, altered gait, biting of the cage, abnormal air righting reflex, bizarre/stereotypic behavior, no hindlimb extension, lack of response to various stimuli, longer catalepsy times, and/or lower mean body temperatures for males and females in the 650 and 2000 mg/kg bw groups. In addition, decreased total and ambulatory locomotor activity were noted for males and females in the 650 and 2000 mg/kg bw groups during the first 20-30 minutes of testing and when the overall session (ambulatory only) was evaluated at the time of peak effect on study day 0. No test substance-related effects were noted for males and females at a dose level of 125 mg/kg bw. Therefore, the NOAEL for male and female neurotoxicity was considered to be 125 mg/kg bw.

B. REVIEWER CONCLUSION'S:

Treatment-related changes in FOB parameters were observed in males and females at all doses. These changes were transient, occurring only at the time of peak effect (4 hours after dosing on Day 0) and resembling pre-test observations on subsequent days. Animals from the low and mid dose groups exhibited similar incidence of treatment-related effects on minor FOB sensory and open field parameters. At the high dose, similar FOB signs were noted at a higher frequency and additional effects not observed at lower doses were seen. Disruption of physiological (increased catalepsy time, decreased body temperature) and locomotor (reduced cumulative ambulatory counts) function only occurred at the mid and high doses. The changes in FOB parameters in combination with a significant depression of cumulative

ambulatory activity counts was considered evidence of an adverse response at the mid and high doses. In the absence of a corroborative effect on motor activity, the FOB effects at the low dose were not considered adverse because the effects were limited to minor behavior aberrations not anticipated to impact quality of life and were of low incidence.

The LOAEL is 650 mg/kg bw based on decreased ambulatory counts in combination with the prevalence of FOB anomalies in males and females. The NOAEL is 125 mg/kg bw.

C. STUDY DEFICIENCIES:

No relevant study deficiencies were noted.