DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.6200 [§82-7a], Chronic Neurotoxicity Screening Battery in Rats

Work Assignment No. 4-01-128 F (MRID 46808228)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Chronic Neurotoxicity – Feeding Study in Rats; OPPTS 870.6200 [§82-7]; OECD None.

PC CODE: 129108 **DP BARCODE:** D331116
TXR#: 0054348

TEST MATERIAL (PURITY): XDE-570 (Florasulam; 99.3% a.i.; Lot # 940714)

SYNONYMS: XR-570, XRD-570, DE-570, N-(2,6-difluorophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-*c*)pyrimidine-2-sulfonamide

CITATION: Shankar, M.R. and K.A. Johnson (1996) XDE-570: Chronic neurotoxicity in Fischer 344 rats. The Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: DR-0312-6565-019N, September 25, 1996. MRID 46808228. Unpublished.

SPONSOR: Dow AgroSciences Canada, Inc., 2100- 450 1 St. SW, Calgary, AB, Canada

EXECUTIVE SUMMARY - In a chronic neurotoxicity study (MRID 46808228), XDE-570 (Florasulam; 99.3% a.i.; Lot # 940714) was administered to 10 young adult Fischer 344 rats/sex/dose in the diet at dose levels of 0, 10, 125 (females only), 250, or 500 (males only) mg/kg/day (time-weighted average test substance intake was 0, 8.6, 216, and 460 mg/kg/day in males and 0, 9, 113, and 266 mg/kg/day in females) for 12 months. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed in all rats at pre-dosing and at 3, 6, 9, and 12 month post-dosing. At study termination, auditory function (auditory brainstem response) was evaluated in 5 rats/sex/dose from the control and high-dose animals (500 mg/kg/day males and 250 mg/kg/day females). After completion of the auditory function examination, a neuropathological examination of perfusion-fixed central and peripheral nervous system tissues was conducted using these control and high-dose animals. All animals were subjected to a gross necropsy at termination. Positive control data were provided.

There were no compound-related effects on mortality, clinical signs, food consumption, FOB parameters, motor activity, and gross or neuropathology observed at any dose. Organ weights were not provided; however, in the concurrently performed 2-year dietary chronic toxicity/ oncogenicity study (MRID 46808236), brain weight was unaffected after 12 and 24 months of treatment.

At 500 mg/kg/day, body weights were decreased ($p<0.05$) by 9-15% in the males at 6, 9, and 12 months. Additionally, body weight gains were decreased by 61-67% at 3-12 months and overall (0-12 months) gains were decreased by 27% compared to controls. Food consumption was similar to controls in these animals.

No treatment-related effects were observed at 250 mg/kg/day and below in either sex.

No evidence of neurotoxicity was observed at any dose in either sex.

The systemic LOAEL is 500 mg/kg/day, based on decreased body weight (9-15%) and body weight gain in males (61-67%). The systemic NOAEL is 250 mg/kg/day.

The neurotoxicity LOAEL was not observed. The neurotoxicity NOAEL is 250 mg/kg/day, the highest dose tested in females.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.6200 for a chronic neurotoxicity feeding study in the rat.

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

NOTE: This DER summarizes EPA conclusions regarding effects observed in the chronic neurotoxicity study in rats. A detailed DER completed by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

COMMENTS: EPA concurs with the PMRA toxicology evaluation, no conclusions have been changed.

46808228.PMRA.der
.pdf

Reviewer: Tom Morris , Date June 1, 2000.

STUDY TYPE: Sub-chronic Neurotoxicity Study, OPPTS 870.6200 [feeding]-[rat]; (No OECD guideline).

TEST MATERIAL (PURITY): XDE-570 (Purity - 99.3%).

SYNONYMS: XR-570, XRD-570, DE-570, florasulam.

CITATION: Shankar, M.R. and Johnson, K.A. September 25, 1996. **XDE-570; Chronic Neurotoxicity in Fischer 344 Rats.** Performing Laboratory: The Toxicology Research Laboratories, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: DR-0312-6565-019N. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a sub-chronic neurotoxicity screening study XDE-570 (Purity - 99.3%) was administered to 10 young adult Fischer 344 rats/sex/dose in the diet (*ad libitum*) at dose levels of 0, 10, 125 (females only), 250 or 500 (males only) mg/kg bw/d (time-weighted average test substance intake was 0, 8.6, 216 and 460 mg/kg bw/d for males and 0, 9.0, 113 and 266 mg/kg bw/d for females) for approximately 12 months. Neurobehavioural assessment (functional observation battery [FOB] and motor activity testing) was performed in 10 animals/sex/group once prior to exposure and at 3, 6, 9 and 12 months post-dosing. At 12 months, auditory function (auditory brainstem response) was evaluated in 5 rats/sex/dose from the control and high-dose groups. After completion of the auditory function examination, a neuropathological examination of perfusion-fixed central and peripheral nervous tissues was conducted on these control and high-dose animals. All animals were subjected to a gross pathological examination following 12 months of treatment.

There were no treatment-related effects on mortality, clinical signs or food consumption. In the high-dose males, body weight and body-weight gain were lower compared to controls while food consumption was comparable to controls. FOB evaluations did not indicate any significant treatment-related findings in either sex, however, there was an increased incidence of urinary perineal soiling in males at 250 and 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d. This was consistent with clinical observations and findings at necropsy. The increased incidence may be associated with a possible tendency towards a slight increase in urination in the open field in these animals, however, the relationship, if any, is uncertain. The increased incidence of perineal soiling was considered to be a secondary or indirect effect related to lack of grooming possibly due to urinary acidification or to the presence of excretory products of the test substance in the urine. Motor activity, fore-limb and hind-limb grip strength, landing foot splay, rectal temperature and auditory brainstem response were not affected by treatment in either sex. No organ weights were provided in the study report. However, brain weight was unaffected by treatment after 12 and 24 months in the concurrent 2-year dietary oncogenicity study in Fischer 344 rats and there were no corroborating treatment-related gross pathological, histopathological or neuropathological findings to suggest a treatment-related effect on brain weight. There were no treatment-related gross pathological or histopathological findings and no treatment-related neuropathological findings in the central or peripheral nervous system. There was no evidence of neurotoxicity in either sex.

The LOAEL for systemic toxicity was 500 mg/kg bw/d based on lower body weight and body-weight gain (males) in the absence of any treatment-related effect on food consumption. The NOAEL for systemic toxicity was 250 mg/kg bw/d.

The LOAEL for neurotoxicity was not determined. There was no evidence of neurotoxicity in either sex at

any dose level; therefore, the NOAEL for neurotoxicity was 250 mg/kg bw/d, the highest dose tested in females.

This study is classified as acceptable / guideline as a subchronic neurotoxicity study in rats (870.6200).

This neurotoxicity study was conducted concurrent with the conduct of the first year of the two-year chronic toxicity / oncogenicity study of XDE-570 in Fischer 344 rats (see DACO 4.4.4 - Johnson, K. H., Haut, K. T. and Stebbins, K. E. November 24, 1997. XDE-570; Two-Year Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Laboratory Project Study ID 960004).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-570 as named in the study. Chemical Name (CA nomenclature): N-(2,6-diflurophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulphonamide

Description: White powdery solid

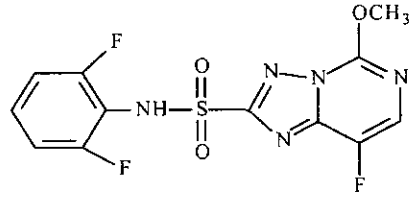
Lot/Batch #: Test Substance # TSN100511 / Lot # 940714

Purity: 99.3 % a.i. (determined by HPLC).

Compound Stability: The test substance was re-assayed after study determination and was confirmed at 99.3% (Knowles, et al., 1997, Lab Report Code GHE-P-6448)

CAS #: 145701-23-1

Structure



2. **Vehicle and/or positive control:** Dietary admixture.

3 **Test animals:**

Species: Male and female rats

Strain: Fischer 344

Age/weight at study initiation: At study initiation, the rats were ≈8 weeks of age (date of birth - Dec 12/1994) with a body weight range of 164.6-188.4 g for males and 109.1-132.4 g for females.

Source: *Charles River Laboratories, Kingston, New York.*

Housing: The animals were housed 2/cage (same sex) in stainless steel cages which had wire mesh floors.

Diet: Certified Rodent Chow #5002 (Purina Mills Inc., St. Louis, MO) in meal form *ad libitum*

Water: Tap water *ad libitum*

Environmental conditions:
Temperature: 22 ± 0.31 °C (range: 21.1-24.1 °C)
Humidity: 50.9 ± 2.8% (range: 38-66 °C)
Air changes: Not provided
Photoperiod: 12 hrs dark/12 hrs light

Acclimation period: At least 1 week.

B. STUDY DESIGN:

1. **In life dates** Start: February 9, 1995. End: February 13, 1996 (necropsy date).

2. **Animal assignment**: Animals were randomly assigned to the study groups using a computer-generated

randomization program based on body weights as summarized in Table 1. The test substance was administered *ad libitum* in the feed for approximately 12 months. The control group animals received untreated diet over the same time period. A functional observational battery (FOB) and motor activity evaluation (MA) were conducted on all animals (10 animals/sex/dose) once prior to exposure and at 3, 6, 9 and 12 months post-dosing. At 12 months, auditory function (auditory brainstem response) was evaluated in 5 rats/sex/dose group from the control and high-dose groups. After the completion of the auditory brainstem response (ABR) examination, a neuropathological examination of perfusion-fixed central and peripheral nervous tissues was conducted on these control and high-dose animals. All animals were subjected to a gross pathological examination following 12 months of treatment (study days 369 and 370).

This neurotoxicity study was conducted concurrent with the conduct of the first year of the two-year chronic toxicity / oncogenicity study of XDE-570 in Fischer 344 rats (see DACO 4.4.4 - Johnson, K. H., Haut, K. T. and Stebbins, K. E. November 24, 1997. XDE-570; Two-Year Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Laboratory Project Study ID 960004).

TABLE 1: Study design.

Test Group	Dose Level (mg/kg bw/d)		Time-Weighted Average Test Substance Intake (mg/kg bw/d) (a)		Number of Animals							
					Initial		Neurobehavioural Studies (b)		Necropsy		Neuropathology (c)	
	M	F	M	F	M	F	M	F	M	F	M	F
Control	0	0	0	0	10	10	10	10	10	10	5	5
Low	10	10	8.6	9	10	10	10	10	10	10	-	-
Mid	250	125	216	113	10	10	10	10	10	10	-	-
High	500	250	460	226	10	10	10	10	10	10	5	5

(a) Time-weighted average test substance intake obtained from pages 140 (males) and 144 (females) in the study report.
(b) FOB and motor activity evaluations were determined pretest, and at 3, 6, 9 and 12 months post-dosing.
(c) Auditory function (auditory brainstem response) was evaluated in 5 rats/sex/dose from the control and high-dose group at 12 months, following completion of the auditory brainstem response (ABR) examination, a neuropathological examination of perfusion-fixed central and peripheral nervous tissues was conducted on these 5 animals/sex/dose in the control and high-dose group.

3. Dose Selection Rationale: The dose levels were selected based on data from the acute oral, 2-week dietary and 13-week dietary (with 4-week reversibility period) studies with Fischer 344 rats. In the 2-week dietary study, 5 rats/sex/dose received XDE-570 *ad libitum* in the diet at dose levels of 0, 100, 500 or 1,000 mg/kg bw/d (Szabo, J.R. and Davis, N.L. Laboratory Project Study ID: TXT:DR-0312-6565-003, study submitted but a full review was not completed). At 1,000 mg/kg bw/d lower food consumption suggestive of minor unpalatability of the diet with subsequent lower body weights and secondary organ weight changes was observed in both sexes. At ≥500 mg/kg bw/d histopathological alterations characterized as nuclear pleomorphism of the renal proximal tubule epithelial cells were observed in both sexes. The NOAEL was 100 mg/kg bw/d. In the 13-week dietary study (with 4-week reversibility period), 10 rats/sex/dose received XDE-570 *ad libitum* in the diet at dose levels of 0, 20, 100, 500, 800 (♀ only) or 1,000 (♂ only) mg/kg bw/d (see DACO 4.3.1 - Redmond, J.M. and Johnson, K.A. Laboratory Project Study ID: DR-0312-6565-001). The LOAEL was 500 mg/kg bw/d based on lower body weight and body-weight gain (♂/♀), decreased RBC parameters (↓ RBC counts, HCT and HGB) possibly indicative of mild anaemia (♂), urine acidification (♂/♀), increased kidney weights (♂/♀) and histopathological findings in the kidneys including hypertrophy of epithelial cells in collecting duct (♂/♀) and decreased degeneration/regeneration of descending portion of proximal tubules (♀). The NOAEL was 100 mg/kg bw/d. In males, the high-dose level of 500 mg/kg bw/d was expected to produce lower body-weight gain. In females, the high dose of 250 mg/kg bw/d was expected to produce lower body-weight gain and to induce histopathologic kidney effects in both sexes of rats. In addition, the high-dose level of 500 mg/kg bw/d was expected to provide evidence of haematological and urinalysis effects in male rats. The intermediate dose was expected to produce minimal signs of toxicity and the low dose was expected to be a no-observed-effect-level.

4. Diet preparation and analysis: Test diets were prepared by serially diluting a concentrated test substance-feed mixture (pre-mix) with ground feed. The pre-mix was mixed for an appropriate length of time to ensure a homogeneous mixture. Premixes were prepared approximately every 2-4 weeks. Diets were prepared weekly during the first 13 weeks of the study and at least once every 2 weeks for the remainder of the dosing period. Initial concentrations of the test substance in the diet were calculated from pre-study body weights and food consumption data. Subsequently, the concentrations of test substance in the diets was adjusted weekly for the first 13 weeks and monthly thereafter based on the most recent body weight and food consumption data. Stability of the test substance was established concurrent with the start of the study for the low dose of 10 mg/kg bw/d. Homogeneity testing of the test substance in the feed was initiated prior to the study and also at two additional time points during the study. Analyses to verify the concentration of the test substance in the feed were conducted at the start and at approximately 3-month intervals thereafter. For these analyses, aliquots of the appropriate diet concentration(s) were solvent extracted, diluted if necessary and analysed by HPLC using UV detection.

Results - Homogeneity Analysis: The analyses showed that the test material was adequately distributed in the feed for all six samples with relative standard deviations (RSD) of 2.6% for the pre-mix, 2.6% and 0.9% for the 500 mg/kg bw/d male diet and 14.3, 11.2 and 8.6% for the 10 mg/kg bw/d female diet. An RSD of 15% or less was deemed to denote a homogenous mixture.

Date Mixed	2/8/95	2/8/95	5/17/95	5/17/95	5/31/95	5/31/95
Dose Level (mg/kg bw/d)	10 (♀)	500 (♂)	10 (♀)	3.0% Pre-mix	10 (♂)	500 (♂)
Concentration Range (%w/w)	0.00833 - 0.0136	0.697 - 0.745	0.0157 - 0.0232	2.90 - 3.15	0.0120 - 0.0163	1.01 - 1.03
Mean Concentration (%w/w)	0.0113	0.719	0.0188	3.06	0.0148	1.02
Standard Deviation	0.00161	0.0185	0.0021	0.08	0.00127	0.0089
%RSD	14.25	2.57	11.17	2.61	8.58	0.87

Stability Analysis: Stability data was determined for the 10 mg/kg bw/d dose group (females). Based on these findings, the test substance was found to be stable in rodent chow for at least 30 days. Since the premix and various dietary levels were mixed at least once every 4 weeks (28 days), stability data was not needed beyond 30 days.

Female - 10 mg/kg bw/d		
Days Elapsed	Observed Amount (% w/w)	% of Initial Day (day 0)
0	0.00113	-
8	0.00126	112
15	0.00123	109
30	0.0011	97

Concentration Analysis: The actual doses that the rats received were estimated at 98.2-103.2% of target based on calculations that used food consumption data from all rats for each dose and sex.

Dose level (mg/kg bw/d)	Range (% of target concentration)		Mean ± SD (% of target concentration)	
	Males	Females	Males	Females
10	83-133	95-115	99.0 ± 20	101.2 ± 8
125	-	91-105	-	98.2 ± 6

Dose level (mg/kg bw/d)	Range (% of target concentration)		Mean ± SD (% of target concentration)	
	Males	Females	Males	Females
250	97-115	97-104	103.2 ± 7	99.4 ± 3
500	99-115	-	102.8 ± 7	-
Premix	101-120		104.8 ± 9	

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

5. Statistics - Statistical analyses were conducted on body weights, rectal temperature, grip performance, landing foot splay, motor activity and ranked FOB observations. The average of the three grip performance trials, for each animal, was used for statistical analyses. Motor activity counts were reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment. The incidences of ranked FOB observations, between control and each treated group (for each sex and time point separately), were evaluated by a test of proportions (Bruning and Kintz, 1977). Only those values which met the critical z-score for an $\alpha = 0.02$ were reported. There were 16 mandatory graded observations leading to a minimum of 480 z-tests (2 sexes x 16 observations x 1 severity level x 5 time periods x 3 dose level comparisons). A *post-hoc* z-test of proportions was conducted on incidences of urinary soiling recorded during clinical and FOB evaluations. For overall FOB summarization, ranked scores for each FOB observation (for males and females at each dose level) were converted into average ranked scores for that observation. Ranks were given values from 1 (lowest level of a behaviour) to 5 (highest rank for that behaviour). The average rank score for a test group was the sum of (rank x incidence) divided by the sample size for that group. The average ranked scores were subjectively evaluated. Auditory brainstem response (ABR) waveforms were also subjectively evaluated for robustness of Peak I (acoustic nerve response). Individual and composite waveforms from control and high-dose group rats were evaluated. For continuous data, means and standard deviations were calculated and homogeneity of variance was evaluated with the F-max test ($\alpha = 0.01$). No extreme departures from homogeneity of variance were found. Initial statistical analyses of continuous data were factorial repeated-measure analyses to account for data from both sexes at all time periods in one statistical analysis. Factors were treatment, sex and time (repeated for time). By using sex as a factor, the statistical power of the test was increased by increasing the degrees of freedom. This statistic uses information from both sexes, but does not blend that data and thus, one objectively tests whether or not sexes responded equally to treatment. In factorial repeated-measure tests (a dependent variable repeated over time, i.e., at pre-exposure, 3, 6, 9 and 12 months), the inclusion of pre-exposure data in the analysis makes only the analyses which include factors of both treatment and time. In case of statistical significance, subsequent analysis were conducted to identify the time interval and the exposure level that caused the significance. The following interactions were studied:

Treatment x time	- a significant p value indicates that, taken together, both males and females were affected by treatment at some time interval.
Treatment x time x sex	- a significant p value indicates that treatment effects were different between males and females at some time interval.
Treatment x time x epoch	- a significant p value indicates that treatment effects were different amongst the different epochs at some time interval. (motor activity only)

To reduce the rate of false declarations, the Type I error rate (α) per comparison was set at 0.02 for body weight, grip performance, landing foot splay and motor activity data and at 0.02 for the z test proportions for the ranked FOB observations. Step-down analyses following a statistically significant primary analysis also were conducted at an α of 0.02. The corrections for multiple statistical analyses were applied to alpha only, and the probability were reported without correction.

Number and Type of Statistical Tests (a)			
Dependent Variable		Number of Primary Tests	Type of Test (b)
Body weight		2 (T x D & T x D x S)	Rep-ANOVA
Grip performance	- forelimb - hindlimb	2 (T x D & T x D x S) 2 (T x D & T x D x S)	Rep-ANOVA
Temperature		2 (T x D & T x D x S)	Rep-ANOVA
Landings food splay		2 (T x D & T x D x S)	Rep-ANOVA
Motor activity	- total counts - Epochs (nested by day)	2 (T x D & T x D x S) 1 (T x D x E)	Rep-ANOVA
FOB observations		>480 (T)	Z-test of proportions

Factors: T = treatment; D = day; S = sex; E = Epoch. Repeated across months. For motor activity, repeated across months and across epochs.
(a) Table extracted from page 75 of the study report.
(b) Rep-ANOVA (repeated-measure analysis of variance) was calculated by rep=MANOVA with the multivariate model. This format avoids the requirement of sphericity of the variance / covariance matrix of the Rep-ANOVA. The multivariate index was the Pillai Trace statistic.

C. METHODS:

1. Clinical Observations: Cage-side observations were conducted at least twice daily (once daily on holidays and weekends) for morbidity, moribundity, mortality and availability of food and water. Additionally, all animals were removed from their cages and hand-held clinical examinations were conducted prior to the start of the study and weekly thereafter. Examinations included a thorough evaluation of skin and fur, swelling / masses, mucous membranes, respiration, nervous system and behaviour pattern of each animal. Examination also included evaluation of the animals for signs of tremor, convulsions and diarrhea. The observer conducting these hand-held clinical examinations was aware of treatment status (non-blind).

2. Body weight: Body weights were recorded during the pre-exposure period and weekly during the first 13 weeks, and at approximately monthly intervals thereafter. Body weights taken nearest in time to each FOB were statistically evaluated in this report (at pre-exposure, and at 3, 6, 9 and 12 months).

3. Food consumption and compound intake: Food consumption data were collected for all animals weekly for the first 13 weeks and for a one week period each month thereafter by weighing the feeders at the start and end of a measurement cycle. From these data, food consumption (g/animal/d) was calculated. Compound intake (mg/kg bw/d) values were calculated as time-weighted averages from the food consumption and body-weight gain data.

4. Neurobehavioural Evaluations: The neurobehavioural evaluation consisting of an FOB and determination of motor activity, was carried out on 10 animals/sex/dose at pre-exposure, and at 3, 6, 9 and 12 months post-dosing. An auditory screen was conducted after 12 months of exposure on 5 animals/sex from the control and high-dose groups by evaluation of tone-pip auditory brainstem response (ABR). All FOB and motor activity evaluations were performed by trained observers who did not know the treatment status of the animal (blind observations).

a. Motor Activity Evaluation: Twenty-four motor activity cages, visually isolated from each other, were located in a quiet dimly lit room. Each motor activity cage consisted of a clear plastic circular alley. An infra-red photo-beam dissected the cage so that the beam crossed the alley in 2 locations. The cages were calibrated prior to testing each day. Calibration was performed with a rod (attached to a rotary motor) that broke the infra-red beam. The time of beam interruption average for all test units was recorded, and any photocell showing a difference exceeding 4 centi-seconds was readjusted to ensure equivalence of devices. The animals were allocated to the motor activity cages in such a way that counterbalancing of treatment groups and sexes across cages and across times was

maximized. The experimental design is referred to as a split-plot factorial design with two between-block (sex and dose) treatments and two within-block (epoch and month) treatments. Each animal was tested individually for motor activity. All test sessions consisted of six 8-minute epochs, totalling 48 minutes of testing for each animal. The duration was chosen based on the results of a validation study indicating that performance of the control animals approached asymptote in 30-40 minutes. Total activity counts for each epoch were recorded. Each beam break that lasted for more than 100 msec constituted an activity count (minimum duration was set to discount activities such as tail-flicking, rearing, head-bobbing, etc). Motor activity was monitored by a computerized system located in an adjoining room.

b. FOB Evaluations: The FOB included home cage, handheld and open field observations, reflex / physiological observations, and measurement of grip performance, landings food splay and rectal temperature. Scoring criteria and explicitly-defined scales were used to rank the severity of observations that do not readily lend themselves to quantitation. For ranked observations, there were five levels (ranks) of observations that ranged from lowest intensity or lowest amount to highest (i.e., 1 -none; 2 - minimal; 3. moderate; 4 - pronounced and 5 - exaggerated). The procedures used to determine landing foot splay and grip strength were based on established methods. The following FOB parameters were evaluated:

HOME CAGE OBSERVATIONS	**OPEN FIELD OBSERVATIONS**
Posture	*Piloerection*
Piloerection	Respiration abnormalities
Gait abnormalities	Posture
Clonic / clonic movements	Clonic / tonic movements
Vocalizations	Stereotypic / bizarre behaviour
Other abnormal observations	Gait abnormalities
HAND-HELD OBSERVATIONS	Vocalizations
Ease of removal from cage	Arousal
Reaction to handling	Rearings / 2 minutes
Muscle tone	Defecation
Palpebral closure	Urination
Pupil size	Other abnormal observations
Lacrimation	**MEASUREMENTS**
Salivation	*Hindlimb grip strength*
Staining (eyes, oral, nasal, anal, urine)	Forelimb grip strength
Other abnormal observations	Landing footsplay
REFLEX / PHYSIOLOGICAL OBSERVATIONS	Body weight
Approach response	Body temperature
Touch response	
Auditory response	
Righting reflex	
Tail pinch response	

The FOB evaluation was conducted by the same observer on all rats pre-exposure and during the 3 and 6 month examinations. A second observer conducted the FOB on all rats during the 9 and 12 months examinations. All examinations were conducted at about the same time each day (morning). The study report indicated that a very high degree of inter-observer reliability existed between the two observers. Positive control data demonstrating the proficiency of the two observers were included in the study report.

c. Auditory Brainstem Response: An auditory screen was conducted by evaluation of tone-pip auditory brainstem response (ABR). Tone-pip ABRs provide information on auditory function at higher frequencies, and are used to screen for mid- to high-frequency hearing loss. Auditory brainstem responses were collected according to the

following procedures. The animals were tested under light isoflurane gas anaesthesia. Small needle electrodes (0.4 mm diameter human sub-dermal electrodes) were utilized. While the animal was anaesthetised, the needle electrodes were placed subcutaneously at three specific sites (transverse over the cerebellum, transverse across the bridge of the nose and over one shoulder). Testing required about 12 minutes/animal, and coordinated locomotion returned in about 2 minutes post-dosing. The following frequencies were tested: 4 kHz (ABR4), 8 kHz (ABR8), 16 kHz (ABR16) and 30 kHz (ABR30). Multiple frequencies were used to evaluate different parts of the cochlea, since ototoxicity characteristically affects hearing at some (usually high) frequencies more than other frequencies. The electrodiagnostic system used was a Nicolet Pathfinder II. Data sweeps (msec of EEG) were digitally sampled 512 times and averaged by an online computer. A computer routine (Nicolet Biomedical Instruments) was used to digitally filter each of the waveforms that was collected at a bandpass of 0.1 to 3. kHz.

Evaluation of ABR: Ototoxicity, via damage to the cochlea (usually cochlear hair cells) will cause a reduced response in the acoustic nerve (VIII). Peak I of the ABR reflects activity in the acoustic nerve. Consequently, ABR's at each frequency, from each animal, were evaluated subjectively for the robustness of Peak I. Similarly, composite (average) ABR for each group was evaluated for robustness of Peak I.

5. Sacrifice and Pathology After completion of the exposure period at approximately 12 months, a gross pathological examination was conducted on all rats. Following an overnight fast, 5 animals/sex/dose were anaesthetized with methoxyflurane and perfused *in situ* using the following method. Animals were heparinized at least 10 minutes before perfusion with 0.2 mL heparin/100 g bw intraperitoneally (10,000 USP units/mL) and were anaesthetized with methoxyflurane vapour inhalation. Animals were perfused intracardially with 0.05 M phosphate buffer containing 0.7% sodium nitrate, followed by a phosphate buffered solution of 1.5% glutaraldehyde - 4% formaldehyde. Necropsy consisted of an examination of the external tissues and orifices. The head was removed, the cranial cavity opened and the brain, pituitary and the adjacent cervical tissues were examined. The nasal cavity was flushed with phosphate buffered solution of 1.5% glutaraldehyde - 4% formaldehyde. The skin was reflected from the carcass, the thoracic and abdominal cavities were exposed and the viscera were examined in situ. All visceral tissues were dissected from the carcass and re-examined. The brain, spinal column with spinal cord, fore- and hind-limbs and tail were trimmed and immersed in fixative (phosphate buffered solution of 1.5% glutaraldehyde - 4% formaldehyde). Muscles from the hind-limb were reflected to expose the nerves. Thoracic and abdominal viscera were also saved in fixative. The 5 remaining animals/sex/dose were fasted overnight, anaesthetized by methoxyflurane inhalation and decapitated, but were not perfused. However, these animals were necropsied in a similar manner as indicated for the perfused animals. Tissues marked with an (X) in the following table (see Tissues Collected and Preserved at Necropsy) were collected from all animals (10 animals/sex/group) and preserved during necropsy. No organ weight data was obtained for these animals, however, organ weights were determined for all animals in the concurrent chronic toxicity / oncogenicity study (see DACO 4.4.4 - Johnson, K. H., *et al.* November 24, 1997. Laboratory Project Study ID: 960004).

Tissues Collected and Preserved at Necropsy					
	DIGESTIVE SYSTEM		**CARDIOVASC./HAEMAT.**		**NEUROLOGIC**
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	*Eyes (optic n.)*

X	Jejunum	X	Thymus		**GLANDULAR**
X	Ileum		**UROGENITAL**	X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal / Harderian glands
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	X	Auditory sebaceous glands
X	Pancreas	X	Seminal vesicle		**OTHER**
	RESPIRATORY	X	*Ovaries*	X	Bone (including joint)
X	Trachea	X	Uterus	X	Skeletal muscle
X	Lung	X	Cervix	X	Skin
X	Nose	X	Coagulating glands	X	All gross lesions and masses
X	Pharynx	X	Oviducts		
X	Larynx	X	Vagina		

Tissues for neuropathological evaluation, marked with an (X) in the following table (see Nervous System Tissue Collection and Disposition), were prepared from all perfusion-fixed animals in the control and high-dose groups. Tissues from the central nervous system and sections of skeletal muscle were embedded in paraffin, sectioned approximately 6 µm thick and stained with hematoxylin and eosin. Spinal root nerves (cervical and lumbar), peripheral nerves sciatic, tibial and sural) and dorsal root ganglia (cervical and lumbar) were osmicated, embedded in epon/araldite plastic, sectioned approximately 2 µm thick and stained with toluidine blue. All tissues were examined by a veterinary pathologist using a light microscope.

Nervous System Tissue Collection and Disposition			
Paraffin		***Plastic***	
Brain		**Dorsal root ganglia**	
- *olfactory bulb*	X	- cervical swelling (C_3 - C_6)	X
- cerebrum, frontal, parietal, temporal, occipital	X	- lumbar swelling (L_1 - L_4)	X
- thalamus / hypothalamus	X	**Dorsal and ventral roots**	
- midbrain	X	- cervical swelling (C_3 - C_6)	X
- pons	X	- lumbar swelling (L_1 - L_4)	X
- cerebellum	X	**Peripheral nerves (cross and longitudinal section)***	
- medulla including nucleus gracilis / cuneatus	X	- proximal sciatic	X
Pituitary gland	X	- tibial	X
Trigeminal ganglia with nerve	X	- peroneal (saved)	X
Spinal cord (cross and oblique section) - cervical swelling (C_3 - C_6)	X	- sural	X
- Lumbar swelling (L_1 - L_4)	X	- caudal (saved)	X
Eyes	X	- optic (longitudinal only)	X
Skeletal muscle - anterior tibial and gastrocnemius	X		
* Fore- and hind-limbs are saved, but nerves were collected only from the right hind-limb.			

6. Positive control validation studies:

FOB - Positive control studies for FOB evaluations were conducted with saline (0.15 mL, i.p.), chlorpromazine (4 mg/kg i.p.), d-amphetamine (8 mg/kg i.p.) or atropine + physostigmine sulfate (2 mL/kg atropine i.p. followed in 5 min with 0.75 mg/kg physostigmine s.c.) to establish the sensitivity, reliability, and validity of these test procedures, the adequacy of training of technical personnel and to serve as a historical control. Head weaving and piloerection were observed in animals treated with d-amphetamine. Chlorpromazine treated animals were observed to hold fixed postures. Atropine-physostigmine treated animals exhibited tremors and decreased response to tail pinch. Similar appropriate observations were made for ranked observations, and for measurements of temperature, grip performance and landing foot splay. Positive control studies with untreated animals and with animals treated with reference substances that evaluate FOB proficiency, adequately established the sensitivity, reliability and validity of these test procedures. It was determined subjectively that C.M. Clements made the appropriate observations for the specific pharmacologic syndromes. Objectively, the Pearson's cross-correlation coefficient of Clements' observational scores vs template (expected) scores yielded r = 0.924, and Pearson's r measurements for temperature, grip and splay was 0.890. These high correlations demonstrated objectively that Clements' observations and measurements were a high match to those expected. The combination of subjective and objective evaluations of FOB proficiency for Clements indicate a high degree of FOB proficiency with positive control substances. The proficiency demonstrated by C.M. Clements (FOB observer) and the FOB positive control study and data were provided on pages 459-585. The raw data and supporting documentation were filed under laboratory ID: HET T1.05-022-000-008.

Motor activity - Positive control studies with untreated animals (0.5 mL physiological saline, i.p. and a non-injected control group) and with rats treated with reference substances that increase (d-amphetamine at final concentrations of 0.06, 0.196 or 0.65 mg/mL) and decrease (chlorpromazine at final concentrations of 0.3, 1.33 or 3.0 mg/mL) motor activity established the sensitivity, reliability and validity of these test procedures. Motor activity positive control study and data were provided on pages 439-458 of the study report. Raw data and supporting documentation were filed under laboratory ID: HET T1.05-018-002-REV.

Neuropathology - Neuropathology proficiency of K. A. Johnson (Study Director, Pathologist) was adequately demonstrated in the following published articles included with the study report.

(1) Eisenbrandt, D. L. et al (1990). Spontaneous Lesions in Subchronic Neurotoxicity Testing of Rats. Toxicologic Pathology 18; 154-164.
(2) Mattson, J. L., Johnson, K. A. and Albee, R. R. (1986). Lack of Neuropathologic Consequences of Repeated Dermal Exposure to 2,4-Dichlorophenoxyacetic Acid in Rats. Fundamental and Applied Toxicology 6; 175-181.
(3) Johnson, K. A., et al (1986). Chronic Toxicity and Oncogenicity Study on Acrylamide Incorporated in the Drinking Water of Fischer 344 Rats. Toxicology and Applied Pharmacology 85; 154-168.

II. RESULTS

A. Observations :

1. Clinical signs of toxicity - There were no significant treatment-related clinical signs. An increased incidence of perineal soiling was observed in males at 250 and 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d. The soiling appeared to be urine dried to the fur of the perineum. The increased incidence may be associated with a possible tendency towards a slight increase in urination in the open field in these animals, however, the relationship, if any, is incertain. The increased incidence of perineal soiling was considered to be a secondary or indirect effect related to lack of grooming possibly due to urinary acidification or to the presence of excretory products of the test substance in the urine.

TABLE 2: Clinical observations (expressed as number of animals having specified observation at least once in 52 weeks/number of animals observed). (a)

Observation		Dose Level (mg/kg bw/d)				
		0	10	125	250	500
Males	Perineal soiling	1/10	4/10	-	8/10 *	10/10 *
Females	Perineal soiling	1/10	0/10	9/10 *	9/10 *	-

(a) Data obtained from page 80 of the study report. Number represent rats having at stated observation at least once in 52 weeks.
* Statistically different from control, $p \leq 0.01$. These differences were tested, *post-hoc*, by a z-test of proportions.

2. *Mortality* - *There were no treatment-related mortalities.* One male at 500 mg/kg bw/d died on study day 362 just prior to the scheduled necropsy on study days 369 and 370. There were no remarkable findings in this animal. This was considered to be a spontaneous death.

B. Body weight and weight gain: Body weight was significantly lower in males at 500 mg/kg bw/d at 6, 9 and 12 months. In the high-dose males, body-weight gain was lower compared to controls from 3 months onwards (≈63, 61 and 67% lower at 3-6 months, 6-9 months and 9-12 months, respectively). Overall body-weight gain in the high-dose males was approximately 27% lower compared to controls. Food consumption in the high-dose males was comparable to controls throughout the study. Body weight and body-weight gain were unaffected by treatment in males at 10 and 250 mg/kg bw/d and in females at 10, 125 and 250 mg/kg bw/d. Body weight and body-weight gain data are summarized in Table 3 (males) and Table 4 (females).

TABLE 3. Mean body weights and body-weight gains (males). (a)

Dose Level (mg/kg bw/d)	0	10	250	500
Body Weights (g ± SD) (n = 10 animals/dose level)				
Pre-dosing	175.4 ± 6.7	176.8 ± 7.6	176.3 ± 6.2	176.7 ± 6.7
3 months	315.6 ± 11.5	327.5 ± 15.8	320.9 ± 16.5	315.9 ± 11.9
6 months	367.8 ± 14.3	375.3 ± 15.7	364.4 ± 21.4	335.4 ± 14.3 *
9 months	394.0 ± 14.5	403.1 ± 16.7	390.0 ± 19.9	345.6 ± 12.5 *
12 months	419.3 ± 12.5	423.0 ± 23.4	415.9 ± 23.8	355.2 ± 15.0 * (n = 9)
Body-Weight Gain (g ± SD) (n = 10 animals/dose level)				
0-3 months	140.0 ± 11.1	150.7 ± 13.3	144.6 ± 14.7	139.2 ± 13.0
3-6 months	52.3 ± 8.4	47.8 ± 6.2	43.5 ± 6.9	19.4 ± 7.8
6-9 months	26.1 ± 6.1	27.8 ± 6.8	25.6 ± 5.7	10.2 ± 6.3
9-12 months	25.3 ± 5.6	19.9 ± 9.6	25.9 ± 7.4	8.3 ± 22.0
0-12 months	243.8 ± 11.2	246.2 ± 21.5	239.6 ± 21.5	177.2 ± 16.8

(a) Data obtained from page 104 of the study report for body weights. Body-weight gains were calculated by reviewer from individual quarterly body weight data obtained from pages 250-253 of the study report. No statistical analyses done for body-weight gain.
* *Significantly different* ($p \leq 0.01$) from the control.

TABLE 4. Mean body weights and body-weight gains (females). (a)

Dose Level (mg/kg bw/d)	0	10	125	250
Body Weights (g ± SD) (n = 10 animals/dose level)				
Pre-dosing	118.4 ± 5.6	121.2 ± 7.3	119.4 ± 4.9	121.5 ± 5.9
3 months	179.5 ± 4.7	182.1 ± 10.9	183.9 ± 8.4	179.8 ± 5.1
6 months	197.4 ± 4.4	197.4 ± 12.1	200.1 ± 7.5	194.1 ± 6.8
9 months	205.2 ± 6.4	204.8 ± 12.0	208.1 ± 9.3	202.6 ± 8.4
12 months	214.2 ± 4.8	216.2 ± 1.32	216.8 ± 8.4	211.3 ± 9.8
Body-Weight Gain (g ± SD) (n = 10 animals/dose level)				
0-3 months	61.1 ± 7.7	60.9 ± 6.0	64.5 ± 9.9	58.3 ± 7.3
3-6 months	17.9 ± 4.9	15.4 ± 5.1	16.2 ± 5.3	15.1 ± 3.4
6-9 months	7.8 ± 4.8	7.4 ± 2.7	8.0 ± 4.1	7.7 ± 3.5
9-12 months	9.0 ± 5.1	11.4 ± 4.0	8.7 ± 5.4	8.7 ± 3.9
0-12 months	95.8 ± 7.2	95.0 ± 9.0	97.4 ± 9.9	89.8 ± 9.7

(a) Data obtained from page 105 of the study report for body weights. Body-weight gains were calculated by reviewer from individual quarterly body weight data obtained from pages 250-253 of the study report. No statistical analyses done for body-weight gain.

C. Food consumption and compound intake:

1. Food consumption - Food consumption was comparable between the treatment groups and controls throughout the study for both sexes.

2. Compound consumption - Time-weighted average test substance intakes (mg/kg bw/d) are summarized in Table 1.

3. Food efficiency - Food efficiency was not provided in the study report. However, in the high-dose males body-weight gain was lower compared to controls from 3 months onwards (≈63, 61 and 67% lower at 3-6 months, 6-9 months and 9-12 months, respectively) while food consumption was comparable to controls suggesting that food efficiency was decreased in these animals. Body weight, body-weight gain and food consumption were unaffected by treatment in males at 10 and 250 mg/kg bw/d and in females at 10, 125 and 250 mg/kg bw/d.

D. Neurobehavioural Evaluations:

1. Motor Activity Evaluations: There were no treatment-related differences in measures of motor activity in males or females at any dose level or at any time during the study (Table 5). The count distribution across epochs did not change as a consequence of treatment at any test period. For both sexes during all test times, measures of activity decreased fairly rapidly after the first epoch of each test session. There was no evidence of a treatment-related effect on habituation at any dose level. The profile of habituation for motor activity during the test session did not change with age or test experience in either sex.

TABLE 5: Motor Activity. (a)

Sex	Month	Dose Level (mg/kg bw/d)				
		0	10	125	250	500
Motor Activity (total counts ± SD)						

Sex	Month	Dose Level (mg/kg bw/d)				
		0	10	125	250	500
Male (n = 10 rats/dose)	Pre-dosing	10.93 ± 1.51	10.46 ± 1.46	-	11.51 ± 1.13	11.55 ± 1.56
	3 months	13.70 ± 1.63	12.36 ± 1.75	-	14.15 ± 1.16	13.43 ± 2.09
	6 months	12.60 ± 0.85	11.37 ± 1.74	-	13.30 ± 1.93	12,36 ± 1.48
	9 months	11.46 ± 0.99	10.71 ± 1.31	-	11.74 ± 2.04	10.28 ± 2.22
	12 months	9.69 ± 1.35	10.30 ± 1.43	-	10.27 ± 2.30	10.40 ± 1.28
Female (n = 10 rats/dose)	*Pre-dosing*	12.99 ± 1.83	13.20 ± 1.23	13.14 ± 2.29	13.15 ± 1.88	-
	3 months	14.94 ± 1.58	14.13 ± 1.54	14.09 ± 1.93	14.07 ± 1.82	-
	6 months	13.27 ± 1.66	13.55 ± 1.16	13.01 ± 1.83	13.00 ± 2.51	-
	9 months	13.36 ± 1.74	13.18 ± 2.23	12.90 ± 2.07	13.12 ± 1.42	-
	12 months	12.45 ± 1.53	11.93 ± 2.39	12.34 ± 1.94	11.69 ± 1.55	-

(a) Data obtained from pages 118-130 in the study report for motor activity and pages for locomotor activity. All test sessions consisted of six 8-minute epochs, totalling 48 minutes of testing for each animal.

2. FOB Evaluations: There were no significant treatment-related findings in the FOB evaluations. FOB evaluations suggest an increased incidence of urinary perineal soiling in males at 250 and 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d. The increased incidence of urinary perineal soiling is consistent with clinical observations and findings at necropsy. The increased incidence may be associated with a possible tendency towards a slight increase in urination in the open field in these animals, however, the relationship, if any, is uncertain. The increased incidence of perineal soiling was considered to be a secondary or indirect effect related to lack of grooming possibly due to urinary acidification or to the presence of excretory products of the test substance in the urine. FOB evaluations are summarized in Table 6 (males) and Table 7 (females).

Fore-limb and hind-limb grip strength, landing foot splay and rectal temperature, were not affected by treatment in males or females at any dose level (Table 8).

TABLE 6: FOB Incidence Summary - males (expressed as # animals with specified observation/# animals examined) (a)

Month	*Observation*		Dose Level (mg/kg bw/d)			
			0	10	250	500
Pre-dosing	- excessive perineal soiling (urine)		0/10	0/10	0/10	0/10
	- urination	- none	3/10	3/10	4/10	2/10
		- minimal	3/10	2/10	2/10	8/10
		- moderate	4/10	5/10	4/10	0/10
3 *months*	- excessive perineal soiling (urine)		0/10	0/10	5/10	8/10
	- urination	- none	3/10	3/10	2/10	0/10
		- minimal	7/10	5/10	8/10	9/10
		- moderate	0/10	1/10	0/10	1/10
		- pronounced	0/10	1/10	0/10	0/10
6 months	- excessive perineal soiling (urine)		2/10	0/10	3/10	5/10
	- urination	- none	6/10	4/10	3/10	3/10
		- minimal	4/10	5/10	4/10	4/10
		- moderate	0/10	1/10	3/10	3/10
9 months	- excessive perineal soiling (urine)		1/10	3/10	6/10	9/10

Month	Observation		Dose Level (mg/kg bw/d)			
			0	10	250	500
	- urination	- none	3/10	4/10	1/10	3/10
		- minimal	6/10	5/10	8/10	4/10
		- moderate	1/10	1/10	1/10	1/10
		- pronounced	0/10	0/10	0/10	2/10
12 months	- excessive perineal soiling (urine)		1/10	3/10	7/10	9/9
	- urination	- none	3/10	1/10	1/10	2/9
		- minimal	1/10	4/10	1/10	2/9
		- moderate	2/10	1/10	5/10	1/9
		- pronounced	4/10	4/10	3/10	4/9

(a) Data obtained from pages 88-102 in the study report.

TABLE 7: FOB Incidence Summary - females (expressed as # animals with specified observation/# animals examined) (a)

Month	Observation		Dose Level (mg/kg bw/d)			
			0	10	125	250
Pre-dosing	- excessive perineal soiling (urine)		0/10	0/10	0/10	0/10
	- urination	- none	5/10	6/10	5/10	5/10
		- minimal	2/10	3/10	2/10	4/10
		- moderate	3/10	1/10	3/10	1/10
3 months	- excessive perineal soiling (urine)		1/10	0/10	4/10	8/10
	- urination	- none	3/10	6/10	7/10	5/10
		- minimal	6/10	3/10	3/10	3/10
		- moderate	1/10	1/10	0/10	2/10
		- pronounced	0/10	0/10	0/10	0/10
6 months	- excessive perineal soiling (urine)		0/10	0/10	3/10	5/10
	- urination	- none	6/10	4/10	3/10	4/10
		- minimal	3/10	5/10	7/10	4/10
		- moderate	1/10	1/10	0/10	2/10

Month	Observation		Dose Level (mg/kg bw/d)			
			0	10	125	250
9 months	- excessive perineal soiling (urine)		0/10	0/10	2/10	8/10
	- urination	- none	9/10	7/10	9/10	6/10
		- minimal	1/10	2/10	1/10	3/10
		- moderate	0/10	1/10	0/10	1/10
		- pronounced	0/10	0/10	0/10	0/10
12 months	- excessive perineal soiling (urine)		0/10	0/10	1/10	8/10
	- urination	- none	5/10	3/10	4/10	3/10
		- minimal	4/10	5/10	4/10	5/10
		- moderate	1/10	2/10	2/10	1/10
		- pronounced	0/10	0/10	0/10	1/10

(a) Data obtained from pages 88-102 in the study report.

TABLE 8: FOB Measurements. (a)

Sex	Month	Dose Level (mg/kg bw/d)				
		0	10	125	250	500
Hind-limb Grip Strength (g ± SD)						
Male (n = 10 rats/dose)	Pre-dosing	232.6 ± 33.9	230.8 ± 36.0	-	223.0 ± 44.0	220.0 ± 35.9
	3 months	296.7 ± 64.8	328.0 ± 68.0	-	330.8 ± 48.5	289.2 ± 41.9
	6 months	303.5 ± 46.1	298.8 ± 49.5	-	309.5 ± 62.9	293.8 ± 39.0
	9 months	522.5 ± 86.2	479.0 ± 49.6	-	507.5 ± 98.9	472.2 ± 79.4
	12 months	503.2 ± 79.4	489.8 ± 35.9	-	513.6 ± 55.0	472.5 ± 42.8 (n = 9)
Female (n = 10 rats/dose)	Pre-dosing	156.5 ± 34.8	156.9 ± 25.3	173.5 ± 42.9	151.8 ± 26.0	-
	3 months	219.6 ± 52.0	189.7 ± 31.5	229.0 ± 40.0	198.1 ± 28.8	-
	6 months	232.6 ± 76.1	192.4 ± 46.7	211.1 ± 23.1	208.0 ± 34.4	-
	9 months	368.3 ± 45.8	346.6 ± 33.3	362.8 ± 61.5	346.1 ± 39.6	-
	12 months	362.5 ± 36.9	346.8 ± 57.8	368.9 ± 31.5	343.8 ± 45.0	-
Fore-limb Grip Strength (g ± SD)						

Sex	Month	Dose Level (mg/kg bw/d)				
		0	10	125	250	500
Male (n = 10 rats/dose)	Pre-dosing	263.0 ± 99.6	257.2 ± 36.2	-	267.4 ± 36.0	252.2 ± 77.1
	3 months	326.7 ± 65.6	320.6 ± 56.2	-	352.2 ± 76.4	308.7 ± 54.6
	6 months	322.3 ± 104.9	288.2 ± 64.0	-	359.2 ± 79.6	297.9 ± 31.6
	9 months	408.7 ± 142.6	455.4 ± 158.3	-	453.2 ± 121.2	432.3 ± 114.0
	12 months	490.1 ± 124.4	487.4 ± 124.8	-	527.7 ± 159.2	461.2 ± 72.6 (n = 9)
Female (n = 10 rats/dose)	Pre-dosing	243.9 ± 66.1	206.4 ± 59.8	240.9 ± 64.6	275.5 ± 41.0	-
	3 months	272.6 ± 76.2	232.5 ± 98.0	240.7 ± 90.1	247.1 ± 44.6	-
	6 months	246.1 ± 60.5	209.7 ± 83.4	232.2 ± 96.7	236.4 ± 60.0	-
	9 months	390.6 ± 97.8	337.1 ± 104.5	393.3 ± 101.1	402.6 ± 83.8	-
	12 months	382.7 ± 95.1	366.7 ± 129.8	479.5 ± 84.7	450.9 ± 74.4	-
Landing Foot splay (cm ± SD)						
Male (n = 10 rats/dose)	Pre-dosing	6.28 ± 0.59	6.38 ± 0.50	-	6.20 ± 1.06	5.84 ± 0.78
	3 months	6.28 ± 0.69	6.21 ± 0.93	-	6.25 ± 0.52	6.52 ± 1.01
	6 months	6.52 ± 0.71	6.40 ± 0.81	-	5.92 ± 0.90	6.49 ± 0.81
	9 months	7.28 ± 1.05	7.08 ± 1.22	-	7.03 ± 1.39	6.71 ± 0.77
	12 months	7.24 ± 0.71	6.90 ± 1.43	-	6.45 ± 1.23	6.37 ± 0.64 (n = 9)
Female (n = 10 rats/dose)	Pre-dosing	5.81 ± 0.88	5.39 ± 0.56	6.02 ± 0.72	6.05 ± 1.00	-
	3 months	5.16 ± 0.59	4.99 ± 0.82	5.43 ± 0.84	5.03 ± 0.97	-
	6 months	5.02 ± 0.69	4.94 ± 0.77	5.31 ± 0.95	4.93 ± 0.49	-
	9 months	5.93 ± 0.71	5.82 ± 0.68	6.26 ± 0.49	5.98 ± 0.83	-
	12 months	5.64 ± 0.84	5.76 ± 0.75	5.87 ± 0.82	5.69 ± 0.69	-
Rectal Temperature (°C ± SD)						
Male (n = 10 rats/dose)	Pre-dosing	38.11 ± 0.33	37.91 ± 0.32	-	37.75 ± 0.33	37.97 ± 0.45
	3 months	38.13 ± 0.39	38.11 ± 0.28	-	38.18 ± 0.48	38.35 ± 0.40
	6 months	37.14 ± 0.54	37.38 ± 0.47	-	37.00 ± 0.53	36.85 ± 0.59
	9 months	37.79 ± 0.54	37.75 ± 0.43	-	37.66 ± 0.39	37.77 ± 0.38
	12 months	37.73 ± 0.33	37.65 ± 0.43	-	37.70 ± 0.24	37.69 ± 0.41 (n = 9)
Female (n = 10 rats/dose)	Pre-dosing	37.93 ± 0.50	37.90 ± 0.59	38.25 ± 0.64	38.09 ± 0.64	-
	3 months	38.07 ± 0.65	38.49 ± 0.35	38.46 ± 0.51	38.34 ± 0.51	-
	6 months	37.25 ± 0.50	37.38 ± 0.49	37.44 ± 0.44	37.73 ± 0.54	-
	9 months	37.59 ± 0.50	37.69 ± 0.36	37.82 ± 0.65	38.00 ± 0.46	-
	12 months	38.14 ± 0.30	37.88 ± 0.40	38.01 ± 0.42	37.98 ± 0.27	-

(a) Data obtained from pages 106-117 in the study report.

3. Auditory Brainstem Response - No treatment-related differences were found in auditory brainstem response between high-dose and control animals for males or females.

E. Sacrifice and Pathology

1. Organ weight - No organ weights were provided in the study report. In the concurrent rat 2-year dietary chronic toxicity / oncogenicity study (see DACO 4.4.4 - Johnson, K. H., et al. November 24, 1997. Laboratory Project Study ID: 960004), brain weight was unaffected by treatment after 12 and 24 months. There were no corroborating treatment-related gross pathological, histopathological or neuropathological findings to suggest a treatment-related effect on brain weight.

2. Gross pathology - There were no treatment-related gross pathological findings. An increase in perineal soiling was evident in males at 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d at necropsy (incidence: 0/5, 1/5, 1/5 and 5/5 at 0, 10, 250 and 500 mg/kg bw/d, respectively, in males and 0/5, 0/5, 4/5 and 5/5 at 0, 10, 125 and 250 mg/kg bw/d, respectively, in females). These findings are consistent with clinical observations and the FOB evaluation. The increased incidence of perineal soiling was considered to be a secondary or indirect effect related to lack of grooming possibly due to urinary acidification or to the presence of excretory products of the test substance in the urine.

3. Microscopic pathology - There were no treatment-related histopathological findings in the tissues examined. There were no treatment-related neuropathological findings in the central or peripheral nervous system. In the concurrent 2-year dietary study in Fischer 344 rats, treatment-related non-neoplastic findings observed after 12 months were generally limited to the kidney and included hypertrophy of the epithelial cells of the collecting ducts in males at ≥250 mg/kg bw/d and in females at 250 mg/kg bw/d, a possible slight decrease incidence of age-related tubular degeneration/regeneration in males at ≥250 mg/kg bw/d and a possible slight increase incidence of age-related tubular degeneration/regeneration in females at 250 mg/kg bw/d.

III. DISCUSSION

A. Investigators' conclusions (extracted from page 10 of the study report): "A treatment-related effect was seen in male body weights. Male high-dose rats had significantly decreased body weights and body weight gains compared to controls at months 6, 9 and 12. Urinary perineal soiling was a treatment-related effect seen in both sexes in the rats from the middle and high-dose groups. Effects of XDE-570 on body weight and urinary perineal staining were attributed to non-specific effects of toxicity. XDE-570 had no effect at any time on hindlimb or forelimb grip performance , landing foot splay or rectal temperature, either in males or in females. XDE-570 also did not affect any aspect of either motor activity or auditory brainstem response, either in males or in females. There were no treatment-related gross or histopathologic findings following one year of exposure. In summary, there were no effects of XDE-570 on any parameter that would suggest a neurotoxic effect. Consequently, the NOEL for neurotoxicity was the high dose level tested, which was 250 mg/kg/day for females and 500 mg/kg/day for males".

B. Reviewer comments: There were no treatment-related effects on mortality, clinical signs or food consumption. In the high-dose males, body weight and body-weight gain were lower compared to controls while food consumption was comparable to controls. FOB evaluations did not indicate any significant treatment-related findings in either sex, however, there was an increased incidence of urinary perineal soiling in males at 250 and 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d. This was consistent with clinical observations and findings at necropsy. The increased incidence may be associated with a possible tendency towards a slight increase in urination in the open field in these animals, however, the relationship, if any, is uncertain. The increased incidence of perineal soiling was considered to be a secondary or indirect effect related to lack of grooming possibly due to urinary acidification or to the presence of excretory products of the test substance in the urine. Motor activity, fore-limb and hind-limb grip strength, landing foot splay, rectal temperature and auditory brainstem response were not affected by treatment in either sex. No organ weights were provided in the study report. However, brain weight was unaffected by treatment after 12 and 24 months in the concurrent 2-year dietary oncogenicity study in Fischer 344 rats and there were no corroborating treatment-related gross pathological, histopathological or neuropathological findings to suggest a treatment-related effect on brain weight. There were no treatment-related gross pathological or histopathological findings and no treatment-related neuropathological findings in the central or peripheral nervous system. There was no evidence of neurotoxicity in either sex.

The LOAEL for systemic toxicity was 500 mg/kg bw/d based on lower body weight and body-weight gain (males) in the absence of any treatment-related effect on food consumption. The NOAEL for systemic toxicity was 250 mg/kg bw/d.

The LOAEL for neurotoxicity was not determined. There was no evidence of neurotoxicity in either sex; therefore, the NOAEL for neurotoxicity was 250 mg/kg bw/d, the highest dose tested in females.

C. Study deficiencies: No organ weight data was obtained in the study report; therefore, brain weights were not provided in the study report. Currently accepted guidelines for subchronic neurotoxicity studies, OPPTS 870.6200 (Neurotoxicity Screening Battery), does not indicate that brain weights are required. However, in the concurrent rat 2-year dietary chronic toxicity / oncogenicity study (DACO 4.4.4 - Johnson, K. H., et al. November 24, 1997. Laboratory Project Study ID: 960004), brain weight was unaffected by treatment after 12 and 24 months and there were no corroborating treatment-related gross pathological, histopathological or neuropathological findings to suggest a treatment-related effect on brain weight. There were no study deficiencies which would impact on the outcome of the study; therefore, this study is classified as acceptable as a subchronic neurotoxicity study in rats (870.6200; no OECD guideline).