DATA EVALUATION RECORD

IMAZOSULFURON

Study Type: Non-guideline; Positive Control Studies

Work Assignment No. 6-1-226 (MRIDs 47766816 through 47766819)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer:
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 10/01/09

Secondary Reviewer:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 10/01/09

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 10/01/09

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 10/01/09

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EPA Reviewer: Sheila Healy **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 12/23/10
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 12/23/10

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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Positive Control Studies.

PC CODE: 118602 **DP BARCODE:** 366664
TXR #: 0055236

TEST MATERIAL (PURITY): Acrylamide (97%); Amphetamine sulfate (>= 99%); Chlorpromazine HCl (>= 98%); Chlordiazepoxide HCl (100%); and Morphine sulfate (100%)

SYNONYMS: Not applicable

CITATIONS: Draper, C. (2007) Acrylamide positive control study for neuropathology in adult HAN Wistar rat (acute neurotoxicity study age at termination). Syngenta Central Toxicology Laboratory, Cheshire, UK. Laboratory Project ID: WR0626-REG, January 10, 2007. MRID 47766816. Unpublished.

Chivers, S. (2007) Amphetamine sulfate and chlorpromazine hydrochloride positive control study for motor activity in the HAN Wistar rat. Syngenta Central Toxicology Laboratory, Cheshire, UK. Laboratory Project ID: WR0578-REG, March 2, 2007. MRID 47766817. Unpublished.

Chivers, S. (2007) Chlordiazepoxide hydrochloride positive control for grip strength in adult HAN Wistar rat. Syngenta Central Toxicology Laboratory, Cheshire, UK. Laboratory Project ID: WR0579-REG, February 27, 2007. MRID 47766818. Unpublished.

Chivers, S. (2007) Morphine sulfate positive control study for tail flick response in the HAN Wistar rat. Syngenta Central Toxicology Laboratory, Cheshire, UK. Laboratory Project ID: WR0580-REG, February 27, 2007. MRID 47766819. Unpublished.

SUBMITTER: Valent USA Corporation, 1600 Riviera Ave, Suite 200, Walnut Creek, CA

EXECUTIVE SUMMARY: Four non-guideline neurotoxicity studies (MRIDs 47766816, 47766817, 47766818, and 47766819) were provided to validate the ability of the procedures and observers of Syngenta Central Toxicology Laboratory (Cheshire, UK) to detect the effect of chemicals on FOB parameters, motor activity, behavior, neuropathological lesions, and other parameters indicative of neurotoxicity.

In the first study (MRID 47766816), acrylamide (97% a.i., Batch # 148571, Lot 16111HB) was administered in the diet to 10 HAN Wistar rats/sex/group at dose levels of 0, 250, and 500 ppm (equivalent to 0/0, 35.1/30.5, and 69.7/58.5 mg/kg/day [M/F], respectively) for 16-19 days (250 ppm) or 9-12 days (500 ppm), and the animals were observed for up to 29 days. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all surviving rats at Week 4. At study termination, all surviving animals were anesthetized and perfused *in situ* for collection of tissues for neuropathological examination. The tissues from all control animals and 5 rats/sex in the 250 and 500 ppm groups were subjected to histo-pathological evaluation of brain and peripheral nervous system tissues.

In the second study (MRID 47766817), amphetamine sulfate (>=99% a.i., Batch # 101K3351) and chlorpromazine HCl (>=98% a.i., Batch # 122K2522), both in deionized water, were each administered once via i.p. injection (5 mL/kg) to separate groups of 10 HAN Wistar rats/sex/dose at doses of 0, 0.1, 1, or 10 mg/kg. Motor activity testing was performed at approximately 1 hour post-dosing.

In the third study (MRID 47766818), chlordiazepoxide HCl (100% a.i., Batch # 94H1023) in deionized water was administered once via i.p. injection (5 mL/kg) to 10 HAN Wistar rats/sex/dose at doses of 0, 10, 20, or 40 mg/kg. Fore- and hind-limb grip strength were assessments were performed approximately 1 hour post-dosing.

In the final study (MRID 47766819), morphine sulfate (100% a.i., Batch # L1730/1) in physiological saline was administered once via i.p. injection (5 mL/kg) to 10 HAN Wistar rats/sex/dose at doses of 0, 10, 50, or 100 mg/kg. Time to tail-flick assessment was performed approximately 30 minutes post-dosing.

Acrylamide (250 and 500 ppm, dietary exposure for 16-19 or 9-12 days, respectively) caused mortality (500 ppm only), decreases in body weight and food consumption, clinical signs of toxicity (abnormal gait and reduced hindlimb function), FOB effects (increased landing foot splay and reduced time to tail-flick), reduced motor activity, and dose-dependent (incidence and severity) demyelination/nerve fiber degeneration of peripheral nerves. **Amphetamine sulfate** (0.1, 1, and 10 mg/kg, single i.p. dose) dose-dependently increased locomotor activity in both sexes. **Chlorpromazine HCl** (10 mg/kg, single i.p. dose) reduced locomotor activity in both sexes. **Chlordiazepoxide HCl** (10, 20, and 40 mg/kg, single i.p. dose) caused dose-dependent decreases in fore- and hind-limb grip strength. **Morphine sulfate** (10, 50, and 100 mg/kg, single i.p. dose) caused a dose-dependent increased latency in time to tail-flick.

These studies are classified as **acceptable/non-guideline** and satisfy the purpose of generating positive control data that demonstrate the performing laboratory's ability to detect neurotoxic effects on FOB parameters, motor activity, behavior, neuropathological lesions, and other parameters indicative of neurotoxicity, and validate the methodology used in these assessments.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided for all studies. It was stated that the studies were conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106 as amended 2004, Statutory Instrument No.

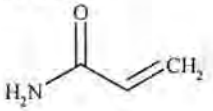
994), and that these Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material #1: Acrylamide

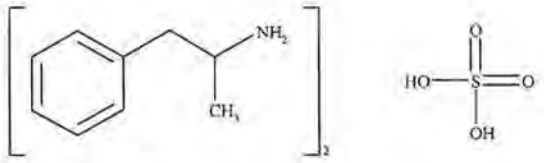
Description: White solid
Batch #: 148571, Lot 16111HB
Purity: 97% a.i.
Stability: Not reported
CAS # of TGAI: 79-06-1
Structure:



Vehicle: Diet

Test material #2: Amphetamine sulfate

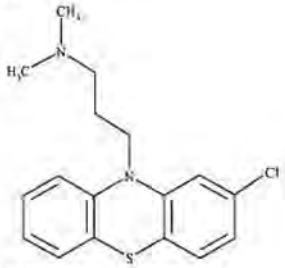
Description: White solid
Batch #: 101K3351
Purity: ≥99% a.i.
Stability: Not reported
CAS # of TGAI: 60-13-9
Structure:



Vehicle: Deionized water

Test material #3: Chlorpromazine hydrochloride

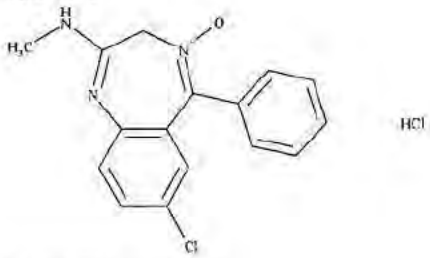
Description: White solid
Batch #: 122K2522
Purity: ≥98% a.i.
Stability: Not reported
CAS # of TGAI: 69-09-0
Structure:



Vehicle: Deionized water

Test material #4: Chlordiazepoxide hydrochloride

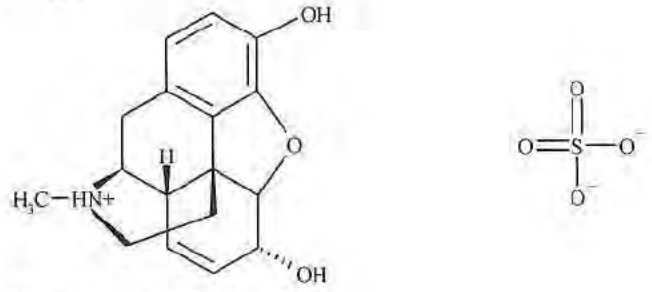
Description: Off-white solid
Batch #: 94H1023
Purity: 100% a.i.
Stability: Not reported
CAS # of TGAI: 438-41-5
Structure:



Vehicle: Deionized water

Test material #5: Morphine sulfate

Description: White powder
Batch #: L1730/1
Purity: 100% a.i. (assumed)
Stability: Not reported
CAS # of TGAI: 64-31-3
Structure:



Vehicle: Physiological saline

2. Test animals

Species: Rat
Strain: HsdRccHan: WIST
Age at dosing: Approximately 4-5 weeks (acrylamide), 6 weeks (amphetamine and chlorpromazine), 8-9 weeks (morphine) and 9 weeks (chlordiazepoxide)
Mean weight at Day 1: 78.6-79.4 g males and 89.5-91.5 g females in the acrylamide study (body weights were not provided in the other 3 studies)
Source: Harlan UK Limited (Bicester, Oxon, England)
Housing: Groups of 5 rats of the same sex/cage (cage type not reported)
Diet: Rat and Mouse No.1 Maintenance Diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*, except during neurobehavioral testing
Water: Tap water, *ad libitum*, except during neurobehavioral testing
Environmental conditions
- **Temperature:** 22±3°C
- **Humidity:** 30-70%
- **Air changes:** At least 15/hr
- **Photoperiod:** 12 hrs dark/ 12 hrs light

Acclimation period: At least 5 days

B. STUDY DESIGN

1. **In-life dates:** The in-life dates were not reported; however, the experimental start and termination dates reported for the 4 studies were as follows:
   May 24, 2005 to June 1, 2005 (MRID 47766819)
   May 24, 2005 to June 3, 2005 (MRID 47766818)
   August 9, 2005 to August 19, 2005 (MRID 47766817)
   May 16, 2006 to October 30, 2006 (MRID 47766816)

2. **Animal assignment:** For each study, the animals were randomly assigned to the test groups noted in Table 1 using body weight and a Latin Square design.

TABLE 1. Study design [a]

Acrylamide (MRID 47766816)			
Experimental parameter	Dose (ppm)		
	0	250	500
Achieved dose (mg/kg/day; M/F)	0/0	35.1/30.5	69.7/58.5
Total number of animals	10/sex	10/sex	10/sex
Behavioral testing (FOB, Motor activity)	10/sex	10/sex	10/sex
Neuropathology	10/sex	5/sex	5/sex

Amphetamine sulfate (MRID 47766817)				
Experimental parameter	Dose (mg/kg)			
	0	0.1	1	10
Motor activity	10/sex[b]	10/sex	10/sex	10/sex

Chlorpromazine hydrochloride (MRID 47766817)				
Experimental parameter	Dose (mg/kg)			
	0	0.1	1	10
Motor activity	10/sex[b]	10/sex	10/sex	10/sex

Chlordiazepoxide hydrochloride (MRID 47766818)				
Experimental parameter	Dose (mg/kg)			
	0	10	20	40
Grip strength	10/sex	10/sex	10/sex	10/sex

Morphine sulfate (MRID 47766819)				
Experimental parameter	Dose (mg/kg)			
	0	10	50	100
Tail-flick response	10/sex	10/sex	10/sex	10/sex

a Data were extracted from pages 14, 16, and 18 of MRID 47766816, page 12 of MRID 47766817, page 12 of MRID 47766818, and page 12 of MRID 47766819.
b The same 10 rats/sex served as the controls for the amphetamine sulfate and chlorpromazine HCl studies.

3. **Test substance preparation, dosing, and analysis:** The test diets for the **acrylamide** study were prepared as 20 kg batches and stored at room temperature until used. For each concentration, the appropriate amount of acrylamide was mixed with 500 g of diet to form a premix. Each premix was diluted with additional RM1 diet to prepare the 250 and 500 ppm test diets. The rats were provided the test diets *ad libitum* (except during behavioral testing). The duration of exposure was scheduled for 29 days; however, due to low body weight and food consumption, the animals were removed from the test diets after 16-19 (250 ppm) or 9-12 days (500 ppm), and given control diet until termination. The **amphetamine sulfate** and **chlorpromazine HCl** dosing solutions were each prepared by mixing the appropriate amount

of test material with deionized water to form concentrations of 0.02, 0.2, and 2 mg/mL. Animals received a single i.p. injection of the test material at a dose volume of 5 mL/kg. The **chlordiazepoxide HCl** dosing solutions were prepared by mixing the appropriate amount of test material with deionized water to form concentrations of 2, 4, and 8 mg/mL. Animals received a single i.p. injection of the test material at a dose volume of 5 mL/kg. The **morphine sulfate** dosing solutions were prepared by mixing the appropriate amount of test material with physiological saline to form concentrations of 2, 10, and 20 mg/mL. Animals received a single i.p. injection of the test material at a dose volume of 5 mL/kg. It was not reported if the test diets or formulations were analyzed for stability, homogeneity, or actual concentration.

4. **Statistics:** The data were analyzed using the following methods, and sexes were analyzed separately. Body weight was analyzed using analysis of covariance (ANCOVA) on initial (Day 1) body weights. Weekly food consumption, motor activity, time to tail-flick, landing foot splay, and grip strength (fore- and hind-limb) were analyzed using Analysis of variance (ANOVA). ANOVA and ANCOVA were carried out using the MIXED procedure in SAS (2004). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group's least-squares mean with the control group's least-squares mean using a two-sided Student's t-test, based on error mean square analysis. Significance was denoted at $p \leq 0.05$ and 0.01. The reviewers consider the statistical methods to be appropriate.

C. METHODS / OBSERVATIONS

1. **Mortality and clinical observations:** In all studies, animals were examined prior to dosing to ensure that they were physically normal and exhibited normal activity. Cage-side observations were performed daily for mortality and clinical signs of toxicity. Detailed physical examinations were performed at least weekly in the acrylamide study, and prior to dosing in all other studies. During Week 4 in the acrylamide study, the detailed physical examination was performed as part of the functional observation battery (FOB).

2. **Body weight:** In all studies, body weight was recorded on Day 1 (prior to initiation of dosing). Additionally in the acrylamide study, animals were weighed weekly during the exposure period until termination.

3. **Food consumption:** In the acrylamide study, food residues and the amount of food given were recorded at weekly intervals or more frequently if required, and the food consumption calculated, at weekly intervals, as a mean value (g food/rat/day) for each cage. Mean chemical intake (mg/kg/day) was calculated from the nominal dietary concentration, food consumption, and body weight data, and is reported in Table 1 of this DER. Food consumption was not recorded in any other study.

4. Neurobehavioral assessment

a. Functional observational battery (FOB): In the acrylamide study, all animals were subjected to a FOB during Week 4. The FOB was conducted by one observer who was unaware as to the treatment status of the animals. The scoring criteria for the FOB were not provided, and the time in the open-field was not reported. The following CHECKED (X) parameters were examined:

	HOMECAGE OBSERVATIONS
X	Vocalization
X	Bizarre behavior
	SENSORY OBSERVATIONS
X	Righting reflex
X	Startle response
X	Splay reflex
X	Visual placing
X	Pupil response
X	Palpebral membrane reflex
X	Corneal reflex
X	Pinna reflex
X	Pain response (toe-pinch)
X	Approach response
X	Touch response

	HANDLING OBSERVATIONS
X	Reactivity
X	Convulsions
X	Vocalization
X	Tremors
X	Piloerection
X	Skin color
X	Appearance / grooming
X	Hyper- / hypothermia
X	Lacrimation / chromodacryorrhea
X	Ptosis
X	Eye prominence
X	Miosis / mydriasis
X	Staining
X	Salivation
X	Respiratory abnormalities
X	Thin appearance
X	Muscle tone
X	Urination / defecation

	OPEN FIELD OBSERVATIONS
X	Arousal / general activity level
X	Posture
X	Bizarre behavior
X	Convulsions (clonic / tonic)
X	Vocalization
X	Mobility
X	Tremors
X	Gait abnormalities
X	Reduced limb function
X	Curvature of the spine
X	Piloerection
X	Appearance / grooming
X	Urination / defecation
	NEUROMUSCULAR OBSERV.
X	Forelimb grip strength
X	Hindlimb grip strength
X	Landing foot splay
X	Time to tail-flick

b. Locomotor activity: Locomotor activity was evaluated approximately 1 hour post-dosing in the amphetamine sulfate and chlorpromazine HCl studies, and following the FOB in the acrylamide study. Activity was monitored by an automated Coulbourn Lab Linc Infra-red Motion Activity System. Animals were individually placed in stainless steel cages with an infra-red sensor attached. Each test session consisted of ten 5-minute intervals, totaling 50 minutes of testing per animal. It was stated that treatment groups were counter-balanced across test times and devices, and that motor activity was assessed in a separate room to minimize disturbances.

c. Grip strength: Muscle weakness (fore- and hind-limb grip strength) was assessed approximately 1 hour post-dosing in the chlordiazepoxide HCl study,. Details of the methodology were not provided.

d. Tail-flick response: Tail-flick response was assessed using the UGO Basile Tail Flick Unit approximately 30 minutes following dosing in the morphine sulfate study,. The testing device was calibrated before use on each study day, and was adjusted if necessary to produce an energy output of nominally 250 mW/cm^2. Once the animal's tail was positioned over the heat source the test was started, activating the heat source and timer. The equipment

recorded the time (sec) from the start until the animal flicked/removed its tail from the heat source. A cut-off time of 22.3-22.4 sec was used to prevent any burns to the animal. If the animal did not tail-flick before the cut-off time, this value was used as the animal's latency to tail-flick. The reviewers assume the same equipment was used to determine the time to tail-flick in the acrylamide study FOB.

5. **Sacrifice and pathology:** All animals in the amphetamine sulfate, chlorpromazine HCL, chlordiazepoxide HCL, and morphine sulfate studies survived to scheduled termination and were sacrificed by over-exposure to halothane Ph Eur. Vapor followed by exsanguination, and were discarded without further examination.

   In the acrylamide study, rats killed *in extremis* during the study were sacrificed by over-exposure to halothane Ph Eur. Vapor followed by exsanguination. All animals that were found dead, sacrificed *in extremis*, or sacrificed at scheduled termination were subjected to a gross necropsy. Tissues were not collected from animals found dead or sacrificed *in extremis*. At scheduled termination, all surviving animals were anesthetized with barbiturate (compound not specified) by intraperitoneal injection, and killed by *in situ* perfusion fixation with formol saline. The brain, spinal cord (cervical and lumbar swellings with dorsal and ventral root fibers and dorsal root ganglia), eyes (with optic nerves and retinas), selected bilateral peripheral nerves (proximal sciatic, and proximal and distal tibial), and gastrocnemius muscle were collected and perfused in an appropriate fixative. The brains of the control group animals were weighed following overnight fixation, and the brains from the 250 and 500 ppm animals were stored in formol saline without further processing.

   The following CHECKED (X) tissues from all controls (10/sex) and from 5 rats/sex/dose in the 250 and 500 ppm groups were further processed and examined microscopically:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
	Forebrain		Mid-thigh
	Midbrain	X	Sciatic notch
	Cerebrum		
	Cerebellum		
	Pons		
	Medulla oblongata		**OTHER**
			Sural nerve
	SPINAL CORD	X	Tibial nerve (proximal and distal)
X	Cervical swelling		Peroneal nerve
	Thoracic swelling	X	Cervical dorsal root ganglion
X	Lumbar swelling	X	Cervical dorsal root fibers
		X	Cervical ventral root fibers
	OTHER	X	Lumbar dorsal root ganglion
	Gasserian ganglion	X	Lumbar dorsal root fibers
X	Optic nerves	X	Lumbar ventral root fibers
X	Eyes		
X	Gastrocnemius muscle		

The sciatic and tibial nerves were embedded in resin, sectioned, and stained in toluidine blue. All other tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** In the acrylamide study, 2 males and 1 female in the 500 ppm group (animals #s 25 & 29, and 60, respectively) were killed *in extremis* during the study due to body weight loss and severe clinical signs (including abnormal gait, reduced hindlimb function, reduced stability, sides pinched in, and piloerection). Additionally, one 500 ppm male (# 23) was found dead on Day 11. This male displayed similar signs as those reported above prior to death. All other animals survived to scheduled sacrifice.

2. **Clinical signs:** In the acrylamide study, increased incidences (# affected/10 vs. 0/10 controls, unless otherwise stated) of the following treatment-related clinical signs were observed in the 250 and 500 ppm groups (Table 2): (i) reduced hindlimb function (8-10 males and all treated females); (ii) abnormal (frog-like) gait (7-9 males vs. 1 control and 9-10 females); (iii) tip toe gait (4-5 males vs. 2 controls and 8 females each dose); (iv) reduced stability (1-3 males and 3-8 females); (v) hunched posture (1-2 males and 6-7 females); (vi) piloerection (4 males at 500 ppm and 1-9 females); (vii) sides pinched in (2 males and 6 females at 500 ppm only); and (viii) subdued (4 males at 500 ppm). Clinical signs data were not provided for any other study.

TABLE 2. Incidence (# affected/# of times observed) of selected clinical signs in rats exposed to acrylamide in the diet for up to 19 days. [a]

Observation	Dose (ppm)		
	0	250[b]	500[b]
Males			
Reduced hindlimb function	0/0	8/81	10/65
Abnormal gait (frog-like)	1/1	7/60	9/84
Tip toe gait	2/2	5/15	4/17
Reduced stability	0/0	1/7	3/7
Hunched posture	0/0	2/11	1/3
Piloerection	0/0	0/0	4/11
Subdued	0/0	0/0	4/7
Sides pinched in	0/0	0/0	2/3
Females			
Reduced hindlimb function	0/0	10/102	10/144
Abnormal gait (frog-like)	0/0	9/94	10/143
Tip toe gait	0/0	8/55	8/48
Reduced stability	0/0	3/20	8/62
Hunched posture	0/0	6/46	7/63
Piloerection	0/0	1/2	9/30
Sides pinched in	0/0	0/0	6/26

a Data were obtained from Table 1 on pages 25-30 of MRID 47766816; n=10.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).

B. BODY WEIGHT AND FOOD CONSUMPTION: In the acrylamide study, decreases ($p<0.05$) in body weights were observed at 250 ppm in the males (↓10-18% throughout the study) and females (↓6-10% on Days 15-29), and at 500 ppm in both sexes (↓13-21%) throughout the study (Table 3). Food consumption was decreased ($p<0.05$) by 10-21% in the 250 ppm animals during Week 2, by 25-38% in the 500 ppm males and females during Weeks 1 and 2, and by 23% in the 500 ppm females during Week 4. Body weight and food consumption data were not reported in the other studies.

TABLE 3. Mean body weight (g) and mean (±SD) food consumption (g/rat/day) in rats exposed to acrylamide in the diet for up to 19 days. [a]

Interval	Dose (ppm)					
	0	250[b]	500[b]	0	250[b]	500[b]
	Males			Females		
Body weight						
Day 1	79.4±12.7	78.8±11.6	78.6±12.5	91.5±7.6	91.4±8.8	89.5±9.0
Day 8	118.4	106.5**(↓10)	93.7**(↓21)	112.7	110.1	98.5**(↓13)
Day 15	157.0	128.0**(↓18)	128.1**(↓18)	129.6	117.0**(↓10)	103.6*(↓20)
Day 29	226.8	199.6**(↓12)	194.5**(↓14)	157.6	148.8*(↓6)	135.5**(↓14)
Food consumption						
Week 1	16.3±1.0	14.2±0.3	12.0±0.8*(↓26)	14.6±0.4	13.7±0.7	10.9±0.2**(↓25)
Week 2	18.9±1.1	14.9±0.6*(↓21)	11.9±0.2**(↓37)	13.6±0.2	12.3±0.3*(↓10)	8.6±0.5**(↓38)
Week 4	20.0±1.1	18.4±0.6	18.6±2.1	14.8±0.4	13.8±0.7	11.4±0.0**(↓23)

a Data were obtained from Tables 2 and 3 on pages 32-35 of MRID 47766816; n=10 for body weight and 2 cages for food consumption. Percent difference from controls (calculated by reviewers) is presented parenthetically. Body weight data for Days 8, 15, and 29 are reported as adjusted means based on intergroup differences in initial group mean body weight.

b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

C. NEUROBEHAVIORAL RESULTS

1. FOB findings: In the acrylamide study, the animals were only exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm). The FOB was performed on Day 28, and the animals had shown signs of recovery, so the effects were not measured at peak effect levels. In the treated groups, abnormal gait was observed in 4-5 males and 3-5 females vs. 1 control male (Table 4a). Other treatment-related clinical signs observed previously (reduced hindlimb function, reduced stability, upward curvature of the spine, and piloerection) were only observed in 1 or 2 treated animals during the FOB.

Landing foot splay was increased ($p<0.01$) by 66-98% in both sexes at 250 ppm (Table 4b). Additionally, foot splay was increased by 38% in the 500 ppm males, but did not attain statistical significance. It was stated that this finding was likely related to the fact that the 250 ppm group was exposed to the test diet for a longer period (16-19 days) than the 500 ppm group (9-12 days). FOB evaluations were not performed in the other studies.

TABLE 4a. Incidence (# affected) of selected FOB findings in rats exposed to acrylamide in the diet for up to 19 days. [a]						
Observation	Dose (ppm)					
	0	250[b]	500[b]	0	250[b]	500[b]
	Males			Females		
Abnormal gait	1	4	5	0	5	3
Reduced hindlimb function (slight)	0	1	1	0	0	0
Reduced stability	0	0	0	0	2	0
Upward curvature of the spine (slight)	0	0	0	0	1	2
Piloerection	0	0	1	0	0	0

a Data were obtained from Table 5 on pages 38-44 of MRID 47766816; n=7-10.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).

TABLE 4b. Mean (±SD) landing foot splay (mm) in rats exposed to acrylamide in the diet for up to 19 days. [a]			
Sex	Dose (ppm)		
	0	250[b]	500[b]
Males	54.7±19.0	90.9±24.8**(↑66)	75.3±25.6 (↑38)
Females	50.1±10.8	99.0±23.8**(↑98)	55.7±27.4

a Data were obtained from Table 6 on page 45 of MRID 47766816; n=7-10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).
** Significantly different from controls at p<0.01

2. **Motor activity:** In the acrylamide study, total session motor activity was decreased (p<0.05) by 25% in the 250 ppm males (Table 5a). Decreases (↓34-35%, p<0.05) in interval motor activity were observed in this group during the 16-20 minute and 21-25 minute intervals. Increases (p<0.05) in motor activity were noted during the 36-40 minute interval in the 250 ppm females and 500 ppm males, and during the 41-45 minute interval in the 500 ppm females. However, these increases did not result in significant increases in overall motor activity counts in these groups.

TABLE 5a. Mean (±SD) overall motor activity (counts) in rats exposed to acrylamide in the diet for up to 19 days. [a]			
Sex	Dose (ppm)		
	0	250[b]	500[b]
Males	381.3±110.1	285.2±82.8*(↓25)	371.6±91.5
Females	256.8±110.7	284.1±78.6	279.0±96.7

a Data were obtained from Table 10 on pages 49 and 50 of MRID 47766816; n=7-10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).
* Significantly different from controls at p<0.05

In the amphetamine sulfate study, total session motor activity was increased (p<0.01) in both sexes by 114-117% at 1 mg/kg and by 132-246% at 10 mg/kg at 1 hour post-dosing (Table 5b). Interval motor activity was also increased (p<0.05) at most intervals in these groups. In the chlorpromazine HCl study, total session motor activity was decreased at 10 mg/kg in the males (↓35%, not statistically significant) and females (↓53%, p<0.01) at 1 hour post-dosing.

Decreases ($p<0.05$) in interval motor activity were limited to the 1-5 and 6-10 minute interval in the 10 mg/kg females.

TABLE 5b. Mean (±SD) overall motor activity (counts) at 1 hour post-dosing in rats dosed once via i.p. with amphetamine sulfate or chlorpromazine HCl. [a]

Sex	Dose (mg/kg)			
	0[b]	0.1	1	10
Amphetamine sulfate				
Males	207.7±83.7	327.1±108.2	449.7±107.9**(↑117)	718.5±285.6**(↑246)
Females	279.8±131.9	324.8±148.2	599.3±128.2**(↑114)	649.4±298.5**(↑132)
Chlorpromazine HCl				
Males	207.7±83.7	295.1±86.4	279.4±114.1	134.7±110.2 (↓35)
Females	279.8±131.9	306.4±76.1	289.4±98.2	132.1±134.8**(↓53)

a Data were obtained from Tables 1 and 2 on pages 18-21 of MRID 47766817, n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b The same 10 rats/sex served as the controls for the amphetamine sulfate and chlorpromazine HCl studies.
** Significantly different from controls at $p<0.01$

3. **Tail-flick response:** In the morphine sulfate study, the time to tail-flick was increased ($p<0.01$) at 10 mg/kg and above by 120-386% in the males and by 97-191% in the females at 30 minutes post-dosing (Table 6a).

TABLE 6a. Mean (±SD) time to tail-flick (sec) at 30 minutes post-dosing in rats dosed once via i.p. with morphine sulfate. [a]

Sex	Dose (mg/kg)			
	0	10	50	100
Males	4.4±2.4	9.7±6.4**(↑120)	21.4±3.5**(↑386)	20.5±3.8**(↑366)
Females	7.5±3.9	14.8±7.0**(↑97)	19.8±5.6**(↑164)	21.8±1.9**(↑191)

a Data were obtained from Table 1 on page 17 of MRID 47766819, n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
** Significantly different from controls at $p<0.01$

In the acrylamide study, the time to tail-flick was decreased in both sexes at 250 ppm (↓34-48%, $p<0.05$) and in the 500 ppm females (↓52%, $p<0.01$; Table 6b). The time to tail-flick was also decreased by 27% in the 500 ppm males, but it did not attain statistical significance.

TABLE 6b. Mean (±SD) time to tail-flick (sec) in rats exposed to acrylamide in the diet for up to 19 days. [a]

Sex	Dose (ppm)		
	0	250[b]	500[b]
Males	6.0±4.2	3.1±1.3*(↓48)	4.4±1.1 (↓27)
Females	5.0±2.0	3.3±1.0*(↓34)	2.4±1.0**(↓52)

a Data were obtained from Table 7 on page 46 of MRID 47766816, n=7-10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).
* Significantly different from controls at $p<0.05$
** Significantly different from controls at $p<0.01$

4. **Grip strength:** In the chlordiazepoxide HCl study, hind-limb grip strength was decreased ($p<0.01$) at 10 mg/kg and above by 30-50% in both sexes (Table 7). Fore-limb grip strength was decreased ($p<0.01$) by 25-44% at 10 mg/kg and above in the females, and by 35% ($p<0.05$) in the 40 mg/kg males. It was stated that some animals were unable to grip the grip strength assessment bars (an effect of the test material); therefore, no values were obtained for these animals, and some test groups had less than 10 subjects. There were no effects of treatment on fore- or hind-limb grip strength at Day 28 in the acrylamide study.

TABLE 7. Mean (±SD) fore- and hind-limb grip strength (g) at 1 hour post-dosing in rats dosed once via i.p. with chlordiazepoxide HCl. [a]

Sex	Dose (mg/kg)			
	0	10	20[b]	40[b]
Hind-limb				
Males	365±60	257±84**(↓30)	245±70**(↓33)	209±42**(↓43)
Females	342±97	228±81**(↓33)	185±48**(↓46)	171±59**(↓50)
Fore-limb				
Males	699±183	644±191	663±126	457±53*(↓35)
Females	753±128	564±131**(↓25)	486±132**(↓35)	424±162**(↓44)

a Data were obtained from Tables 1 and 2 on page 17-18 of MRID 47766818, n=10, except as noted below. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b n=9 in the 20 mg/kg females, n=4 in the 40 mg/kg males, and n=8 in the 40 mg/kg females
* Significantly different from controls at $p<0.05$
** Significantly different from controls at $p<0.01$

D. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** No treatment-related gross lesions were noted in any animal in the acrylamide study. Animals in the other studies were not subjected to a gross necropsy.

2. **Neuropathology:** In the acrylamide study, increased incidence (# affected/5 vs. 0/10 controls, unless otherwise stated) of minimal to slight demyelination/nerve fiber degeneration was observed at 250 and 500 ppm as follows (Table 8): (i) distal tibial nerve (all treated animals); (ii) proximal tibial nerve (all treated animals vs. 1 male and 2 female controls); and (iii) proximal sciatic nerve (all treated animals vs. 1 control of each sex). Microscopic examinations were not performed in the other studies.

TABLE 8. Incidence (# affected) of demyelination/nerve fiber degeneration in rats exposed to acrylamide in the diet for up to 19 days. [a]

Tissue	Severity	Dose (ppm)		
		0	250[b]	500[b]
Males				
Distal tibial nerve	Total	0	5	5
	Minimal	0	3	3
	Slight	0	2	2
Proximal tibial nerve	Total	1	5	5
	Minimal	1	3	4
	Slight	0	2	1
Proximal sciatic nerve	Total	1	5	5
	Minimal	1	1	4
	Slight	0	4	1
Females				
Distal tibial nerve	Total	0	5	5
	Minimal	0	1	2
	Slight	0	4	3
Proximal tibial nerve	Total	2	5	5
	Minimal	2	1	1
	Slight	0	4	4
Proximal sciatic nerve	Total	1	5	5
	Minimal	1	1	3
	Slight	0	4	2

a Data were obtained from Table 14 on pages 54-56 of MRID 47766816, n=10 for controls and 5 for treated groups.
b Animals were exposed to the test material for 16-19 (250 ppm) or 9-12 days (500 ppm).

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The Investigators concluded that **acrylamide** (250 and 500 ppm) in the diet caused decreases in body weight and food consumption, clinical signs of toxicity (abnormal gait and reduced hindlimb function), FOB effects (increased landing foot splay, reduced time to tail-flick), reduced motor activity, and dose-dependent (incidence and severity) demyelination/nerve fiber degeneration of peripheral nerves. **Amphetamine sulfate** (0.1, 1, and 10 mg/kg, single i.p. dose) dose-dependently increased locomotor activity, whilst **chlorpromazine HCl** (10 mg/kg, single i.p. dose) reduced locomotor activity. **Chlordiazepoxide HCl** (10, 20, and 40 mg/kg, single i.p. dose) caused dose-dependent decreases in fore- and hind-limb grip strength, which validated the technique used to measure grip strength. **Morphine sulfate** (10, 50, and 100 mg/kg, single i.p. dose) caused a dose-dependent increased latency in time to tail-flick, which validated the technique used to determine sensory perception in rats.

B. **REVIEWER COMMENTS:** The reviewers agree with the Investigators conclusions, but would also add that acrylamide caused mortality at 500 ppm.

The positive control data generated by these studies demonstrate the performing laboratory's ability to detect neurotoxic effects on FOB parameters, motor activity, behavior, neuropathological lesions, and other parameters indicative of neurotoxicity, and validate the methodology used in these assessments.

C. **STUDY DEFICIENCIES:** None