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

JUL 20 1999

Office of Prevention, Pesticides
and
Toxic Substances

MEMORANDUM

SUBJECT: **TERBUFOS**: Subchronic Neurotoxicity Study

FROM: Linda L. Taylor, Ph.D. 7/19/99
Reregistration Branch I,
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D. 7/19/99
Branch Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

TO: Pamela Noyes
Special Review and Reregistration Division (7508C)

Registrant: American Cyanamid Company
Chemical: S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl phosphorodithioate [IUPAC}; S-(*t*T-butylthio)methyl O,O-diethyl-phosphorodithioate; S-tert-butylmercaptomethyl O,O-diethyl dithiophosphate
Synonym: Terbufos; Counter®; Contraven; AC 92100
Caswell No.: 131A
P.C. Code: 105001
DP Barcode: D256766
Submission: S563401

COMMENT: The subchronic neurotoxicity study in rats [MRID 44842302] has been reviewed, and the Data Evaluation Record [DER] is appended. Although there was a separate report on the 21-day range-finding study [MRID 44842301] performed to determine the dietary concentrations for the definitive study, a separate DER was not generated.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 44842302), Terbufos [AC 92100] (89.7% a.i.) was administered in the diet to 20 Crl:CD (SD)IGS BR rats/sex/dose at

dose levels of **0 ppm, 0.5 ppm, 0.8 ppm, or 5.0 ppm [males]/3.0 ppm [females]** for at least 85 days [10 rats/sex/cholinesterase group] or 13 weeks [10 rats/sex/neurobehavioral group]. These dose levels corresponded to **0.036, 0.059, and 0.369 mg/kg/day, respectively, in males; 0.042, 0.064, and 0.251 mg/kg/day, respectively, in females.**

No adverse or treatment-related effects were observed on mortality or clinical signs. The high-dose males displayed slightly decreased body weights [94%-97% of control] throughout the study compared to the controls, but the high-dose females displayed body weights that were comparable to or greater than the controls throughout the study. Body-weight gains were decreased [86%-93% of control] in the high-dose males throughout the study, although the magnitude of the deficit diminished with time. There was no adverse effect on body-weight gain in the females. Food consumption was comparable to/greater than the control in the treated groups [both sexes].

There were no treatment-related effects observed on ophthalmoscopy, and motor activity was comparable among the groups for both sexes throughout the study. There were no significant and treatment-related differences relative to the controls in any of the parameters monitored in the functional observational battery in either sex.

There was a dose-related inhibition of plasma cholinesterase activity [males ≈70%/females ≈90% inhibition] and RBC acetylcholinesterase activity [≈100% inhibition] throughout the study in both sexes. At study termination, brain cholinesterase activity [males 55%-58%/females 68%-71% inhibition] was decreased at the high-dose level in both sexes, with the magnitude of the inhibition greater in the females than in the males.

No treatment-related macroscopic and microscopic lesions were observed in either sex. Brain weight data were not provided.

The NOAEL for systemic toxicity is 0.8 ppm [0.059 mg/kg/day], and the LOAEL for systemic toxicity is 5 ppm [0.369 mg/kg/day], based on decreased body weight and body-weight gains in males. No neurobehavioral or neuropathological effects were observed in either sex.

The NOAEL for inhibition of plasma cholinesterase and RBC acetylcholinesterase activity [both sexes] is 0.5 ppm [0.036 (males)/0.042 (females) mg/kg/day] and the LOAEL for inhibition of plasma cholinesterase and RBC acetylcholinesterase activity [both sexes] is 0.8 ppm [0.059 (males)/0.064 (females) mg/kg/day].

The NOAEL for brain cholinesterase activity is 0.8 ppm [0.059 (males)/0.064 (females) mg/kg/day] and the LOAEL for brain cholinesterase activity is 5 (males)/3 (females) ppm [0.369 (males)/0.251 (females) mg/kg/day].

This guideline subchronic neurotoxicity study is **Acceptable [OPPTS 870.6200; §82-7],** and it satisfies the guideline requirement for a subchronic neurotoxicity study in rats.

EPA Reviewer: Linda L. Taylor, Ph.D. 7/14/99
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. 7/14/99
Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)

013572

DATA EVALUATION RECORD

STUDY TYPE: Subchronic neurotoxicity - rat OPPTS 870.6200 [§82-7]

DP BARCODE: D256766 SUBMISSION CODE: S563401

P.C. CODE: 105001 TOX. CHEM. NO.: 131A

TEST MATERIAL (PURITY): Terbufos; AC 92100 [89.7% a.i.] ACTION: Organophosphate insecticide

CHEMICAL: S-[[(1,1-dimethylethyl)thio]methyl]O,O-diethyl phosphorodithioate [IUPAC}; S-(*t*T-butylthio)methyl O,O-diethyl-phosphorodithioate; S-tert-butylmercaptomethyl O,O-diethyl dithiophosphate; Terbufos

SYNONYMS: Counter®; Contraven; AC 92100 CAS Registry No: 1113071-79-9

CITATION: Mandella, R. (1999). 13-Week Dietary Neurotoxicity Study with AC 93100 in the Rat. Huntingdon Life Sciences, New Jersey. Study No. 98-4521 [Protocol No. 971-98-102]. May 26, 1999. MRID 44842302. Unpublished.

SPONSOR: American Cyanamid Company

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No adverse or treatment-related effects were observed on mortality or clinical signs. The high-dose males displayed slightly decreased body weights [94%-97% of control] throughout the study compared to the controls, but the high-dose females displayed body weights that were comparable to or greater than the controls throughout the study. Body-weight gains were decreased [86%-93% of control] in the high-dose males throughout the study, although the magnitude of the deficit diminished with time. There was no adverse effect on body-weight gain in the females. Food consumption was comparable to/greater than the control in the treated groups [both sexes].

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The NOAEL for brain cholinesterase activity is 0.8 ppm [0.059 (males)/0.064 (females) mg/kg/day] and the LOAEL for brain cholinesterase activity is 5 (males)/3 (females) ppm [0.369 (males)/0.251 (females) mg/kg/day].

This guideline subchronic neurotoxicity study is **Acceptable [OPPTS 870.6200; §82-7]**, and it satisfies the guideline requirement for a subchronic neurotoxicity study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Terbufos [AC 92100]
Description: colorless to pale yellow liquid with a mercaptan-like odor
Lot #: AC 9429-26
Purity: 89.7% a.i. [concentrations adjusted to correct for purity]
Stability of compound:
CAS #: 13071-79-9
Chemical Formula: $C_9H_{21}O_2PS_3$

2. Vehicle: *Acetone and -60 mesh Grit-O'Cobs*

3. Test animals: Species: rat
Strain: albino rats (outbred) VAF/Plus® CD® (Sprague-Dawley derived) [Crl:CD®(SD)IGS BR]
Age and weight at initiation of dosing: ≈43 days old; males 181 [144-207] grams; females 153 [128-178] grams
Source: Charles River Laboratories, Kingston, NY
Housing: individually
Diet: Certified Rodent Diet, No. 5002 [meal] PMI Nutrition International, MO *ad libitum*
Water: Tap water ad libitum
Environmental conditions: **standard**
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates: - Start: October 27, 1998; end: January 29, 1999

2. Animal assignment: In order to equalize group means between neurobehavioral and cholinesterase subgroups, the rats were distributed into 8 groups of 10 rats/sex by a computerized random sort program so that body weight means for each group were comparable. Groups were then combined so that each study group contained 20 rats/sex.

TABLE 1. STUDY DESIGN FOR 13-WEEK NEUROTOXICITY STUDY IN RATS

Test Group Conc. in Diet (ppm) M/F	Number of Rats Assigned [#/sex]				
	initial	neurobehavioral evaluation♪	blood cholinesterase✂ [wks 4 & 8]	blood/brain cholinesterase [wk 13]✖	neuropathology
Control (0/0)	20	10	10	15	5
Low (0.5/0.5)	20	10	10	15	5
Mid (0.8/0.8)	20	10	10	15	5
High (5.0/3.0)	20	10	10	15	5

♪ motor activity and FOB assessments performed pretest, and at weeks 4, 8, & 13 and 5/sex of these were sacrificed by perfusion and nervous tissues were processed for neuropathological evaluations; ✖ includes the 10/sex examined at weeks 4 & 8 plus 5/sex from the neurobehavioral group not used for neuropathology; ✂ also examined at week 13

3. Dose Selection: No details were provided in the report. However, there is a 21-day range-finding study [MRID 44842301; Study No. 98-2582], which was performed to determine dietary concentrations to be used in this study. The dose levels were 1.0 ppm [0.11mg/kg/day], 5.0 ppm [0.55 mg/kg/day], and 6.0 ppm [0.67 mg/kg/day] for the males and 0.5 ppm [0.06 mg/kg/day], 3.0 ppm [0.33 mg/kg/day], and 4.0 ppm [0.43 mg/kg/day] for the females. The NOAEL for significant decreases in plasma, erythrocyte, and brain cholinesterase was 1.0 ppm for males and 0.5 ppm for females. At 5 ppm, the percent inhibition in males was 72.5% [plasma], 98.4% [erythrocyte], and 63.5% [brain]. At 3 ppm, the percent inhibition in females was 84.1% [plasma], 98.6% [erythrocyte], and 67.9% [brain]. At the highest dose tested [HDT; 6.0 ppm], the percent inhibition in males was 85.2% [plasma], 99.3% [erythrocyte], and 81.1% [brain]. At the HDT [4.0 ppm], the percent inhibition in females was 92.1% [plasma], 100% [erythrocyte], and 83.4% [brain]. In the males, body weight and body-weight gains were comparable to controls at the 1 ppm and 5 ppm dose levels during the study but were significantly decreased from control at 6 ppm [body weight 88%-90% of control; body-weight gain 60%-77% of control]. In the females, body weights were only slightly lower [92%-95% of control] than control throughout the study at the high-dose level. Body-weight gains were decreased significantly at the 4 ppm at weeks 1 [56% of control] and 2 [68% of control] and nonsignificantly at weeks 3 and 4 [74% of control] compared to the control. At the 3 ppm dose level in females, body-weight gain was decreased [89% of control] compared to the control only during the first week [Table 2].

Table 2. Range-Finding Study - Body Weight/Body-Weight Gain [grams] and Cholinesterase [IU/mL]

Sex/Dose /Time	0 ppm	1 ppm [♂]/0.5 ppm [♀]	5 ppm [♂]/3 ppm [♀]	6 ppm [♂]/4 ppm [♀]
body weight MALES				
week 0	170.0±10.3	169.6±10.5	169.4±8.8	168.6±8.4
week 1	223.0±11.1	219.4±17.0	217.2±7.7	200.2*±6.6 [90]♪
week 2	277.6±17.2	274.6±25.3	270.8±6.7	244.0*±18.1 [88]
week 3	317.6±20.1	319.8±23.9	314.0±8.4	281.6*±25.0 [89]
week 4	331.2±21.9	330.8±23.6	323.8±7.6	289.6*±27.1 [87]
body weight FEMALES				
week 0	146.8±6.8	146.2±8.7	147.6±8.6	148.4±7.3
week 1	170.4±5.5	171.0±14.6	168.6±13.0	161.6±7.0 [95]
week 2	194.2±9.8	193.0±20.3	192.8±13.8	180.8±11.3 [93]
week 3	209.4±8.1	213.0±23.5	209.2±18.3	194.6±12.5 [93]
week 4	216.0±9.7	217.6±22.2	216.6±19.9	199.6±12.5 [92]
body-weight gain MALES				
weeks 0-1	53.0±1.9	49.8±8.5	47.8±4.7 [90]	31.6**±6.5 [60]
weeks 0-2	107.6±8.5	105.0±17.4	101.4±7.0 [94]	75.4**±11.5 [70]
weeks 0-3	147.6±12.2	150.2±16.5	144.6±11.5 [98]	113.9**±17.3 [77]
weeks 0-4	161.2±12.9	161.2±16.5	154.4±11.3 [96]	121.0**±19.4 [75]
body-weight gain FEMALES				
weeks 0-1	23.6±5.6	24.8±6.1	21.0±5.3 [89]	13.2*±1.8 [56]
weeks 0-2	47.4±8.7	46.8±12.0	45.2±5.4 [95]	32.4*±5.0 [68]
weeks 0-3	62.6±8.7	66.8±15.5	61.6±10.6 [98]	46.2±6.5 [74]
weeks 0-4	69.2±10.3	71.4±14.3	69.0±11.8	51.2±6.5 [74]
cholinesterase [IU/mL] MALES				
plasma (% I)	0.494±0.077	0.463±0.102	0.136**±0.023 [72.5]	0.073**±0.024 [85.2]
RBC (% I)	1.130±0.149	0.730±0.277	0.018**±0.021 [98.4]	0.008**±0.017 [99.3]
brain (% I)	18.607±0.184	17.444±1.019	6.797**±1.062 [63.5]	3.513**±0.686 [81.1]
cholinesterase [IU/mL] FEMALES				
plasma (% I)	1.376±0.148	1.335±0.494	0.219±0.042 [84.1]	0.109±0.016** [92.1]
RBC (% I)	1.085±0.139	1.143±0.229	0.015*±0.027 [98.6]	0.000±0.000 [100]✖
brain (% I)	18.983±0.259	18.716±0.689	6.087**±0.854 [67.9]	3.160**±0.337 [83.4]

♪ [% of control]; * $p<0.05$; ** $p<0.01$; ✖ all 5 females in the 4 ppm group showed complete inhibition
data from Tables 2, 3, 7, and 8 [pages 39, 40, 42, 43, 54, 55, 57, 58] of the report [MRID 44842301]

4. Diet preparation and analysis: Diet was prepared weekly. Appropriate amounts of test material were dissolved in acetone, then mixed with -60 mesh Grit-O'Cobs® and mixed into standard laboratory diet. Each dose level was prepared separately. Control rats received standard laboratory diet mixed with acetone and -60 mesh Grit-O'Cobs® at the same levels used to prepare the test diets. Prepared diets were stored at room temperature.

Homogeneity Analysis: Results of analyses of preliminary mixes confirm that the mixing procedures produced homogeneous diets.

Stability Analysis: It was stated that results from another study [21-day range-finding study, Study No. 98-2582, MRID44842301] showed that the test material was stable in the diet at room temperature and when stored refrigerated or frozen for at least 14 days.

Concentration Analysis: The diets were found to contain the intended concentrations.

5. Statistics: Mean values for all groups were compared to mean for the control group at each time interval, with the following exceptions for Methods 1 and 2. Dose groups were eliminated from statistical analysis if their standard deviations was zero. If a dose group was eliminated from statistical analysis, the remaining groups were analyzed using the procedure outlined in Method 1 or Method 2.

METHOD 1: Parameters: weekly body weight [BW]; body-weight change from initial body weight; food consumption [FC]. Method of Analyses: BW and FC data were analyzed initially by Bartlett's test for equal variance. If results were significant at <0.01 level, the data were transformed by Blom's Normalized Rank Test before continuing with any further analysis. The data were then analyzed by a standard two-way ANOVA. The residuals were tested for normality using the Shapiro-Wilk test. If the residual data were normally distributed and ANOVA analysis was significant at 0.5 level, the data were analyzed using Dunnett's test followed by analysis using standard tests for linear regression and lack of fit. If the results of ANOVA were not significant, the data were analyzed using standard tests for linear regression and lack of fit. If the residual data were not normally distributed at the 0.01 level, the data were transformed using Blom's Normalized Rank Test and reanalyzed using ANOVA and regression analysis. If the residuals of the transformed, reanalyzed data were normal, the data were analyzed using Dunnett's test followed by analysis for linear regression and lack of fit. If the residuals of the transformed, reanalyzed data were not normal, the results were noted as suspect and the data were analyzed using Dunnett's test followed by analysis for linear regression and lack of fit.

METHOD 2: Parameters: blood/brain cholinesterase data, FOB BW, forelimb/hindlimb grip strength measurements, landing foot splay measurements. Method of Analyses: Statistical evaluation of equality of means was made by appropriate technique, followed by a multiple comparison procedure, if needed. First, Bartlett's test was performed to determine if groups had equal variances. If equal, parametric procedures were used; if not, nonparametric procedures were used. Parametric : standard one-way ANOVA using the F distribution to assess significance. If significant differences among means were indicated, Dunnett's test was used to determine which means were significantly different from control. If nonparametric procedure was needed, the Kruskal-Wallis test was used, and if differences were indicated, Dunn's summed rank test was used to determine which treatments differed from control. A statistical test for trend in the dose levels was performed also. In the parametric case, standard regression techniques with a test for trend and lack of fit were used. In nonparametric case, Jonckheere's test for monotonic trend was used. Bartletts test was conducted at the 1%, two-sided risk level, and all other tests were conducted at the 5% and 1%, two-sided level.

METHOD 3: Parameters: motor activity counts. Method of Analysis: Motor activity counts were collected in 12 consecutive, 5-minute intervals on each test day. The set of counts over these 12 intervals gave a "profile" of the rat's motor activity. These profiles were then compared within each study period using a multivariate model that assessed whether the profiles for different groups had the same shape [the *parallel* hypothesis]. If the data passed on the parallel hypothesis, active-treatment groups were compared with control with respect to total motor activity [the *level* hypothesis]. The Wilks' Lambda, a variant of the Hotelling T^2, was used to test the parallel hypothesis; two-way ANOVA with interaction was used

to test for the overall treatment effect with respect to the level hypothesis, followed, if necessary, by Dunnett's t-test to find groups that differed from control. The Shapiro-Wilk test was used to assess normality of the residuals from the multivariate model; when $p<0.01$ for this test, the Blom inverse normal transformation was applied to achieve normality of the residuals. Other tests were deemed significant at $p=0.05$ level.

C. METHODS

1. **Observations**

Each rat was observed twice daily in his/her cage for mortality, general appearance, and signs of severe toxic or pharmacologic effects. Physical examinations were performed twice pretest and weekly during the study. These consisted of removal from the cage and observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, as well as evaluations of respiration and palpation for tissue masses.

2. **Body weight**

Each rat was weighed twice pretest, weekly during dosing, and at study termination [just prior to necropsy].

3. **Food consumption and compound intake**

Food consumption was determined weekly, beginning one week prior to study initiation. Each rat was provided with a full feeder every 6 days. After 6 days, the feeder was weighed and the amount of feed consumed was calculated. Compound intake (mg/kg/day) values were calculated weekly from the food consumption data and dietary inclusion levels of test material.

4. **Ophthalmoscopic examination**

Ophthalmoscopic examinations were performed pre-test on all rats, and the eyes of all neurobehavioral rats were examined at study termination. Lids, lacrimal apparatus and conjunctiva were examined visually. The cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disc were examined by indirect ophthalmoscopy. Mydriacyl 1% was used to induce mydriasis.

5. **Neurobehavioral studies**

Testing was staggered over four sessions with approximately equal numbers of rats per sex per group in each session. Temperature, humidity, noise level, and illumination of the testing rooms were monitored.

Functional Observational Battery: All rats were subjected to the Functional Observational Battery [FOB], with observations being made pre-test and during weeks 4, 8, and 13 of dosing. Testing was performed "blind"; i.e., observer did not know the identity of the group. The time of testing was counter-balanced across treatment groups. The following evaluations were performed.

1. Home cage observations: posture, vocalization, and palpebral (eyelid) closure.
2. Observations during handling: reactivity to general stimuli [removal/handling]; assessment of signs of autonomic function [lacrimation, salivation, altered fur appearance, red/crusty deposits around eyes.
3. Observations in the open field: arousal level, locomotion and gait, count of urination and defecation, tremors/convulsions, abnormal movements or behaviors, excessive or repetitive actions, piloerection and exophthalmos.
4. Reflex assessments: response to visual (approach response) and auditory (finger snap) stimuli, response to tail pinch, pupillary function.
5. Grip strength: grip strength [hind- and forelimb] was measured using a Grip Strength Meter [two measurements/interval]
6. Landing foot splay: A small amount of paint was applied to the hindpaws and the rat was dropped onto a flat surface. The distance between the marks of paint was measured [two measurements/interval].
7. Air righting ability: Each rat was held upside down and dropped [onto bedding material] from a height of two feet, and the landing position of each rat was recorded.
8. Body weight: Body weights were recorded.

Locomotor Activity: The motor activity of each rat was monitored using a modified version of Schulze's procedures [1990] and an automated Photobeam Activity System. Sessions were 60 minutes in length, twelve 5-minute intervals. The time of testing was balanced across treatment groups.

6. **Cholinesterase determinations**: Blood samples were collected *via* venipuncture of the orbital sinus from anesthetized [CO_2/O_2] rats at weeks 4, 8, 13/14. At study termination, the brain was dissected and divided into left and right halves. The right halves were assayed for acetylcholinesterase activity and the left halves were stored as backup samples. Cholinesterase assays were performed on a Hitachi 717 chemistry analyzer using a modified Ellman assay.

7. **Sacrifice and Pathology**: At the scheduled sacrifice, rats not designated for neuropathology were anesthetized with carbon dioxide and exsanguinated. A complete necropsy was performed on all rats immediately after sacrifice and included examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal, and pelvic cavities and neck; and the remainder of the carcass for the presence of macroscopic morphologic abnormalities. The brain was excised from the skull; and whole brain weight was recorded for each rat.

Histopathology: The tissues listed below were preserved and examined for 5 neuropathology rats/sex in the control and high-dose groups.

Brain - forebrain/cerebral cortex/hippocampus/basal ganglia/midbrain/cerebellum and pons/medulla
Spinal cord - cross/longitudinal sections [cervical, thoracic, lumbar]
Eyes with optic nerve
Nerves - sciatic, tibial, sural [cross and longitudinal sections]
Trigeminal ganglia
Dorsal root ganglion [from C_3-C_6 and L_1-L_4]
Dorsal and ventral root fibers [from C_3-C_6 and L_1-L_4]
Tissues w/ microscopic findings including tissue masses

RESULTS

A. Observations

1. Survival: There were no treatment-related deaths in either sex.
2. Clinical signs: There were no signs of toxicity observed in either sex at any dose level.
3. Ophthalmoscopic examinations: No treatment-related ocular abnormalities were observed in either sex.

4. Body weight and body-weight gains: Body weight: Males at the high-dose level displayed slightly lower body weights [94%-97% of control] throughout the study [Table 3], but the females at the high-dose level displayed body weights that were comparable to the control initially and greater than the control from week 4 on. Body-weight gains were decreased slightly [86%-94% of control] at the high-dose level in males throughout the study although the magnitude of the decrease diminished with time. The high-dose females displayed greater body-weight gains than the control throughout the study.

Table 3. Body Weight/Body-weight Gain [grams]

Sex/Group/Time	0 ppm	0.5 ppm	0.8 ppm	5 ppm [males]/3 ppm [females]
body weight MALES				
week 0	180.2±11.7	181.5±10.5	183.1±14.1	180.3±9.4
week 1	235.0±15.7	239.3±15.8	240.6±18.6	227.5±11.5 [97]♪
week 2	284.7±24.7	287.4±20.7	291.7±24.2	273.0±16.4
week 3	325.3±33.3	328.7±26.5	335.0±30.3	310.6±21.2 [96]
week 10	472.3±64.7	486.5±54.3	490.7±62.2	462.1±41.8
week 13	501.1±72.9	527.8±66.8	528.2±40.9	472.3±50.4 [94]
body weight FEMALES				
week 0	152.1±10.3	152.5±8.8	154.0±10.8	152.6±11.3
week 1	178.4±11.9	178.4±10.6	181.4±12.9	177.5±15.3
week 2	204.8±15.5	202.0±11.8	209.0±14.3	204.5±18.9
week 4	239.7±19.5	230.7±14.6	243.3±18.9	241.9±23.9
week 13	290.2±30.4	276.8±21.2	297.3±37.1	309.4±30.7
body-weight gain MALES				
weeks 0-1	54.8±7.4	57.9±7.3	57.5±6.6	47.2**±7.9 [86]
weeks 0-2	104.5±17.0	106.0±13.7	108.6±13.6	92.7*±12.4 [89]
weeks 0-3	145.1±27.2	147.3±19.9	151.9±21.0	130.3±18.0 [90]
weeks 0-6	225.5±45.1	233.8±33.5	237.3±39.7	210.5±30.3 [93]
weeks 0-13	**321.1±66.1**	**347.5±61.7**	**345.9±44.8**	**293.7±50.1 [92]**
weeks 1-2✔	49.7	48.1	51.1	45.5 [92]
weeks 2-3✔	40.6	41.3	43.3	37.6 [93]
body-weight gain FEMALES				
weeks 0-1	26.3±4.2	25.9±4.0	27.4±4.0	24.9±5.6
weeks 0-2	52.7±8.0	49.5±6.1	55.1±6.0	51.9±9.2
weeks 0-3	71.8±11.1	65.2±7.2	72.1±9.8	71.0±11.0
weeks 0-6	108.3±15.8	95.3*±11.1	106.8±16.1	113.3±16.6
weeks 0-13	**137.4±20.9**	125.7±14.2	**142.9±26.3**	**157.3±24.2 [115]**

✔ calculated by reviewer using data from Table 3 (from means); ♪ [% of control]; * p<0.05; ** p<0.01
Data from Tables 3 and 4 [pages 77-89] of the report

5. Food/water consumption and compound intake

a. Food consumption -: There were no adverse effects on food consumption in either sex. Significant increases in food consumption were observed in the mid- and high-dose males at week 2 [not dose-related] and in the high-dose females at weeks 1, 2, and 3 [Table 4].

Table 4. Food Consumption

Week/Dose/Sex	0 ppm	0.5 ppm	0.8 ppm	5 [males]/3 [females] ppm
MALES [grams]				
week 0	146.0±7.3	146.8±7.5	147.4±9.1	145.9±8.9
week 1	119.9±5.8	119.9±4.8	122.0±4.8	123.8±6.2
week 2	102.4±7.1	102.4±4.7	108.0*±8.1	106.3*±4.1
week 5	78.3±4.5	77.0±4.6	77.2±4.9	74.8±3.6
week 13	52.3±4.2	50.3±2.9	50.5±4.3	52.6±3.5
FEMALES [grams]				
week 0	136.1±9.8	137.6±7.4	138.7±8.8	137.9±7.8
week 1	115.1±5.0	119.8*±5.1	115.1±5.5	124.6**±5.2
week 2	100.8±5.0	104.7±4.6	101.8±4.9	107.0**±4.5
week 3	96.5±5.1	96.3±4.0	94.8±4.4	101.2±6.0
week 13	75.6±30.4	66.7±6.8	59.8±4.6 [79]♪	60.3±4.0 [80]

Data from pages Table 6 [pages 98-103] of the report; ♪ [% of control]; * p<0.05; ** p<0.01

b. Compound consumption - Because the test material was administered at a constant concentration throughout the study, the amount of test material ingested on a mg/kg/day basis decreased with time on study [Table 5].

Table 5. Compound Intake [mg/kg/day]

Week/Sex	0.5 ppm	0.8 ppm	5/3 ppm
MALES			
1	0.060	0.098	0.619
2	0.051	0.086	0.532
3	0.045	0.074	0.451
6	0.035	0.058	0.344
13	0.025	0.040	0.263
mean intake overall	**0.036**	**0.059**	**0.369**
FEMALES			
1	0.060	0.092	0.374
2	0.052	0.081	0.321
3	0.048	0.076	0.304
6	0.042	0.064	0.247
13	0.033	0.048	0.181
mean intake overall	**0.042**	**0.064**	**0.251**

Data from page 38 and Table 7 [pages 105-110 of the report]

B. **Motor activity and FOB**

1. Motor activity - There were no apparent, treatment-related, changes in motor activity in either sex at any time point [Table 6]. At week 4 for the high-dose males and week 8 for the high-dose females, total

counts over the 60-minute session were 85% and 83% control counts, respectively. At 13 weeks, the males displayed a dose-related increase in total counts compared to the control male group. There was no apparent difference in the "settling down" effect over the course of the 60-minute observational period in either sex at any dose level.

Table 6. Motor Activity Values [total]

Dose/sex/interval	0 ppm	0.5 ppm	0.8 ppm	5/3 ppm
MALES				
pretest	425.8	429.4	436.7	424.1
week 4	732.8	776.9	795.7	626.0 [85]♪
week 8	854.6	959.5	884.3	846.9
week 13	564.8	614.3	712.6	743.9 [132]
FEMALES				
pretest	404.5	479.9	476.0	459.0
week 4	687.7	616.3	707.7	632.7
week 8	982.5	853.0	853.9	813.0 [83]
week 13	749.3	667.1	584.8	695.5

♪ [% of control]; Data from Table 8, pages 114-121 of the report

2. Functional observational battery: There were no statistically significant and treatment-related differences relative to the controls in forelimb or hindlimb grip strength or in landing foot splay in either sex [Tables 7-9], although both sexes at the high-dose level displayed a slight decrease in forelimb grip strength compared to the control values at some measurement intervals. All other evaluations with respect to reflexes, alertness, handling, open field assessments were comparable among the groups.

Table 7. FOB [forelimb grip strength and body weight - grams]

Dose Sex Parameter/Interval	MALES				FEMALES			
	0 ppm	0.5 ppm	0.8 ppm	5 ppm	0 ppm	0.5 ppm	0.8 ppm	3 ppm
pretest forelimb grip strength								
trial 1	469±63	442±82	494±72	479±98	465±63	486±68	489±48	478±78
trial 2	461±53	485±53	489±84	451±87	471±65	481±72	491±51	496±55
week 4 forelimb grip strength								
trial 1	696±130	700±135	706±104	653±138	686±123	680±153	734±147	652±127
trial 2	626±63	781±172*	742±129	608±159	610±161	618±82	703±120	590±87
week 8 forelimb grip strength								
trial 1	1110±200	936±158	1135±306	864±332	866±248	947±189	1032±291	976±124
trial 2	1103±264	872±221	1131±286	951±326	764±274	895±262	977±354	896±188
week 13 forelimb grip strength								
trial 1	1170±427	1211±226	1323±355	1083±215	1049±282	977±181	1117±347	872±319
trial 2	1229±398	1207±249	1360±362	1069±255	966±307	932±215	1101±458	859±273
body weight								
pretest	138±16	140±13	137±18	139±15	126±12	125±11	127±12	124±13
week 4	338±41	349±38	354±16	317±27	230±21	217±17	232±20	232±19
week 8	431±61	450±52	455±31	408±42	266±28	251±19	271±28	279±25
week 13	483±74	519±67	518±40	467±51	284±31	270±20	294±37	306±29

Values rounded by reviewer; * p<0.05; data from Table 9, pages 123-130 of the report

Table 8. FOB [hindlimb grip strength - grams]

Dose/Sex Parameter/Interval	MALES				FEMALES			
	0 ppm	0.5 ppm	0.8 ppm	5 ppm	0 ppm	0.5 ppm	0.8 ppm	3 ppm
pretest hindlimb grip strength								
trial 1	364±61	319±52	385±57	377±82	353±73	376±96	366±46	354±61
trial 2	326±56	307±34	364±70	312±80	309±66	365±66	353±51	343±69
week 4 hindlimb grip strength								
trial 1	717±130	682±153	770±165	667±131	575±82	626±100	669±102	601±128
trial 2	682±168	668±136	766±180	650±187	529±86	648*±96	658*±111	596±93
week 8 hindlimb grip strength								
trial 1	997±228	865±268	1218±125	1027±257	862±341	833±291	926±267	874±250
trial 2	887±227	873±261	1118±336	986±299	733±176	954±229	883±221	842±317
week 13 hindlimb grip strength								
trial 1	1056±250	1015±183	1126±270	959±399	831±214	726±169	831±241	789±267
trial 2	1056±285	920±190	1187±405	1036±322	702±179	798±166	927±245	774±188

Values rounded by reviewer; * p<0.05; data from Table 9, pages 123-130 of the report

Table 9. FOB [landing foot splay (cm)]

Dose Sex Parameter Interval	MALES				FEMALES			
	0 ppm	0.5 ppm	0.8 ppm	5 ppm	0 ppm	0.5 ppm	0.8 ppm	3 ppm
pretest landing foot splay								
trial 1	5.6±2.0	5.2±2.0	5.5±2.0	5.2±1.3	4.1±1.0	4.3±1.1	4.8±1.9	5.1±1.7
trial 2	5.9±1.7	5.1±1.6	5.8±1.7	5.1±0.9	4.0±1.4	4.2±1.6	5.6±1.2	5.2±1.5
week 4 landing foot splay								
trial 1	6.6±2.4	5.1±2.7	6.6±2.4	5.2±1.8	4.5±1.9	3.4±1.1	5.0±2.4	5.2±1.6
trial 2	6.8±2.3	5.4±2.2	6.0±1.9	5.9±1.8	4.4±1.5	3.8±0.9	5.5±2.2	5.5±1.7
week 8 landing foot splay								
trial 1	7.8±2.1	7.2±2.3	8.6±2.6	7.5±1.0	4.8±1.9	5.4±1.6	6.8±2.4	6.5±1.8
trial 2	7.2±1.9	6.6±2.4	7.8±2.5	7.1±1.7	5.0±2.1	5.2±0.9	7.0±2.4	6.2±1.6
week 13 landing foot splay								
trial 1	7.3±1.8	7.2±2.1	6.6±2.9	6.5±1.8	5.1±2.1	4.7±1.7	6.0±2.7	5.7±1.7
trial 2	7.2±2.1	6.4±2.0	6.5±2.7	5.9±2.0	5.2±1.6	4.9±1.3	6.2±1.9	6.0±1.3

data from Table 9, pages 123-130 of the report

C. **CHOLINESTERASE ASSESSMENT** - There was a dose-related inhibition of both plasma and RBC cholinesterase activities in both sexes throughout the study [Table 10]. **PLASMA**: The high-dose rats displayed significant decreases in plasma cholinesterase activity [males ≈70%; females ≈90%] throughout the study. Additionally, the mid-dose females [neuropathology rats] displayed decreased activity [40%] at study termination, although statistical significance was not attained. The inhibition in females was considered as a treatment-related effect because the inhibition was above the limit of variability in plasma cholinesterase determination [≈20%], and the RBC cholinesterase was significantly decreased at this dose level. **ERYTHROCYTES**: At week 4 [earliest time point measured], erythrocyte acetylcholinesterase inhibition at the high-dose level was 99.7% in males and 100% in females. At weeks 8 and 13, erythrocyte acetylcholinesterase inhibition remained nearly complete [males 97.2%-100%; females 99.9%-100%] in both sexes at the high-dose level. At the mid-dose level, males displayed a non-significant decrease [≈30% inhibition] at 4 weeks and a statistically significant decrease thereafter [35%-48% inhibition]. Mid-dose females did not displayed a statistically-significant inhibition until week 13 [≈33%].

Table 10. Cholinesterase [plasma and RBC]				
Dose/Sex/Week	0 ppm	0.5 ppm	0.8 ppm	5/3 ppm
MALES				
PLASMA				
4	0.472±0.059	0.492±0.073	0.463±0.075	0.125**±0.027 [73.5]√
8	0.464±0.052	0.489±0.093	0.474±0.066	0.135**±0.026 [70.9]
13	0.456±0.048	0.488±0.083	0.475±0.085	0.139**±0.420 [69.5]
13✖	0.514±0.074	0.532±0.065	0.475±0.064	0.142**±0.031 [72.4]
ERYTHROCYTE				
4	0.958±0.136	0.832±0.117	0.673±0.231 [29.7]	0.003**±0.008 [99.7]
8	1.355±0.415	0.968±0.211 [28.6]	0.699**±0.118 [48.4]	0.038**±0.047 [97.2]
13	1.252±0.199	1.104±0.191	0.789**±0.077 [37]	0.018**±0.037 [98.6]
13✖	1.135±0.263	1.115±0.204	0.738*±0.161 [35]	0.000±0.000 [100]
FEMALES				
PLASMA				
4	1.322±0.254	1.217±0.275	1.160±0.173 [12.3]	0.120**±0.039 [90.9]
8	1.807±0.454	1.753±0.456	1.496±0.136 [17.2]	0.150**±0.040 [91.7]
13	2.098±0.444	2.076±0.462	1.669±0.204 [20.4]	0.200**±0.037 [90.5]
13✖	2.830±0.730	2.535±0.642	1.686±0.406 [40.4]	0.236**±0.090 [91.7]
ERYTHROCYTE				
4	1.084±0.266	1.000±0.370	1.158±0.437	0.000±0.000 [100]
8	1.349±0.685	1.335±0.450	1.052±0.336 [22.0]	0.001**±0.004 [99.9]
13	1.309±0.286	1.063±0.161 [18.8]	0.878**±0.150 [32.9]	0.001**±0.004 [99.9]
13✖	1.130±0.170	1.210±0.583	0.825±0.249 [27.0]	0.000±0.000 [100]

n=10 except for ✖ neuropathology rats not used for neuropathology (n=5); √ [% inhibition]; * p<0.05; ** p<0.01
data from Table 10, pages 156-163 of the report

D. Sacrifice and Pathology

1. Brain cholinesterase - There was a marked decrease in brain cholinesterase activity in both sexes at the high-dose level at study termination [Table 11]. The females [3 ppm] displayed a greater inhibition [68%-71% *vs* 55%-58%] than the males [5 ppm].

Table 11. Brain Cholinesterase				
Dose/Sex	0 ppm	0.5 ppm	0.8 ppm	5/3 ppm
MALES				
cholinesterase rats	18.137±0.905	17.428±0.609	17.610±1.227	7.668**±1.449 [57.7]✱
neuropathology rats	17.540±1.035	16.937±1.371	17.700±0.738	7.893**±1.184 [55]
FEMALES				
cholinesterase rats	17.390±0.458	17.723±1.001	17.628±0.718	4.982**±0.557 [71.4]
neuropathology rats	17.403±1.255	16.833±0.601	17.680±0.449	5.560**±1.175 [68.1]

✱ [% inhibition]; ** p<0.01; data from Table 10, pages 160-163 of the report

3. Gross pathology - There were no treatment-related findings in either sex. No organ weight data were generated. The brain was not weighed.

4. Microscopic pathology: There were no treatment-related findings in either sex. 013572

III. DISCUSSION

A. In the subchronic neurotoxicity study in rats, no adverse or treatment-related effects were observed on mortality or clinical signs of toxicity. The high-dose males displayed slightly decreased body weights [94%-97% of control] throughout the study compared to the controls, but the high-dose females displayed body weights that were comparable to or greater than the controls throughout the study. Body-weight gains were decreased [86%-93% of control] in the high-dose males throughout the study, although the magnitude of the deficit diminished with time. There was no adverse effect on body-weight gain in the females. Food consumption was comparable to/greater than the control in the treated groups [both sexes].

There were no treatment-related effects on ophthalmoscopy, and motor activity was comparable among the groups for both sexes throughout the study. There were no significant and treatment-related differences relative to the control in any of the parameters monitored in the functional observational battery in either sex.

There was a dose-related inhibition of plasma cholinesterase activity and RBC acetylcholinesterase activity throughout the study in both sexes. At study termination, brain cholinesterase activity was decreased at the high-dose level in both sexes, with the magnitude of the inhibition greater in the females than in the males.

Organ-weight data [including the brain] were not provided. No treatment-related macroscopic and microscopic lesions were observed in either sex.

The NOAEL for systemic toxicity is 0.8 ppm [0.059 mg/kg/day], and the LOAEL for systemic toxicity is 5 ppm [0.369 mg/kg/day], based on decreased body weight and body-weight gains. NO neurobehavioral or neuropathological effects were observed in either sex.

The NOAEL for inhibition of plasma cholinesterase and RBC acetylcholinesterase activity [both sexes] is 0.5 ppm [0.036 (males)/0.042 (females) mg/kg/day] and the LOAEL inhibition of plasma cholinesterase and RBC acetylcholinesterase activity [both sexes] is 0.8 ppm [0.059 (males)/0.064 (females) mg/kg/day].

The NOAEL for brain cholinesterase activity is 0.8 ppm [0.059 (males)/0.064 (females) mg/kg/day] and the LOAEL for brain cholinesterase activity is 5 (males)/3 (females) ppm [0.369 (males)/0.251 (females) mg/kg/day].

B. Study deficiencies - With the exception of the lack of brain weignt data and pretest cholinesterase values, none that would adversely affect study interpretation.

This subchronic neurotoxicity study in the rat is **Acceptable/guideline** [OPPTS 870.6200; §82-7] and satisfies the guideline requirement for a subchronic neurotoxicity study in rats.